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Research Paper

Isotopic evidence for the origin of dimethylsulfide and dimethylsulfoniopropionate-like compounds in a warm, monomictic freshwater lake

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Environmental context. The volatile sulfur compound, dimethylsulfide (DMS), plays a major role in the global sulfur cycle by transferring sulfur from aquatic environments to the atmosphere. Compared to marine environments, freshwater environments are under studied with respect to DMS cycling. The goal of this study was to assess the formation pathways of DMS in a freshwater lake using natural stable isotopes of sulfur. Our results provide unique sulfur isotopic evidence for the multiple DMS sources and dynamics that are linked to the various biogeochemical processes that occur in freshwater lake water columns and sediments.

Abstract. The volatile methylated sulfur compound, dimethylsulfide (DMS), plays a major role in the global sulfur cycle by transferring sulfur from aquatic environments to the atmosphere. The main precursor of DMS in saline environments is dimethylsulfoniopropionate (DMSP), a common osmolyte in algae. The goal of this study was to assess the formation pathways of DMS in the water column and sediments of a monomictic freshwater lake based on seasonal profiles of the concentrations and isotopic signatures of DMS and DMSP. Profiles of DMS in the epilimnion during March and June 2014 in Lake Kinneret showed sulfur isotope ($\delta^{34}S$) values of $+15.8 \pm 2.0$ per mille (‰), which were enriched by up to 4.8 ‰ compared with DMSP $\delta^{34}S$ values in the epilimnion at that time. During the stratified period, the $\delta^{34}S$ values of DMS in the hypolimnion decreased to −7.0 ‰, close to the $\delta^{34}S$ values of coexisting H_2S derived from dissimilatory sulfate reduction in the reduced bottom water and sediments. This suggests that H_2S was methylated by unknown microbial processes to form DMS. In the hypolimnion during the stratified period DMSP was significantly ^{34}S enriched relative to DMS reflecting its different S source, which was mostly from sulfate assimilation. In the sediments, $\delta^{34}S$ values of DMS were depleted by 2–4 ‰ relative to porewater (HCl-extracted) DMSP and enriched relative to H_2S. This observation suggests two main formation pathways for DMS in the sediment, one from the degradation of DMSP and one from methylation of H_2S. The present study provides isotopic evidence for multiple sources of DMS in stratified water bodies and complex DMSP–DMS dynamics that are linked to the various biogeochemical processes within the sulfur cycle.

Additional keywords: assimilatory sulfate reduction, dissimilatory sulfate reduction, sediment, sulfur isotope.

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Introduction

Methylated sulfur compounds are important sources of biogenic sulfur to the atmosphere[1] with dimethylsulfide (DMS) being the major form of biogenic sulfur emitted to the atmosphere from the ocean.[2] DMS emitted from natural sources contributes to the formation and growth of sulfate aerosols in the atmosphere, and therefore has a potential effect on the climate system through reflection and scattering of solar radiation, water droplet formation and hence cloud albedo.[3] The main precursor of DMS in oxic saline environments such as the surface ocean is dimethylsulfoniopropionate (DMSP), a common osmolyte in marine algae. Other precursors can also contribute to microbial DMS production. These include methionine, an amino acid present in many living organisms, dimethyl sulfoxide (DMSO) and methanethiol (MT).[4] The dominant mechanism for the formation of DMS in anaerobic environments was suggested to be MT methylation, with MT being formed either from H_2S methylation or from methionine degradation.[5–7]

DMS concentrations in the water column of fresh water lakes and ponds (0.05–4.4 nM) are similar to those in typical oceanic

water (2–4 nM).[4,8–10] This is surprising given that freshwaters have much lower concentrations of sulfate (100–200 μM) compared to ocean water (28 mM),[11] and that DMSP, the major precursor of DMS in seawater, is not thought to be prevalent in freshwater plankton, although it has been reported in some lakes.[8,12] Given the widespread occurrence of significant DMS concentrations in freshwater environments,[8] and the potential for its emission to the atmosphere, it is important to learn about its sources and sinks in freshwater habitats.

Stable sulfur isotopes have the potential to provide information on the natural sources and sinks of compounds like DMS without experimental manipulation. Sulfur has four stable isotopes (^{32}S, ^{34}S, ^{33}S and ^{36}S) of which the most abundant are ^{32}S and ^{34}S. Usually during microbial processes there is a discrimination against the heavy isotope, leaving the product isotopically enriched in the light one and the residual pool enriched in the heavy one. Anaerobic dissimilatory sulfate reduction (respiratory electron accepting process) is the main sulfur metabolism process on Earth and is characterised by large isotopic fractionation (up to 72 per mille (‰)) with the light isotope favoured in the H_2S product.[13–16] The assimilatory sulfate reduction process, i.e. direct uptake of sulfate for cell biosynthesis, controls the sulfur cycle in oxygenated parts of the water column. The fractionation during assimilatory sulfate reduction is small relative to dissimilatory sulfate reduction with fractionation values ranging between ~1 and 3 ‰ causing the biomass to be ^{34}S slightly depleted relative to sulfate.[13,14,17]

The different sources of DMS may have distinct $^{34}S/^{32}S$ ratios as they are affected by different sulfate reduction routes (dissimilatory *v.* assimilatory). Analysis of $\delta^{34}S$ of organic sulfur species may thus help identify production mechanisms of benthic *v.* planktonic DMS. The $\delta^{34}S$ values of DMSP of marine surface water around the globe is very homogeneous ranging between +18.9 and +20.3 ‰ and the fractionation between DMSP and DMS is <+1 ‰.[18] Macroalgae seem to produce ^{34}S depleted DMSP (+18.2 ‰) as measured in intertidal or shallow subtidal systems, and culture experiments with macroalgae showed ^{34}S depletion of DMS relative to DMSP by up to −1.6 ‰.[19] The $\delta^{34}S$ values of DMS and other volatile organic sulfur (VOS) compounds in sediments and anaerobic water bodies are usually ^{34}S depleted, as is their putative precursor, H_2S, that is produced by sulfate reducing microorganisms.[20]

The aim of the present study was to study the sulfur isotope composition of DMS and DMSP in Lake Kinneret, Israel, a freshwater lake, with the ultimate goal of inferring information about production routes in different compartments of the lake. Lake Kinneret is a monomictic lake that is stratified from April to January. Sulfate concentration in the water column is ~0.5 mM and the hypolimnion and upper 3 cm of sediment are dominated by dissimilatory sulfate reduction.[21] Previous research showed that DMS in the water column of the lake may be derived from DMSP produced by *Peridinium gatunense*, a freshwater dinoflagellate that dominates the bloom in Lake Kinneret.[12] Dinoflagellates are also known to produce unique dimethyl sulfur species[22] that could release DMS in the common protocol used to measure DMSP, which involves treatment of samples with strong alkali and quantifying the released DMS as a proxy for DMSP.[23] Thus, the identity of DMSP in lake samples is still somewhat uncertain.

