DISSERTATION

# Biogeochemistry of sulfur in forest ecosystems with special focus on investigation of sulfur stable isotopes

zur Erlangung des Doktorgrades unter der Leitung von

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Dedicated in the memory of my grandmother, Hana

## Abstract

Understanding of the biogeochemistry of sulfur and the natural sulfur cycle is essential when the impact of sulfur emitted by combustion of fossil fuels on the environment or when sulfur deficiency in agricultural soils is investigated. Mutual relationships among sulfur species, like adsorbed sulfate, organic sulfur compounds or ester-sulfates, give first insights into biogeochemical processes of sulfur. As some of the processes (like immobilization of sulfate, mineralization of organic sulfur or weathering of sulfur-bearing minerals) cause significant changes in $^{34}S/^{32}S$ isotope ratios of (sulfate-)sulfur in soil solution, whereas other processes (like adsorption and desorption of sulfate) have no significant impact on the isotopic composition of sulfur, investigation of sulfur stable isotopes is a key to identify the predominant processes of the sulfur cycle in soil.

Multicollector inductively coupled plasma mass spectrometry (MC ICP-MS) is a technique enabling a direct measurement of $^{34}S/^{32}S$ isotope ratios in e.g. natural water samples and soil extracts with high sample throughput and relative combined measurement uncertainty of 0.02 %, which is sufficient for the investigation of sulfur biogeochemistry. As some elements, like Ca, K, Li or Na, that can be found in the matrix of natural samples (e.g., soil, soil solution), cause above given threshold concentrations significant fractionation of the measured sulfur stable isotopes, a sulfate-matrix separation is required prior to analysis. A method, applying an anion exchange resin on a plastic membrane was developed and validated for sulfate separation from water (e.g., soil solution) samples and subsequent analysis of S isotope ratios by MC ICP-MS.

A method, diffusive gradients in thin films (DGT), for a direct assessment of readily available and reversibly adsorbed soil sulfate was developed and validated. The method was compared to classical soil tests, and it showed significant correlation to water-extractable soil sulfate. The combination of the DGT technique and analysis by MC ICP-MS was demonstrated to be a suitable method for sulfate sampling with simultaneous matrix (e.g., Ca, K, Li, Na) separation and subsequent $^{34}S/^{32}S$ measurement.

A quantitative analysis of sulfur species obtained by sequential extraction indicated mineralization to be the predominant process of sulfur biogeochemistry in the investigated nutrient-rich Austrian forest soil. This was confirmed by measurement of $^{34}S/^{32}S$ isotope ratios of sulfate-sulfur in rain water and soil solution originating from the same area, purified by anion exchange resin on a plastic membrane. The DGT MC ICP-MS method applied to European soils indicated mineralization of soil S already during sulfate sampling.

The application to these samples demonstrated the usefulness of the methods developed within this thesis.

## Kurzfassung

Das Verständnis der Schwefelbiogeochemie und des Schwefelzyklus ist erforderlich, um den Einfluss vom Schwefel, der während Verbrennung von fossilen Brennstoffen entsteht, auf die Umwelt zu untersuchen, oder um den Schwefelmangel in Agrarböden zu erforschen. Die Zusammenhänge zwischen Schwefelspezien, wie dem adsorbierten Sulfat, organischen Schwefelverbindungen oder Ester-Sulfaten, können den ersten Einblick in die Biogeochemie geben. Da einige von den biogeochemischen Prozessen, wie Schwefel-Immobilisierung, Mineralisierung organischer Schwefelverbindungen oder Verwitterung von schwefelhaltigen Mineralen, die $^{34}S/^{32}S$ Isotopenverhältnisse von Sulfat-Schwefel in der Bodenlösung signifikant beeinflussen, während andere Prozesse, wie Adsorption und Desorption von Sulfat, keinen signifikanten Einfluss auf die Schwefelisotopie haben, stellt die Untersuchung der stabilen Schwefelisotope eine optimale Methode zur Identifizierung der vorwiegenden biogeochemischen Prozesse dar.

Multikollektor induktiv gekoppelte Plasma - Massenspektrometrie (MC ICP-MS) ist die Technik, die direkte Messung von $^{34}S/^{32}S$ Isotopenverhältnissen in Wasserproben und Bodenextrakten mit hohem Probendurchsatz ermöglicht. Die relative Messunsicherheit beträgt 0.02 %, was für Untersuchung der Schwefelbiogeochemie ausreichend ist. Da einige Elemente, wie Ca, K, Li oder Na, die z. B. in der Matrix von Bodenwasser oder Bodenextrakten zu finden sind, ab gewisser Konzentration signifikante Fraktionierung der gemessenen Schwefelisotopen verursachen, ist eine Sulfate - Matrix Trennung notwendig. Die optimale Methode für Wasserproben, mit hohem Probendurchsatz, ist die Trennung mittels eines Anionentauschers auf einer Kunststoffmembrane.

Eine passive Probenmethode, die Diffusionsgradienten in dünnen Filmen (DGT), wurde für direkte Entnahme von reversibel adsorbierten Bodensulfat entwickelt und validiert. Die Methode wurde mit klassischen Bodentests verglichen. Der DGT-S korrelierte signifikant mit Wasser-extrahierbarem Bodensulfat. Die Kopplung von DGT mit MC ICP-MS stellt eine Methode für Matrixtrennung während Bodensulfatentnahme mit anschließender $^{34}S/^{32}S$ Analyse dar.

Die quantitative Analyse von Schwefel in Spezien gewonnen durch die sequentielle Extraktion hat die Mineralisation als den vorwiegenden Prozess der Schwefelbiogeochemie in den untersuchten nahrungsreichen Böden angedeutet. Dies wurde durch die $^{34}S/^{32}S$ Analyse von Sulfat-Schwefel im Regenwasser und Bodenwasser aus dem gleichen Gelände bestätigt. Die Isotopenverhältnisse wurden nach Trennung von Sulfat von der Matrix durch Anionentauscher auf einer Kunststoffmembrane mittels MC ICP-MS bestimmt. Die DGT MC ICP-MS Methode hat Mineralisierung bereits während der S Entnahme angedeutet.

Die Anwendungen haben den Nutzwert der entwickelten Methoden bewiesen.

# Table of Contents

# 1 Introduction

## 1.1 Sulfur in the Environment

As "*the Lord rained on Sodom and Gomorrah sulfur*" [1], inter alia, the high oxidation-reduction reactivity of the element was demonstrated. Sulfur (S) has been known since ancient times, used as fumigant in Greece, to bleach clothes in Sicily etc. [2]. In the Middle Ages, sulfur was believed to be present in all metals and it represented one of the three (together with mercury and salt) cornerstones of alchemy [3]. Figure 1-1 represents the alchemical symbol for sulfur. However, first in 1777, Antoine Lavoisier declared sulfur to be an element and not a compound [2].

**Figure 1-1** Alchemical symbol for sulfur.

Although sulfur (S) is not present in all metals as believed by alchemists, it can be mostly found in compound with metals (e.g., $FeS_2$, $Fe_2S_4Cu_2$, PbS, $CaSO_4$, etc.) in the nature [4]. Sulfur is by weight the fifth most abundant element in the universe [5]. The high reactivity of S is given by the electron configuration of the S atom in ground state ($[Ne]3s^23p^4$) which enables S to occur in valence states from -2 to +6. Sulfur has four stable ($^{32}S$, $^{33}S$, $^{34}S$ and $^{36}S$) and 20 radioactive isotopes (from $^{26}S$ to $^{49}S$) [6]. The most common stable S isotope is $^{32}S$ (94.99 %), followed by $^{34}S$ (4.25 %), $^{33}S$ (0.75 %) and $^{36}S$ (0.01 %) [7]. From the radioactive isotopes, only $^{35}S$ has a half-life in days (87.4 days, [6]) and exists in nature [8]. The half-life of the other radioactive S isotopes lies between ns and hours and they all are anthropogenic.

On the Earth, sulfur occurs in numerous compounds, forms and phases. Inorganic (e.g., $H_2S$, $SO_2$) and organic (e.g., $CH_3SH$, $CH_3SCH_3$) gases containing S are present in the atmosphere, both of natural and anthropogenic origin [9]. Dissolved sulfates are predominant in natural waters, although thiosulfate or organic S compounds also can become the major S specie, e.g. in mine tailing or peat effluents, respectively [10]. In the soil, about 90 % of S is in the organic form [9, 11]. A schema of S cycle in the environment is displayed in Figure 1-2.

The speciation (biogeochemistry) of S in soil is driven mainly by microbial activity and associated chemical reactions and is discussed in following chapters in more detail. Sulfur is present in sulfide form (mainly with Fe, but also with other chalcophile elements like Pb or Zn) in metamorphic rocks. Sedimentary rocks (e.g., gypsum, sandstones) and sediments of biogenic origin (coal, oil) contain even more S than metamorphic rocks [9].

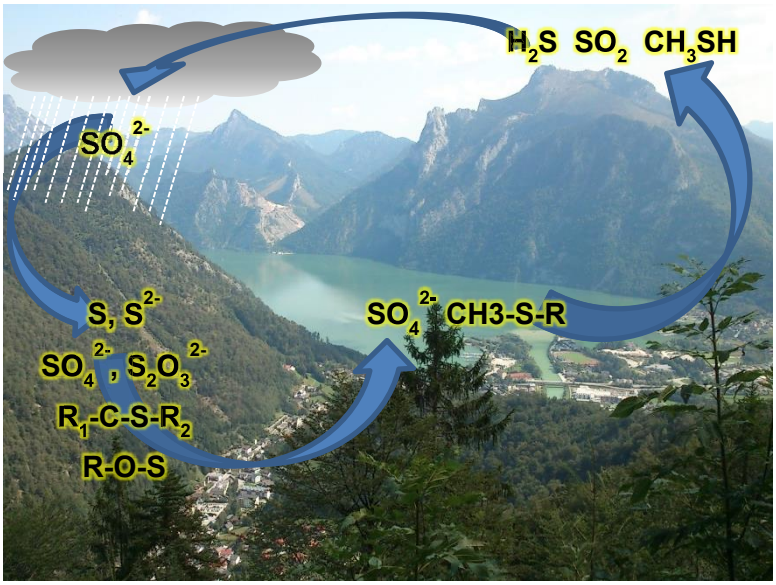


**Figure 1-2** Sulfur in the environment.

### 1.1.1 Sulfur as a pollutant

Combustion of biogenic sediments (coal, oil, and subsequently petroleum products), which are rich on S, causes large and extensive emissions of $SO_2$ [9, 12]. Only combustion of coal was responsible for about 60 % of total atmospheric pollutant $SO_2$ [12]. Natural sources (e.g., forest fires, volcanic activity) do not contribute significantly to the total $SO_2$ emissions [13].

Sulfur dioxide acts at higher concentrations (in average over 0.35 mg $SO_2$ $m^{-3}$ air) as a phytotoxin. It affects the pH of the leaf cells thus hampering the enzyme activity and oxidation-reaction processes, competes with $CO_2$ (reducing thus photosynthesis), and disrupts the opening and closure of stomata, leading to water stress. As a consequence, plants exposed to $SO_2$ may exhibit general chlorosis, necrotic spots and large blotches on leafs or even general growth reduction [14]. Moreover, most of the released $SO_2$ is oxidized to sulfate in the atmosphere. In this form, sulfur returns by wet or dry deposition to the Earth's surface [15]. Elevated sulfate deposition is, together with deposition of nitrate, the main cause of the "acid rain", which leads to soil acidification, mobilization of toxic metals and an increase in base cations losses from soil [16]. Sulfate partly binds to oxides, especially of Al and Fe, and may form Al-OH-$SO_4$ minerals which both leads to storage and delayed leaching of sulfate from soil.

Due to the negative impact of $SO_2$ and $SO_4^{2-}$ on vegetation, legislative restrictions were applied internationally. Thus, the $SO_2$ emissions (and, consequently, the $SO_4^{2-}$ amounts in wet and dry deposition) were reduced by 77 % in Austria between 1977 and 2013 [13].

### 1.1.2 Sulfur as a macronutrient

Sulfate is, on the other hand, the principal source of S for plants [9]. Sulfur is an essential macronutrient for all living species. It is part of amino acids (methionine, cysteine) and their proteins, coenzymes or sulfolipids. Sulfur compounds are involved in metabolism, electron transfer or in response to oxidative stress in plants [9]. Most of S is taken up from soil and distributed through the plant to target organs in $SO_4^{2-}$ form [17]. Due to the high solubility of the most of $SO_4^{2-}$ salts, sulfate is taken up by mass flow (dissolved in the pore water). Additionally, sulfur can be taken up as $SO_2$ directly through stomata [14]. In the cell organs, sulfur is metabolized (reduced) and immobilized. Unlike other macronutrients (e.g. N), the immobilized S can be oxidized back to sulfate (mobile form). During the decay of plant litter or of dead plants, sulfur metabolites can be oxidized and mineralized by soil microflora back to $SO_4^{2-}$, the phytoavailable S form.

According to some authors, S represents one of the most limiting factors in agricultural production nowadays [18, 19] and side- and plant-specific S fertilizing will be necessary. This is a new issue as in the last decades as the S ($SO_4^{2-}$) supply was guaranteed by S present in the atmosphere. However, the abovementioned regulation of $SO_2$ emissions, along with application of S-free fertilizers and pesticides and use of low-S energy sources (natural gas) led to S deficit in soils [18, 20].

## 1.2 Biogeochemistry of sulfur in forest soils

Atmospheric immissions are the main source of S (sulfate) in forest soils. Due to their negative impact on the environment, the immissions have been strongly reduced in the last decades (see above). These reductions led to negative input (bulk deposition) - output (seepage) S balances in many monitored watersheds [9, 21, 22]. As reported by Novak *et al*., sites with net S export are located in rather polluted areas, indicating a slow release of the historical high S loads [22].

### 1.2.1 Biogeochemical sulfur cycle

The negative input-output S balance can be caused by microbial mineralization of previously immobilized sulfur or desorption of previously adsorbed sulfate. Total S amount in living biomass increased in the second half of the 20$^{th}$ century [9]. As vegetation uptake and return to the soil is one of the major processes of the ecosystem cycling, the release (mineralization) of the plant S compounds can contribute to the net S export, as well. Weathering of S-bearing minerals could represent an additional S source. However, the contribution of weathering to the overall S balance was reported to be rather negligible [9].

Plants and soil microflora do not only use S to build amino acids, proteins or sulfolipids. *Desulfovibrio* is one of bacteria genera that use $SO_4^{2-}$ as an electron acceptor in anaerobic

soils. Along with S, the bacteria reduce Fe-oxides, leading to generation of Fe-sulfides or elemental S [23, 24].

The Fe-sulfides and elemental S are oxidized back to sulfate as soon as the soil is aerated, e.g. by *Thiobacillus ferrooxidans*. In pH-neutral soils, Fe-oxides and $H_2SO_4$ are generated. At low pH, however, sulfate minerals like $KFe^{3+}{}_3(OH)_6(SO_4)_2$ (jarosite) can be created. Other products of the oxidation of sulfide are gypsum and anhydrite, hardly soluble sulfate salt ($CaSO_4$ x 2 $H_2O$ and $CaSO_4$, $K_{sp}$ = 3.14 x $10^{-5}$ and 4.93 x $10^{-5}$ $mol^2$ $L^{-2}$, respectively) [25]. These minerals are subject to weather and the released sulfate to contribute to the overall S budget.

### 1.2.2 Sulfur stable isotope fractionation

Many natural processes are mass dependent, i.e. isotopes of different masses participate in these processes at different rates. Thus, when these reaction processes (transformations) are not complete, the reaction product has a different (fractionated) composition of isotopes from that of the reactant [26]. As a result of the fractionation, the relative abundance of S stable isotopes varies in nature.

The fractionation of S isotopes accompanied already the formation of Earth's mantle and core [27]. As about 97 % of Earth's sulfur is present in the core, the silicate mantle exhibits fractionated $^{34}S/^{32}S$ ratios according to the relevant metal–silicate partition coefficients [27]. Further fractionation was observed by seawater evaporation and crystallization of halite. Both halite and the related brine were depleted in $^{34}S$ as compared to the original seawater [28]. The uptake and mineralization of S compounds by microbes [29] and plants [17] have led to further fractionation of S stable isotopes, because - in general - the soil microflora prefers the lighter $^{32}S$ isotope [29]. Metabolic processes in plants lead to isotopic fractionation of S within the plant and to different S isotope ratios in different plant parts [17]. Such fractionation is reflected in the isotopic composition of matured plant material (peat, coal, oil) [12]. For comparison, differences in S stable isotopes in oil of different age are related to oil aging (and aging of the reservoir rocks), e.g. due to evaporation [12].

The weathering of S-bearing rocks and minerals which have S isotopic composition different from that of the surrounding soil may influence the isotope ratios of the soil S as well [30] (see Figure 1-3). However, as typically no correlation is found between $^{34}S/^{32}S$ isotope ratios in the runoff and in bedrock [22], the contribution of weathering to S runoff is - generally - negligible. Likens *et al*. reported that 1.28 kg S $ha^{-1}$ $yr^{-1}$ is released into the ecosystem by mineral weathering in Hubbard Brook experimental forest, contributing thus to less than 3 % of the overall S input [31].

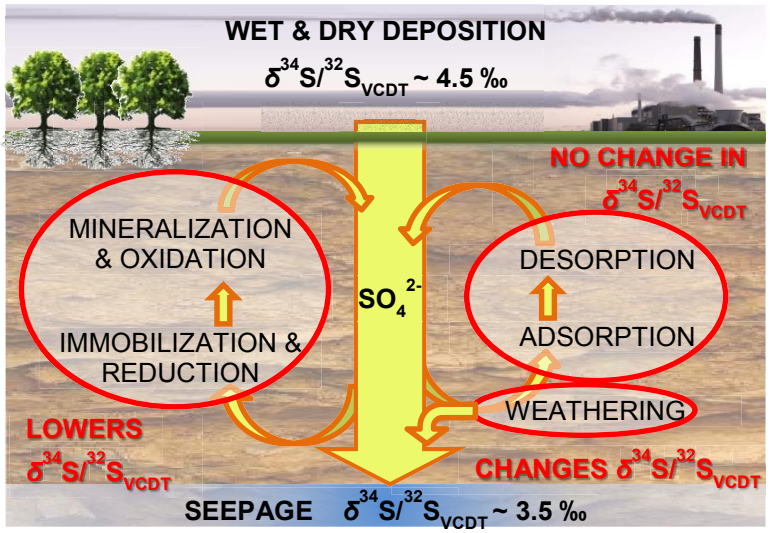


**Figure 1-3** Sulfur cycle and S isotopic fractionation in soil (adapted from [30]).

In contrast to biochemical reactions (metabolization, mineralization) performed by soil flora and mineral weathering of S-bearing minerals, adsorption and desorption of sulfate on soil (clay) particles and Fe/Al-sesquioxides in soil does not lead to a measurable isotopic fractionation [9, 29]. Thus, if the net positive S export from the catchments investigated e.g. by Novak *et al*. ([22], see above) was governed by desorption of the historically adsorbed S, the isotopic composition of S in the runoff (also termed “seepage”, see Figure 1-3) would correspond to the S isotopic composition of deposition.

Considering the abovementioned processes and their impact on the isotopic composition of soil S, analysis of S stable isotope ratios in natural samples (e.g., precipitation, soil, pore water) can reveal the soil S past biogeochemistry, as was investigated e.g. by Mitchell [29] or Likens [31]. Due to changes in S isotopic composition, the latter could state that the majority of the deposited $SO_4^{2-}$ entering the forest ecosystem is cycled through plants and microbes before being released back to the pore water and seepage. According to Mitchell *et al*., asides the mineralization of organic S compounds, mineral weathering contributes at least to some extend to the S cycle and its release into pore water [29].

According to Krouse *et al*., sulfur cycle should not be interpreted without performing isotopic analysis of S [26]. Besides the investigation of S stable isotopes, radionuclide tracers (e.g., $^{35}S$) can be used as markers to follow the S cycle as well [18]. However, the analysis and interpretation of stable isotopes data and use of their natural variation is more versatile and globally applicable tool for monitoring of biogeochemistry of S [26].

The investigation of S isotopes is mainly applied in environmental studies. Analysis of groundwater sulfate can be used to track sources of S bearing pollutants [32]. It was observed that pyrite-derived sulfate dominate the deep groundwater, while the groundwater sampled on the surface was controlled by acidic anthropogenic depositions. However, further

application making use of the natural variation of S isotope ratios have appeared e.g. in archaeology. Determination of S stable isotope ratios was used to determine the origin of cinnabar in ancient burial mounds [33]. Another field of application represents geology. Sulfur isotope ratios can be helpful in e.g. characterization of genesis of a gold deposit [34]. The natural local variation of S stable isotopes has been used in provenance studies of food products. Crittenden *et al*. demonstrated the power of a multi-element isotopic analysis for determining the geographic origin of dairy products [35], with S isotope ratios being significantly different in European and Australian milk. A pilot study on beer showed significant differences between e.g. Spanish and German beer [36].

## 1.3 Analysis of soil sulfur

The total S amount in soil (sum of S in its all organic and inorganic compounds) can be assessed by various approaches. Dry chemical methods like fusion of soil with $Na_2CO_3/Na_2O_2$ are labor intensive and operator sensitive [10]. The same applies for most of the wet chemical methods. These include e.g. digestion of soil using NaOBr [37] or using $HClO_4$ and $HNO_3$ [38]. Moreover, soil has to be predigested with $HNO_3$ before $HClO_4$ is added for safety reasons. Alternatively, addition of HF (instead of $HClO_4$) leads to a complete soil digest. However, as S is not considered to be bound in silicates, soil (acid microwave-assisted) digestion with $HNO_3/H_2O_2$ (as oxidizing agent) should be sufficient for total soil S determination. The instrumental determination of total S (combustion of soil) is described below.

The content of sulfate in soil is often evaluated as sulfate is the dominant S form in aerobic soils [12] and the main S source for plants [9]. Often, the assumed equilibrium between soil and soil porewater is used and the sulfate content is determined in soil solution [9, 30, 39]. Soil solution is usually sampled under vacuum using a lysimeter [39] or suction cups. The solutes (e.g., sulfate) therein can be analyzed directly. This sampling method is, however, not only time-consuming, but it also causes disruption and alteration of soil structure and pores [40]. Alternatively, the sulfate in the soil can be assessed by extraction procedures (see below). Diffusive gradients in thin films (DGT) technique represent third, relatively novel method for sampling and analysis of ions in soils.

### 1.3.1 Determination of sulfur fractions

Inorganic S represents normally less than 10 % of the total S [11] and contains mainly $SO_4^{2-}$. Sulfate is generally divided into readily available, adsorbed and “carbonate occluded” $SO_4^{2-}$. Sulfides are present only in negligible amounts in well-aerated soils [10]. The readily available (also termed “water-soluble”, e.g. [18]) S fraction represents non-specifically adsorbed (electrostatic attraction to e.g. organic matter) sulfate that easily passes into the

soil solution. Higher amounts can be expected in topsoil. Usually, this fraction is obtained by extraction of soil by water [18, 20]. Tabatabai recommended use of a salt solution (0.1 mol $L^{-1}$ LiCl) to overcome dispersion difficulties [10]. The adsorption of sulfate depends on soil pH (it is negligible at pH over 6.5 and rises with decreasing pH [10]), on charge and amount of clay minerals, and on the concentration of sulfate and other anions in the soil solution [18]. Most of sulfate is adsorbed on Fe and Al oxides and organic matter [41]. Due to its higher adsorption potential [10], a phosphate solution is usually applied for extraction of adsorbed sulfate [11, 18, 20, 38, 42].

The term “carbonate occluded S” is often used in literature for S fraction soluble in 1 mol $L^{-1}$ HCl [20, 38]. According to some authors, this fraction corresponds to sulfate co-precipitated with $CaCO_3$ as gypsum or anhydrite [25]. However, Morche observed an increase of the “carbonate occluded S” fraction although carbonates in the soil were dissolved during her study [18]. The author concluded that a considerable portion of organic S was extracted by HCl along the occluded sulfate. Moreover, some other HCl-soluble minerals (e.g. Fe and Al sulfates), mainly in non-calcareous, acidic soils, can contribute to this S fraction as indicated by Chen [42]. Thus, the term “HCl-soluble S” [42] is more appropriate than the “carbonate occluded S”.