Sediments are likely to receive DMSP in sinking detritus, and indeed high concentrations of DMSP have been reported in some marine sediments.[24,25] These studies utilised the indirect, alkaline hydrolysis method to quantify sedimentary DMSP, and because a significant fraction of the sedimentary 'DMSP' pool appears to be refractory to biodegradation on time scales of weeks or longer,[4] it may be more correct to refer to the sedimentary pool as 'base-hydrolysable DMS', as suggested by Kiene.[26] This particle-associated pool could include intercellular DMSP or adsorbed DMSP or DMS and possibly other sulfonium compounds. DMS in sediments could arise from the base-hydrolysable DMS (hereafter referred to as bound DMSP; $DMSP_{bound}$) or it could arise from sequential methylation of H_2S and MT. Depletion of sulfate in the hypolimnion and surficial sediments[21,27] of Lake Kinneret should provide ample H_2S for the methylation route. Such a mechanism could result in DMS with a distinct isotopic composition from that produced from water-column-derived DMSP.[20]

In this study we carried out sampling for in-situ profiles of DMS and DMSP concentrations and sulfur isotopic ratios ($\delta^{34}S$) in the lake water column and sediments using compound-specific sulfur isotopic analyses.[28,29] Samples were taken during several different times of the year, encompassing different water-column mixing regimes. We use the isotope results to infer probable DMS production routes in oxic and anoxic compartments of the lake.

Methods

Study site

Lake Kinneret is located in northern Israel (Fig. 1) and it is a warm monomictic subtropical lake. In the spring, the newly formed epilimnion is characterised by increasing temperatures and enhanced phytoplankton development, whereas in the hypolimnion oxygen is depleted gradually by heterotrophic microorganisms followed by depletion of nitrate. The organic matter degradation below the thermocline continues in the summer by bacterial iron and manganese reduction. By the end of the stratification period, sulfate reduction occurs in the bottom water. In the upper part of the sediment sulfate reduction is the dominant microbial process year round, and below depths of 5 cm sulfate reduction is limited by sulfate availability and degradation processes shift mainly to methanogenesis.[21,27]

Water sampling

The water column and the sediment of Lake Kinneret were sampled between March 2014 and January 2015 on four occasions: one during early stratification when the whole water column was still oxic, and three during the stratified period. Samples were collected from Station A, located at the deepest part of the lake (38–39-m water depth, Fig. 2) using a Niskin bottle. The physiochemical conditions in the water column at the specified sampling dates were characterised by the temperature, dissolved oxygen (DO), chlorophyll-*a* (Chl-*a*) and oxidation reduction potential (ORP) profiles measured by the autonomous profiling unit (Eureka, Austin, Texas) that operates at the lake's centre (Fig. 3). Sulfide and sulfate data were taken from the Lake Kinneret Data Base (LKDB) with permission of Yaron Beeri-Shlevin (Fig. 4). Both solutes are measured biweekly according to standard methods.[30]

The sample processing scheme for the water column, sediment pore water and sediment solid phase is presented in Fig. 2. Samples for DMS analysis were transferred immediately from the Niskin bottle into 40-mL amber glass vials (two for each depth) equipped with Teflon septa and filled to the top (no head space). Samples for DMSP analysis were taken the same way,

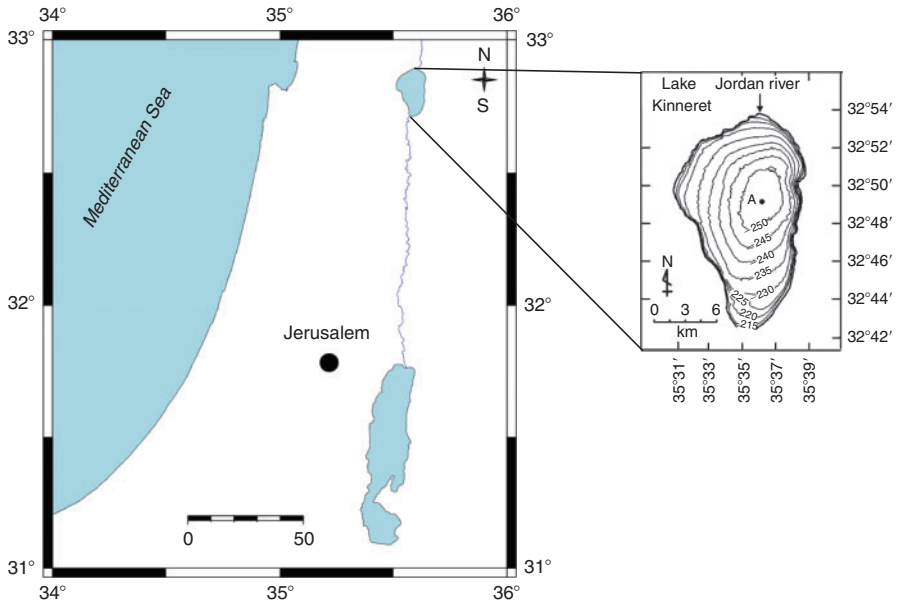

Fig. 1. Lake Kinneret, Israel, located in the northern Dead Sea rift (left). Station A ~40-m depth (right).

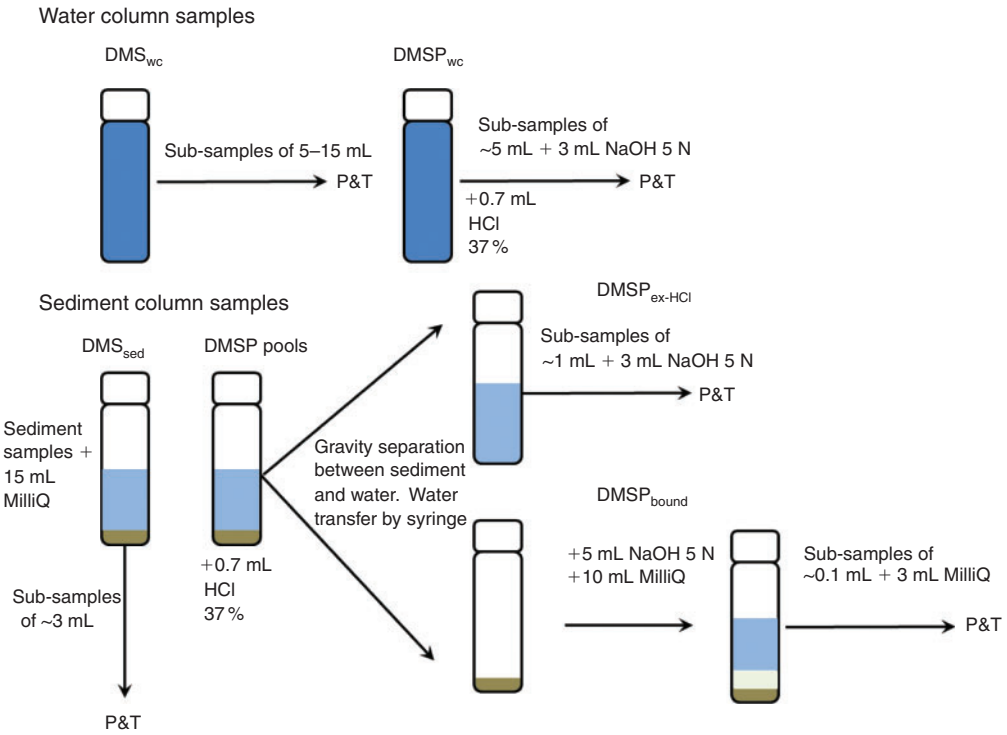

Fig. 2. Sample treatment scheme for water column and sediment samples for both concentration and sulfur isotope ratio measurements.

with vials (two for each depth) being pre-filled with 0.7 mL of 37 % w/w HCl.[29] Addition of acid to seawater samples to preserve DMSP is a known method for preservation of DMSP for at least several months.[31] We used HCl and not H_2SO_4 because we did not want to have another sulfur source in our water samples while we were measuring S isotopes.[29] We specifically use a high HCl concentration (1.5 % final concentration) to minimise the enzymatic conversion of phytoplankton DMSP into DMS after acidification.[31] However, if colonial *Phaeocystis* spp. are present, then this method underestimates the concentration of DMSP in the lake water.[31] These species were not present in the Lake Kinneret water samples, therefore underestimation is unlikely. All vials were kept at the temperature of surface lake water (~25 °C) until processing.

Sub-samples (5–15 mL) for DMS analysis were transferred to a separate vial using a syringe. For DMSP, sub-samples of ~5 mL were transferred using a syringe to a separate vial and then 3 mL of 5 M NaOH was added. Typically we diluted the

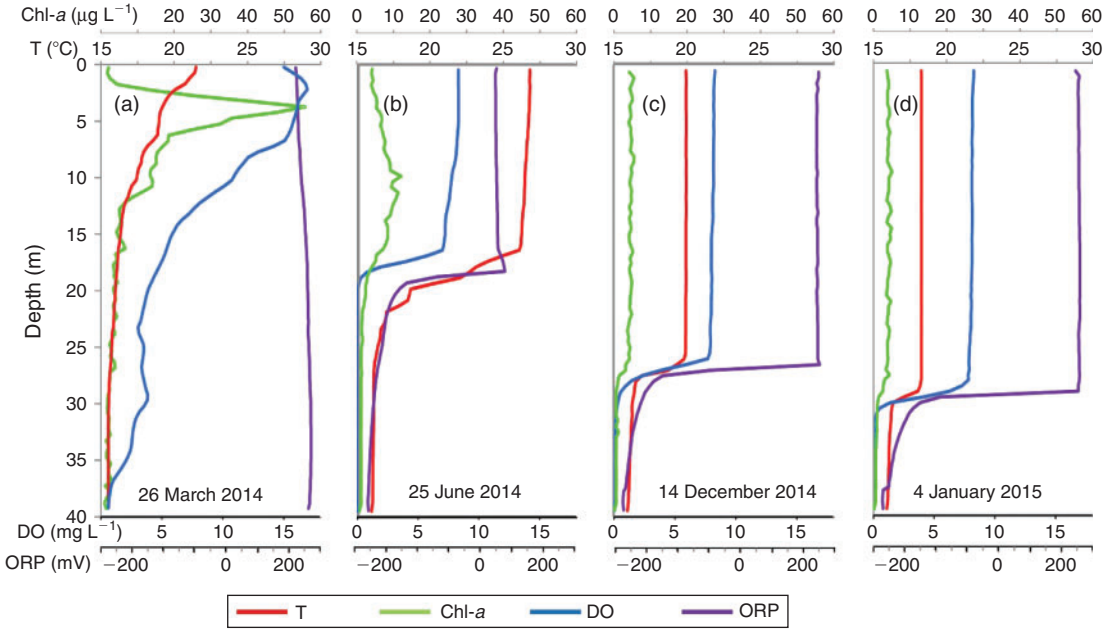

Fig. 3. Water column profiles of temperature, chlorophyll concentration, oxygen concentration and oxidation reduction potential (ORP) in (a) 26 March 2014; (b) 25 June 2014; (c) 14 December 2014; (d) 4 January 2015 (data from the Lake Kinneret Data Base (LKDB)).

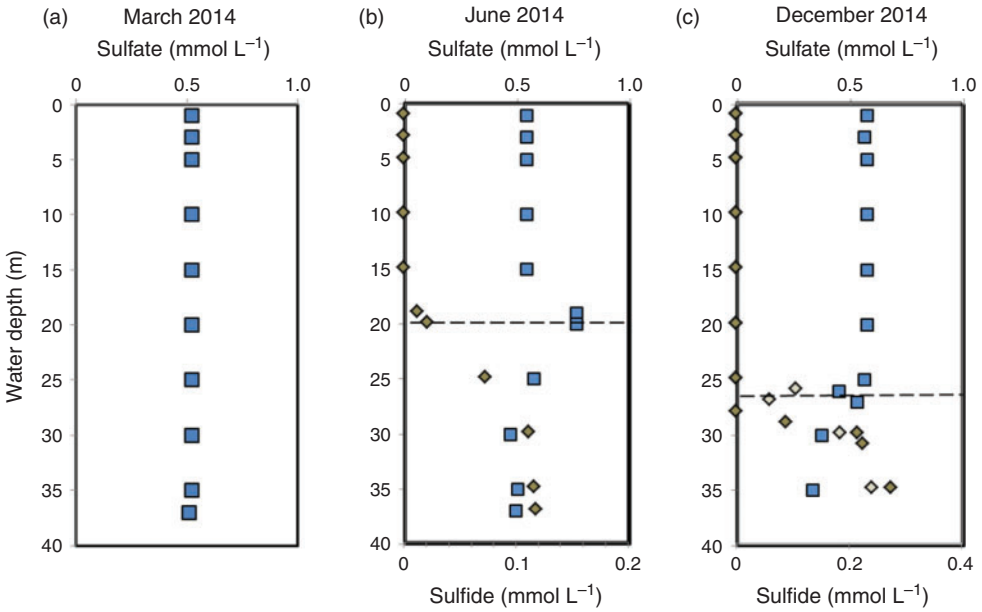

Fig. 4. Sulfate (squares) and hydrogen sulfide (diamonds) concentrations during (a) March 2014; (b) June 2014 and (c) December 2014 (squares and light diamonds), January 2015 (dark diamonds). Dashed lines represent the depth of the thermocline at the time of sampling. Data from the Lake Kinneret Data Base, courtesy of Yaron Beeri-Shlevin.

original sample (before addition of NaOH) by a factor of two with MilliQ water. After at least 4 h of the base-hydrolysis reaction, the released DMS was quantified as described below.

Sediment sampling

Cores of ~30-cm length, with side holes sealed with rubber stoppers, were obtained from the sediment and sampled by means of a custom-made gravity corer. Subsamples from selected depths of the cores were obtained through the side holes.

The sample processing scheme for sediment pore water and sediment solid phase is presented in Fig. 2.

Sediment samples (1–2 g of wet sediment) for DMS analysis (DMS_{sed}), were transferred to N_2-preflushed 40-mL amber vials filled with 15 mL of sparged MilliQ water (two vials for each depth were taken). Sub-samples (~3 mL) of slurry from the original vial were then transferred using a syringe to a separate vial equipped with a Teflon septum and purge and trap as described later.

For DMSP analysis, ~1 g of wet sediment was transferred to 40-mL vials (two vials from each depth were taken) prefilled