A sequential extraction is applied to obtain all three inorganic S fractions [18, 20, 38, 42]. These extraction methods (water, phosphate, HCl) are, however, not specific and it cannot be excluded that hydrophilic organic S compounds are co-extracted.

Organic S compounds represent in general over 90 % of the total S in soil [11]. The organic S is often divided in two groups: ester sulfates (a variety of alkyl sulfates, aryl sulfates, phenol sulfates etc., [10] and carbon bonded S (mainly amino acids, but also sulfoxides etc., [10]). Ester sulfates represent in general the majority of organic S [18]. The turnover of organic S compounds into sulfate (mineralization) depends on many parameters (soil temperature, humidity, pH, use of soil) that affect the microbial activity. It also depends on the vegetation type growing on the soil whether ester sulfates or carbon bonded S is mineralized [18]. In general, ester sulfates are considered as the non-stable organic S fraction: after hydrolysis, the ester sulfates join the inorganic sulfate pool [18]. The organic S can be calculated as a difference between total S and inorganic S (sum of water-extractable, adsorbed and HCl-soluble S). To divide it into ester sulfate and carbon bonded S, the soil is reduced by HI as described by [38] and modified by [18]. All the listed fractions were proved to be of importance in S dynamics and biogeochemistry [42] or in availability of S for plants [18].

### 1.3.2 Diffusive Gradients in Thin Films (DGT) technique for sulfate analysis

DGT represents a technique that is applicable to assess a labile (primarily sorbed) fraction of elements, generally involved in a rather short-term cycling in the soil system. DGT is an advanced sink technique, in which the ion-binding layer is covered by a hydrogel disc and a protective membrane that prevents particle contamination and acts as well-defined diffusive layer. Due to the well-defined diffusion geometry of the DGT, the time-averaged analyte concentration can be calculated using:

$$c_{DGT} = \frac{M \cdot \Delta g}{D \cdot A \cdot t} \qquad \text{(Equation 1-1, [43])}$$

where *M* is the mass of the solute eluted from the resin gel, Δ*g* is the diffusive layer thickness (sum of the diffusive gel and protective membrane thicknesses), *D* is the diffusive coefficient, *A* is the sampling window surface area and *t* is the sampling time (see Figure 1-4).

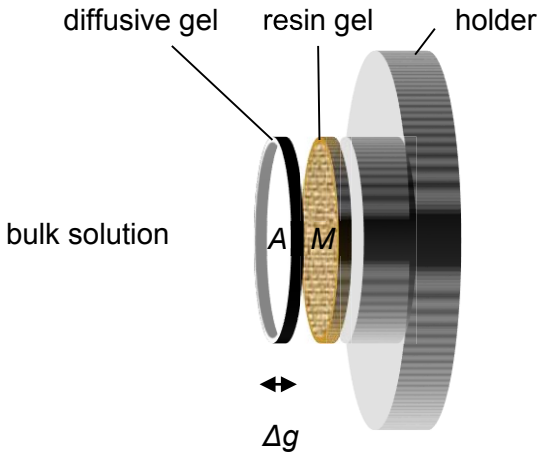


**Figure 1-4** DGT device. *A* stands for the sampling window surface area, *M* for the mass of the solute passed thorough the diffusive layer with diffusion coefficient *D* in time *t*, and ⊿g for the diffusive layer thickness.

As DGT (an advanced sink technique) works on a different principle as compared to a soil extraction (based on a quasi-equilibrium between soil and the extracting solution), it can be assumed that the two methods assess different soil pool of the analyte. It was shown for P that the DGT technique corresponds better to the plant uptake than standard soil extractions [44, 45]. Agbenin *et al*. reported that DGT correlated better than a “plant root simulator” (which applies an ion exchange resin membrane without any diffusive layer) with the plant uptake for Cu, Pb and Zn [46].

The fraction of an element taken up by DGT represents the portion of this element that is re-soluble in (soil) solution when the equilibrium between soil and solution is affected. This is

a consequence of a concentration gradient induced by a diffusion process. In sum, the DGT-labile fraction stands for reversibly adsorbed and dissolved mineral part of the element in soil. If the plant uptake is primarily (e.g. Ca, Sr) or partly (e.g. $SO_4^{2-}$) controlled by mass transport, the DGT-labile fraction represents the portion of elements in soil that is easily desorbed from the solid phase into soil solution within relatively short time and, thus, theoretically available to plants.

### 1.3.3 Quantitative analysis of sulfur

There are several methods available for determination of S amount in the soil solution, S pool assessed by soil extraction, by DGT or for determination of the total S content in soil (see Table 1-1 summarizing the analytical methods and the corresponding limits of detection (LOD) for S). Classical methods represent the precipitation and gravimetrical determination of dissolved / extracted sulfate as $BaSO_4$ as described in [47]. The pH of the extract has to be set to 3-5 prior to addition of 10% $BaCl_2$ solution, to exclude co-precipitation of $BaCO_3$. However, phosphate present in the extract may skew the measurement results by precipitation of $Ba_3(PO_4)_2$ as well. This is the case e.g. when phosphate solution is used for extraction of adsorbed sulfate as suggested by Tabatabai ([10], see above). Moreover, the time-consuming gravimetric analysis is not suited for environmental studies, where usually a large number of samples is investigated.

Alternatively, the sulfate content in the extract can be determined turbidimetrically as described by Sheen *et al*. [48] and modified by Santelli *et al*. [49]. However, as $Pb(NO_3)$ is used for analysis, chloride present in the extract (e.g. in the 1 mol $L^{-1}$ HCl extract, see above) could lead to a positive false result in determination of sulfate. The standard method of the US environmental agency (EPA) applies therefore precipitation of $BaSO_4$ [50].

Johnson and Nishita [51] developed a 'methylene blue' method for colorimetric determination of soil S. The extracted S or the soil S (all but carbon bonded S) is reduced to $H_2S$ in the "Johnson-Nishita Apparatus" using the mixture of HI (45%), HCOOH (88%) and $H_3PO_2$ (50%) (4:2:1). The $H_2S$ is trapped in a NaOAc buffer and reacts with p-aminodimethylaniline in the presence of $Fe^{3+}$ to form 'methylene blue'. The intensity (absorbance) of "methylene blue" color is determined at the wavelength of 670 nm [52]. According to Tabatabai [10], the Johnson-Nishita method is the most sensitive and precise technique under colorimetric procedures and has therefore found its place in S studies. However, the reaction of $H_2S$ to "methylen blue" is not quantitative as reported by [10]. Moreover, also this method is labor-intensive and not suited for a large number of samples.

Instrumental analysis is therefore routinely applied nowadays, as it allows for automatization and reduces sample preparation steps. The most common instrumental method is ion chromatography (IC) applicable for the determination of the readily available, adsorbed and

HCl-extractable sulfate content and for determination of sulfate content in soil solution [9, 10, 38, 42, 53]. Dual-column IC have been applied for sulfate determination, combining a strong base low-capacity anion exchange resin packed in the analytical column and a strong acid high-capacity cation exchange resin packed in a suppressor column [42]. The sample cations are exchanged for $H^+$ in the suppressor. Thus, the separated anions leave the IC as acids leading to low background signals. The conductivity detector is commonly used in the IC [10, 38, 42]. The IC has been shown to perform well for soil extracts [9, 38, 42] with detection limits down to 0.1 mg $L^{-1}$ [10].

Conversion of S to $SO_2$ with subsequent determination in an infrared (IR) cell represents another instrumental method for S content determination. The technique is designed for analysis of solid matter and is thus mainly used for determination of total S [10]. Also dried S fractions (extracted $SO_4^{2-}$) can be analyzed [54]. The solid sample is introduced in a mixture with a combustion accelerator (e.g. Fe) in a ceramic boat and heated to more than 1100 °C under $O_2$ stream in an inductive furnace. The amount of the generated $SO_2$ is determined by the amount of adsorption at the wavelength of 284 nm in the IR cell [10, 55].

The IC and the combustion (IR-based) method are powerful techniques for measurement of S in soils. However, they both have some drawbacks: neutral (organic) S compounds cannot be determined by the IC and further processing of the soil extract is required for the combustion method. Therefore, inductively coupled plasma – optical emission spectrometry (ICP-OES) has established under the methods of choice for S analysis.

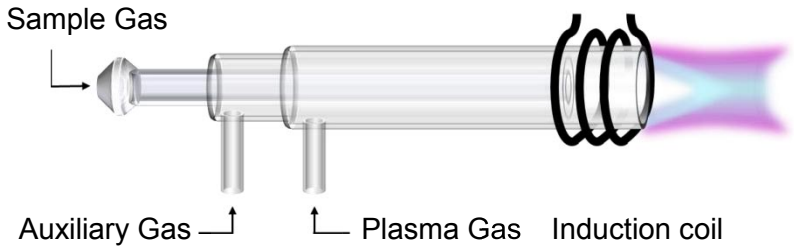


**Figure 1-5** Inductively coupled plasma.

Inductively coupled plasma (ICP) on a stream of gas was observed by Babat already in 1947 [56]. For ICP generation is required: a gas (usually Ar) streaming through a quartz tube (torch), which is placed in an inductive coil, and a high-voltage spark [57] (see Figure 1-5). The spark initiates electron stripping from some of gas atoms. These electrons are trapped in the electro-magnetic field caused by the radio-frequency power of 750 - 1700 W [57] applied to the inductive coil. The trapped (inductively coupled) electrons collide with other gas atoms, inducing stripping of other electrons in a chain reaction. These collisions are responsible for the high temperature of the plasma (to 10 000 K). In total, the

plasma ball at the top of the torch consists of neutral gas atoms, cations and electrons, and is assumed to be “quasi-neutral”. Generally, the gas flowing through the torch is comprised of three streams: the plasma (also termed “cool”) gas, which mainly contributes to the generation of plasma and protects the walls of the torch, the sample (also termed “nebulizer”) gas, which carries the sample into the plasma, and auxiliary gas, which enables to optimize the plasma profile (see Figure 1-5).

The use of the spectra emitted by ICP (i.e., the ICP-OES technique) was first described by Wendt and Fassel in 1965 [58], and quickly was followed by others, on both sides of the Iron Curtain (e.g., Kleinmann [59], or Fassel [60]). The wavelengths relevant for S are 180.731 nm and 182.037 nm [53]. Use of the 180.731 nm line leads to lower detection limits (0.2 mg S $L^{-1}$, as compared to 0.3 mg S $L^{-1}$ at 182.037 nm line [53]), on the other hand, spectral interferences must be considered [38]. Using ICP-OES, both organic and inorganic S species can be analyzed in the soil extract or soil solution [38], theoretically without any further processing of the sample. However, as shown by Shan *et al*., in some types of soil extracts (e.g., in 1 mol $L^{-1}$ HCl extract) separation of sulfate by IC prior to analysis can be required in order to not overestimate the inorganic S fraction [38].

The argon ICP used in ICP-OES and quadrupole mass spectrometer (MS) used e.g. as a detector in gas chromatography were coupled to become ICP-MS for the first time in 1980 [61]. Some modifications had to be made to enable the ICP working with the grounded mass spectrometer. Since then, ICP was coupled with sector field or time-of-flight mass spectrometers and developments in sample introduction, ion transmission rate, dynamic detection range or interference removal efficiency have been made. The removal / reduction of interferences is highly relevant when analyzing S by ICP-MS. The ions generated in the ICP are detected according to their mass-to-charge (*m*/*z*) ratio. As the *m*/*z* of the most abundant S isotopes, $^{32}S$ and $^{34}S$, corresponds – among others – to the *m*/*z* of $0_2^+$ ($^{16}O^{16}O^+$ and $^{16}O^{18}O^+$, respectively), high background signal can be expected on the *m*/*z* 32 and 34, hampering analysis of low amounts of S. Strategies and mechanisms to overcome this problem are discussed below.

The application of ICP-MS enables for determination of S in low concentrated solutions and soil extracts. The limits of detection are down to 0.5 µg S $L^{-1}$ [62] and 0.01 µg S $L^{-1}$ [63] for quadrupole and sector field based instruments, respectively.

The abovementioned methods for quantitative S analysis, including the corresponding achievable limits of detection, are summarized in Table 1-1. All methods are applicable for S concentration as low as 1 mg $L^{-1}$. However, if lower amounts of S in environmental samples need to be detected, ICP-MS is clearly the analytical method of choice.

**Table 1-1** Analytical methods for determination of S.

| Method | Type | LOD | Reference |
|---|---|---|---|
| Gravimetric | $BaSO_4$ precipitation | > 0.3 mg $L^{-1}$ | [64] |
| Turbidimetric | $BaSO_4$ precipitation | 1 mg $L^{-1}$ | [50] |
| Colorimetric | “methylene blue" | 0.1 mg $L^{-1}$ | [52] |
| Ion chromatography | Dual-column | 0.1 mg $L^{-1}$ | [10] |
| Absorption spectrometry | 284 nm | 1 mg $L^{-1}$ | [65] |
| ICP-OES | 180.731 nm | 0.2 mg $L^{-1}$ | [53] |
| | 182.037 nm | 0.3 mg $L^{-1}$ | [53] |
| ICP-MS | QQQ | 0.5 µg $L^{-1}$ | [62] |
| | sector field | 0.01 µg $L^{-1}$ | [63] |

### 1.3.4 Sulfur isotope ratio analysis

Sulfur isotope analysis is applied more and more in environmental analyses, geologic studies, archaeology, food provenance studies etc. as can be demonstrated on the increasing number of related scientific publications (see Figure 1-6). The number has almost doubled within the last 15 years (283 publications in 2002, 511 in 2015; source: scopus.com).

The analysis of stable S isotopes in soil solution sulfate is usually performed by isotope ratio mass spectrometry (IRMS) [17, 26, 40]. The technique is applied for more than 90 % of studies related to S isotope analysis (see Figure 1-6). Pretreatment of the sample is necessary. Either is the dissolved sulfate precipitated to $BaSO_4$ using $BaCl_2$ in acidified (pH = 2 - 2.5) soil solution or extract sample. The precipitate is then mixed e.g. with oxidizing agents $V_2O_5$ and $SiO_2$ (1:10:10) [66] and combusted in an elemental analyzer (900 °C). The amounts of the resulting $^{32}S^{16}O$ and $^{34}S^{16}O$ are measured on *m*/*z* 48 and 50 by the connected IRMS. Due to the high amounts of $V_2O_5$ and SiO2, the possible variations of oxygen isotopes (that would influence the measured signal intensities) in sulfate samples are suppressed [66]. The possible co-precipitation of $Ba_3(PO_4)_2$ (see section Quantitative analysis), however, might further represent a problem. Alternatively, $SF_6$ can be prepared of $BaSO_4$ by reaction with $BrF_5$ at 300 °C [67]. As F has only one isotope, the measurement is interference-free. Although this method enables for high measurement precision and for analysis of $^{36}S$, the sulfate pretreatment is still sample and time consuming. Moreover, the precipitation of $BaSO_4$ can become challenging when soil solution samples or soil extracts are limited in volume or in sulfate concentration.

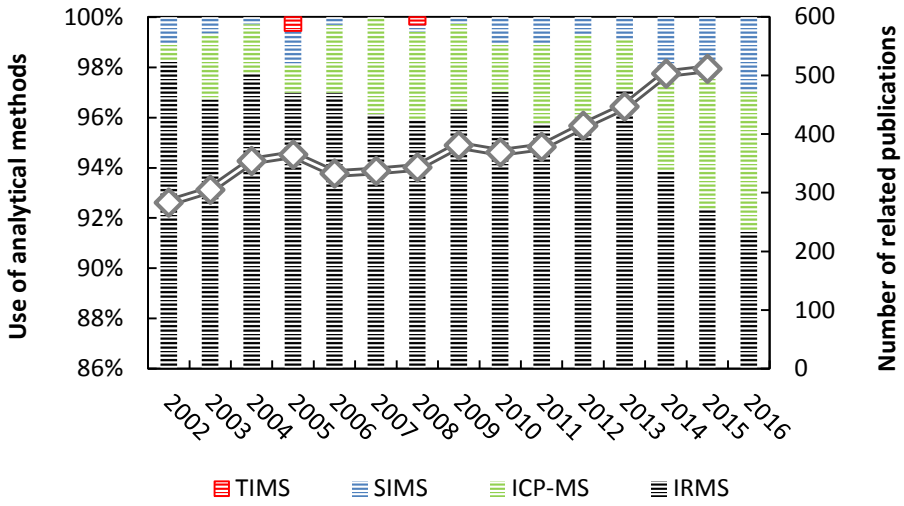


**Figure 1-6** Number of publications related to analysis of S stable isotopes (line) and proportional use of different mass spectrometry techniques applied in these publications. Source: scopus.com.

Therefore, other measurement techniques have appeared in literature. Mann *et al*. developed $^{33}S$-$^{36}S$ double-spike thermal ionization mass spectrometry (TIMS) technique and applied it to sulfur isotope study of high elevation snow pits [68, 69]. However, also the double-spike TIMS method requires chemical treatment of sulfate before analysis – a reduction of sulfate to $H_2S$ and reaction with As-$NH_3$ to $As_2S_3$ [68]. Attempts were made to use Fourier transform infrared spectroscopy to measure $^{34}S/^{32}S$ [70]. Standards and samples were oxidized to $SO_2$ by adding $V_2O_5$ and $SiO_2$ [66] and heating to 1000 °C. Although the method was accurate (0.4 % deviation from the reference method, IRMS), the measurement precision was poor (> 1 %, RSD) [70]. Moreover, as in the case of IRMS or TIMS, pretreatment of the sample was necessary.

Secondary ion mass spectrometry (SIMS) has been applied for *in-situ* high-resolution S isotope analysis of geological samples [71]. $^{34}S/^{32}S$ measurement precision of 0.02 % (2 SD) and spatial resolution of 10 µm can be achieved. The technique can be applied e.g. for S isotope thermometry with precision of ± 50 °C [71]. However, there is no use of SIMS in investigation of the S biogeochemistry.

So far, only inductively coupled plasma mass spectrometry (ICP-MS) enables direct measurement of sulfur S isotopes of dissolved sulfate. The use of a multicollector ICP-MS (MC ICP-MS) allows for a high precise analysis. Therefore, the use of MC ICP-MS for S isotopic studies has been increasing steadily in the last 15 years (see Figure 1-6). While only in 2 scientific publications (1 % of all publications related to S isotopic analysis) MC ICP-MS was applied in 2002, it was 30 in total (6 % of all publications related to S isotopic analysis)

in 2015. Though the benefits and increasing popularity of MC ICP-MS, some analytical aspects have to be considered.

### 1.3.5 $^{34}S/^{32}S$ analysis by MC ICP-MS: aspects to consider

*Spectral interferences*

Several spectral interferences may occur in ICP-MS. Along with the abovementioned oxygen-based interferences, nitrogen-, calcium- or chlorine-based interferences may be found in the mass spectra (see Table 1-2). The interferences can be resolved on the one hand by applying higher mass resolution in sector field mass spectrometers (both single [63] and multicollector ICP SFMS instruments [72]). A mass resolution (defined as full width at half maximum, see [73]) of 1800 is sufficient to resolve oxygen-based interferences, while a mass resolution of 4300 is required to eliminate all spectral interferences at the different S isotope *m*/*z* [74].

**Table 1-2** A selection of the main spectral interferences on *m*/*z* of interest for S isotope ratio measurements

| $^{32}S^+$ | $^{34}S^+$ | $^{32}S^{16}O^+$ | $^{34}S^{16}O^+$ |
|---|---|---|---|
| ***m*/*z* 32** | ***m*/*z* 34** | ***m*/*z* 48** | ***m*/*z* 50** |
| $^{16}O^{16}O^+$ | $^{18}O^{16}O^+$ | $^{48}Ti^+$ | $^{50}Ti^+$ |
| $^{18}O^{14}N^+$ | $^{17}O^{16}O^1H^+$ | $^{48}Ca^+$ | $^{50}Cr^+$ |
| $^{18}O^{13}C^1H^+$ | $^{18}O^{15}N^1H^+$ | $^{47}Ti^1H^+$ | $^{32}S^{18}O^+$ |
| $^{15}N^{16}O^1H^+$ | $^{18}O^{14}N^2H^+$ | $^{46}Ca^2H^+$ | $^{38}Ar^{12}C^+$ |
| $^{18}O^{12}C^2H^+$ | $^{17}O^{15}N^2H^+$ | $^{36}Ar^{12}C^+$ | $^{36}Ar^{14}N^+$ |
| $^{17}O^{13}C^2H^+$ | $^{33}S^1H^+$ | $^{31}P^{17}O^+$ | $^{37}Cl^{13}C^+$ |
| $^{15}N^{15}N^2H^+$ | $^{68}Zn^{2+}$ | $^{18}O^{16}O^{14}N^+$ | $^{35}Cl^{15}N^+$ |
| $^1H^{18}O^{13}C^+$ | | $^{17}O^{17}O^{14}N^+$ | $^{37}Cl^{12}C^1H^+$ |
| $^1H^{17}O^{14}N^+$ | | $^{18}O^{15}N^{15}N^+$ | $^{36}Ar^{13}C^1H^+$ |
| $^{64}Ni^{2+}$ | | $^{17}O^{16}O^{15}N^+$ | $^{35}Cl^{14}N^1H^+$ |
| $^{64}Zn^{2+}$ | | | $^{35}Cl^{13}C^2H^+$ |

Alternatively, a pressurized cell can be applied in quadrupole-based ICP-MS instruments. The cell (an individual multipole) can be pressurized with a reactive gas (e.g., $O_2$ [62]) and the product is detected (i.e., $SO^+$). A collision gas can be used as well (e.g., He [75], Ne or Xe [76]) for collisional focusing and suppressing the amount of the interference. Depending on the manufacturer, such system is referred to as “DRC” (dynamic reaction cell, where “dynamic” refers referring to a bandpass filter that eliminates interfering species within the cell; Perkin Elmer, e.g. instruments *Elan* or *Nexion*), “CC” (collision cell; Agilent Technologies, e.g. instruments *7500* or *7700*), “iCRC” (integrated collision reaction cell;

Analytik Jena instrument *PlasmaQuant*), "QCell" ("flatapole" (quadrupole) collision cell; Thermo Scientific, e.g. instrument *iCAP*) or "QQQ" (a quadrupole-octopole-quadrupole system, Agilent Technologies instrument *8800*). The major difference between the single reaction cell (DRC, iCRC, QCell etc.) and the "QQQ" instrument lies in the number of mass analyzers. In the "QQQ", one scanning quadrupole is placed in front of the octopole cell, where the reaction / collision takes place, and one between the cell and the detector. Thus, the "QQQ" can be operated as a MS/MS instrument, reducing effectively interferences at *m*/*z* 48 and 50 (see Table 1-2) [62].

Suppression of the oxygen-based interferences can be achieved by use of an appropriate introduction system. Membrane desolvation units like APEX-ACM, APEX-spiro TMD (both Elemental Scientific) or Aridus II (Teledyne Cetac) reduce solvent loading to the plasma efficiently and thus reduce the amount of oxygen in plasma as compared to a conventional sample introduction system. Another strategy is the reduction of solvent vapor by cooling the cyclonic or the double-pass glass spray chambers to 2 °C.

*Matrix effects (non-spectral interferences)*

Elements of the sample (soil solution, extract, etc.) matrix can change plasma conditions and thus cause "non-spectral" interferences. These effects were observed for easy-to-ionize elements like Na (although the authors incorrectly ascribed the effect to $Cl^-$ [77]), for Fe, Ni, Mo, Sn, Sr [78] and for Ca [78, 79]. According to [79], the matrix effects are dependent mainly on the absolute matrix element concentration rather than its relative concentration ratio (or molar ratio) to S. Although such conclusion - based on the presented data - is questionable, care has always to be taken to deal with the possible matrix effect. Three strategies can be applied here:

(i) use of an internal standard. As the properties (mass, ionization energy) of the standard should be as close as possible to those of S, silicon is the element of choice [36, 72]. Modern MC ICP-MS instruments (*Nu Plasma II*, Nu Instruments Inc., or *Neptune*, Thermo Scientific) allow for simultaneous detection of S and Si isotopes. Sulfur represents a four-isotopic system. Therefore, a double spike ($^{33}S$, $^{36}S$) could be used as an internal standard as applied by [68] for TIMS. However, the application of a double spike technique in ICP MS is hampered by the high background signal at *m*/*z* 36 caused by $^{36}Ar^+$ (plasma gas) that cannot be resolved by commercial ICP-MS instruments.

(ii) calibration of S isotope ratios by a matrix-matched standard. This strategy is easily applicable when samples of constant matrix are investigated (e.g., marine water, [77]). However, this is not the case when S isotope ratios in soil solution and soil extracts from different sites and seasons should be determined, as the matrix of these samples is likely to vary strongly.

(iii) separation of matrix. Use of a strong cation exchange resin (e.g. AG50-X8, [78]) is recommended as all the described interfering matrix elements are cations. However, ion chromatography employing strong base anion exchange resin is also applicable.

A selective sampling of sulfate from the soil would be an ideal solution as none of the listed strategies would be required.

The matrix effects contribute to the instrumental isotopic fractionation (IIF, [80]), commonly referred to as instrumental “mass bias” (see below).

*Measurement precision*

In general, the signal of two ($^{32}S$, $^{34}S$) or more ($^{28}Si$, $^{29}Si$, $^{30}Si$, $^{32}S$, $^{33}S$, $^{34}S$) isotopes is accumulated for a given time. A ratio of these integrated signals results in an isotope ratio. The average and standard deviation is usually calculated from a number of replicate measurements. The basic difference in operating principles of single collector and multicollector ICP-MS instruments is the scanning and detection of multiple isotopes at one detector vs. the simultaneous measurement of isotopes at multiple detectors, respectively. The duration of one measurement cycle is the result of scanning-, settling- and measurement (dwell) time [81]. A product of the dwell time and number of measurement cycles (named e.g. repetition, sweep or run, depending on the manufacturer) gives the total integration time. Usually, the scanning and settling times are fixed by the instrument as they are functions of the mass analyzer and detector electronics [81]. The measurement cycle duration should be dominated by the dwell time, which can be optimized for each measured mass separately for single collector instruments [81].

Narrower mass regions (up to single point peak hopping [82]), shorter dwell times and larger number of measurement cycles are chosen to approximate simultaneous detection and achieve a fast scan between analyzed isotopes in a single collector instrument [81, 83]. Under these conditions, the best achievable precision values are obtained. On the other hand, an insufficient number of ions is counted if dwell times are chosen too short. If so, the counting statistics $\sigma_{cs}$ (caused by random generation of ions in ICP; proportional to the square root of the number $N$ of counts in a measurement, see equation 1-2) increases while the measurement precision decreases.

$$\sigma_{CS} = \sqrt{N} \qquad \text{(Equation 1-2)}$$

The relative counting statistics noise is lower when more ions of a measurand are counted [82]. Thus, the higher the sensitivity of an instrument, the lower the relative counting statistics noise - and the shorter dwell times can be set.

Besides the counting statistics, the measurement precision is affected by sample introduction and plasma fluctuations in the ICP source. These sources dominate over the counting statistics when a higher amount of ions (> 100 000 cps [82]) is counted. They can be reduced e.g. by use of optimized sample introduction systems and by optimization of plasma conditions (gas flows, torch vs. cones position) but they do not disappear completely.

The precision of the measured isotope ratio can be calculated using different statistical approaches. For example, if an isotope ratio is determined 100 times in one measurement, the resulting measurement precision can be expressed (i) as standard deviation (SD) of the 100 determined ratios, (ii) as SD of e.g. 10 blocks à 10 measurements or (iii) as a standard error of the mean of 10 blocks à 10 measurements (the latter is the case of, e.g., the software provided by Nu Instrument Inc.). Since different “precision values" are obtained by the different calculations, the chosen approach should be stated always.

Reporting the measurement precision is a conventional way to express the ICP-MS $^{34}S/^{32}S$ measurement performance: Menegario reached 1 % RSD when using ICP-QMS [84]; the application of an ICP-SFMS led to significantly more precise measurements, down to 0.04 % (1 SD) [63]. The best measurement precision was reported as expected for MC ICP-MS, usually between 0.02 % (2 SD) [85] and 0.05 % [86], alternatively 0.01 % (standard error) [87].

The measurement precision together with the limit of detection is usually the deciding criterion when the suitability of the ICP-MS instrumentations for $^{34}S/^{32}S$ measurement is compared. San Blas *et al*. obtained measurement precision of 0.01 %, 0.1 % and 0.4 % when applying Faraday cups and ion counters in MC ICP-MS and using single collector ICP-MS, respectively [87]. The use of Faraday cups, however, led to high LOD as compared to ion counters. Giner Martinez-Sierra *et al*. observed 4-times higher measurement precision when comparing MC ICP-MS with ICP-SFMS (0.05 % and 0.2 %, respectively) [86].

Reporting only the measurement precision (the measurement repeatability or the within-lab reproducibility), however, leads in general to an underestimation of the true measurement uncertainty as was demonstrated by [72] and [36]. Asides the measurement precision, the precision of measurement of the calibration for the isotope ratios (or measurement precision of the internal standard) and the uncertainty of the background signal subtraction should be taken into account. The resulting S isotope ratio should be then given with its standard (expanded) combined uncertainty.

*Background S signal*

The background signal (blank) contribution to the uncertainty is minimized when the measured signal of the isotopes is minimally affected by the background noise. Usually, this

is achieved by analyzing solutions of sufficiently high S concentration (e.g., 20 - 85 µg $mL^{-1}$ [78, 84]). Alternatively, the signal-to-noise ratio can be increased significantly by use of a heated desolvation unit [88, 89].

*Instrumental isotopic fractionation (IIF)*

The sum of all effects within the instrument that lead to a deviation of the detected S isotope ratio from the real S isotope ratio in the sample is referred to as “mass bias” or as “instrumental isotopic fractionation” [80], and include:

- the matrix effect,
- fluctuation of the ion source (plasma),
- mass fractionation within the plasma (mass-independent collisions in the plasma and mass-dependent diffusion, i.e., lighter isotopes tend to diffuse more easily further from the center of the ion beam than the heavier isotopes),
- space-charge effects (affecting more likely lighter isotopes as they are rather in the outer regions of the plasma than the heavier isotopes),
- mass fractionation within the instrument (heavier isotopes are less accelerated by the ion optics than lighter isotopes),
- ion repulsion within the ion beam,
- collisions and reactions in pressurized cell (if applied),
- and other effects, like isotope-dependent oxide formation [90], which are discussed e.g. in [80]

Calibration of the measurement by a matrix-matched and S concentration-matched standard of known (certified) S isotopic composition is often applied for correction of the IIF [78]. This calibration is referred to as “standard-sample bracketing” [78] or as “external intra-elemental correction” [91]. Linear correction law is used for bracketing [91].

Internal standardization (or “internal inter-elemental correction” [91]) using certified Si isotope ratio standard appears also often in the literature [36, 72]. Correction law applying the ratio of the atomic masses as in [36] is used in this case. Alternatively, when no certified Si isotope ratio standard is available, a combination of internal standardization and external calibration can be used as described e.g. by Irrgeher *et al*. [91]. The Si isotope ratios as defined by IUPAC can be then applied.

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# 2 Targeted sampling of sulfur and its (isotopic) fractionation in soils

The aim of this thesis was the development and validation of analytical methods enabling to study the soil S biogeochemistry, and the application of these methods to Austrian, European and Australian soils.

A sequential extraction, followed by HI-reduction was modified, validated and applied to soil profiles to obtain all relevant S fractions (**Section 2.1**). Statistical analysis was used to interpret the S biogeochemistry. A special emphasis was put on the investigation of S stable isotopes. A suitable analytical method was chosen by comparison of the performance of different, fully validated ICP-MS platforms (**Section 2.2**). This method was applied to study S isotopic composition in rain water and in soil solution after purification by an anion-exchange resin membrane (**Section 2.3**). The changes in the isotopic composition were used to identify the dominant processes of the S cycle in the investigated soils.

In a further work, a novel passive sampling technique (DGT) for “labile” (i.e., readily available, reversibly adsorbed) sulfate was co-developed (**Section 2.4**) and compared with classic soil tests. The novel DGT technique was subsequently successfully applied for analysis of soil S stable isotopes (**Section 2.5**).

This chapter consists of research papers that were published, submitted or are foreseen to be published in scientific peer-reviewed journals. The layout of these papers was adapted to match with other parts of this work. Permission to reproduce the papers was obtained by the copyright holders.

## 2.1 Fractionation of sulfur in beech (*Fagus sylvatica*) forest soils in relation to distance from the stem base

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**ABSTRACT**

The investigation of the fractionation of S compounds in forest soils is a powerful tool for interpreting S dynamics and S biogeochemistry in forest ecosystems. Beech stands on nutrient-rich sites on Flysch and on nutrient-poor sites on Molasse were selected for testing the influence of the stemflow, which represents a significant input of water to the soil, on spatial patterns of sulfur (S) fractions.

Soil cores were taken at six distances from a beech stem per site at 55 cm uphill and at 27, 55, 100, 150 and 300 cm downhill from the stem. The cores were divided into the mineral soil horizons 0-3, 3-10, 10-20, 20-30 and 30-50 cm. Soil samples were characterized for pH and S fractions.

Sequential extraction by $NH_4Cl$, $NH_4H_2PO_4$ and HCl yielded readily available sulfate-S (*RAS*), adsorbed sulfate-S (*AS*) and HCl-soluble sulfur (*HCS*). Organic sulfur (*OS*) was estimated as the difference between total sulfur (*ToS*, acidic digestion) and inorganic sulfur (*RAS* + *AS* + *HCS*). Organic sulfur was further divided into ester sulfate-S (*ES*, HI-reduction) and carbon bonded sulfur (*CS*).

On Flysch, *RAS* represented 3-6 %, *AS* 2-12 %, *HCS* 4-18 % and *OS* 72-89 % of *ToS*. On Molasse, *RAS* amounted 1-6 %, *AS* 1-60 %, *HCS* 0-28 % and *OS* 33-94 % of *ToS*. Spatial S distribution patterns with respect to the distance from the tree stem base could be clearly observed at all investigated sites. Desorption of sulfate in response to stemflow is put forward to explain reduced contents of *AS* at the stem basis (27 cm). The presented data is a contribution to current reports on negative input - output S budgets of forest watersheds, suggesting that mineralization of *OS* on nutrient rich soils and desorption of historic *AS* on nutrient-poor soils are the dominant S sources.

## 2.2 The performance of single and multicollector ICP-MS instruments for fast and reliable $^{34}S/^{32}S$ isotope ratio measurements

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**ABSTRACT**

The performance and validation characteristics of different single collector inductively coupled plasma mass spectrometers based on different technical principles (ICP-SFMS, ICP-QMS in reaction and collision mode, and ICP-MS/MS) were evaluated in comparison to the performance of MC ICP-MS for fast and reliable S isotope ratio measurements.

The validation included the determination of LOD, BEC, measurement repeatability, within-lab reproducibility and deviation from certified value as well as a study on instrumental isotopic fractionation (IIF) and the calculation of the combined standard measurement uncertainty. Different approaches of correction for IIF applying external intra-elemental IIF correction (aka standard-sample bracketing) using certified S reference materials and internal inter-elemental IIF (aka internal standardization) correction using Si isotope ratios in MC ICP-MS are explained and compared.

The resulting combined standard uncertainties of examined ICP-QMS systems were not better than 0.3 – 0.5 % ($u_{c, rel}$), which is in general insufficient to differentiate natural S isotope variations. Nonetheless, single collector ICP-SFMS has the potential to perform sufficiently well for e.g. provenance studies (single measurement $u_{c, rel}$ = 0.08 %). However, the measurement reproducibility (> 0.2 %) was the major limit of this system and leaves room for improvement.

MC ICP-MS operated in edge mass resolution mode, applying bracketing correction of IIF, provided isotope ratio values with highest quality (relative combined measurement uncertainty: 0.02 %; deviation from the certified value: < 0.002 %).

## 2.3 MC ICP-MS $\delta^{34}S_{VCDT}$ measurement of dissolved sulfate in environmental aqueous samples after matrix separation by means of an anion exchange membrane

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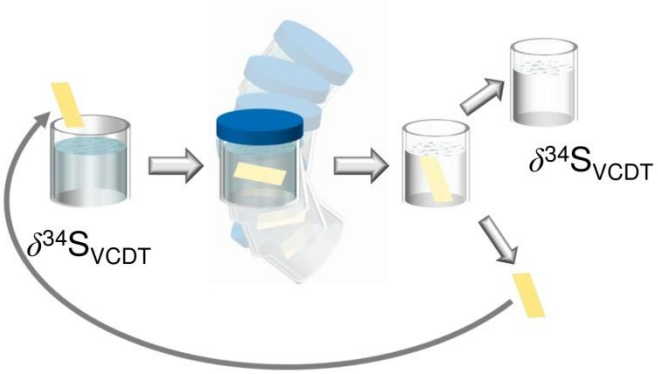
**ABSTRACT**

Analysis of $^{34}S/^{32}S$ of sulfate in rainwater and soil solutions can be seen as a powerful tool for the study of the sulfur cycle. Therefore, it is considered as a useful means, e.g., for amelioration and calibration of ecological or biogeochemical models. Due to several analytical limitations, mainly caused by low sulfate concentration in rainwater, complex matrix of soil solutions, limited sample volume, and high number of samples in ecosystem studies, a straightforward analytical protocol is required to provide accurate S isotopic data on a large set of diverse samples. Therefore, sulfate separation by anion exchange membrane was combined with precise isotopic measurement by multicollector inductively coupled plasma mass spectrometry (MC ICP-MS). The separation method proved to be able to remove quantitatively sulfate from matrix cations (Ca, K, Na, or Li) which is a precondition in order to avoid a matrix-induced analytical bias in the mass spectrometer. Moreover, sulfate exchange on the resin is capable of preconcentrating sulfate from low concentrated solutions (to factor 3 in our protocol). No significant sulfur isotope fractionation was observed during separation and preconcentration. MC ICP-MS operated at edge mass resolution has enabled the direct $^{34}S/^{32}S$ analysis of sulfate eluted from the membrane, with an expanded uncertainty $U$ ($k$ = 2) down to 0.3 ‰ (a single measurement). The protocol was optimized and validated using different sulfate solutions and different matrix compositions. The optimized method was applied in a study on solute samples retrieved in a beech (*Fagus sylvatica*) forest in the Vienna Woods. Both rainwater (precipitation and tree throughfall) and soil solution $\delta^{34}S_{VCDT}$ ranged between 4 and 6 ‰, the ratio in soil solution being slightly lower. The lower ratio indicates that a considerable portion of the atmospherically deposited sulfate is cycled through the organic S pool before being released to the soil solution. Nearly the same trends and variations were observed in soil solution and rainwater $\delta^{34}S_{VCDT}$ values showing that sulfate adsorption / desorption are not important processes in the studied soil.



## INTRODUCTION

Various processes led to the sulfur isotope ($^{34}S/^{32}S$) fractionation such as bacterial $SO_4^{2-}$ reduction, fractional crystallization, or evaporation of seawater [1, 2]. Regional differences in $^{34}S/^{32}S$ ratios were applied in archaeology [3], anthropology [4], or food authenticity studies [5]. Further, the isotopic system of S was applied in geochronology [6] or marine sciences [7], also with a focus on mass independent $^{33}S/^{32}S$ fractionation [8]. However, environmental studies represent the main field of application of $^{34}S/^{32}S$ analyses to shed light on the environmental sulfur cycle [1, 9, 10].

In the environment, sulfur acts as an essential nutrient for vegetation. It is a constituent of amino acids, proteins, coenzymes, or sulfolipids of plants. At the same time, sulfur (in the form of sulfate) is co-responsible for the 'acid rain' phenomenon, which causes soil acidification and associated leaching of base cations from the soil [10]. Therefore, the understanding of the environmental sulfur cycle is of highest interest. Sulfur enters an ecosystem mainly in the form of sulfate (by wet and dry deposition). Sulfate is a mobile anion, which passes easily via seepage through the soil [10]. However, part of the sulfate can be taken up by plants and microbes and reduced to build organic sulfur compounds. Another part might be adsorbed on soil particles. In a reverse process, organic sulfur can be mineralized into sulfate and adsorbed sulfate can be desorbed. To a generally small extent, weathering of sulfur-bearing minerals contributes to the sulfate flow, as well [10]. Some of these processes (immobilization/mineralization, weathering) are known to result in a change of the isotopic composition of dissolved sulfate [1]. Thus, the change of the isotopic composition can serve as basis for ecological/biogeochemical modelling, helps in fertilization planning, and allows for prediction of soil recovery from acid rain effects [11]. (Throughout this publication, the term rainwater summarizes terms precipitation (rainwater above a forest canopy) and tree throughfall, i.e., precipitation after the passage through the canopy.)

The sulfur cycle can be dependent on seasonal trends and conditions, like humidity or temperature. Therefore, a long-term study of biogeochemical processes of sulfur in soil is advantageous. This requires the periodical sampling of rainwater and soil solution, considering the following analytical challenges: depending on the season, the amount of dissolved matrix elements (cations, anions, organic compounds) varies, and the amount of a water sample or the concentration of dissolved sulfate can be low (<5 mL, <0.002 mmol $L^{-1}$, respectively). Classical method (gas source isotope ratio mass spectrometry, IRMS) requires sufficient sulfate concentration in solution, or high sample volume for precipitation of few milligrams of solid sulfate ($BaSO_4$) and might therefore not be able to cope with the challenges straightforward [12].

Paris *et al*. have shown the capability of (multicollector) inductively coupled plasma mass spectrometry ((MC) ICP-MS) for the isotopic analysis of small amounts of dissolved

sulfate [8]. When introducing only 5 to 40 nmol sulfur into the instrument, the authors reported a reproducibility (2 *SD*) below 0.15 ‰ for natural marine samples. Applying a matrix-matched standard, Bian *et al*. estimated 0.13 ‰ 'external precision' (within-lab reproducibility, 2 *SD*) in their in-house sulfur standard [13]; Lin *et al*. from the same working group reached even 0.07 ‰ (2 *SD*) [7]. A long-term reproducibility (2 *SD*) of less than 0.45 ‰ was estimated for laser ablation MC ICP-MS [14, 15]. Authors using a single collector ICP-MS reported a measurement repeatability (*SD*) of 0.4 ‰ in 100 ng $g^{-1}$ S standard applying medium mass resolution [16] and 0.7 ‰ (*SD*) in a seawater standard in low resolution [17]. Although the latter instrumentation is still applicable for biogeochemical studies, where $^{34}S/^{32}S$ is expected to vary in the per mill range, MC ICP-MS devices are the method of choice when small isotopic differences are targeted. The main limitation during data reduction includes mainly correction for blank and instrumental isotopic fractionation (IIF). None of the authors provided combined uncertainties. However, when reporting measurement reproducibility or repeatability only, the main method limitations including correction for blank or IIF are not considered properly.

Usually, external calibration of isotope ratios by standard – sample bracketing is applied [7, 8, 13, 14]. Correction applying internal standardization (interelemental internal IIF correction) is less common [5, 18]. The general drawback of this approach is the assumption that both elements (analyte and standard) undergo the same isotopic fractionation. In the case of sulfur, Clough has shown that the $^{30}Si/^{28}Si$ isotopic system can be used to correct measured $^{34}S/^{32}S$ ratios even in natural samples with high matrix content [18]. Other applicable correction procedures like combination of bracketing and internal standardization or double spike calibration are described, e.g., in [19].

A proper consideration of the sample composition is necessary since matrix elements can cause a significant bias in measured $^{34}S/^{32}S$ ratios. Craddock reported a shift of up to 0.7 ‰ caused by elements contained in sulfur-bearing minerals (Ca, Fe, As, Ni, Mo, Sn) [14]. Paris described the dependence of the detected sulfur signal on the $Na^+$ concentration in the measured solution [8]. The effect of NaCl addition to a sulfur standard on measured $^{34}S/^{32}S$ ratio (shift by up to −2 ‰) was shown by Lin *et al*. [7]. To eliminate the matrix effect, Craddock and Paris used cation exchange columns (which worked well, with the exception of Mo), and Lin applied matrix-matched standards. In general, the latter is less time consuming when the matrix of studied samples can be considered as almost equal (usually within 10 % variation of the elemental content). This is the case for e.g. marine samples, where dissolved $Na^+$ and $Cl^-$ are the major constituents. However, the amount of dissolved matrix elements usually changes from sample to sample in rainwater and soil solution. Therefore, a separation technique is required, which is fast, robust, and reliable to allow quantitative separation and high sample throughput.

Ion exchange resins on plastic membranes have been used since the 1960s for sampling of dissolved analytes from soil [20]. When combined with a semipermeable layer, the ion exchange membrane acts as a plant root simulator (PRS). PRS is a simple and cost-saving method and, therefore, it has found a wide range of applications in soil science [21, 22]. The easiness of application, quickness, and possibility to re-use the membrane several times make the anion exchange resin on a plastic membrane an ideal candidate for sulfate separation in a high number of water samples. Kwon *et al*. tested an anion exchange resin placed on a polystyrene matrix for isotopic analysis of oxygen and sulfur in sulfate by IRMS [9]. They observed that the sampling method does not cause a significant isotopic fractionation of sulfur, even in the presence of other anions (competitive anion exchange). Although their method worked well, the sampled sulfate still had to be precipitated as $BaSO_4$ for the subsequent isotopic analysis by IRMS. To circumvent this, a direct analysis of the sulfur isotopes by MC ICP-MS had to be validated for further application.

In this study, we demonstrate the necessity of matrix separation for reliable isotope ratio analysis of sulfur in rainwater and soil solution. We further combined the separation by means of an anion exchange resin on plastic membrane with direct $^{34}S/^{32}S$ ratio analysis by MC ICP-MS. The tested and validated method was applied to natural rainwater and soil solution samples from a 1-year study in Austrian forest ecosystems.

## METHODS

### Sample and sample preparation

All consumables were double acid washed (10 and 1% $HNO_3$ *m*/*m* prepared from concentrated $HNO_3$ (p.a., Merck, Darmstadt, Germany), diluted with laboratory water type I (0.055 µS $cm^{-1}$; TKA-GenPure, Niederelbert, Germany), and rinsed with laboratory water type I. Laboratory water type I and nitric acid were further purified by using a sub-boiling distillation system (Milestone Inc., Shelton, CT, USA) and were used for dilution of standards and preparation of reagents. $(NH_4)_2SO_4$ salts (AnalaR, VWR, Leuven, Belgium, further named as 'V'; p.a., Merck, further named as 'M') were used for method development and optimization of method parameters (e.g., anion exchange time, tuning of instruments). $NaHCO_3$ was used for regeneration of anion exchange membranes. Isotope certified reference materials (CRMs) IAEA-S-1, silver sulfide and IAEA-S-2, silver sulfide (both IAEA, Vienna, Austria) were used for calibration and validation of the MC ICP-MS measurement. The solid CRMs were dissolved by microwave-assisted acid digestion (Multiwave 3000, Anton-Paar, Graz, Austria): 6 mL sub-boiled $HNO_3$ was added to 75 mg of a CRM. The digested material was diluted with sub-boiled water to obtain a 3.1mmol $L^{-1}$ S stock solution.