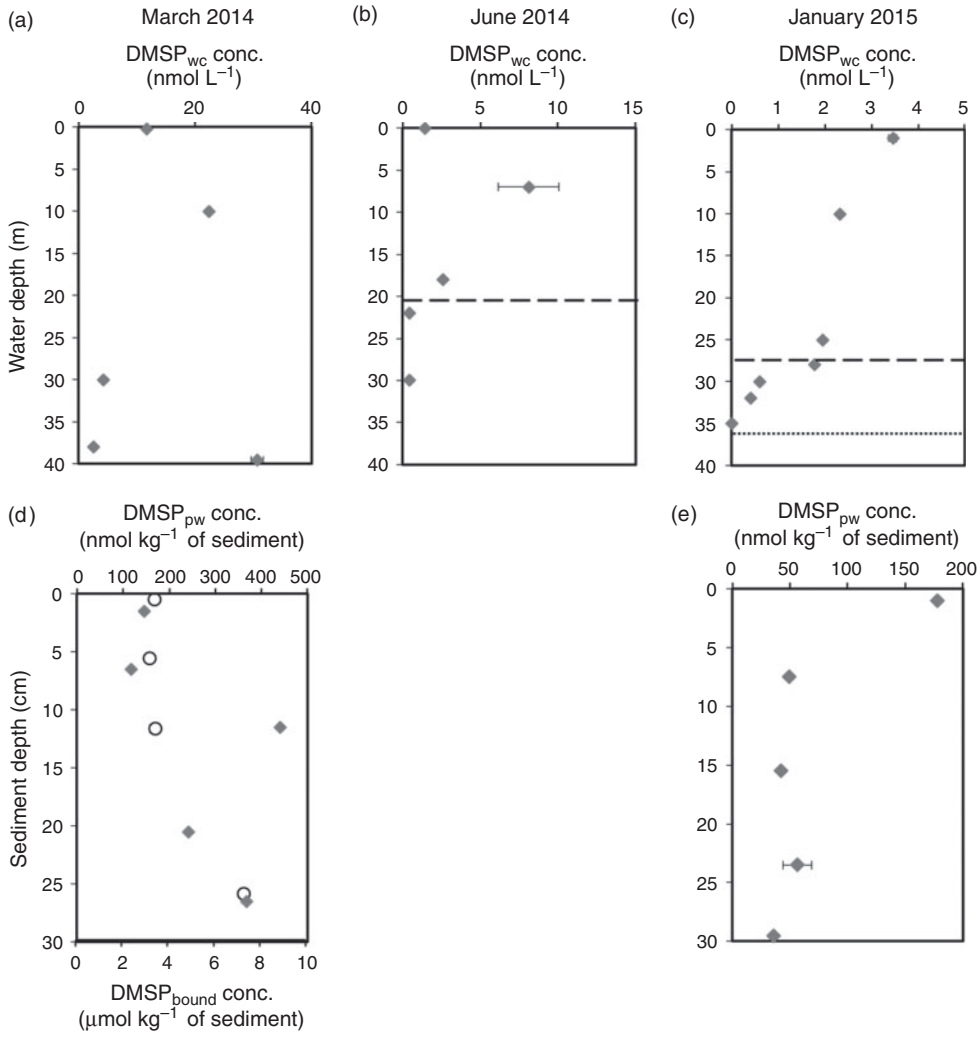

Fig. 5. Dimethylsulfoniopropionate (DMSP) concentrations in the water column (upper row) and sediments (bottom row), $DMSP_{pw\text{-}HCl}$ (grey diamond) and bound DMSP ($DMSP_{bound}$) (empty circles) during mixed (d) and stratified (e) periods. Dashed lines represent the depth of the thermocline at the time of sampling. Dotted line represents sediment–water interface depth during January 2015. Error bars (1σ standard deviation) are shown for samples with duplicates.

with ~0.7 mL of concentrated (37 % w/w) HCl and 15 mL of MilliQ water. The vials were left for gravity separation between water and sediment for at least 24 h. These samples were then divided into two DMSP pools: HCl extracted DMSP ($DMSP_{ex\text{-}HCl}$) and 'bound' DMSP ($DMSP_{bound}$). Sub-samples (~1 mL) of the water phase, HCl-extracted DMSP ($DMSP_{ex\text{-}HCl}$), were transferred using a syringe to a separate vial and then 3 mL of 5 N NaOH were added. After 4 h or more the released DMS was quantified as described below. The remaining sediment, after the removal of the water phase water (Fig. 2), was treated with 5 mL of 5 N NaOH for at least 4 h before further treatment and termed $DMSP_{bound}$. Because $DMSP_{bound}$ was very concentrated, we took only ~0.1 mL sub-samples (Fig. 2) of the slurry after base treatment to a separate vial prefilled with 10 mL of MilliQ water and the released DMS was analysed as described below.

It is important to note that the distinction between $DMSP_{ex\text{-}HCl}$ and $DMSP_{bound}$ is at this stage only operational, based on our acid extraction and phase separation of water from solid (see Fig. 2). In addition the DMSP pool may represent trapped or adsorbed DMSP and both pools could include other sulfonium compounds that can release DMS under base treatment.

DMSP concentrations and isotopic measurements during December 2014 were poorly reproducible because of sampling issues. Therefore, in January 2015, 2 weeks after the December 2014 sampling, we sampled again for DMSP in the water column and sediments. The thermocline and oxicline during that time deepened by 1 m and the water temperature remained the same, therefore DMS results from December 2014 and DMSP results from January 2015 are represented in the same graph (Figs 5c,e, 6c, 7c,f).

Quantification of DMS and DMSP

DMS was analysed using a system consisting of a Teledyne Tekmar (Atomx) purge and trap sample concentrator with autosampler coupled to a Perkin Elmer Clarus 580 GC and equipped with flame photometric detector (FPD), a specific detector for sulfur compounds. The purge and trap sample concentrator was equipped with a 5-mL sparge vial. Samples were sparged with He for 11 min, and the DMS trapped with an analytical trap (Tenax absorbent). The trap was then heated to 250 °C to desorb the DMS to the GC column through a heated transfer line (150 °C), and separated on an Gs-Tek column

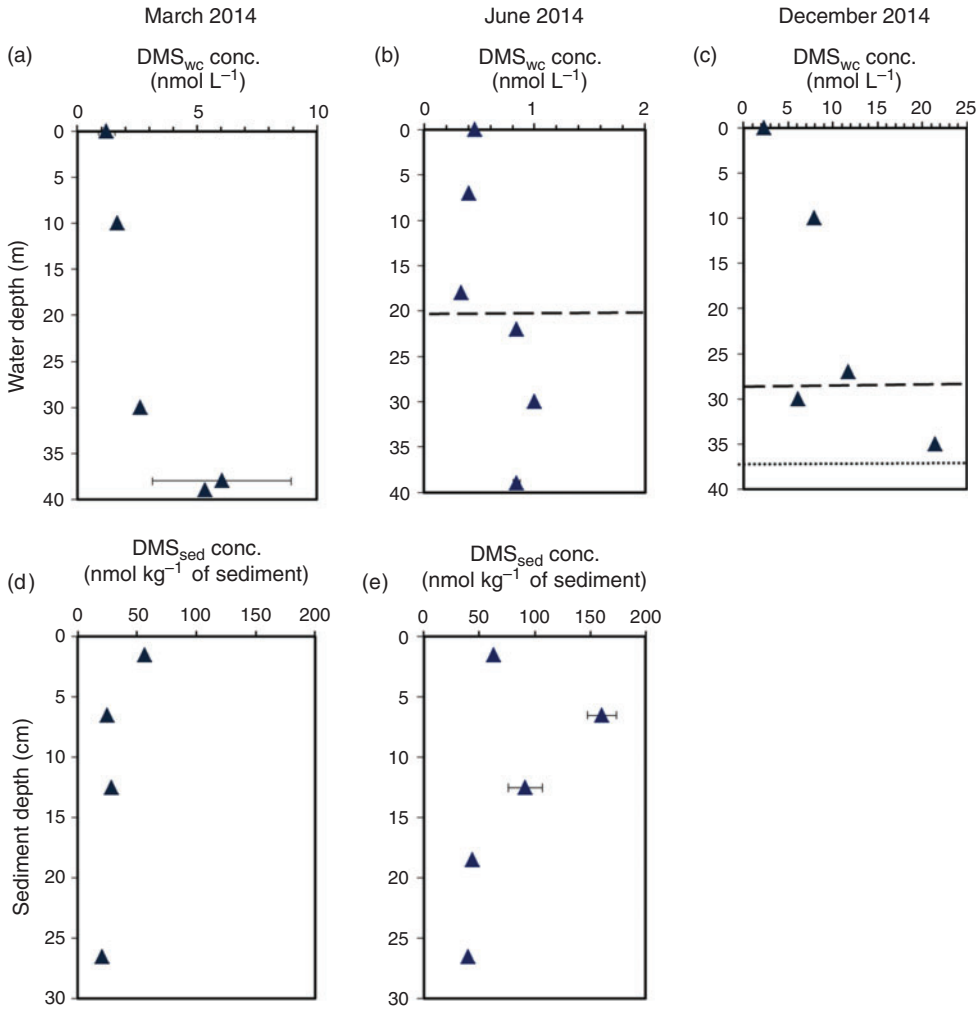

Fig. 6. Dimethylsulfide (DMS) concentrations in the water column (upper row) and sediment porewater (bottom row) during mixed and stratified periods. Dashed lines represent the depth of the thermocline at the time of sampling. Dotted line represents sediment–water interface depth during December 2014. Error bars (1σ standard deviation) are shown for samples with duplicates.