**Investigation of matrix effects**

Dissolved LiCl, $NH_4Cl$, $NH_4H_2PO_4$, $NH_4NO_3$ (all p.a., Merck) and KCl (p.a., Sigma-Aldrich, Buchs, Switzerland) salts, single-element standards (Fe, Na (both CertiPur, Merck), Al, Ca, Mg, Mn (all Inorganic Ventures, Christiansburg, VA, USA)), and 2-propanol (Merck) were used to investigate the matrix effect of elements occurring in the investigated samples on the measured $^{34}S/^{32}S$ ratio. Investigations were performed element per element using a 60 µmol $L^{-1}$ S solution of dissolved IAEA-S-2 certified reference material. The selection of the S concentration was based on the determined optimal S concentration for a reliable MC ICP-MS measurement (see below). $Li^+$ was studied, since LiCl is often used for soil extractions. Ammonium salts were used to investigate the effect of $Cl^-$ and $PO_4^{3-}$. Nitrate was not investigated, since 2 % $HNO_3$ is the measurement matrix and thus matrix matching of standards and samples is given. 2-Propanol was used for simulation of dissolved organic compounds. The concentration of cations, anions, and organic carbon in the simulated matrix was based on the median and the maximum concentrations found in natural soil solution samples under investigation (4 and 204 µmol $L^{-1}$ Al; 98 µmol $L^{-1}$ and 2.5 mmol $L^{-1}$ Ca; 2 and 159 µmol $L^{-1}$ Fe; 56 µmol $L^{-1}$ and 2.9 mmol $L^{-1}$ K; 41 and 535 µmol $L^{-1}$ Mg; 2 and 98 µmol $L^{-1}$ Mn; 30 and 357 µmol $L^{-1}$ Na; 550 µmol $L^{-1}$ and 5.6 mmol $L^{-1}$ $NH_4^+$; 50 and 705 µmol $L^{-1}$ $Cl^-$; 5 and 51 µmol $L^{-1}$ $PO_4^{3-}$; 42 and 83 mmol $L^{-1}$ C). Li concentration was based on the frequently applied extractant concentrations (1 and 10 mmol $L^{-1}$ LiCl). In more detail, Ca, K, Li, Na, and Cl− were investigated: increasing concentrations (0.1, 0.5, 0.8, 1.0, and 2.5 mmol $L^{-1}$ Ca; 0.3, 0.6, 1.0, and 3.1 mmol $L^{-1}$ K; 1.3, 2.5, 5.0, and 10.0 mmol $L^{-1}$ Li; 0.2, 1.1, 2.2, 4.4, and 10.9 mmol $L^{-1}$ Na; 0.3, 0.7, and 1.4 mmol $L^{-1}$ $Cl^-$) were added to the S reference solution and $^{34}S/^{32}S$ ratios were measured. The resulting variations of $^{34}S/^{32}S$ ratios with increasing matrix content were used to establish correlations and to estimate a lower limit of Ca/S, K/S, and Li/S ratios where no significant bias in the isotope ratio measurements can be expected. Effects of $Na^+$ and $Cl^-$ were studied to relate our observations with published literature sources. The anion exchange resin membrane procedure was tested to separate the interfering elements from sulfate.

**Anion exchange on resin membranes**

Commercially available anion exchange resin membranes (551642S, VWR) were cut in 2×3 cm pieces. Membranes were placed in 0.5 mol $L^{-1}$ $HNO_3$ for 1 h for cleaning, rinsed with sub-boiled water, and regenerated for 4 h in a 0.5 mol $L^{-1}$ $NaHCO_3$ (p.a., Sigma-Aldrich) solution. The regenerated membranes were rinsed with sub-boiled water and placed into 15 mL of standard solution or sample. These solutions (containing the membranes) were shaken for 16 h. The membranes were rinsed with sub-boiled water, and the adsorbed sulfate was extracted from the membrane in 15 mL 2 % $HNO_3$ within 1 h of shaking.

Recovery of sulfate was tested for the $SO_4^{2-}$ concentration range found in our soil solution samples. The recovery was tested for actual samples, as well. Since other anions can be found in the soil solution in significant amounts, the influence of anion competition on $SO_4^{2-}$ exchange on the membrane was investigated: $Cl^-$ and $NO_3^-$ anions in concentrations of 0.1, 1.0, 5.0, 10, and 15 mmol $L^{-1}$ were added to the sulfate standard (0.6 mmol $L^{-1}$ $SO_4^{2-}$). The kinetics of anion exchange on the membrane were investigated by placing regenerated resin membranes into a standard solution (0.9 mmol $L^{-1}$ $SO_4^{2-}$) for 10 min, 30 min, and 1, 2, 4, 8, and 16 h. In order to test for sulfate preconcentration by the anion exchange membrane, we reduced the volume of the elution solution to 10 mL and to 5 mL 2 % (m/m) $HNO_3$.

**Environmental samples**

The study sites Jubiläumswarte, Exelberg, and Windischhütte are situated along a distance gradient (8, 10, and 13 km, respectively) from the city of Vienna, Austria, in the Vienna Woods. All sites are pure beech (*Fagus sylvatica*) stands on nutrient-rich soils with a high clay content, developed on Flysch bedrock. More details are given in [23]. Throughfall and precipitation (at an open field adjacent to each stand) samples were collected using polyethylene funnels. Soil solutions were sampled via tension lysimeters (Soilmoisture Equipment Corp., CA, USA) with a manually applied suction of −50 kPa, installed at 10, 30, and 50 cm depth in the mineral soil. Solute samples were taken monthly from May 2010 to May 2011 for $^{34}S/^{32}S$ ratio analysis. All water samples were transported to the laboratory in clean polyethylene bottles and frozen until analysis. The major quantity of the sampled soil solution is collected by the lysimeter immediately after the suction is applied. Hence, we matched rainwater chemistry of the antecedent period with chemistry of soil solution, pumped at the end of this period.

**Quantitative analyses**

The content of dissolved elements in analyzed environmental samples was determined by ICP-OES (Optima 8300, PerkinElmer,Waltham, MA, USA) using external calibration. The content of dissolved anions was determined by liquid anion chromatography (ICS-900, Dionex, Sunnyvale, CA, USA). Total organic carbon was measured by TOC-L analyser (Shimadzu, Kyoto, Japan). Sulfate contents in standards and elemental composition of simulated matrix before and after sulfate separation were determined by single-collector ICP-MS (Element XR, Thermo Fisher Scientific, Bremen, Germany) operated at medium resolution ($R$ = 4000), using external calibration and internal normalization (1 ng $mL^{-1}$ In) prior to isotope ratio analysis.

### $^{34}S/^{32}S$ ratio analyses

A Nu Plasma HR (Nu Instruments, Wrexham, UK) MC ICP-MS was used with a desolvating nebulization system (DSN, Nu Instruments) as sample introduction system for $^{34}S/^{32}S$ ratio analyses. Measurement was performed in edge mass resolution mode ($R$ ~ 2700), resolving spectral interferences (e.g., $^{16}O^{16}O^{+}$, $^{18}O^{16}O^{+}$) from the analyte signal and allowing for measurement on a flat peak shoulder at the same time. For further details on edge mass resolution and the peak shape, see, e.g., [18]. The concentration of sulfur in all samples and standards was adapted to 60 µmol $L^{-1}$ for isotope ratio measurements. At this concentration, the best signal to noise ratio was reached. Gas flow rates and lens system voltages were optimized to reach a sensitivity of minimum 0.1 V / (µmol $L^{-1}$) total S prior to each measurement batch. The operating parameters are summarized in Table 2.3-1. Blank correction was performed automatically by on-peak zero measurement. IIF was corrected by sample – standard bracketing. The bracketing standard IAEA-S-1 was measured before and after each sample at a concentration of 60 µmol $L^{-1}$. All measured ratios have been expressed as delta values, relative to a VCDT $^{34}S/^{32}S$ ratio reference value according to [24]. Accuracy of measurement was assessed by measurement of IAEA-S-2 isotopic certified reference material (certified value, 22.66 ± 0.20 ‰; long-term average of measured values, 22.53 ± 0.51 ‰, 2 *SD*, *n* = 22) (Table 2.3-1).

**Table 2.3-1** Operating parameters of Nu Plasma HR. Gas flow rates were optimized prior to each measurement batch.

| | |
|---|---|
| RF power | 1300 W |
| Auxiliary gas flow rate | 0.91 L $min^{-1}$ |
| Cool gas flow rate | 13 L $min^{-1}$ |
| DSN nebulizer pressure | ~ 30 psi |
| DSN hot gas flow | ~ 3.1 L $min^{-1}$ |
| DSN membrane gas flow | ~ 0.3 L $min^{-1}$ |
| DSN spray chamber temperature | ~ 112 °C |
| DSN membrane temperature | ~ 118 °C |
| Sample uptake rate | ~ 110 mL $min^{-1}$ |
| Axial mass / mass separation | 33.002 / 0.167 |
| Applied Faraday cup detectors | L4: $^{32}S$ |
| | Ax: $^{33}S$ |
| | H5: $^{34}S$ |
| Measurement statistics | 6 blocks |
| | 10 measurements per block |
| Measurement time / sample | ~ 10 min |
| Instrumental background | ~ 1 µmol $L^{-1}$ (total S) |

### Uncertainty estimation

Quantitative measurements are expressed with an estimated uncertainty based on the standard deviation (*SD*) of the measurement. The combined uncertainty of the isotope ratio

measurement was calculated according to the ISO Guide to the Expression of Uncertainty in Measurement [25]. The uncertainties of blank correction (including correlation of blank $^{34}S$ and $^{32}S$ signals), $^{34}S/^{32}S$ measurement precision (*SD*), and IIF correction by standard – sample bracketing were propagated using the Kragten spreadsheet method [26].

## RESULTS

### Matrix constitution and matrix effects on S isotope ratio measurements

The matrix constitution (dissolved cations, anions, and organic carbon compound concentrations) of soil solution, precipitation, and throughfall samples is summarized in Table 2.3-2.

**Table 2.3-2** Mass concentration range and the median concentration of dissolved cations, anions and organic carbon compounds in soil solution, precipitation and throughfall samples. Number of analyzed samples: 1298.

| Component | Concentration / (µmol $L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Soil solution | | Precipitation | | Throughfall | |
| | Median | Range | Median | Range | Median | Range |
| Al | 4 | 0 - 204 | 0 | 0 - 30 | 0 | 0 - 26 |
| Ca | 98 | 10 - 2550 | 23 | 8 - 315 | 50 | 15 - 428 |
| Fe | 2 | 0 - 159 | 0 | 0 - 4 | 0 | 0 - 7 |
| K | 56 | 0 - 2897 | 13 | 0 - 354 | 74 | 8 - 1105 |
| Mg | 41 | 0 - 535 | 4 | 4 - 95 | 21 | 4 - 140 |
| Mn | 2 | 0 - 98 | 0 | 0 - 18 | 0 | 0 - 29 |
| Na | 30 | 0 - 357 | 4 | 0 - 335 | 13 | 0 - 252 |
| $Cl^-$ | 51 | 3 - 705 | 11 | 3 - 412 | 14 | 6 - 370 |
| $NO_3^-$ | 223 | 2 - 3242 | 29 | 3 - 1606 | 77 | 3 - 1123 |
| $PO_4^{3-}$ | 4 | 2 - 67 | 4 | 1 - 27 | 5 | 1 - 54 |
| $SO_4^{2-}$ | 89 | 2 - 1015 | 16 | 4 - 200 | 34 | 4. - 1289 |
| TOC | 642 | 100 - 47000 | 325 | 183 - 1150 | 650 | 83 - 3050 |

Since the soil solutions represent a higher matrix content among the investigated sample types, the effect of the matrix on the S isotope ratio was tested based on concentrations (the median and the maximum concentration) in these samples (see ‘Methods’ section). Figure 2.3-1 shows the influence of cations, anions, and organic carbon on the measured sulfur isotope ratios. The values are expressed as a relative shift from the reference value (grey range).

It was observed that only Ca, K, and Li caused a significant bias of the isotope ratio (i.e., the measured ratio differed from the reference value even under consideration of the expanded

uncertainty). The influence of these elements on the analysis was investigated in more detail by adding stepwise increasing concentrations of these elements to the S standard. The resulting correlations are shown in supplementary Fig. S1 (see bellow). The parameters of these correlations are summarized in Table 2.3-3. The repeatability of the Ca - $\delta^{34}S_{VCDT}$ regression curve within one measurement day was chosen to test the applicability of using a mathematical model for correction of the matrix effect. The relative standard deviation of the slopes of three regression lines was 39 %.

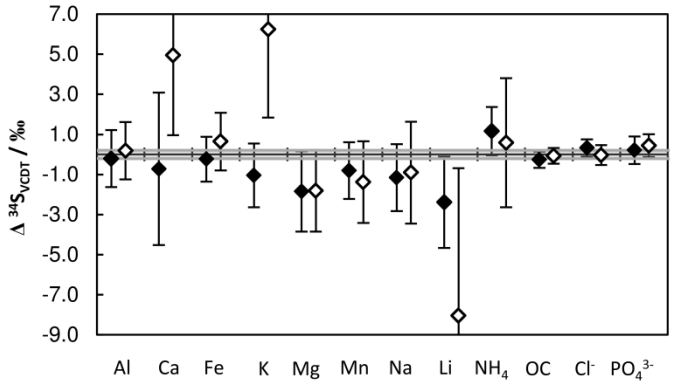



**Fig. 2.3-1** The influence of addition of matrix elements at median (black diamonds) and highest (white diamonds) concentration retrieved in soil solution samples (see Table 2.3-2) on measured $\delta^{34}S_{VCDT}$ values. $\Delta^{34}S_{VCDT}$ represents a relative shift from the reference value (grey range). *OC* stands for organic carbon. Error bars are expanded uncertainties *U* (*k* = 2). The observed increase of uncertainty is explained in following paragraphs

**Table 2.3-3** Regression curves parameters for the dependence of measured $\delta^{34}S_{VCDT}$ values on increasing amount of Ca, K or Li in a S standard. Element/S rat. stands for the lowest Ca/S mass ratio already leading to a significant bias in measured $\delta^{34}S_{VCDT}$ ratios and K/S or Li/S mass ratio leading to imprecise (*U* > 2 ‰, (*k*=2)) measurement.

| Element | Ca | K | Li |
|---|---|---|---|
| Regression type | Linear | Linear | Linear |
| Slope | 0.146 | -0.019 | -0.093 |
| $R^2$ factor | 0.866 | 0.925 | 0.936 |
| Repeatability | 39%, (*n* = 3) | N/A | N/A |
| Element/S rat. | 5 | 5 | 1 |

The observed decrease in the detected signal intensity of $^{32}S$ and the increase of the combined measurement uncertainty with increasing cation concentration in the S standard is

shown in Fig. 2.3-2 on the example of K. The main contributor to the uncertainty is the correction for instrumental background. Since S signal is suppressed significantly by the matrix, the contribution of the instrumental background to the total combined uncertainty increased with increasing matrix concentration. The combined measurement uncertainty increased by a factor of about 5 within the observed concentration range.

Our observations were not fully consistent with previous findings [7], where a possible bias was explained by the presence of $Cl^-$ in the solution (added as NaCl) from a level of 0.3 mmol $L^{-1}$ $Cl^-$ in 0.3 mmol $L^{-1}$ S solution. Therefore, the influence of $Cl^-$ and $Na^+$ on the final $\delta^{34}S_{VCDT}$ value was investigated in more detail. No significant bias in measured $\delta^{34}S_{VCDT}$ ratios was observed when adding up to 1.4 mmol $L^{-1}$ $Cl^-$ (added as $NH_4Cl$) (Cl / S mass ratio = 25). In contrast, the addition of Na caused a significant decrease of $\delta^{34}S_{VCDT}$ ratios of the S standard, from a level of 1.1 mmol $L^{-1}$ (which is far above the concentration range in the investigated samples). The bias effect followed a linear ($R^2$ = 0.974) dependence on the increasing Na concentration (see supplementary Fig. S1). The addition of both $Cl^-$ and $Na^+$ caused an increase of the combined measurement uncertainty, since a suppression of the analyte signal was observed in both cases.

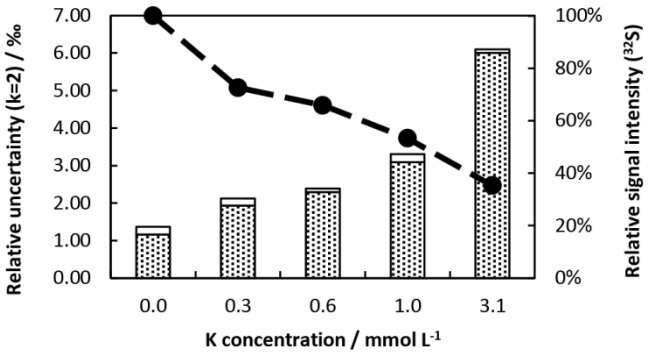



**Fig. 2.3-2** A relative decrease of the signal intensity on $^{32}S$ (dashed line) and increase of the expanded uncertainty of the measurement (bars) with increasing concentration of K in a S standard. White bars show the summarized contribution of measurement precision and calibration of S isotope ratios, and dotted bars show the contribution of blank correction to the uncertainty

**Anion exchange on resin membranes**

The efficiency of sulfate separation by the anion exchange resin on a membrane was tested for the $SO_4^{2-}$ concentration range of the investigated solution samples (see Table 2.3-2 and supplementary Table S1 (see bellow)). One hundred percent recovery (±1 %, *SD*, *n* = 14) was accomplished for all samples in a pH range of 2–11. The influence of $Cl^-$ and $NO_3^-$ on the sulfate exchange efficiency was negligible at a concentration of less than 5 mmol $L^{-1}$

(which corresponds to the concentration ranges in the investigated samples). At a $Cl^-$ and $NO_3^-$ concentration of 10 and 15mmol $L^{-1}$, the recovery of sulfate decreased to 65 and 55 %, respectively. The kinetics were studied by using a 0.9 mmol $L^{-1}$ sulfate standard. It was observed that sulfate from the immerse solution was exchanged quantitatively within 1 h. Addition of Ca, K, Li, or Na changed the kinetics (see supplementary Table S1) leading to slower exchange rates (or lower sulfate recovery). Moreover, an enrichment factor of about three was obtained under routine laboratory conditions when starting with an initial volume of 15 mL and an elution volume of 5 mL (which corresponds to the volume needed for the subsequent direct isotope ratio measurement). Laboratory tests are summarized in supplementary Table S1 (see bellow).

Quantitative matrix separation was obtained for all elements under investigation: Ca (up to 2.5 mmol $L^{-1}$), K (up to 4.2 mmol $L^{-1}$), Li (up to 7.2 mmol $L^{-1}$), Na (up to 3.9 mmol $L^{-1}$), organic carbon (up to 12.5 mmol $L^{-1}$), and Ca (up to 2.5 mmol $L^{-1}$).

Isotope ratio analysis showed no significant difference in $\delta^{34}S_{VCDT}$ values between the initial solution and the eluate for both tested ammonium sulfate solutions (Fig. 2.3-3). Seven replicate analyses were performed for the $(NH_4)_2SO_4$ salt 'V' (V1–V7) and five for the $(NH_4)_2SO_4$ salt 'M' (M1–M5) solutions, following the procedure described in the 'Methods' section. Sulfate enrichment by elution in reduced elution volume (10 or 5 mL) did not show an isotopic effect (see supplementary Fig. S2). Furthermore, no effect was observed in a simulated matrix solution, when the concentration of dissolved anions ($NO_3^-$ or $Cl^-$ accompanying added matrix elements) did not exceed 5 mmol $L^{-1}$ in Ca- and K-enriched solutions (see supplementary Table S1).

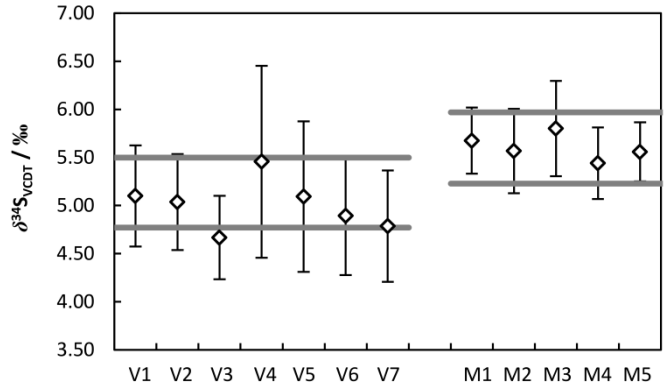



**Fig. 2.3-3** Reproducibility of the sulfate separation procedure in combination with MC ICP-MS on the example of V1–V7 and M1–M5 $(NH_4)_2SO_4$ solutions. Horizontal grey lines show upper and lower $\delta^{34}S_{VCDT}$ limits of the corresponding initial solution (mean of three measurements ± $U$ ($k$ = 2)). Error bars are expanded uncertainties $U$ ($k$ = 2)

### Precipitation, throughfall, and soil solution samples

The results are presented as averaged values from the three sampling sites. The $\delta^{34}S_{VCDT}$ of precipitation as well as throughfall samples $\delta^{34}S_{VCDT}$ ranged between 4.0 and 6.5 ‰. The maximum was reached in December 2010 and April 2011. $\delta^{34}S_{VCDT}$ values of soil solution sampled at 10, 30, and 50 cm ranged between 3.6 and 4.7 ‰. No significant difference was determined between different soil depths. Therefore, the $\delta^{34}S_{VCDT}$ values of soil solutions averaged over all three soil depths, precipitation, and throughfall were plotted against sampling months in Fig. 2.3-4.

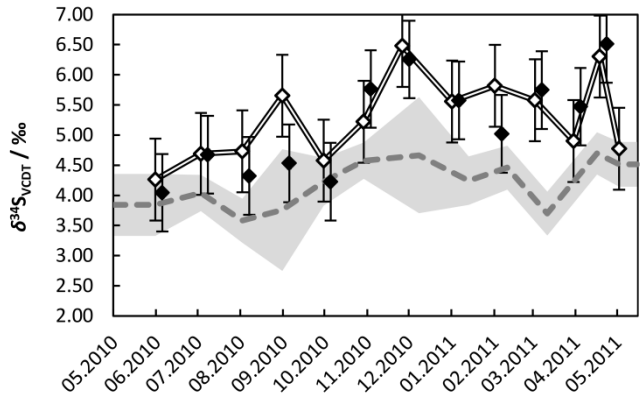



**Fig. 2.3-4** Mean sulfur isotopic composition of rainwater and soil solution sulfate ($n$ = 3 for each data point). Precipitation (open diamonds) corresponds well with throughfall (black diamonds). Soil solution (dashed line, mean of three soil depths) follows the trend of rainwater. Error bars and the grey area width represent combined uncertainties

## DISCUSSION

### Matrix effects

MC ICP-MS enables the direct analysis of $^{34}S/^{32}S$ ratios in dissolved sulfate. However, sample matrix elements can influence the precision and accuracy of the measurement [7, 14]. This is an important issue especially in soil solutions as they show distinctly higher concentration levels as compared to precipitation samples. Primarily, the high contents of dissolved Ca or K (both reach more than 2.5 mmol $L^{-1}$) might question the applicability of MC ICP-MS for a direct and reliable analysis of $^{34}S/^{32}S$ ratio without any matrix effects correction.

Ammonium, Al, Fe, Mg, Mn, Na, organic C, $Cl^-$, or $PO_4^{3-}$ did not cause a significant shift in measured $\delta^{34}S_{VCDT}$ ratios for the concentration ranges found in the investigated samples (see Fig. 2.3-1). Contrary to [14], we did not observe a matrix effect caused by Fe in our simulated matrix, although one of our tested Fe / S mass ratios (concentrations of 2 and 159 µmol $L^{-1}$ Fe correspond to Fe / S mass ratios of 0.05 and 4.4, respectively) was above

the ratio published by Craddock (Fe / S=0.9). Addition of Ca, K, and Li resulted in a significant shift in the measured $\delta^{34}S_{VCDT}$ ratios (up to −8 ‰) and led also to a pronounced increase in measurement uncertainty (e.g., a $U$ ($k$ = 2) of 7.4 ‰ was reached when adding 10 mmol $L^{-1}$ Li). Lin [7] observed a strong effect of $Cl^-$ on the $\delta^{34}S_{VCDT}$ measurement when adding NaCl to his in-house S standard. Since our observations were different and $NH_4Cl$ addition caused no bias in our measurement, we could relate this effect mainly to the presence of Na in the solution, since addition of Na led to decrease of the measured $\delta^{34}S_{VCDT}$ values similarly to [7].

[7] and [13] suggest a matrix-matched bracketing standard to correct for matrix effects. It proved that this is hardly possible in the study of a large number of soil solutions with a high variation of matrix elements. Even though the matrix effect can be approximated by a linear function (starting from Ca / S or K / S mass ratios higher than 5 or Li / S mass ratio of 1), the poor repeatability of the regression curve (39 % on the example of Ca) shows that a simple mathematical correction of matrix effects is not conductive as this correction leads to increased measurement uncertainties. Moreover, the decrease of the signal intensity leads likewise to a significant increase in the measurement uncertainty. The use of an internal standard (e.g., Si) [5, 18] is mainly hampered by the decrease of the analyte signal intensity as well. In addition, it cannot be assumed a priori that internal standard and analyte are subject of the same IIF. As a consequence, a sulfate / matrix separation has proven to be a precondition for accurate S isotope ratio analysis by MC ICP-MS.