(60 m × 0.32 mm ID × 1.0 µm, helium carrier gas) held at 40 °C and operated in constant flow mode at 7 mL min^{-1}. The detector was heated to 300 °C, with flows of air (100 mL min^{-1}) and hydrogen (50 mL min^{-1}). A calibration curve of DMS standard (usually 0.2–13 nmol L^{-1}) was prepared before every sequence run of water samples. DMS standard (1–3 nM) and a blank MilliQ water sample (pre-purged for 30 min), were analysed every five samples.

Analytical precision for analysis of DMS and DMSP standards was usually better than 5 % (relative standard deviation, RSD). However, precision of duplicate or triplicate analyses of Lake Kinneret water samples were in the range of 10–20 %, possibly because the lake water was not homogeneous, a phenomenon that was most apparent during stratification periods. However, this could also be the result of other issues such as plankton disturbance during sparging etc.

Sulfur isotope analysis of DMS and DMSP

The sulfur isotopic composition of DMS and DMSP was measured by a purge and trap system that was connected to a gas chromatograph coupled to a multicollector (MC)–inductively coupled plasma (ICP) mass spectrometer (MS)[29] (Neptune Plus, Thermo Fischer Scientific). Water column samples or sediment sub-samples diluted in anoxic MilliQ water were collected from the original amber vial using a syringe (1–30 mL) with minimal disturbance, and injected gently into a new 40-mL sparging vial equipped with a Teflon septum. The vial was then sparged with He (40 mL min^{-1}) for 12 min. Water vapour was removed by a Nafion-membrane dryer (Perma pure LLC, NJ, USA) using dry N_2 as the counter flow. A Teflon sample loop was inserted into a dewar of liquid N_2 to trap DMS. After sparging, the six-port valve (Valco Instrument Co, TX, USA; heated to 80 °C) was turned to the inject position and the sample loop transferred quickly from the liquid N_2 to hot water and the trapped gases were injected into a Agilent J&W GC column (DB1, 60 m × 0.32-mm internal diameter × 1.0 µm), connected directly to the six-port valve. At the same time the gas chromatograph (Perkin Elmer 580) and the MC-ICP-MS were started. After separation by the GC, analytes were transferred to the MC-ICP-MS through a heated (200 °C) transfer line. The sulfur-isotopic composition of individual gas chromatograph peaks were measured by MC-ICP-MS. A home-built reference gas injector allowed the introduction of SF_6 peaks

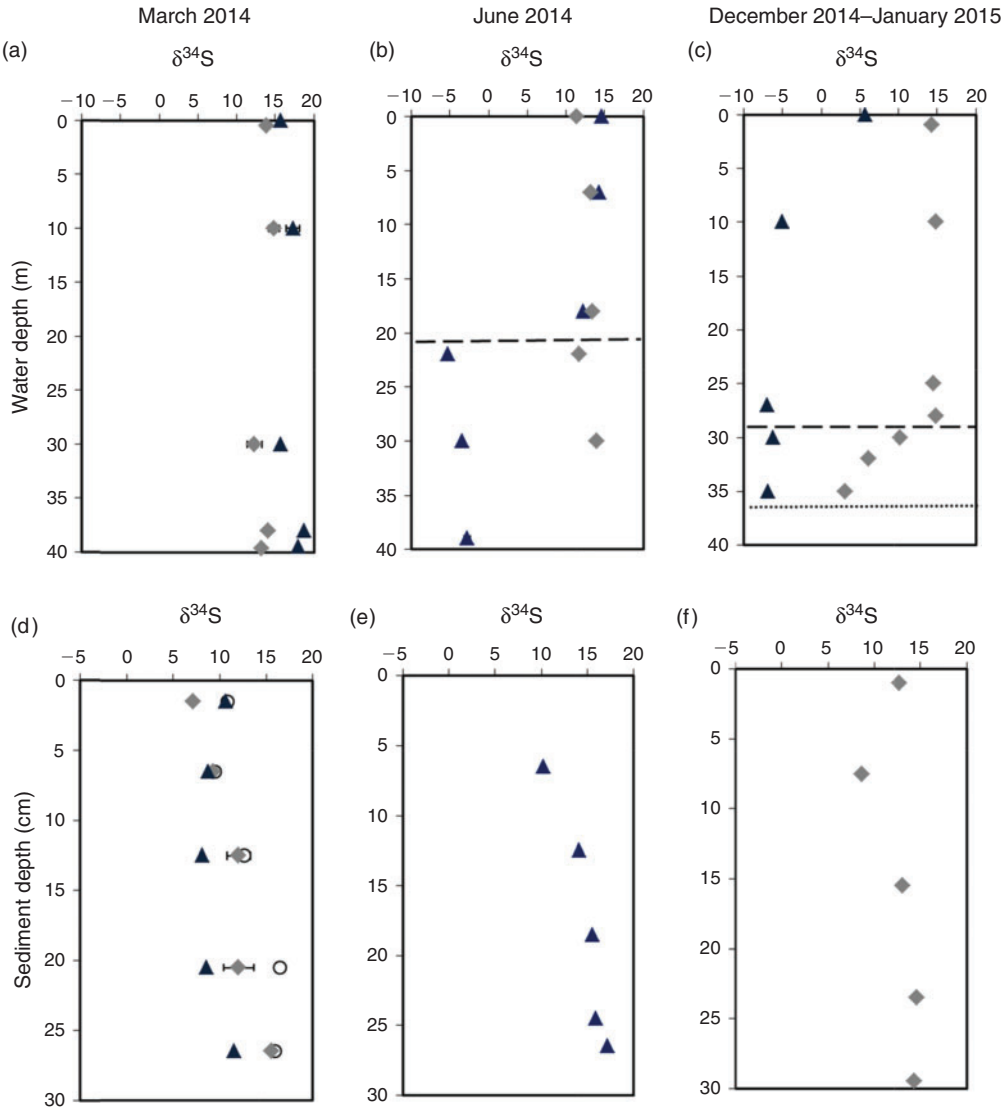

Fig. 7. The $\delta^{34}S$ of dimethylsulfide (DMS) (triangles), dimethylsulfoniopropionate water column ($DMSP_{wc}$) or $DMSP_{pw}$ (diamonds) and 'bound' DMSP (circles) in the water column (upper row) and sediments (bottom row) during mixing (March 2014) and stratification (June 2014, December 2014–January 2015). Note that the depths in the water column are in meters and in the sediments in centimeters. Error bars (1σ standard deviation) are shown for samples with duplicates.