**Anion exchange on resin membranes**

The applied anion exchange resin on plastic membrane proved its suitability for sulfate sampling from ammonium sulfate solution, as well as from a simulated soil solution matrix. Sulfate was taken up quantitatively by the membrane under the investigated parameter (simulating natural conditions). At the same time, all studied matrix elements (cations and organic carbon) remained completely in the initial solution. Addition of Ca, K, Li, and Na slowed down the exchange rate of sulfate on the resin significantly. As all these elements were added as salt solutions to a S standard, the deceleration can be explained by the presence of dissolved anions (up to 32 mmol $L^{-1}$ $NO_3^-$ when adding 2.5 mmol Ca single-element standard). Due to this observed reduction of anion exchange rates caused by co-dissolved anions and in accordance with literature [9], an exposure time of 16 h was chosen. The anion exchange proved to be robust in a pH range between 2 and 11 which covers well the range which is expected in natural precipitation and soil solution samples.

Kwon *et al*. tested competitive anion exchange on their plant root simulator [9]. They observed that nitrate occupied a significant portion of the exchange sites and hampered the exchange of sulfate. In our experiments, the sulfate exchange was slowed down significantly

first at $Cl^-$ and $NO_3^-$ concentration of 5.0 mmol $L^{-1}$, corresponding to $Cl^-$ / S, resp. $NO_3^-$ / S molar ratio of 8.

Due to the large variation of sulfate in natural samples, preconcentration via the anion exchange resin is an asset. Depending on the initial volume and the final elution volume, a significant preconcentration is achievable by the use of an anion exchange membrane under routine laboratory conditions without compromising quantitative S / matrix separation.

Isotope ratio analyses of initial S standard solutions and eluates proved that no significant isotopic fractionation during sulfate separation occurred for both low (40 µmol $L^{-1}$ $SO_4^{2-}$) and high (1.25 mmol $L^{-1}$ $SO_4^{2-}$) sulfate concentrations independent of the initial volume / elution volume ratio (see Fig. 2.3-3). Separation of sulfate from a simulated matrix was not accompanied by isotopic fractionation either (see supplementary Fig. S2). Only $NO_3^-$ and $Cl^-$ added together with the investigated matrix elements in concentrations higher than 5 mmol $L^{-1}$ caused a significant fractionation of sulfur stable isotopes during the sulfate separation (see supplementary Table S1). This was accompanied with significantly lower recovery (down to 12 % when $NO_3^-$ concentration reached 32 mmol $L^{-1}$). However, such a high anion concentration was not found in any of the more than 1000 analyzed natural water samples (see Table 2.3-2). Therefore, we state that the separation technique is suitable for $^{34}S/^{32}S$ analysis of dissolved sulfate in natural water samples.

**Precipitation, throughfall, and soil solution samples**

The developed method was applied for a study on water samples from forest ecosystems in the Vienna Woods. Wet and dry depositions of atmospheric sulfur are the main sources of sulfate in the environment [10]. Elemental composition of throughfall is given by elements present in precipitation, by material deposited as particles, gases, or cloud droplets being washed off during a precipitation event, and by exchange processes within the canopy (including foliage, woody parts, epiphytes, and microorganisms). Canopy exchange includes both leaching (efflux from the canopy) and uptake or retention (influx to the canopy) [27]. Therefore, precipitation and throughfall were compared in this study. No significant difference in $\delta^{34}S_{VCDT}$ values was observed between the two water types. This indicates that neither dry deposition on the leaf surface nor canopy exchange processes affect the isotopic composition of S significantly. However, S isotope fractionation may be hidden behind the combined uncertainty of the measurement. Higher $\delta^{34}S_{VCDT}$ values during winter months (December) and spring (April) might be caused by the change in emission sources (e.g., elevated central and domestic heating during the winter), because, depending on fuel used, the emitted $SO_2$ - $\delta^{34}S_{VCDT}$ values can vary strongly [1].

No significant change in soil solution $\delta^{34}S_{VCDT}$ values was observed for different soil depths (10, 30, and 50 cm). From this point of view, the ecosystem seems to be homogeneous within the first 50 cm soil depth in our study sites.

When comparing throughfall with soil solution, lower absolute values of $\delta^{34}S_{VCDT}$ were observed in the soil solutions even though the results overlap within their uncertainties. Depletion in $^{34}S$ of $SO_4^{2-}$ in soil solution in comparison to $SO_4^{2-}$ in throughfall may indicate S mineralization as a potential $SO_4^{2-}$ source, because the soil microflora prefers the lighter $^{32}S$ isotope [11]. Furthermore, it has been suggested for aerobic, forest soils that the mineralization of labile organic S produces $SO_4^{2-}$ that is more depleted in $^{34}S$ compared to adsorbed $SO_4^{2-}$ or the $SO_4^{2-}$ in soil solution. Adsorption / desorption causes no significant isotopic discrimination [1]. The $\delta^{34}S_{VCDT}$ values of this study indicate that the soil solution $SO_4^{2-}$ budget is driven by throughfall chemistry. A considerable portion of the atmospherically deposited sulfate is cycled through the organic S pool before being released to the soil solution. This cycling is reflected in the abovementioned lower $\delta^{0}S_{VCDT}$ values in the soil solutions. During most of the year, the S isotopic composition of the soil solution follows the pattern of throughfall without substantial delay. Adsorption and desorption are, thus, not important processes within the nutrient-rich (high-pH) soils.

## SUPPLEMENTARY MATERIAL

The supplementary Figures S1 and S2 and the supplementary Table S1 are available free of charge via the internet at http://link.springer.com/article/10.1007/s00216-015-9053-z.

## ACKNOWLEDGMENTS

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## *2.4* Novel diffusive gradients in thin films technique to assess labile sulfate in soil

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## ABSTRACT

A novel diffusive gradients in thin films (DGT) technique for sampling labile soil sulfate was developed, based on a strong basic anion exchange resin (Amberlite IRA-400) for sulfate immobilization on the binding gel. For reducing the sulfate background on the resin gels, photopolymerization was applied instead of ammonium persulfate induced polymerization. Agarose cross-linked polyacrylamide (APA) hydrogels were used as diffusive layer. The sulfate diffusion coefficient in APA gel was determined as $9.83 \times 10^{-6} \pm 0.35 \times 10^{-6}$ $cm^2$ $s^{-1}$. The accumulated sulfate was eluted in 1 mol $L^{-1}$ $HNO_3$ with a recovery of 90.9 % ± 1.6 %. The developed method was tested against two standard extraction methods for soil sulfate measurement. The obtained low correlation coefficients indicate that DGT and conventional soil test methods assess differential soil sulfate pools, rendering DGT a potentially important tool for measuring labile soil sulfate.

## INTRODUCTION

Sulfur (S) is a major plant macronutrient as part of e.g. amino acids, proteins and coenzymes. It is involved in the plant metabolism as well as in the response to oxidative stress [1]. Sulfur deficiency in arable soils has been reported to become one of the major limitations in crop production [2]. Plants take up S as sulfate ($SO_4^{2-}$) from the soil porewater. Therefore, determination of labile soil $SO_4^{2-}$ is essential for the investigation of S phytoavailability in soils [1-3].

Batch extraction techniques using different extractant solutions, e.g. $H_2O$, 0.03 mol $L^{-1}$ $KH_2PO_4$ and 1 mol $L^{-1}$ HCl [4,5], are the most common methods to assess readily available, adsorbed, and carbonate-occluded soil sulfate, respectively. Common agricultural S testing methods include the KCl-40 test, which uses 0.25 mol $L^{-1}$ KCl as an extractant [6]. This method was proposed to be more representative for plant available soil S than the MCP-S method (using 0.01 mol $L^{-1}$ $Ca(H_2PO_4)_2$), as KCl-40 provides a measure of adsorbed and soluble $SO_4^{2-}$, including gypsum.

Tension lysimeters or suction cups are alternative methods for assessing dissolved soil S by directly taking soil porewater samples [1,3]. However, they do not account for the reversibly adsorbed fraction of soil $SO_4^{2-}$. Sampling strategies employing ion resins as $SO_4^{2-}$ sinks, which deplete $SO_4^{2-}$ in the soil porewater and thereby induce desorption from the solid phase, have been developed to additionally account for this soil $SO_4^{2-}$ pool [7]. This approach has the additional advantage of pre-concentrating $SO_4^{2-}$ on the resin. However, as the resin is directly exposed to the soil [7,8], it may be easily contaminated with soil particles [8].

Competition of other anions (e.g., phosphate, nitrate) for binding sites of the resin may also lead to sampling artefacts [9].

Diffusive gradients in thin films (DGT) is an advanced sink technique, in which the resin is embedded in a hydrogel layer and is covered by a pure hydrogel disc and a protective membrane. This setup prevents particle contamination effectively and allows for the calculation of the time-averaged analyte ($SO_4^{2-}$) concentration due to the well-defined diffusion geometry [10]:

$$c_{DGT} = \frac{M \cdot \Delta g}{D \cdot A \cdot t} \qquad \text{(Equation 2.4-1)}$$

*M* is the mass of analyte bound on the resin layer, Δ*g* is the diffusive layer thickness, *D* is the diffusion coefficient of the analyte in the diffusive layer, *A* is the sampling area and *t* is the sampling time.

The DGT methodology has been shown to perform exceedingly well in assessing the bioavailable solute fraction if the solute availability is limited by diffusion [11]. Several studies demonstrated that soil phosphate assessed by DGT correlated better with plant phosphate uptake [11-13] and with crop yield responses to applied P [14] compared to conventional batch extractions or other resin-based sampling techniques [11,13]. Guppy and Blair [15] have shown that established methods (KCl-40 and MCP) were poor at predicting maize S uptake and responses to S applications in a short-term glasshouse experiment. Therefore an improved, simple and quick laboratory method like DGT for determining available S could have significant benefits.

No DGT method for $SO_4^{2-}$ sampling is currently available. The only S species for which a DGT method is available is sulfide, which is sampled by the conversion of AgI to $Ag_2S$ [16]. As sulfate sorption to oxide minerals (e.g. ferrihydrite, zirconium oxide) [17,18], which have been used for measuring oxyanions (e.g. $PO_4^{3-}$and $AsO_4^{3-}$) by DGT so far, is weak, a general anion exchange resin is the material of choice for sampling sulfate with DGT.

In this study, we present a novel DGT technique for the sampling of labile soil $SO_4^{2-}$. The developed anion exchange resin gel was characterized (regarding its $SO_4^{2-}$ uptake capacity, pH working range and elution efficiency) for applications in soil. Comparison of its performance with traditional techniques assessing soil $SO_4^{2-}$, the KCl-40 and MCP extractions, shows that DGT samples a different $SO_4^{2-}$ pool and is therefore a potential alternative for soil $SO_4^{2-}$ testing.

## MATERIALS AND METHODS

### General laboratory procedures

All consumables were double acid washed using 10% (*w*/*w*) and 1% (*w*/*w*) $HNO_3$ (p. a., Merck, Darmstadt, DE) and rinsed with laboratory water type I (0.055 µS $cm^{-1}$; TKA-GenPure, Niederelbert, DE) before use. Laboratory water type I was used for preparation of all standard solutions, for soil extractions and for water saturation of soil samples. Laboratory water type I and $HNO_3$ were further purified by a sub-boiling distillation system (Milestone Inc., Shelton, CT, US) and used for the elution of $SO_4^{2-}$ from the resin gel (1 mol $L^{-1}$ $HNO_3$) and for microwave-assisted digestions (Multiwave 3000, Anton Paar, Graz, AT).

### Diffusive and resin gel preparation

Agarose cross-linked polyacrylamide (APA) diffusive hydrogels of 0.8 mm thickness were prepared according to [10] and cut to discs. Amberlite IRA-400 (chloride form, Sigma Aldrich, Buchs, CH) resin was selected as a binding agent for $SO_4^{2-}$. The resin was ground with a ball mill for 10 minutes, passed through a 200 µm sieve and washed in 10% HCl (p.a., Merck), repeating this step twice followed by four rinses with pure water, to reduce the background S on the resin.

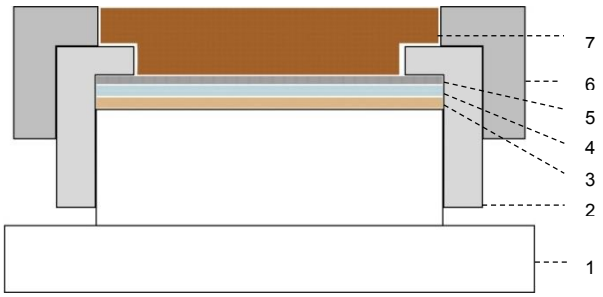
The common acrylamide polymerization technique used for DGT gels applies ammonium persulfate (APS) as initiator [10]. This approach is not suitable for preparing resin gels for the sampling of $SO_4^{2-}$, as elevated background S levels on the binding gel can be expected. To reduce background S levels, photopolymerization using Riboflavin ((-)-Riboflavin, Sigma Aldrich) as photoinitator was applied. The polymerization was started through the decomposition of Riboflavin upon exposition to a light source. A detailed study on the riboflavin-initiated polymerization of acrylamide was published e.g. by Oster *et al*. [19].

Three grams (wet weight) of ground and washed Amberlite IRA-400 were mixed with 10 mL gel solution prepared as described in [10] and 60 µL of riboflavin solution (0.01 g riboflavin in 10 mL $H_2O$) and 20 µL of tetramethylethylendiamine (TEMED; VWR Int., Randor, US) were added. The solution was shaken well and cast between two acid-washed glass plates (6 x 20 cm) separated by a U-shaped acid-washed plastic spacer (0.4 mm thickness). The glass plate with the freshly coated gel solution was left under fluorescent light overnight. Gels appeared to set after about 1 hour. The resin gels produced in this way were relatively weak and subject to tearing. While avoiding the binding of $SO_4^{2-}$ from APS to the resin gels was important for preventing elevated S background levels, such precautions are not necessary for diffusive gels, which have no capability for $SO_4^{2-}$ binding. Therefore the diffusive gels used in this study were produced using the classical procedure [10]. Any residual S

introduced as APS was washed off the diffusive gels during the gel hydration step. A 10 mmol $L^{-1}$ $NaNO_3$ solution was used for storage of all gels (Reagent Plus, Sigma Aldrich).

Polyethersulfone filters (0.45 µm pore size, 0.13 mm thick, Sartorius Stedim, Goettingen, DE) were used as a protective membrane. The filters were washed with 1 mol $L^{-1}$ $HNO_3$ overnight and stored in 10 mmol $L^{-1}$ $NaNO_3$. DGT samplers (DGT Research Ltd., Lancaster, UK) were used for both solution and soil tests. The schematic of the DGT device is pictured in Figure 2.4-1a. Figure 2.4-1b shows the application to soil (see below).

a)




b)

**Figure 2.4-1** DGT sampling device schematic (1a): 1. piston, 2. outer sleeve with sampling window, 3. resin (Amberlite IRA-400) gel, 4. diffusive (APA) gel, 5. protective membrane, 6. plastic frame to hold the soil sample in place, 7. soil sample. Application of the DGT device to soil (1b).

**Evaluation of DGT sampling**

Sulfur background level

The sulfur contents in the protective membrane (acid-washed and unwashed), in the diffusive gel and in the resin gel (S background concentrations) were determined after elution in 10 mL 1 mol $L^{-1}$ $HNO_3$ for 16 h and calculated as S amount per membrane or gel disc. The S content of the eluent was used to determine the instrument limit of detection of the

inductively coupled plasma mass spectrometer (ICP-MS, see below) (*LOD* = average eluent S content + 3 × standard deviation).

The method blank (resin gel, diffusive gel and protective membrane in DGT sampler placed for 4 h in a moist plastic bag at 21 °C) was measured in 10 mL 1 mol $L^{-1}$ $HNO_3$ eluate of the resin gel and calculated as S amount per resin gel disc. The S contents were used to determine the method limit of detection for S by ICP-MS (*MDL* = average method blank S content + 3 × standard deviation).

Resin gel elution efficiency (*R*)

A recovery experiment using a $^{35}S$ radiotracer (New England Nuclear, Perkin Elmer, Waltham, MA, US) was conducted. 10 mL 0.5 mg $L^{-1}$ S ($(NH_4)_2SO_4$, p.a., Merck) and 10 mL 10 mg $L^{-1}$ S ($(NH_4)_2SO_4$) solutions were spiked with 980 Bq $^{35}S$. A resin gel was immersed in each solution. The solutions were shaken for 4 h and the gels were eluted in 10 mL 1 mol $L^{-1}$ $HNO_3$ for 16 h subsequently. This experiment was repeated 5 times.

Diffusion coefficient (*D*)

The diffusion coefficient *D* of $SO_4^{2-}$ in APA gel was determined using a diffusion cell [20]. The cell consisted of two 110 mL perspex containers, each with a 1.59 cm diameter opening. A 2.5 cm diameter diffusive gel disc was placed between the openings and the containers were clamped together. 100 mL water (pH 5.6) were introduced into one of the containers and the tightness of the clamping was checked. Then, 100 mL $(NH_4)_2SO_4$ (p.a., Merck) solution (pH 5.6) was introduced into the second container. To ensure that *D* is not concentration-dependent, sulfate solutions of 1 mg $L^{-1}$, 10 mg $L^{-1}$ and 45 mg $L^{-1}$ S were used. Each sulfate solution was spiked with 370000 Bq $^{35}S$. Solutions in both containers were stirred continuously. Subsamples were taken from both containers in time intervals of about 30 min to follow the diffusion of S through the APA gel. *D* and its uncertainty were calculated as described in [21]. The final coefficient $D_{25}$ was calculated as a mean value of the diffusion coefficients determined in the 1 mg $L^{-1}$, 10 mg $L^{-1}$ and 45 mg $L^{-1}$ S solutions at 25 °C. The *D* value in further experiments was calculated from $D_{25}$ by temperature adjustment using equation 2.4-2 [10]:

$$\log D_t = \frac{1.37023(t-25)+8.36\times10^{-4}(t-25)^{-2}}{109+t} + \log\frac{D_{25}(273+t)}{298} \qquad \text{(Equation 2.4-2)}$$

pH working range

Sulfate as $(NH_4)_2SO_4$ was dissolved in laboratory water type I to reach a S concentration of 4 - 5 mg $L^{-1}$. The solutions (3 L each) were stirred until equilibrium with air was reached and pH was stable. The pH of the solutions was set to 2.98, 3.48, 3.55, 3.97, 4.00, 5.00, 5.10, 5.60, 6.12, 7.02, 7.40, 8.18, 8.34, and 9.05 using $HNO_3$ and NaOH. Three to four DGT samplers were exposed to each solution for 4 hours. The temperature was monitored throughout the experiments. The calculated $c_{DGT}$ values (Equation 2.4-1) were compared to the S concentration in the corresponding immersion solution, $c_{soln}$.

Gel capacity

A synthetic soil solution was prepared for testing the resin gel capacity for $SO_4^{2-}$ uptake under realistic conditions, *i.e.* taking the competing anion species chloride, nitrate and phosphate into account. The concentration of $SO_4^{2-}$ was chosen based on typical porewater $SO_4^{2-}$ concentrations [22]. For obtaining a realistic and conservative estimate of the gel $SO_4^{2-}$ capacity, concentrations of $Cl^-$, $NO_3^-$ and $PO_4^{3-}$, based on upper level of the concentration ranges of own and literature soil solution data [3, 22], were chosen. Sulfate as $(NH_4)_2SO_4$ was dissolved in laboratory water type I (6 L) to reach a $SO_4^{2-}$ concentration of 15 mg $L^{-1}$. NaCl, $NaNO_3$ and $KH_2PO_4$ were added to the $SO_4^{2-}$ containing solution to reach concentrations of 9.0, 60 and 7.5 mg $L^{-1}$ of $Cl^-$, $NO_3^-$ and $PO_4^{3-}$, respectively. DGT samplers (21 in total) were placed into this solution. After 3, 6, 9, 15, 24, 39 and 48 hours, three samplers were taken out at a time. Temperature and pH were monitored during the experiment. The resin gels were eluted in 1 mol $L^{-1}$ $HNO_3$ subsequently. The content of $SO_4^{2-}$, $Cl^-$ and $PO_4^{3-}$ in the eluates was measured ($NO_3^-$ content could not be measured as $HNO_3$ was used for elution). The gel capacity was estimated as the highest mass accumulated on the gel that did not differ significantly from the theoretical mass uptake according to Eqn. 1.

**Analyses**

ICP-MS

Single collector sector field ICP-MS (Element XR, Thermo Fisher Scientific, Waltham, MA, US) was used for S quantification in standard solutions and resin gel eluates during method development in the VIRIS Laboratory, Tulln (AT). External calibration (0 – 3 mg S $L^{-1}$) and internal standardization (using 1 µg $L^{-1}$ In) were applied. The instrumental limit of detection (LOD) was 3 µg S $L^{-1}$.

$^{35}S$ radiotracer

Isotope dilution using $^{35}S$ was used when very low S concentrations were expected (determination of diffusion coefficient) and for validation of some data obtained by ICP-MS measurement (elution efficiency). A liquid scintillation counter Tri-Carb 2910 TR (Perkin Elmer) was used for measuring the beta radiation emitted by $^{35}S$. Measurements were performed on a comparative basis, *i.e.*, the activity of the eluates was compared to the activity of immersion solutions for the determination of the elution efficiency. The gradual increase of activity in subsamples in time was used for determination of the diffusive coefficient.

ICP-OES

All DGT eluents and extraction solutions from the soil survey (see 2.6) were measured for their S content using ICP-OES (Optima 7000 DV, Perkin-Elmer) at 181.975 nm at the University Adelaide (AU). External calibration (0 – 10 mg S $L^{-1}$) was applied. The instrumental limit of detection was 20 µg S $L^{-1}$.

**Uncertainty estimation**

The calculation of the combined uncertainty of the elution efficiency ($u_R$, equation 2.4-3) was based on the combination of the measurement repeatability ($SD_1$, $n$ = 5) and reproducibility ($SD_2$, $n$ = 3), combining thus both sample heterogeneity and measurement reproducibility:

$$u_R = \sqrt{SD_1^2 + SD_2^2} \qquad \text{(Equation 2.4-3)}$$

The uncertainty estimation of the quantitative measurement was calculated by applying the approach of partial derivatives ($u_{cDGT}$, equation 2.4-4) based on [21]. Its calculation included the uncertainties of S quantification ($u_{MEAS}$, which comprises measurement precision of analyte and of internal standard, blank correction uncertainty and uncertainty of calibration), the uncertainty of the diffusive layer thickness ($u_{DL}$), the uncertainty of the sampling window surface area ($u_A$) and the uncertainty of the diffusion coefficient ($u_D$, comprising the uncertainty of the slope of the mass vs. time line, of the thickness of the diffusive gel, of the surface area of the connection of the diffusion cell halve and of the original concentration of S in the solution), the sampling time ($u_t$) and the elution efficiency ($u_R$):

$$\frac{u_{cDGT}}{c_{DGT}} = \sqrt{\left(\frac{u_{MEAS}}{c_S}\right)^2 + \left(\frac{u_{DL}}{DL}\right)^2 + \left(\frac{u_A}{A}\right)^2 + \left(\frac{u_D}{D}\right)^2 + \left(\frac{u_t}{t}\right)^2 + \left(\frac{u_R}{R}\right)^2} \qquad \text{(Equation 2.4-4)}$$

where $c_S$ is the determined S concentration in the eluate, *DL* is the diffusive layer thickness, *A* is the sampling window area, *D* is the diffusion coefficient, *t* is the sampling time and *R* is the elution efficiency.

Estimation of the uncertainty of the $c_{DGT}/c_{soln}$ ratio, which was e.g. used for determining the pH working range, comprised both the uncertainty of the $c_{DGT}$ value ($u_{cDGT}$, Equation 2.4-4) and the uncertainty of the determination of the S concentration in the immersion solution ($u_{MEAS2}$, which comprises measurement precision of analyte and of internal standard, blank correction uncertainty and uncertainty of calibration):

$$\frac{u(c_{DGT}/c_{soln})}{c_{DGT}/c_{soln}} = \sqrt{\left(\frac{u_{cDGT}}{c_{DGT}}\right)^2 + \left(\frac{u_{MEAS2}}{c_{soln}}\right)^2} \qquad \text{(Equation 2.4-5)}$$

Significance of a difference between mean values ($c_{DGT}/c_{Soln}$ vs. 1.0 line in pH working range determination, and experimantal vs. theoretical DGT uptake in gel capacity determination) was tested with respect to the expanded uncertainties ($U = 2 \times u_X$) of the mean values to cover 95% confidence interval. Two mean values were significantly different, if

$$|m_1 - m_2| > \sqrt{U_{m1}^2 + U_{m2}^2} \qquad \text{(Equation 2.4-6)}$$

where $m_1$ and $m_2$ represent the mean values and $U_{m1}$ and $U_{m2}$ their expanded uncertainties [23].

**Comparison of DGT S with conventional soil S extraction techniques**

We assessed the relation of DGT sampled S and S extracted by two conventional soil extraction methods (KCl-40, MCP) of 8 agricultural soils (see Table 2.4-1) from major cropping regions in Australia. The soil parameters were determined by standard methods following [24]. To determine S by KCl-40, 4.5 g of air-dried soil were extracted in 30 mL 0.25 mol $L^{-1}$ KCl at 40 °C. The mixture was incubated for 3 h at 40 °C and was repeatedly shaken by hand. The supernatant was separated by centrifugation. To determine MCP-S,

20 g of air-dried soil was mixed with 100 mL 0.01 mol $L^{-1}$ $Ca(H_2PO_4)_2$ at pH 4. The mixture was shaken over-head for 17 h. The supernatant was separated by centrifugation [24]. For the DGT technique, soils were moistened to 100 % water holding capacity (WHC) one day prior to deployment. Six DGT devices were deployed on each soil for 6 hours at a constant temperature of 21 °C (see Figure 2.4-1b). After deployment, DGT devices were rinsed with laboratory water type I and the binding gels were retrieved and eluted.

**Table 2.4-1** Soil properties

| Soil name | Abbrev. | State of origin (Australia) | Texture | Clay % | WHC*) % | pH ($CaCl_2$) | $C_{org}$ % | $N_{tot}$ % |
|---|---|---|---|---|---|---|---|---|
| Birchip | BI | VIC | Medium clay | 31 | 24.9 | 7.7 | 0.75 | 0.13 |
| Hart | HA | SA | Medium clay | 38 | 52.8 | 6.4 | 1.49 | 0.16 |
| Karoonda | KA | SA | Sand | 2 | 20.1 | 5.4 | 0.39 | 0.08 |
| Keith | KE | SA | Sandy clay | 15 | 20.2 | 5.0 | 1.94 | 0.17 |
| Lake Bolac | LB | VIC | Sand | 3 | 39.4 | 5.9 | 1.33 | 0.15 |
| Mt Barker | MB | WA | Sand | 12 | 28.7 | 5.6 | 2.49 | 0.20 |
| Otterbourne | OT | ACT | Medium clay | 13 | 32.5 | 5.4 | 3.00 | - |
| Tumby Bay | TB | SA | Sandy clay | 17 | 22.4 | 4.6 | 3.00 | 0.23 |

*) water holding capacity

## RESULTS

### Blank levels, LOD, diffusion coefficient, elution efficiency

The background S signals measured in the eluent (1 mol $L^{-1}$ $HNO_3$), in eluates of the acid-washed membrane, and of the diffusive gel were below the instrument limit of detection of ICP-MS, see Figure 2.4-2. The background S content of the unwashed protective membrane and resin gel reached 0.69 ± 0.17 µg S per membrane disc and 0.34 ± 0.15 µg S per gel disc (average ± 1 SD), respectively.

The method limit of detection (*MDL*) was 0.29 mg S $L^{-1}$. The S loading of the resin gel in the method blank was 1.64 ± 0.63 µg S per gel disc (average ± 1 SD). For some gel discs, the loading reached to 2.23 µg S. However, some batches of the resin gel showed S loadings below the instrument LOD.

The mass S diffused through the APA diffusive gel over time is displayed in Figure 2.4-3. The diffusion coefficient of $SO_4^{2-}$ at 25 °C ($D_{25}$) calculated from these slopes was $9.83 \times 10^{-6} \pm 0.35 \times 10^{-6}$ $cm^2$ $s^{-1}$ ($u_c$). The main contributor (about 90 %) to the combined uncertainty was the uncertainty of the correlation between time and mass diffused through the gel.

The elution efficiency $R$ was 90.9 ± 1.6 % ($u_c$). These values, together with their uncertainties, were applied for further calculations.

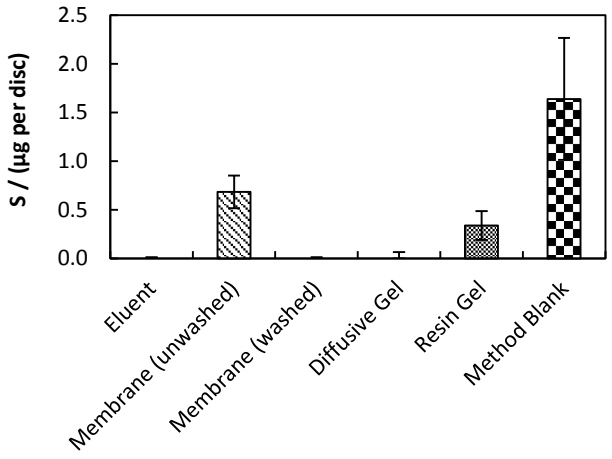



**Figure 2.4-2** Background signal of the eluent (1 mol $L^{-1}$ $HNO_3$) and S loadings of eluted membranes and gels. Washed membrane and diffusive gel are below instrument LOD. Error bars are 1 SD ($n$ = 4).

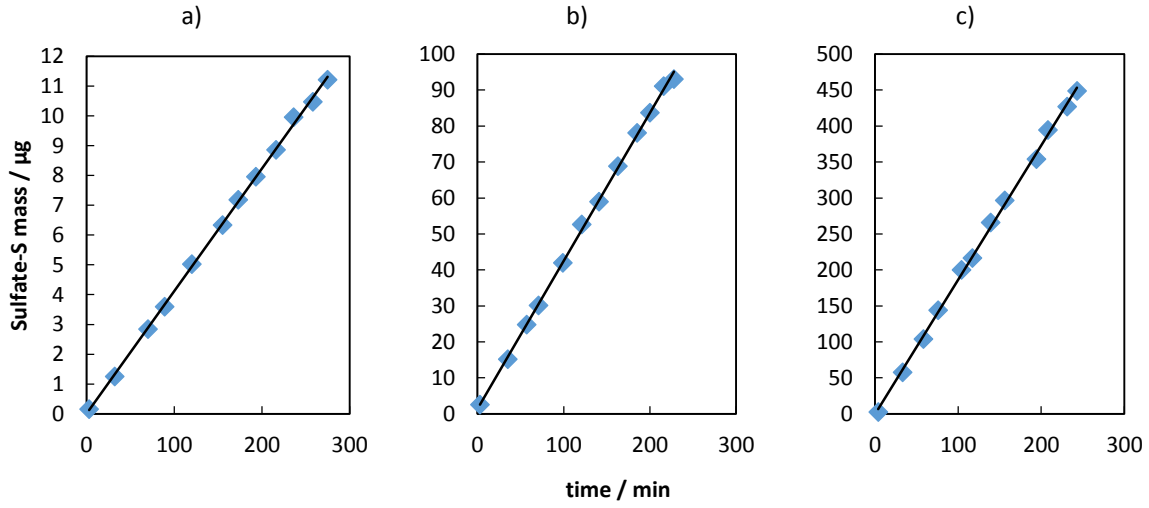



**Figure 2.4-3** Mass transport of sulfate-S through the APA diffusive gel over time. Using the slope of the regression line and the initial S concentration (a: 1 mg $L^{-1}$, b: 10 mg $L^{-1}$, c: 45 mg $L^{-1}$), $D$ was calculated according to [20].

### pH working range

The relative combined uncertainty of the $c_{DGT}/c_{soln}$ ratio was 8.9 %, the major contributor to the uncertainty was the determination of individual $c_{DGT}$ values. The relative combined uncertainty of an individual $c_{DGT}$ value was on average 8 % ($u_c$, $k$ = 1). With respect to the combined uncertainty, the $c_{DGT}/c_{soln}$ was not significantly different from 1 for immersion

solution pH values between 3 and 9 (Figure 2.4-4). Slightly larger deviations of the mean $c_{DGT}/c_{Soln}$ values from 1 were observed in the pH range 3-5 compared to higher pH values.

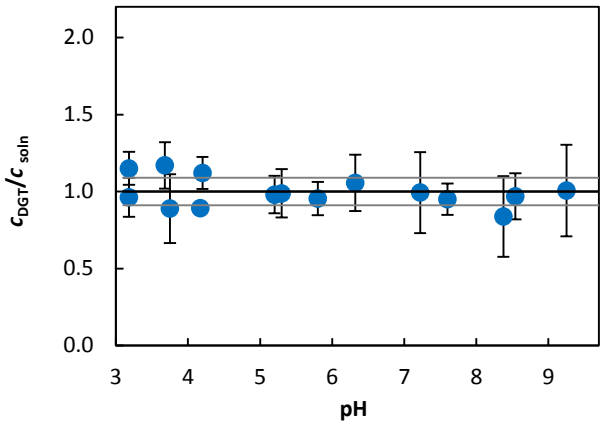



**Figure 2.4-4** Determination of the pH working range. The error bars are $u_c$, the 1.0 line is displayed with its uncertainty ($u_c$ = 0.089).

### Gel capacity

The relative combined uncertainty of the theoretical uptake line was 9.5 %, with the S measurement uncertainty being the main contributor (>75 %). The experimentally determined S uptake onto the gel after 24 h DGT deployment was up to 130 ± 11 µg S per disc and is in agreement with the theoretical value of 144 ± 13 µg S per disc (Figure 2.4-5a). The concentration of $Cl^-$ declined to <LOD (LOD: 10 µg $L^{-1}$) after 15 h of DGT exposure, which was expected as the resin was used in its $Cl^-$ form. The phosphate uptake reached its maximum after 24 h (6.3 ± 1.6 µg P per disc). At deployment times of 39 and 48 h, the P taken up by DGT declined to around half the maximum value (2.8 ± 0.7 µg per disc and 3.3 ± 1.0 µg per disc, respectively) (Figure 2.4-5b).

The characteristics of the method are summarized in Table 2.4-2.

**Table 2.4-2** DGT method characteristics

| Parameter | Abbrev. | Value | Type of uncertainty | Unit |
|---|---|---|---|---|
| Method limit of detection | *MDL* | 0.29 | - | mg S $L^{-1}$ |
| Method blank resin gel loading | - | 1.64 ± 0.63 | SD ($n$ = 4) | µg S per disc |
| Diffusion coefficient | *D* | 9.83 x $10^{-6}$ ± 0.35 x $10^{-6}$ | $u_c$ ($k$ = 1) | $cm^2$ $s^{-1}$ |
| Elution efficiency | *R* | 0.909 ± 0.016 | $u_c$ ($k$ = 1) | - |
| Gel capacity | - | 130 ± 11 | SD ($n$ = 3) | µg S per disc |

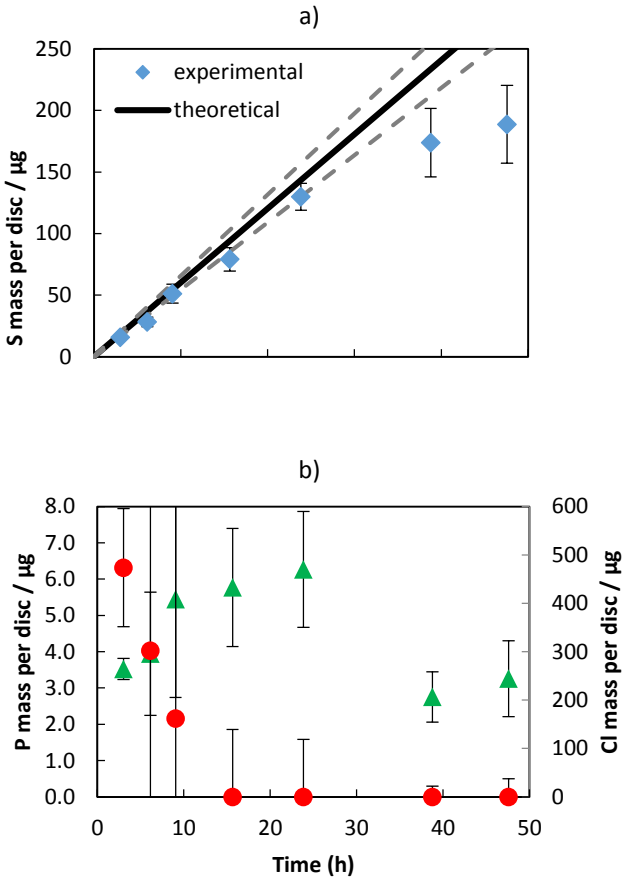



**Figure 2.4-5.** Sulfate uptake capacity. (a) Experimental (blue diamonds) and theoretical (black line ± 9 %, $u_c$) sulfate uptake over time. (b) Release of chloride (red points) and phosphorus uptake (green triangles) by the gel over time. Error bars are $u_c$.

### Comparison of DGT S with conventional soil S extraction techniques

DGT S extracted from the experimental soils ranged between 1.5 and 20.2 µg, corresponding to $c_{DGT}$ values of 0.23 - 3.16 mg $L^{-1}$. The linear correlation coefficients of DGT-S with S extracted by the MCP and KCl-40 methods were low with $r^2 = 0.40$ (MCP) and $r^2 = 0.18$ (KCl-40) (Figure 2.4-6).

## DISCUSSION

The elevated background S levels on the unwashed protective membrane (Figure 2.4-2) indicate the necessity to clean the membrane before use in order to prevent contamination of the sampler. No background S was detectable in the acid-washed membranes as well as in diffusive gel eluates. While S was determined in blank resin gel disc eluates, considerably more S was eluted from discs retrieved from non-deployed method blank DGT units.

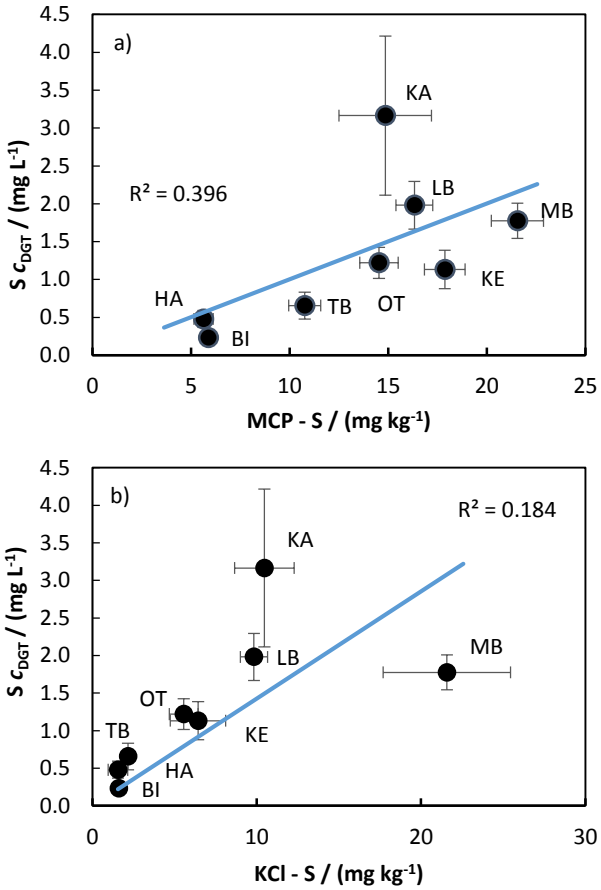



**Figure 2.4-6**: Correlation of DGT-S with S extracted by (a) MCP and (b) KCl-40. The line represents the linear regression line. Error bars are 1 SD.

This indicates, that sampler assembly and handling may increase the background sulfate level on the resin gel, most likely due to sulfate being a very abundant chemical species even in a clean room laboratory setting. Monitoring of the sulfate background on method blanks is therefore a necessity for DGT sulfate analyses. However, the S masses accumulated on the resin gels in the soil experiment were 4 – 55 times higher than the blank S gel loading, indicating that this slightly elevated background S value is not a problem for soil sulfate testing using DGT.

The determined diffusion coefficient value (9.83 x $10^{-6}$ ± 0.35 x $10^{-6}$ cm$^2$ s$^{-1}$) was approximately 91.4 % of the $SO_4^{2-}$ $D_{25}$ values for pure water (10.8 x $10^{-6}$ [25]) or seawater (10.7 x $10^{-6}$ [27]), which is expected as solute diffusion coefficients are generally lower in APA diffusive gels than in water [20,27]. This is caused most likely by tortuous diffusion pathways through the acrylamide gel matrix. *D* was the same for all test solutions (1 mg L$^{-1}$, 10 mg L$^{-1}$ and 45 mg L$^{-1}$ S) indicating that $SO_4^{2-}$ diffusivity is not concentration dependent, which is a pre-requirement for quantification of labile $SO_4^{2-}$ by DGT.

The elution efficiency (90.9 % ± 1.6 %) showed that the sampled $SO_4^{2-}$ is not completely eluted from the resin gel. However, as the elution efficiency is almost constant (low combined uncertainty), a correction factor can be applied for the calculation of $c_{DGT}$. Higher elution efficiency (100 %) can be obtained when the sorbent in the resin gel is dissolved during elution, as is the case for ferrihydrite resin gels that are used for phosphate sampling by DGT [27], or when the resin gel is digested [18]. Application of an ion exchange resin in DGT leads to generally lower recoveries, e.g. when Chelex is used for cation sampling (70 – 82 % recovery [10]).

DGT can be expected to perform well for $SO_4^{2-}$ quantification in the pH range between 3 and 9. This wide pH range enables the application of the developed method to both arable and acidic (e.g., forest) soils. However, a larger uncertainty must be taken into account for the pH range 3-5.

The relative expanded uncertainty of an individual $c_{DGT}$ value estimated in this study (typically 16 %, $U_{rel}$ $k$ = 2) was higher than the 10% expanded uncertainty ($U_{rel}$, $k$ = 2) reported by Kreuzeder *et al*. [21]. The main contributor (up to 40 %) to the 8% combined uncertainty was the uncertainty of the diffusion coefficient. The relative uncertainty of the $D_{25}$ for $SO_4^{2-}$ in hydrogel (4 %) is twofold larger than the relative uncertainties of $D_{gel}$ reported in [21].

The capacity of the gel was 130 µg S per gel disc, which equals 41 µg S $cm^{-2}$ gel (calculated based on the sampling window area of 3.14 $cm^2$) in the synthetic soil solution. As this value was obtained under high sulfate:anion ratios, the reported capacity can be considered a conservative lower limit estimate. Based on the DGT soil analyses done in this study, and some of our ongoing work in which we obtained a maximum DGT S uptake of about 85 µg for 24 h deployments, this capacity is well suited for analyzing soil S using DGT.

In the capacity test, $PO_4^{3-}$ was continuously bound to the gel during the first 24 h. However, the mass of P measured later on declined to around half the maximum value. This behavior indicates, that $SO_4^{2-}$ is taken up preferentially, as it replaced previously sorbed phosphate when the total gel loading was already high. The decrease of the $Cl^-$ concentration in the eluates with time was expected as the chloride form of Amberlite IRA-400 was used and $Cl^-$ was continuously replaced by other anions. The discrepancy between the sum of the bound $SO_4^{2-}$ and $PO_4^{3-}$ and the exchanged $Cl^-$ can be explained by $NO_3^-$ bound on the resin. Although it can be assumed that $NO_3^-$ does not bind strongly to the resin and would be exchanged by $SO_4^{2-}$ present in the solution, its concentration in the synthetic soil solution was four times higher than the $SO_4^{2-}$ concentration. Part of the $Cl^-$ was therefore probably exchanged for $NO_3^-$. It was not possible to confirm this assumption as 1 mol $L^{-1}$ $HNO_3$ was used as the eluting agent.

The developed method was successfully applied for a small set of selected soil samples. Sulfur sampled by DGT did not correlate well with that extracted by MCP and KCl-40. The low correlation coefficients between MCP and DGT ($r^2$ = 0.40) and KCl-40 and DGT ($r^2$ = 0.18) are likely linked to the differential sampling mechanisms of the methods applied. While extractions are based on a quasi-equilibrium between soil and extractant, DGT acts as an infinite sink technique that samples labile soil sulfate [13]. Visual inspection of Figure 2.4-6 suggests that the two soils (KA, MB) might be outliers in the correlation. Excluding the soils KA and MB from the correlation between KCl-40 and DGT would increase the correlation coefficient to $r^2$ = 0.94. If the KA soil was excluded from the comparison of DGT with MCP, the correlation coefficient would increase to $r^2$ = 0.75. However, neither a statistical outlier test (Grubbs' Test, $p > 0.05$) identified KA and MB as outliers, nor does the geochemical composition of the soil samples suggest a different behavior than the other soils. Clearly, a larger set of samples is needed to better understand the relation of the DGT and MCP/KCL-40 sampled sulfate fractions.

It has been shown that neither MCP nor KCl-40 correspond well to the $SO_4^{2-}$ uptake of plants [15], while DGT has been shown to be a good predictor of plant-available nutrients and contaminants [28]. In an agronomical evaluation of the presented DGT method as a soil S test, Mason et al. [29] found that DGT predicted maize relative yield and S uptake better than the two extraction methods. Together with reports that DGT and plants utilize the same soil phosphate pools, while chemical batch extractions do not [12,13], this indicates that DGT S uptake from soil resembles that of plants very well, while batch extraction methods are not efficient predictors of plant S uptake. Our study provides a first indication of the potential of DGT-S in soil testing, however further investigation and validation of this approach is warranted.

**CONCLUSIONS**

The presented DGT method showed great potential for soil $SO_4^{2-}$ sampling. The preference of the applied resin gel towards $SO_4^{2-}$ over other anions typically present in soil and soil solution, and the high capacity of the gel allow for $SO_4^{2-}$ sampling from soils of a pH range between 3 and 9.

As conventional extraction methods are not very representative of plant available soil S, the simple and quick DGT technique can deliver significant benefits. However, it has to be proved whether DGT samples the same soil S pool as plants. Direct comparison of plant and DGT uptake applying isotopic marking or analysis of stable S isotopes will enable this assumption to be tested.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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## 2.5 Diffusive gradients in thin films measurement of sulfur stable isotope variations in labile soil sulfate

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**ABSTRACT**

A diffusive gradients in thin films (DGT) technique, based on a strongly basic anion exchange resin (Amberlite IRA-400), was successfully tested for $^{34}S/^{32}S$ analysis in labile soil sulfate. Separation of matrix elements, that potentially cause non-spectral interferences in $^{34}S/^{32}S$ analysis by MC ICP-MS (Na, K, Ca), during sampling of sulfate was demonstrated. No isotopic fractionation caused by diffusion or elution of sulfate was observed until the resin gel disc was loaded with more than 79 µg S. Above this threshold, fractionation towards $^{34}S$ was observed. The method was applied to 11 different topsoils and one mineral soil profile (0-100 cm depth) and compared with soil sulfate extraction by water. The S amount and isotopic ratio in DGT-S and water-extractable sulfate correlated significantly ($r^2$ = 0.89 and $r^2$ = 0.74 for the 11 topsoils, respectively). The systematically lower $^{34}S/^{32}S$ isotope ratios of the DGT-S were ascribed to mineralization of organic S.

**INTRODUCTION**

Soluble soil sulfate is the most important sulfur (S) species in many isotopic studies, as sulfate is the dominant inorganic S form in most aerobic soils [1]. Fractionation of $^{34}S/^{32}S$ isotopes is caused by thermodynamic and kinetic effects accompanying uptake and mineralization of S compounds by microbes and plants [1,2], evaporation and crystallization of seawater [3], transformation of minerals [4] and other natural processes [1,2]. The resulting variation of $^{34}S/^{32}S$ isotope ratios can be used in environmental studies to study S biogeochemistry [5], in archaeology to determine the origin of findings in burial mounds [6] or to characterize ore genesis [7].