(with known $\delta^{34}S$) in parallel with analytes for isotopic calibration. This reference gas injector is similar to the one described previously by Amrani et al.[28] and Said-Ahmad and Amrani.[29] A standard DMS sample was introduced to the system for calibration every 3–4 samples and we used a bracketing technique to correct for instrumental mass bias. DMS standards were prepared by dissolving varying amounts of liquid DMS (anhydrous, >99 % purity, Aldrich) transferred by syringe into a bottle or vial containing distilled water that was pre-purged for at least 90 min to remove any endogenous DMS. Final concentrations of the reference solutions varied between 0.1 and 10 nmol L^{-1}. These solutions went through the same purge and trap method and sulfur isotope analysis as that described above for lake water samples. These results were compared with direct injection of DMS solution in toluene to the conventional split–splitless injector of the gas chromatograph and calibration with SF_6 reference gas to determine the accuracy of the method.[29]

The results are expressed in conventional $\delta^{34}S$ notation as a per mille deviation from the international standard V-CDT (Vienna Canyon Diablo Troilite) according to Eqn 1.

$$\delta^{34}S = (({}^{34}R_{sample}/{}^{34}R_{std}) - 1) \times 1000 \quad (1)$$

where ${}^{34}R$ is the integrated ${}^{34}S/{}^{32}S$ ion-current ratio of the sample and standard peaks. Analytical precision of analysis of DMS and DMSP standards was usually in the range of 0.1–0.4 ‰ (1σ standard deviation) in agreement with Said-Ahmad and Amrani.[29] However, similar to the concentration reproducibility issues, S isotope analysis precision of duplicate or triplicate sample analysis of Lake Kinneret water and porewater was usually less than 1 ‰ but in some cases reached 2 ‰.

Results

The first profile for this study (26 March 2014; Fig. 3a) was in the partially mixed period, however thermal stratification had

already started with temperatures decreasing gradually from 22 °C near the surface to 15.5 °C near the sediments. A prominent Chl-*a* peak of 55 μg L^{-1} at a 5-m depth is representative for the annual algal spring bloom. As a result of photosynthetic activity, DO concentrations were above 18 mg L^{-1}, well above saturation in the upper 10 m of the water column. Below the photic zone, DO concentrations declined steadily towards the sediment to concentrations below 1 mg L^{-1}. The fairly uniform profile and high values (>271 mV) of the ORP indicates that the whole water column was still oxic. This changed by the time of the second profile, when a sharp chemocline was established at a 20-m depth, separating oxic (ORP = 100 mV) from the sulfide enriched water layers (ORP = −300 mV) (Fig. 3b). At that time the temperature in the epilimnion had increased to 27 °C while Chl-*a* and DO dropped to 10 μg L^{-1} and 8 mg L^{-1} respectively. The last two profiles were measured 3 weeks apart, during the time of destratification in winter. On 14 December 2014 when the water temperature in the epilimnion had decreased to 20 °C, the chemocline dropped to 27 m (Fig. 3c), and on 4 January 2015 it was at 31 m (Fig. 3d). Complete mixing occurred two days later on 6 January 2015 (not shown).

Hydrogen sulfide and sulfate concentration data in the water column are from the LKDB, and the data presented here are from the closest sampling dates to our profile collection dates. On 17 March 2014, H_2S was not detected in the water column, and SO_4^{2-} concentrations were constant throughout the water column (0.52 mM; Fig. 4a). On 22 June 2014, when the hypolimnion was anoxic, H_2S concentrations were already elevated, with maximum concentration at the sediment–water interface (0.12 mM) and decreasing upward towards the thermocline where it is oxidised (Fig. 4b). Sulfate concentrations above the thermocline were 0.54 mM, and there was a sulfate maximum of 0.76 mM at the thermocline from oxidation of upward diffusing H_2S. Sulfate concentrations below the thermocline (0.49 mM) were lower than in the epilimnion as a result of dissimilatory SO_4^{2-} reduction (Fig. 4b). Hydrogen sulfide concentrations were at their maximum values during December 2014 (0.24 and 0.27 mM on 14 and 28 December 2014 respectively; Fig. 4c), and decreased from the sediment–water interface towards the thermocline. Sulfate concentrations above the thermocline in December were 0.56 mM and decreased to 0.33 mM at the sediment–water interface (Fig. 4c).

The DMSP water column ($DMSP_{wc}$) concentration profiles showed peaks of 22.4 and 8.1 ± 1.9 nM at 10 and 7-m depth during March and June 2014 respectively (Fig. 5a–c). During January 2015 the highest concentration (3.5 ± 0.1 nM) was near the surface at 1 m. $DMSP_{wc}$ concentrations varied during the different months (Fig. 5), with minimum concentrations detected during June 2014 and maximum concentrations during March 2014.

$DMSP_{ex\text{-}HCl}$ concentrations in the sediments (Fig. 5d,e) were 1–2 orders of magnitude higher than in the water column. Two profiles of $DMSP_{ex\text{-}HCl}$ concentration were obtained, one during the mixed period (i.e. March 2014; Fig. 5d) and one during stratification (4 January 2015; Fig. 5e). The general trend of $DMSP_{ex\text{-}HCl}$ concentration in March showed increasing concentrations with depth from 146 to 368 nM, with a possible subsurface peak at 12-cm depth during March. During June 2014, the general trend showed exponential decrease with depth, with maximum concentration at 1-cm depth (177 nM).

$DMSP_{bound}$ concentrations measured during March 2014 (Fig. 5d) were at least one order of magnitude higher than $DMSP_{ex\text{-}HCl}$. $DMSP_{bound}$ concentrations were ~3 μmol kg^{-1} of wet sediment and increased up to 7 μmol kg^{-1} of sediment at 20–21-cm depth.

Over the year, DMS concentrations in the water column varied greatly between the different stages of stratification, and maximum concentrations occurred during late stratification (December 2014). DMS_{wc} concentrations during the mixed period were 1.2 nM near the lake surface and increased with depth to a maximum of ~6.0 nM just above the bottom at 38 and 39 m (Fig. 6a). During June 2014, at the beginning of stratification, DMS_{wc} concentrations were low and with no significance difference between them (0.3–0.5 nM) in the epilimnion, decreasing only slightly with depth to the thermocline (Fig. 6b). DMS_{wc} concentrations in the hypolimnion were approximately the same value of ~1 nM. During December 2014 (Fig. 6c), when the lake was still stratified, DMS concentrations in the epilimnion (2–12 nM) were higher than during early stratification and generally increased with depth. The highest concentration (22 nM) was obtained just above the sediment surface in the hypolimnion.

DMS concentrations in the sediments were ~1 order of magnitude greater than in the water column with higher concentrations in June than in March. In March the concentrations decreased slightly with depth (Fig. 6d), whereas during June 2014 (Fig. 6e) there was a maximum of DMS_{sed} (160 nM) at 7-cm depth, with an exponential decrease below this depth.

The $\delta^{34}S_{DMSPwc}$ profiles in the water column during the mixed period, March 2014, showed fairly constant values throughout the water column (+13.6 ± 1.0 ‰), with values ranging between +11.4 to +14.8 ‰ (Fig. 7a). In June 2014, when the lake was stratified, $\delta^{34}S_{DMSPwc}$ values in the epilimnion and hypolimnion were still similar (+12.7 ± 1.0 ‰) to the profiles from the mixed period (Fig. 7b). In January 2015 $\delta^{34}S_{DMSPwc}$ values in the epilimnion were similar to the values measured before (+14.6 ± 0.3 ‰; Fig. 7c), but below the thermocline, in the hypolimnion, $\delta^{34}S_{DMSPwc}$ values were much lower (+3.0 ± 0.1 ‰), just above the sediment.