Multicollector inductively coupled plasma mass spectrometry (MC ICP-MS) has been applied routinely for S isotope ratio analysis [5,8,9]. While high sensitivity (<0.1 µmol S required for analysis [5]) and measurement uncertainty (<0.03 % [5]) can be achieved with MC ICP-MS, non-spectral interferences caused by matrix elements (mainly K, Na, Ca) have been shown to be major limitations [5,9]. Sample purification procedures have been applied successfully for overcoming matrix interferences in measurements of the sulfate-S isotopic composition in soil extracts and soil porewaters [5,9]. Although post-sampling separation procedures are effective, they represent a time-consuming step with the potential to cause method-related isotope fractionation. A targeted sampling procedure for soil sulfate, that separates potential interferents already during the sampling step, would be an ideal alternative to conventional separation procedures. In a recent study, we developed a novel technique for passive sampling of labile soil sulfate [10], based on the diffusive gradients in thin film (DGT) methodology [8,11]. DGT employs a solute binding agent, usually either an ion resin or a mineral binding phase (eg. Fe-oxide, Zr-oxide), immobilized in a thin hydrogel layer, to sample solutes in environmental media like waters, sediments and soils [11]. In a DGT

sampler, the binding gel layer is overlain by a pure hydrogel layer, which prevents particle contamination and acts as diffusion layer for the solutes to be sampled.

Several studies showed the potential to use DGT for the investigation of the isotopic composition of solutes and for isotope dilution studies using radiotracers. Dalqvist *et al*. investigated the isotopic composition of Nd in fresh and marine waters [12], while Turner *et al*. analyzed $^{235}U/^{238}U$ ratios in two river waters [13]. The suitability of DGT for measuring the isotopic composition of Zn and Pb was investigated in laboratory studies [14,15]. Sub-mm isotopic variations in dissolved sulfide-S in sediment porewaters were studied using DGT in combination with laser ablation MC ICP-MS [8,16]. Mason *et al*. [17] and Six *et al*. [18] applied isotope dilution using the radioisotope $^{32}P$ to compare the phosphate pool sampled by DGT and other soil test methods with the phosphate pool available for plant uptake. All of these studies concluded that DGT is well suited for measuring isotope compositions, and that the sampling process does not cause detectable isotope fractionation.

In this study, we tested and validated the measurement of the isotopic composition of labile soil sulfate-S using DGT. The method was applied to analyze the sulfate-S isotope composition of a set of mineral soil samples.

## MATERIALS AND METHODS

### General laboratory procedures

Laboratory tools were double acid washed using 10% (*w*/*w*) and 1% (*w*/*w*) $HNO_3$ (p. a., Merck, Darmstadt, DE) and rinsed with laboratory water type I (0.055 µS $cm^{-1}$; TKA-GenPure, Niederelbert, DE) before use. Laboratory water type I was also used for preparation of all standard solutions, for soil extractions and for water saturation of soil samples. Laboratory water type I and $HNO_3$ were further purified by a sub-boiling distillation system (Milestone Inc., Shelton, CT, US) and used for the elution of sulfate from the resin gel (1 mol $L^{-1}$ $HNO_3$), from resin membranes (2% (*w*/*w*) $HNO_3$) and for microwave-assisted digestions (Multiwave 3000, Anton Paar, Graz, AT).

### DGT sampling

Gel and sampler preparation

DGT samplers (DGT Research Ltd., Lancaster, UK) were used for both solution and soil tests. Polyethersulfone filters (0.45 µm pore size, 0.13 mm thick, Sartorius Stedim, Goettingen, DE) were used as a protective membrane. The membranes were washed in 5% $HNO_3$ (*w*/*w*) and stored in an aqueous 10 mmol $L^{-1}$ $NaNO_3$ solution (Reagent Plus, Sigma Aldrich, Buchs, CH). Agarose cross-linked polyacrylamide (APA) diffusive hydrogels of 0.8

mm thickness were prepared according to [11] and cut to discs. Anion exchange resin hydrogels for S sampling (0.4 mm thickness) were prepared according to [10]. A 10 mmol $L^{-1}$ $NaNO_3$ solution was used for storage of all gels.

Resin gel elution

After application to standards or soils, the samplers were retrieved, and the resin gel was rinsed with water and eluted in 10 mL 1 mol $L^{-1}$ $HNO_3$ for 16 hours. The elution efficiency (90.9 % ± 1.6 %) has already been reported in [10].

DGT performance and matrix separation

The separation of sulfate from the major matrix elements Na, K and Ca, which are present in soil solutions at concentrations of < 1 to 600 mg $L^{-1}$ [19] and may cause non-spectral interferences in $^{34}S/^{32}S$ analysis by MC ICP-MS [5], was tested using $CaSO_4 \times 2\ H_2O$ (p.a., Fluka, Buchs, CH), $K_2SO_4$ (p.a., Fluka) and $Na_2SO_4 \times 10\ H_2O$ (p.a., Merck) dissolved in 3 L $H_2O$ to reach concentrations ($c_{Soln}$) of approximately 100 mg $L^{-1}$ S. All standard solutions had an electrolyte background concentration of 10 mmol $L^{-1}$ $NaNO_3$ (p.a., Sigma Aldrich) and a pH value of 5.6, adjusted using dilute NaOH and $HNO_3$ solutions (both p.a., Merck). DGT samplers (5 replicates) were exposed to the standard solutions for four hours. Each experiment was repeated 5 times. The separation of Na, K and Ca was calculated as difference between the cation mass fraction in standard solution ($M_{Soln}$) and its mass fraction in the eluate ($M_{El}$), divided by the $M_{Soln}$.

To test whether uptake by DGT causes fractionation of S isotopes, two different $(NH_4)_2SO_4$ salt batches (p.a., Merck, labelled as “A” and Normalpure, VWR, Leuven, BE, labelled as “B”) were dissolved to reach concentrations of 100 mg $L^{-1}$ S. The two standard solutions had significantly (see Statistical analysis below) different S isotopic composition ($\delta(^{34}S/^{32}S)_{VCDT}$ “A”: 5.27 ‰ ± 0.87 ‰ ($U$; $k$ = 2); $\delta(^{34}S/^{32}S)_{VCDT}$ “B”: 6.39 ± 0.64 ‰ ($U$; $k$ = 2)). DGT samplers were placed into 3 L of each solution for four hours. $^{34}S/^{32}S$ isotope ratios of the standard solutions were compared with the $^{34}S/^{32}S$ ratio of sulfate sampled by the DGT method.

In a previous study we determined the capacity of the S resin gel to be 130 ± 11 µg S per disc (i.e., 41 ± 3 µg S $cm^{-2}$) [10] by comparing the amount of sulfate-S bound by resin gels to the theoretical uptake onto the DGT sampler according to equation 2.5-1 [11]:

$$c_{DGT} = \frac{M \cdot \Delta g}{D \cdot A \cdot t} \qquad \text{(Equation 2.5-1)}$$

where $\Delta g$ is the diffusive layer thickness (sum of the diffusive gel and protective membrane thicknesses), $D$ is the diffusive coefficient, $A$ is the sampling window surface area and $t$ is the sampling time. Elution efficiency was taken into account.

In addition to the capacity determination, we measured the isotopic composition of these DGT gel eluates to determine potential isotope fractionation in resin gels that approach analyte saturation. To account for the competition of ubiquitous anion species in soil porewaters for binding sites on the resin gel disks, a synthetic soil solution was prepared [10]. DGT samplers were deployed for 3, 6, 9, 12, 15, 24, 36 and 48 hours. The $^{34}S/^{32}S$ ratio of the DGT-S was evaluated against the isotopic composition of "A" salt used for preparation of the synthetic soil solution.

Background S concentration was estimated by placing the DGT sampler into a moist plastic bag for 4 hours. The mass of S eluted from this resin gel was considered a "method blank" and used for blank correction.

**Isotopic composition of labile soil sulfate**

Soil samples

Twelve soils of different origin, pH, texture and total S content (Table 2.5-1) were investigated. Jubiläumswarte, Kobernaußerwald and Brixlegg were forest soils, Santomera originated from a research station, and the other eight samples were arable soils. Brixlegg soil was divided into Ae, B1, B2 and two B3 horizons. For all other samples only topsoil (max. 30 cm soil depth) samples were available. All soil samples were air dried and sieved (2 mm) before the experiment. The soil characteristics shown in Table 2.5-1 were determined according to standard procedures (pH [20], clay [21], $CaCO_3$ [22]). The water holding capacity (WHC) was determined by mixing the dried soil with water until the soil got saturated (no free water was observed). The WHC equals the mass of the water added relative to the mass of the water-saturated soil. The Brixlegg samples (BAe – BB4 samples) were evaluated separately as they represented a compact soil profile with the possibility to follow changes in response to the applied test methods with soil depth.

Total sulfur content

0.1 g of each soil sample was dissolved by acid microwave - assisted digestion (5 mL sub-boiled $HNO_3$ and 1 mL $H_2O_2$ (Suprapur, Merck)). The digestion performance was approved by digestion of the RTS-1 (CANMET, Ottawa, CA) soil reference material, certified for total and extractable S content.

Water extractable sulfate

Three grams of each soil sample was extracted in 18 mL laboratory water type I for 24 hours (shaking over-head). The extract was filtered (Minisart RC 25, Sartorius Stedim) to remove soil particles. Anion exchange resin membranes (551642S, VWR) were applied for sulfate separation from the extract [5]. 0.5 mol $L^{-1}$ $NaHCO_3$ (p.a., Merck) solution was used for membrane regeneration.

**Table 2.5-1** Soil properties and total S content

| Soil sample | Abbr. | Country of origin | pH ($CaCl_2$) | Clay g $kg^{-1}$ | WHC *) % | $CaCO_3$ g $kg^{-1}$ | $S_{tot}$ mg $kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| Aigen | W | AT | 7.1 | 170 | 43 | 20 | 200 |
| Blankenstein | D | DE | 6.2 | 200 | 39 | 0 | 252 |
| Hohes Kreuz | H | AT | 6.1 | n.d. | 42 | 0 | 369 |
| Horn | R | AT | 5.7 | 240 | 27 | 0 | 193 |
| Jubiläumswarte | J | AT | 5.9 | 180 | 49 | 0 | 315 |
| Kobernaußerwald | K | AT | 3.8 | 100 | 40 | 0 | 254 |
| Moosbierbaum | B | AT | 7.6 | n.d. | 48 | 100 | 215 |
| Münchendorf | M | AT | 7.7 | n.d. | 58 | 350 | 626 |
| Santomera | E | ES | 7.8 | 300 | 32 | 500 | 200 |
| Tulln | T | AT | 6.8 | 300 | 33 | 50 | 343 |
| France | F | FR | 4.8 | n.d. | 54 | 0 | 434 |
| Brixlegg – Ae | BAe | AT | 3.9 | 170 | 53 | 0 | 519 |
| – B1 | BB1 | AT | 4.0 | 250 | 42 | 0 | 199 |
| – B2 | BB2 | AT | 4.1 | 130 | 40 | 0 | 214 |
| – B3a | BB3 | AT | 3.9 | 180 | 37 | 0 | 274 |
| – B3b | BB4 | AT | 4.0 | 150 | 40 | 0 | 264 |

*) water holding capacity

Sampling of DGT-labile soil sulfate-S

The soil samples were mixed with laboratory water type I to reach their maximum water holding capacity (WHC, Table 2.5-1). The resulting pastes were incubated for 24 hours at 20° C for equilibration of the soil porewater and the soil solid phase. A 2 mm thick layer of the paste was spread carefully onto the DGT samplers, which were subsequently incubated for another 24 hours at 20° C. The eluates of the resin gels were measured for S concentration by ICP-MS and diluted to 1 mg S $L^{-1}$ for isotopic analysis by MC ICP-MS. Uniform S concentration in samples is required for isotopic analysis to ensure a uniform ion density and exclude between-sample measurement bias. This experiment was replicated 3 times for each soil.

**Analyses**

Single collector ICP-MS (Element XR, Thermo Fisher Scientific, Waltham, MA, US) was used for quantification of S, Na, K and Ca in standard solutions, resin gel eluates, and of S in soil digests and soil extracts. External calibration and internal standardization (In) were applied.

MC ICP-MS Nu Plasma HR (Nu Instruments Ltd, Wrexham, UK) connected to a sample desolvation unit (Aridus II, Teledyne, Omaha, NE, US) was used for S isotope ratio measurement in standards and extracts. The instrument was run in edge mass resolution at $(m/z)/\Delta(m/z)$ ~ 2700 (for more detail see e.g. [9]). The correction for the instrument background signal was performed automatically by on-peak zero measurement. External correction for instrumental isotopic fractionation ("standard-sample bracketing") was accomplished by applying IAEA-S-1 (IAEA, Vienna, AT) as external bracketing standard. All samples and standards were diluted to 1 mg $L^{-1}$ total S for the measurement. The gas flow rates and the lens voltage were optimized daily to reach a sensitivity of at least 5 V (mg $L^{-1}$ sulfur)$^{-1}$. The precision and accuracy of the measurement was assessed by measuring IAEA-S-2 (IAEA) as a sample (measurement precision: 0.2 ‰, 1 *RSD*; accuracy: long-term average of measured values: 22.53 ‰ ± 0.51 ‰ (2 *SD*, $n$ = 22), certified value: 22.66 ‰ ± 0.20 ‰ (SD)). All values are reported as relative to a Vienna Canyon Diablo Troilite (VCDT) standard according to [23].

**Uncertainty estimation**

The uncertainty estimation of the quantitative measurement ($c_{DGT}$) is based on [24]. It takes the uncertainties of S quantification, diffusive layer thickness, sampling window area, sampling time, diffusion coefficient and elution efficiency uncertainties [10] as well as repeatability (SD) of the experiment into account.

The combined uncertainty of the $\delta(^{34}S/^{32}S)_{VCDT}$ measurement is based on [25]. It takes the measurement precision of the sample and of the bracketing standard, the uncertainty of the blank correction and the correlation of $^{34}S/^{32}S$ blank signals into account. The combined uncertainty was calculated for each sample individually.

**Statistical analysis**

Significance of difference between two mean values (DGT-S isotope ratios and standard solution S-isotope ratios, DGT-S isotope ratios and water-extractable S isotope ratios) was tested with respect to the expanded ($U$; $k$ = 2) uncertainties of the mean values. Two mean values were significantly different, if

$$|m_1 - m_2| > \sqrt{U_{m1}^2 + U_{m2}^2} \qquad \text{(Equation 2.5-2)}$$

where m1 and m2 represent the mean values and *U*m1 and *U*m2 their expanded uncertainties [26].

Equation 2.5-3 [27] was used for computing the appropriate *t* value to test significance of a correlation coefficient (between $c_{DGT}$ and water-extractable S amount and between DGT-S and water-extractable S isotope ratios) by Student's *t*-test ($p$ = 0.95):

$$t = r\sqrt{\frac{n-2}{1-r^2}} \qquad \text{(Equation 2.5-3)}$$

where *r* is the correlation coefficient and *n* are the degrees of freedom.

## RESULTS

### Sulfate uptake and matrix separation

Sulfate $c_{DGT}$ values were in good agreement with standard solution S concentration ($c_{Soln}$, see Table 2.5-2). The main contributor to the combined uncertainty of the calculated $c_{DGT}$ was the repeatability of the DGT application (up to 84 %) followed by the uncertainty of the diffusion coefficient *D* (22 % on average; [10]). During the sulfate sampling by the DGT, the investigated matrix elements (Ca, K, Na) remained almost entirely in the standard solution (see Table 2.5-2). The main source of the combined uncertainty was again the repeatability of the method (more than 95 %).

**Table 2.5-2** Agreement between $c_{Soln}$ and $c_{DGT}$ of S and separation of cations (Na, K, Ca) from the sulfate sampled by DGT ($n$ = 5). Values with expanded uncertainties ($U$, $k$ = 2).

| Standard solution | S concentration / mg $L^{-1}$ | | $c_{DGT}/c_{Soln}$ % | Cation concentration / mg $L^{-1}$ | Cation separation % |
|---|---|---|---|---|---|
| | $c_{Soln}$ | $c_{DGT}$ | | $c_{soln}$ | $(M_{Soln} - M_{El})/M_{Soln}$ |
| $Na_2SO_4$ x 10 $H_2O$ | 9.3 ± 0.2 | 8.0 ± 1.4 | 86 ± 15 | 14.3 ± 0.9 | 99 ± 1 |
| $K_2SO_4$ | 8.9 ± 0.1 | 8.4 ± 0.9 | 95 ± 10 | 23.2 ± 1.1 | 99 ± 0 |
| $CaSO_4$ x 2 $H_2O$ | 9.4 ± 0.2 | 8.9 ± 1.8 | 95 ± 19 | 11.5 ± 1.0 | 99 ± 1 |

### Sulfate-S isotope fractionation during DGT uptake

The isotopic composition of sulfate-S sampled by DGT from "A" or "B" standard solution corresponded to that of the standard solution. The typical expanded uncertainty of the $\delta(^{34}S/^{32}S)_{VCDT}$ value was ~0.90 ‰ with measurement precision being the main contributor (up

to 89 %). The different uncertainty of “A” and “B” are caused by different measurement conditions on different measurement days. The results are shown in Figure 2.5-1. The $^{34}S/^{32}S$ isotope ratio is presented as relative to composition of the corresponding immersion solution ($\Delta(^{34}S/^{32}S)$) for better presentation.

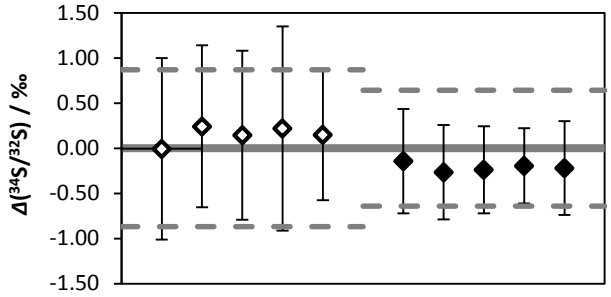


**Figure 2.5-1.** S isotope ratios measured in sulfate sampled by DGT. The values are expressed as relative to the corresponding standard solutions (batch “A” $(NH_4)_2SO_4$: open diamonds, batch “B” $(NH_4)_2SO_4$: black diamonds). The error bars and the dashed lines are expanded uncertainties $U$, $k$ = 2.

The capacity experiment showed that the $^{34}S/^{32}S$ ratio of DGT-S corresponded to that of the synthetic soil solution (salt batch “A”) at gel loadings ≤79 µg S per disk. When the gel S loading reached and exceeded 130 µg S per disc, increased $^{34}S/^{32}S$ ratios were observed in DGT-S (Figure 2.5-2). $\Delta(^{34}S/^{32}S)$, relative to the $\delta(^{34}S/^{32}S)_{VCDT}$ of the standard solution, was 1.79 ± 1.29 ‰, 5.85 ± 3.02 ‰, and 7.64 ± 1.98 ‰ for loadings of 130 µg S per disc, 174 µg S per disc, and 189 µg S per disc, respectively.

**Soil samples**

The water-extractable S ranged between 7.0 (F) and 32.7 (B) mg S $kg^{-1}$ soil. The minimum S $c_{DGT}$ was obtained from the E sample (0.43 mg $L^{-1}$), the maximum from the B sample (3.39 mg $L^{-1}$). The resin gel loadings were in the range between 9.6 µg sulfur per disc (sample E) and 76 µg sulfur per disc (sample B). The S $c_{DGT}$ is plotted against the mass concentration in the soil of water-extractable S in Figure 2.5-3. The correlation between the results was 0.89 ($r^2$) for the 11 studied soils and 0.78 ($r^2$) for the Brixlegg soil profile.

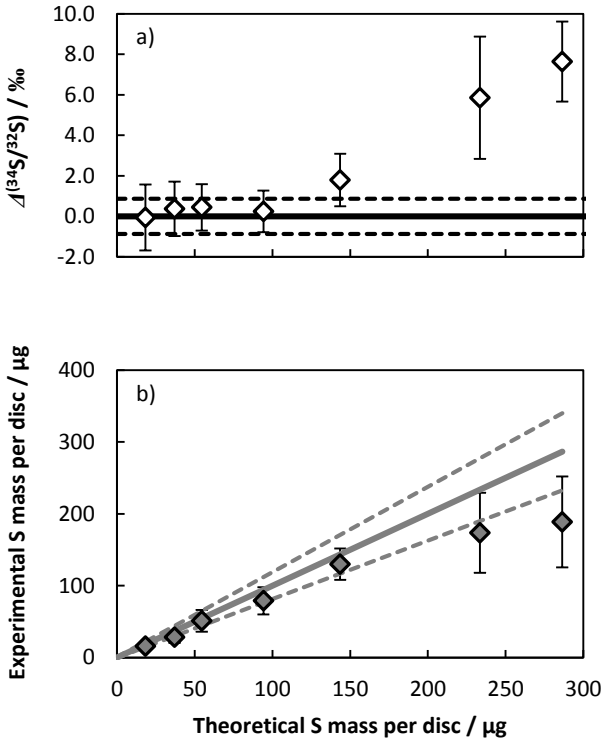


**Figure 2.5-7** a) $\Delta(^{34}S/^{32}S)$ isotope ratios of DTG sulfate-S (open diamonds) relative to synthetic soil solution (salt "A", black line) with increasing resin gel loading, and b) experimental S uptake per disc (grey diamonds) and theoretical 1.0 line depending on the theoretical S uptake per disc. Error bars and dashed lines are expanded uncertainties *U*, *k* = 2. More information on gel loading versus theoretical uptake can be found [10].

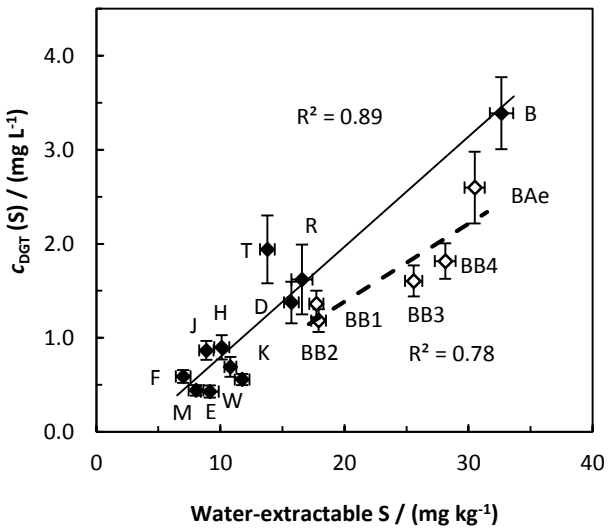


**Figure 2.5-8.** Water-extractable sulfate S concentration against the calculated S $c_{DGT}$ for the 11 studied soils (black diamonds, full line) and Brixlegg soil profile (open diamonds, dotted line). The error bars are expanded uncertainties *U*, *k* = 2.

The results of the isotopic analysis of S in water-extractable sulfate after purification by anion exchanger resin membrane and analysis of S sampled by DGT are summarized in Figure 2.5-4. The expanded uncertainty of the $\delta(^{34}S/^{32}S)_{VCDT}$, values was 1.1 ‰ with repeatability of the experiment being the main contributor (44 %). The correlation of the two methods was 0.74 ($r^2$) for the 11 soils and 0.53 ($r^2$) for the Brixlegg soil profile.

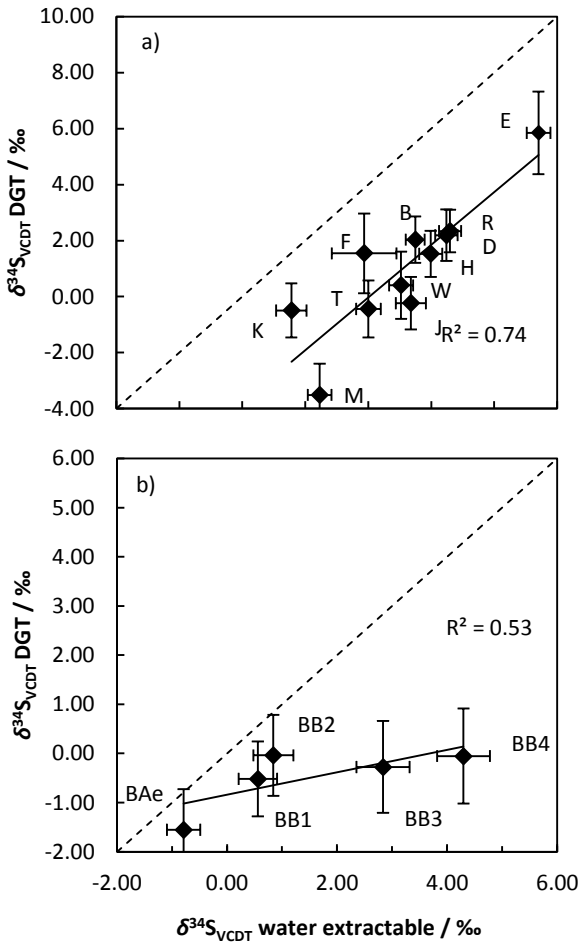


**Figure 2.5-9.** Correlation between $\delta(^{34}S/^{32}S)_{VCDT}$ values of water-extractable soil sulfate S and S sampled by DGT from (a) 11 different soils and (b) Brixlegg soil profile. Dashed line is the theoretical 1:1 line. Error bars are expanded uncertainties *U*, *k* = 2.