The water column $\delta^{34}S_{DMSwc}$ in the March mixed period were 2–5 ‰ ^{34}S enriched relative to $\delta^{34}S_{DMSPwc}$ at that time, with maximum values of +18.7 ‰ close to the sediment–water interface (Fig. 7a). In June 2014, at the beginning of stratification, $\delta^{34}S_{DMSwc}$ values in the surface water (Fig. 7b) were +14.6 ± 0.3 ‰ and decreased slightly to +12.3 ‰ above the thermocline (values slightly lower than those of DMSP). The values in the epilimnion were close to the values during the mixed period, however below the thermocline $\delta^{34}S_{DMSwc}$ decreased sharply to −5.3 ‰ (Fig. 7b) just below the interface, with slightly more ^{34}S-enriched values of −3.4 and −2.7 ‰ at 30- and 39-m depth respectively. In December 2014, at late stratification, $\delta^{34}S_{DMSwc}$ values in the epilimnion decreased sharply from 5.6 ‰ at the surface to values between −5.0 and −7.0 ‰ below 10-m depth (Fig. 7c). The values at 10-m depth and below were ^{34}S depleted even though the thermocline and oxycline were located at 27-m depth.

In the sediment, $\delta^{34}S_{DMSsed}$ values (Fig. 7d,e) were depleted relative to $DMSP_{ex\text{-}HCl}$ and $DMSP_{bound}$ isotopic values (Fig. 7d) below 10-cm depth during March 2014, unlike $\delta^{34}S_{DMSwc}$ values in the upper epilimnion and mixed water column that were enriched by 2–5 ‰ relative to $\delta^{34}S_{DMSP}$. The $\delta^{34}S_{DMSsed}$ was +8.1 to +11.5 ‰ in March and +10.2 to +17.1 ‰ in June 2014. The $\delta^{34}S_{DMSPex\text{-}HCl}$ ranged from +7.2 to +15.5 ‰ in March 2014 and from +8.6 to +14.7 ‰ in January 2015 with no clear trend with depth.

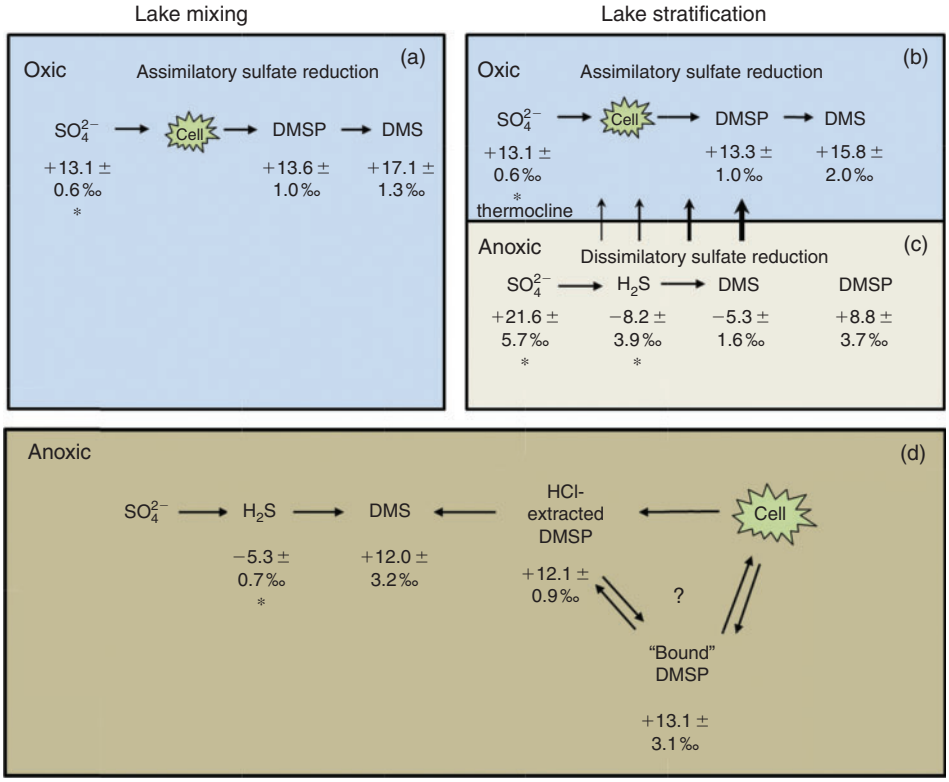

Fig. 8. Illustration of dimethylsulfide (DMS) production pathways in the water column during the mixing period (a) and during stratification (b,c) and sediments (d) as a function of the oxic state of the system. Values marked with an asterisk (*) are taken from Knossow et al.[36] Lake mixing values are average of March 2014 values. Lake stratification values: $\delta^{34}S$ of DMSP water column ($DMSP_{wc}$) and DMS_{wc} are an average of June 2014 and January 2015 for epilimnion and hypolimnion. H_2S $\delta^{34}S$ value in the sediment is from the sediment–water interface according to Knossow et al. [36] All other $\delta^{34}S$ values in the sediment are an average of values from all months of sampling.

Discussion

The DMSP and DMS concentration and sulfur isotopic ratio results show that the DMSP–DMS system in Lake Kinneret is dynamic and dependent on the stratification stage of the lake with a connection between the water column and sediments.

DMSP and DMS sources under oxic conditions

Maximum DMSP concentrations (Fig. 5a–c) in the water column during the mixed period and in the epilimnion during stratification stages correspond to maximum Chl-*a* (Fig. 3). This indicates that in the oxic water column the main source for DMSP is from algae.[32–34] Previous research showed that DMSP, produced by *Peridinium gatunense*, is the main precursor for DMS in the Lake Kinneret water column,[12] where the DMS is present at lower concentrations than DMSP. Indeed, DMS concentration profiles in the water column were almost one order of magnitude less than the DMSP concentration as is typically observed in seawater.[35] Maximum DMS concentrations during the mixed period were detected near the bottom, likely influenced by sediment contribution, probably as a result of high turbidity at that depth during that time.

The uniform values of $\delta^{34}S_{DMSPwc}$ ($+13.3 \pm 1.0$ ‰) under oxic conditions in the water column during the mixed period (March 2014) and early stratification (June 2014) had similar values to those of $\delta^{34}S_{SO4}$ ($+13.1 \pm 0.6$ ‰) measured by Knossow et al. in the epilimnion of Lake Kinneret.[36] The sulfur isotope fractionation during assimilatory sulfate reduction is small, −0.9 to −2.8 ‰,[13] which results in $\delta^{34}S$ of bulk organic matter slightly ^{34}S depleted relative to sulfate values (Fig. 8a,b). Amrani et al.[18] showed a similar pattern in the marine surface water samples around the globe with $\delta^{34}S_{DMSP}$ ranging between +18.9 and +20.3 ‰, close to the $\delta^{34}S$ value of seawater sulfate of +21.1 ‰. Similar results were obtained by Oduro et al.[19] for DMSP $\delta^{34}S$ of macroalgae and phytoplankton. It is important to note that in some cases, ^{34}S enriched values (~1 ‰) of DMSP relative to its source sulfate was observed in deeper marine water during an algal bloom.[18] The isotopic data for DMSP in Lake Kinneret in the mixed period and early stratification in all water column samples is similar, within analytical uncertainties, to its sulfate source and suggests a clear assimilatory sulfate reduction source for DMSP.