## DISCUSSION

### Suitability of DGT for $^{34}S/^{32}S$ analysis

The non-spectroscopic matrix-based interferences in $^{34}S/^{32}S$ analysis by MC ICP-MS were discussed e.g. by [5] or [9]. A purification step (by cation exchange column or anion exchange resin on a plastic membrane, respectively) was applied by the authors to remove matrix elements from dissolved sulfate. However, the DGT method is capable to sample dissolved sulfate selectively, removing thus the interfering matrix elements (see Table 2.5-2).

While $c_{DGT}/c_{Soln}$ ratio was high for sulfate (86 % ± 15 % - 95 ± 19 %), all investigated matrix elements (Ca, K, Na) were separated quantitatively (to more than 99 %) during the sampling by DGT. The main source of the relatively large expanded uncertainty $U_{cDGT}$ (up to 19 %, $k$ = 2) was the repeatability of the method (contributing up to 84 % to the combined uncertainty). This can be explained e.g. by small differences (e.g. in resin amount) between resin gel batches. Thus, the DGT technique for soil S enables for a direct, matrix-free sampling of labile S, and is thus advantageous for S isotope analysis by MC ICP-MS.

The elution efficiency (90.9 % ± 1.6 %) reported in [10] shows that the sulfate sampled by DGT is not completely eluted from the resin gel. Since the elution process can be accompanied by isotopic fractionation, the suitability of the DGT method for S isotopic analysis was proven.

It is evident that the comparison of the $\delta(^{34}S/^{32}S)_{VCDT}$ values in sulfate-S sampled by DGT with the values of the corresponding standard solution ("A" and "B", Figure 2.5-1) shows no significant isotopic fractionation. In this experiment, the mass accumulated on the gels was well below 79 µg per disc. Above this gel loading, fractionation towards $^{34}S$ was observed (Fig. 2.5-2a). In comparison, deviation of the experimental uptake from the theoretical uptake was only observed at gel loadings >130 µg per disc (Fig 2.5-2b, [10]). Obviously, the sulfate-S isotope composition is fractionated when approaching saturation of the resin gel. Isotope fractionation by ion exchange has been reported previously and the enrichment of $^{34}S$ using anion exchange resin was even applied to produce compounds enriched in $^{34}S$ [28]. Therefore, for reliably determining the sulfate-S isotope composition, the resin gel loading must not exceed 79 µg per disc, while quantitative sulfate DGT measurements with this gel are possible up to a gel loading of ≤130 µg per disc [10].

**Soil samples**

The resin gel loadings in the soil deployment were generally lower than the threshold for sulfate isotope composition measurements (max. 76 µg S per disc), and thus well below the threshold for the quantitative determination of DGT-labile sulfate S (130 µg S per disc). The comparison of water-extractable sulfate S and S $c_{DGT}$ showed high correlation ($r^2$ = 0.89) between the two techniques for the 11 different soils investigated. The correlation between the two parameters was somewhat lower ($r^2$ = 0.78) in the mineral soil horizons of the Brixlegg soil profile. This observation indicates, that the water-extractable and DGT-labile sulfate quantities in the set of soils investigated here are closely linked. In our previous work, soil-S measured using stronger extractants (1 mol $L^{-1}$ KCl; 1 mol $L^{-1}$ $Ca(H_2PO_4)_2$) yielded much lower correlations to DGT-measured S ($r^2$ = 0.18 and $r^2$ = 0.40, respectively), probably because those extractants were more efficient in extracting sorbed and mineralizable S than

the $H_2O$ extract. Note that in the present and the earlier work [10], two different sets of soils were investigated.

The results of the $^{34}S/^{32}S$ isotope ratio analysis of water-extractable sulfate S and sulfate S sampled by DGT show that DGT-S was systematically and significantly depleted in $^{34}S$ as compared to water-extractable sulfate S. The S isotope ratios found in sulfate S sampled by the two techniques correlated significantly ($r^2$ = 0.74 for the 11 soils and $r^2$ = 0.53 for soil profile). The expanded uncertainty of the $\delta(^{34}S/^{32}S)_{VCDT}$ values was higher in the soil experiment compared to the experiment in laboratory solutions (0.9 ‰ and 1.1 ‰, respectively). This can be explained by small inhomogeneities of the natural samples as method repeatability was the main contributor to uncertainty of the S isotopic analysis in soils. The difference in the isotopic composition of the sulfur sampled by the two methods is most probably caused by the mineralization of S from organic sources during the DGT experiment, as the soil microflora prefers the lighter $^{32}S$ isotope in metabolism [1,29,30]. Before DGT sampling, the soil pastes are incubated at room temperature for 24 h before, and for additional 24 h during DGT sampler exposure. Therefore a considerable amount of time is available for organic S species to be microbially mineralized to inorganic sulfate by microbial activities. DGT may thus be a very effective tool for measuring sulfate-S mineralized from soil organic matter.

## CONCLUSIONS

The presented DGT technique was shown to be well suitable for sulfate sampling and matrix separation in one step. Even though DGT-S can be quantified up to a resin gel disc loading of ≤ 130 µg S, analysis of $^{34}S/^{32}S$ is only possible up to a gel discs loading of ≤ 79 µg S. Such an effect has not been reported before for DGT-based methods for isotope composition measurements, but it might be important also for other resin/isotope system combinations. Therefore we suggest that isotope fractionation vs. gel loading should be tested. Significantly and systematically lower $^{34}S/^{32}S$ isotope ratios of the DGT-S than of water-extractable sulfate-S in soils indicate mineralization of organic S during DGT application. Therefore, DGT should be a versatile tool to investigate soil S mineralization, as the experimental conditions (soil paste moisture, temperature, soil incubation/exposure time) can be modified easily. However, additional incubation tests and comparison with sulfate uptake by plants are warranted.

## ACKNOWLEDGEMENTS

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# 3 Summary and conclusions

Although regulations of $SO_2$ emissions have been applied in Austria already in 1980s, the impact of the $SO_2$ - and, consequently, sulfate - deposited in the past represents still a current topic. Due to S cycle in soil, the high historical loads of sulfate are released nowadays, causing thus a negative input - output (rain water - stream water) balance of sulfate in many forest ecosystems. At the same time, caused by the regulated $SO_2$ emissions, sulfur is becoming one of the major limitations in the agricultural production. In this thesis, the techniques and methods of the Analytical Chemistry for quantitative analysis of soil S fractions and analysis of stable S isotopes are presented, to investigate the S availability, S cycle and S biogeochemistry. The understanding of these is a prerequisite to solve both ecological and agricultural S-concerning issues.

The sequential extraction of readily available, adsorbed, and HCl-extractable sulfate, organic sulfur and ester-sulfate from beech (*Fagus sylvatica*) forest soil profiles showed clearly differences in distribution of these species in soil, caused by the soil type, by the distance from a beech tree and by the soil depth. For the first time, the influence of the gradual distance from a tree stem (representing the point with the highest water input) on the S biogeochemistry was evaluated. Concentration gradients of the inorganic S fractions caused by stemflow could be clearly observed, even on the pseudogley soil at the clayey, nutrient-rich Flysch soil, where a larger proportion of lateral water flow could be expected. Desorption caused by high amounts of water of low $SO_4^{2-}$ concentrations at the stem is an explanation of the reduced amounts of adsorbed sulfate at the stem base. Thus, the stem area of beech seems to have recovered from high historical $SO_4^{2-}$ loads. High correlations between organic S and adsorbed S indicated high mineralization rates at the Flysch, whereas no such correlation was found on acidic, nutrient-poor Molasse soil, indicating that S immobilization is the predominant process on the latter.

The sequential extraction represents a classical method to assess the different soil S species and, via statistical analysis, gives insights into the soil S cycle. Moreover, as demonstrated in this thesis (Section 2.1), the method enables for a chemical imaging of the distribution of the S species in soil profile. On the other hand, the sequential extraction is a labour intensive and sample consuming method. Moreover, as the extraction of readily available, adsorbed, and HCl-extractable sulfate is not selective and, e.g., organic S species might be co-extracted, ion chromatography is required for analysis. Thus, this method is not applicable, if the amount of the soil sample is limited and/or if the amount of the sulfate species is low. The latter would pose a problem for ion chromatography as dilution of the extract is required (e.g., for the HCl-extractable sulfate) on the one hand, and the limit of detection might not be low enough (0.1 mg $L^{-1}$ S, see Table 1-1) on the other.

A special emphasis was put on the investigation of S stable isotopes, as this is an analytical approach for investigation of S biogeochemistry recommended since 1990s. Therefore, four different ICP-MS platforms were validated for fast and reliable S isotope ratio analysis in a comparative study (Section 2.2).

The comprehensive evaluation of the performance of single collector ICP-MS for accurate S isotope ratios showed clearly the still existing limitations. MC ICP-MS operated in edge mass resolution mode, applying bracketing correction of instrumental isotopic fractionation, provided isotope ratio values with highest quality (relative combined measurement uncertainty: 0.02 %; deviation from the certified value: < 0.002 %). The performance of MC ICP-MS was independent of the approach of the correction of instrumental isotopic fractionation. Internal correction, using Si isotopes, has the advantage to reduce measurement time if high sample throughput is required. Moreover, possible matrix effects on the measurement are corrected.

Ideally, the internal standard should have similar properties (*m*/*z*, ionization energy) as the measurand and the internal standard and the measurand should be measured simultaneously to ensure the same measurement conditions. Silicon isotopes $^{30}Si$ and $^{28}Si$ best fulfil the first condition. However, simultaneous detection of all isotopes of interest ($^{34}S$ and $^{32}S$ as measurand, and $^{30}Si$ and $^{28}Si$ as standard) is challenging and has not been reported yet. Moreover, the potentially different instrumental fractionation of S and Si isotopes within a highly variable matrix has to be taken into account when considering a versatile application of the internal correction of instrumental isotopic fractionation. Besides the necessity to apply a strategy for correction of instrumental isotopic fractionation, one crucial parameter which influences the measurement results and limits possible applications to natural samples is the observed S background level.

Moreover, natural samples of complex and variable matrix, e.g. soil solution (see Section 2.3), require pre-treatment – a separation of matrix elements from S. As demonstrated, higher concentration of cations (e.g., Ca, K, Li, Na) in matrix leads to an additional instrumental isotopic fractionation. The presented method, separation of sulfate by means of an anion exchange resin on a plastic membrane, is a powerful tool if water samples (rain water, soil solution) are investigated and high sample throughput is required. However, due to its limited capacity, the anion exchange resin on a plastic membrane is not applicable universally, e.g. for analysis of adsorbed or HCl-extractable sulfate. In these cases, purification by an analytical column would be required. Any influence of the purification on S isotopic composition would have to be excluded. A hyphenated technique like HPLC-MC ICP-MS would not be suitable as it would require long measurement times.

The developed DGT technique was successfully applied for selective sampling of readily available and reversibly adsorbed soil sulfate (Section 2.4 and 2.5). The resin gel showed

higher preference towards sulfate over $Cl^-$ or $PO_4^{3-}$, and applicability for soils of a pH range between 3 and 9. The low correlation between DGT-S and extractable sulfate-S indicates that different S species are assessed by the DGT method. As the S extraction methods do not correspond to the S plant uptake and as DGT was shown to be a suitable method for sampling of plant-available nutrient pool (e.g., phosphate), it can be assumed that the different S species sampled by DGT correspond to the plant-available S. In contrary to quantitative analysis using weak salts, the water-extractable sulfate S correlated significantly with DGT-S.

The DGT technique was successfully tested for $^{34}S/^{32}S$ analysis in soil sulfate. It was demonstrated that matrix elements, which potentially cause non-spectral interferences in $^{34}S/^{32}S$ analysis by MC ICP-MS (Na, K, Ca), are separated during sampling of sulfate. Isotopic analysis of liquid standards showed that diffusion and elution of sulfate cause no isotopic fractionation. However, when the S gel loading approximated the capacity of the gel, fractionation towards $^{34}S$ was observed. The $^{34}S/^{32}S$ isotope ratios of DGT-S and of water-extractable sulfate correlated significantly. The systematically lower $^{34}S/^{32}S$ isotope ratios of the DGT-S (as compared to water-extractable sulfate) were ascribed to mineralization of organic S.

Although the developed DGT method showed high potential for sampling of plant-available soil sulfate under natural conditions, avoiding thus the use of such strong extractants as 1 mol $L^{-1}$ HCl, further investigations are warranted. A direct comparison of incubation tests and plant uptake of the same soil with subsequent analysis of S stable isotopes should prove whether the DGT soil S fraction corresponds to the plant-available soil S. The assumption that the systematically low S isotope ratios of the DGT-S as compared to water-extractable sulfate-S are caused by mineralization of organic S compounds should be proved by application of DGT to one type of soil at different temperatures or for different long incubation time. After these tests, the DGT can become a method of choice for investigation of sulfate availability in both basic agricultural and acidic forest soils.

In summary, modern analytical methods like measurement of $^{34}S/^{32}S$ isotope ratios in natural samples using MC ICP-MS represent powerful tools for investigation of S biogeochemistry.

However, some aspects still challenge the isotopic analysis. One of these is the strategy for correction of instrumental isotopic fractionation. As matrix-matching of standards is not suitable for natural samples of strongly varying matrix, time-consuming matrix separation prior to analysis and standard-sample bracketing are required. Application of an internal standard (Si) would be therefore of advantage. MC ICP-MS instrumentation needs further development enabling simultaneous detection of wider spectrum of light isotopes (for

*m*/*z* 28 - 34 in this case), e.g. by changes in geometry of the analyzers and by the use of further developed detector blocks.

Reduction of S background signal would widen the applicability of MC ICP-MS to water samples and soil extracts with S concentration below 1 mg $L^{-1}$ without an increase in the combined measurement uncertainty.

The developed DGT MC ICP-MS protocol should be further tested for suitability for assessing the plant-available soil S. In that case, the DGT MC ICP-MS method should be applied in food provenancing studies, where the origin of agricultural raw products would be tested using $^{34}S/^{32}S$ isotope ratios.

The analytical methods applying either matrix separation by means of anion exchange resin on a plastic membrane or DGT using suitable immobilizing resin with subsequent isotopic analysis by MC ICP-MS could be applicable also for other, similar isotopic systems, e.g. Si, and investigation of plant-soil element cycling.

# 4 Appendices

## 4.1 List of Abbreviations

| | |
|---|---|
| $A$ | sampling window surface |
| APA | agarose cross-linked polyacrylamide |
| APS | ammonium persulfate |
| AS | adsorbed sulfate |
| B | Moosbierbaum (soil, Section 2.5) |
| BAe | Brixlegg – Ae (soil, Section 2.5) |
| BB1 | Brixlegg – B1 (soil, Section 2.5) |
| BB2 | Brixlegg – B2 (soil, Section 2.5) |
| BB3 | Brixlegg – B3a (soil, Section 2.5) |
| BB4 | Brixlegg – B3b (soil, Section 2.5) |
| BI | Birchip (soil, Section 2.4) |
| CC | collision cell |
| $c_{DGT}$ | concentration of an analyte in solution calculated using mass $M$ of the analyte taken up by DGT |
| CS | carbon bonded sulfur |
| $c_{Soln}$ | concentration of an analyte in solution |
| $D$ | diffusive coefficient |
| D | Blankenstein (soil, Section 2.5) |
| $\Delta$g | diffusive lyer thickness |
| DGT | diffusive gradients in thin films |
| DGT-S | concentration of an sulfur in solution calculated using mass $M$ of sulfur taken up by DGT |
| DL | diffusive layer thickness |
| DRC | dynamic reaction cell |
| E | Santomera (soil, Section 2.5) |
| ES | ester-sulfate |
| F | France (soil, Section 2.5) |
| H | Hohes Kreuz (soil, Section 2.5) |
| HA | Hart (soil, Section 2.4) |
| HCS | HCl-extractable sulfur |
| HPLC | high performance liquid chromatography |
| HPLC-MC ICP-MS | high performance liquid chromatography multicollector |
| IC | ion chromatography |
| ICP | inductively coupled plasma |

| | |
|---|---|
| ICP-MS | inductively coupled plasma mass spectrometer |
| ICP-OES | inductively coupled plasma optical emission spectrometer |
| iCRC | integrated collision reaction cell |
| IIF | instrumental isotopic fractionation |
| IR | infrared |
| IRMS | isotope ratio mass spectrometry |
| IUPAC | International Union of Pure and Applied Chemistry |
| J | Jubiläumswarte (soil, Section 2.5) |
| *k* | coverage factor |
| K | Kobernaußerwald (soil, Section 2.5) |
| KA | Karoonda(soil, Section 2.4) |
| KE | Keith (soil, Section 2.4) |
| $K_{sp}$ | solubility product constant |
| LB | Lake Bolac (soil, Section 2.4) |
| *LOD* | limit of detection |
| *M* | mass (of an analyte) |
| *m* | mass (of an ion) |
| M | Münchendorf (soil, Section 2.5) |
| MB | Mt Barker (soil, Section 2.4) |
| MC | multicollector |
| MC ICP-MS | multicollector inductively coupled plasma mass spectrometer |
| MCP | monocalcium phosphate |
| *MDL* | method limit of detection |
| MS | mass spectrometer |
| *N* | number of counts |
| OC | organic carbon |
| OS | organic sulfur |
| OT | Otterbourne (soil, Section 2.4) |
| Qcell | quadrupole collision cell |
| QMS | quadrupole-based mass spectrometer |
| QQQ | “triple-quadrupole”; a MS/MS instrument equipped with a collision / reaction cell |
| *R* | recovery |
| R | Horn (soil, Section 2.5) |
| RAS | readily available sulfate |
| RSD | relative standard deviation |
| SD | standard deviation |

| | |
|---|---|
| SFMS | sector field mass spectrometer |
| SIMS | secondary ion mass spectrometry |
| $t$ | sampling time |
| T | Tulln (soil, Section 2.5) |
| TB | Tumby Bay (soil, Section 2.4) |
| TEMED | tetramethylethylendiamine |
| TIMS | thermal ionization mass spectrometry |
| ToS | total sulfur |
| $U$ | expanded uncertainty |
| $u_c$ | combined uncertainty |
| VCDT | Vienna Canyon Diablo Troilite |
| W | Aigen (soil, Section 2.5) |
| WHC | water holding capacity |
| $z$ | charge |

## 4.2 Curriculum Vitae

### PERSONAL DATA


| | |
|---|---|
| **Name** | **Ondrej Hanousek** |
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### WORK EXPERIENCES

UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA — since 9/2012
**Research Assistant**

NET4GAS, PRAGUE, CZECH REPUBLIC — 10/2011 – 6/2012
**Trainee in Sales Department**

### EDUCATION

UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA — 12/2012 - 11/2016 (estimate)
**Doctoral program (Wood and Forest Sciences)**
- Doctoral Thesis: Biogeochemistry of sulfur in forest ecosystems with special focus on investigation of sulfur stable isotopes

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE, CZECH REPUBLIC — 9/2009 - 6/2012
**Master of Science (Analytical Chemistry and Quality Engineering)**
- Master Thesis: Speciation analysis of mercury in plants

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE, CZECH REPUBLIC — 9/2006 - 6/2009
**Bachelor of Science (Synthesis and Production of Pharmaceuticals)**
- Bachelor Thesis: The Volume Resistivity of Original and Generic Pharmaceuticals Comparison

### FURTHER EDUCATION AND RESEARCH EXCHANGES

HELMHOLTZ CENTRE GEESTHACHT, GERMANY — 12/2015
- Measurements of stable S isotopes using ICP-MS/MS

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE, CZECH REPUBLIC — 12/2014
- Speciation of soil sulfur (HI-reducible S)

ROBERT BOSCH GMBH, CESKE BUDEJOVICE, CZECH REPUBLIC — 9/2011
- Summer School

PHARMACHEM – ENVIRONMENTAL LABORATORY, SKOPJE, FYROM — 7/2011 – 8/2011
- Air, dust and water analyses; participation in proficiency testing

EBERHARD KARLS UNIVERSITÄT TÜBINGEN, GERMANY — 9/2009 – 6/2010
- Internship in Analytical Chemistry: Intra- and inter chip reproducibility of biosensor surfaces

## LANGUAGES

| | |
|---|---|
| Czech | mother tongue |
| German | fluent |
| English | fluent |
| Spanish | limited working proficiency |

## PUBLICATIONS (PEER-REVIEWED)

Prohaska, T; Irrgeher, J; Hanousek, O: ICP Mass Spectrometry using Sector Field Analyzers. In: Montaser, A (Ed.): Inductively Coupled Plasma Mass Spectrometry (planned publication date in 2016)

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Hanousek, O; Rottmann, L; Prohaska, T (2015) CHAPTER 5 Mass Resolution. In: Sector Field Mass Spectrometry for Elemental and Isotopic Analysis. The Royal Society of Chemistry, pp 97-106

## ORAL PRESENTATIONS

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Hanousek, O; Santner, J; Mason, S; Wenzel, WW; Prohaska, T (2015): “Analysis of sulfate $\delta^{34}S$ in soil by DGT MC ICP-MS”. DocDay 2015, Tulln, AT

Hanousek, O; Santner, J; Berger, TW; Mason, S; Prohaska, T (2015): “Measurement of sulfur stable isotope variations in bioavailable sulfate in soils by DGT MC ICP-MS”. DGT Conference 2015 "From DGT Research to Environmental Assessment", AZTI, San Sebastian, ES

Hanousek, O; Santner, J; Berger, TW; Prohaska, T (2015): “Spolehlivá analýza stabilních izotopů síry v environmentálních vzorcích pomocí MC ICP-MS”. 8. kurz ICP-MS/OES 2015, Spektroskopicka spolecnost Jana Marka Marci, Brno, CZ (**invited speaker**)

Hanousek, O; Santner, J; Berger, TW; Prohaska, T (2015): “Measurement of sulfur stable isotope ratios in dissolved sulfate by DGT MC ICP-MS”. 11th ASAC JunganalytikerInnenforum, Innsbruck, AT

Hanousek, O; Santner, J; Berger, TW; Prohaska, T (2015): “Reliable analysis of sulfur stable isotopes in environmental samples using MC ICP-MS”. European Winter Conference on Plasma Spectrochemistry, Münster, DE

Hanousek, O; Berger, TW; Prohaska, T (2014): “Study of stable isotopes of sulfur in forest soils using MC ICP-MS – analytical challenges and solutions”. PANGEO Austria 2014, Graz, AT

Prohaska, T; Zitek, A; Irrgeher, J; Horsky, M; Hanousek, O (2014): “Application of non-traditional isotopes in analytical ecogeochemistry”. Winter Conference on Plasma Spectrochemistry, Amelia Island, FL, USA

Hanousek, O; Prohaska, T; Berger, TW (2014): “Analytical Challenges in Determination of Sulfur Stable Isotopes by MC-ICP-MS”. Pumpaya 2014, Mosonmagyarovar, HU

Hanousek, O; Berger, TW; Prohaska, T (2013): “Stable isotopes of sulfur to study Austrian forest ecosystems”. 9th ASAC JunganalytikerInnenforum 2013, Vienna, AT

Hanousek, O (2012): “Predicting reversibility of soil acidification in beech stands – sulfur isotope ratios measurement”. Pumpaya 2012, Podersdorf am See, AT

**POSTER PRESENTATIONS**

Prohaska, T; Zitek, A; Irrgeher, J; Horsky, M; Tchaikovsky, A; Draxler, J; Sturm, M; Hanousek, O (2015): “(Netradiční) izotopové systémy v analytické ekogeochemii“ [Poster]. 8. kurz ICP-MS/OES 2015, Brno, CZ

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