The $\delta^{34}S$ values of the DMS in the water column during the mixed period, in March (Figs 7a, 8a), support its origin from water column DMSP, as proposed previously by Ginzburg et al.[12] The $\delta^{34}S$ values of DMS in the lake were enriched by 2–5 ‰ relative to DMSP. This is a greater enrichment for DMS relative to DMSP compared to that observed in the water column of the Gulf of Aqaba and the Mediterranean Sea, which showed DMS to be ^{34}S enriched by ~1 ‰.[18,29] However, incubation experiments with degrading macroalgae under dark conditions showed a different trend, with DMS being ^{34}S depleted by up to 1.6 ‰ relative to DMSP.[19] The reason for the significantly ^{34}S enriched values for DMS in Lake Kinneret relative to seawater is not yet clear and may point to different enzymatic cleavage mechanisms for DMSP in fresh water relative to seawater or

algal *v*. bacterial enzymatic cleavage of DMSP to produce DMS.[37] It is also possible that biological DMS consumption fractionates DMS differently in freshwater or that other, non-biological controls (e.g. photochemical oxidation) on the removal of DMS from the water are responsible for this fractionation between DMSP and DMS. Evaporation of DMS from the surface water is not likely to cause this fractionation as it has been shown that the water–air transfer of DMS has a very small (−0.5 ‰) fractionation factor.[18]

The $\delta^{34}S$ values of DMS in the epilimnion at early stratification (Figs 7b, 8b) were similar to those of the DMSP and not slightly enriched as in the March samples. This observation suggests that there might be another small source of DMS in this month that is ^{34}S depleted. The most probable source for this ^{34}S depleted sulfur source is H_2S from dissimilatory sulfate reduction formed when anoxic conditions developed in the lower water column. This H_2S may be methylated to form MT and eventually DMS.[7,38] Further evidence for this mechanism is the accumulation of ^{34}S-depleted DMS to high concentrations deep in the water column during late stratification (i.e. December 2014) (Figs 6c, 7c), and its diffusion upward could explain the ^{34}S depleted values of the DMS in the whole epilimnion during December. The concentration of DMS decreased from near the bottom where it was likely produced at high rates to the surface water where it is likely being lost. Loss mechanisms for DMS may include ventilation to the atmosphere, and biological and photochemical oxidation. Approximate mass balance calculations shows that ∼15 % contribution of DMS from methylation of H_2S in June is required in order to achieve the measured values, taking $\delta^{34}S$ of DMSP as 13 and 3 ‰ enrichment in DMS production from DMSP and $\delta^{34}S$ of H_2S of ∼–5 to −7 ‰[36] and with no fractionation in the DMS production from H_2S. This mass balance remains uncertain because we currently lack information on fractionation of sulfur isotopes during the various biotransformations, e.g. methylation of sulfide and MT, and the consumption of DMS. Oduro et al. reported 4–5 ‰ ^{34}S enrichment of VOS compounds relative to their proposed H_2S source. However, this observation was on a complex mixture of VOS compounds and not specifically for DMS.[20]

At the end of the stratification period, virtually all the DMS in the epilimnion may be derived from the upward flux of ^{34}S-depleted (i.e. -6.3 ± 0.8 ‰) DMS as its $\delta^{34}S$ is similar to that of the hypolimnetic DMS (Figs 7c, 8b). This period coincides with a decrease in the phytoplankton community in the winter and thus the DMSP concentrations (Fig. 5) and its potential as a DMS source. However, in the surface water (0-m depth, Fig. 7c), the higher $\delta^{34}S$ values for DMS (+5.6 ‰) indicate a greater contribution from DMSP degradation, with the contribution of sulfide-derived DMS being lower because most of the sulfide is oxidised deeper in the water column (Fig. 6c). Mass balance calculation shows that the contribution of ^{34}S depleted DMS from upward diffusion is reduced to ∼50 % in the surface water.

DMSP and DMS under anoxic conditions

During stratification (June and December 2014) $\delta^{34}S_{DMS}$ in the anoxic conditions of the hypolimnion showed significant ^{34}S depleted sulfur isotopic values (−2.7 to −7.0 ‰, Fig. 7b,c), supporting a DMS origin from H_2S methylation (via MT). Moreover, DMS maximum concentrations were below the thermocline during stratification or at the sediment–water interface during the mixed period. This suggests that the main source for DMS in Lake Kinneret in the anoxic water column is not from DMSP cleavage but rather from H_2S, through biomethylation that occurs below the thermocline (Fig. 8c). Sulfate and H_2S concentration profiles and $\delta^{34}S$ values[36] during the stratified period support active dissimilatory sulfate reduction in the anoxic water column (Fig. 4b,c), providing a reduced sulfur source for methylation. The maximum SO_4^{2-} concentration at the thermocline during early stratification, June 2014 (Fig. 4b), may indicate oxidation of sulfide and intermediate sulfur species found in the thermocline.[39]

Hydrogen sulfide and MT methylation was suggested as the main route for DMS formation in freshwater environments.[5,39,40] Biogenic methylation of inorganic sulfides and polysulfides were shown to occur in Lake Kinneret even under oxic conditions.[41] However, until recently the main evidence for the routes came from correlation between DMS production and addition of methyl-donors in incubation experiments[12,42,43] or correlation between DMS and H_2S in-situ profiles in the absence of DMSP.[39] Oduro et al. provided isotopic evidence for a methylation route to produce VOS compounds in a freshwater lake.[20] They measured $\delta^{34}S$ of total VOS compounds (in which MT was the most abundant species by far) of ∼–25 ‰ in a meromictic freshwater lake with high sulfate concentrations (12–16 mmol L^{-1}), and showed that the difference in $\delta^{34}S$ between VOS compounds and H_2S was +4 to +5 ‰. Their suggested route for DMS and MT production was methylation with methyl groups derived from methoxylated aromatic compounds. Here we present evidence of a H_2S methylation route for DMS production specifically (not as a mixture of VOS compounds) in the freshwater anaerobic hypolimnion of Lake Kinneret.

The depleted ^{34}S DMSP values below the thermocline during late stratification (Fig. 7c) are intriguing. Oduro et al. could not detect DMSP in the anaerobic part of Fayettville Green Lake and they verified their findings with electrospray ionisation (ESI)-MS analysis.[20] We did not perform ESI-MS analysis, and thus cannot directly confirm the presence of DMSP. Our 'DMSP' might be one or more other dimethylsulfonium compounds that release DMS upon addition of strong base (e.g. NaOH) or even possibly a complex of DMS that releases DMS under alkaline conditions. If indeed it is DMSP, it may imply a different formation route for DMSP under these anaerobic conditions. A possible formation pathway for ^{34}S depleted DMSP can be assimilatory uptake of MT and possibly other sulfide or polysulfides species (organic and inorganic) to produce ^{34}S depleted methionine, the biosynthetic precursor of DMSP.[43,44] However, further study is needed to explore this speculative pathway.

DMS and DMSP concentrations in the sediments were one order of magnitude higher than in the water column. Thus, the isotope values of the DMSP in the sediment should be affected by its main algal sources and sinks to the sediment and less so by assimilation of H_2S methylation products in the hypolimnion, as is indeed observed (Fig. 7f). $DMSP_{ex\text{-}HCl}$ concentrations in the sediment increased with depth in March 2014 (Fig. 5d). This increase in $DMSP_{ex\text{-}HCl}$ concentration in the sediment may indicate an ongoing source for HCl-extracted DMSP. The sedimentation rate in Lake Kinneret is high (4 mm $year^{-1}$), therefore organic matter formed in the upper water column can be preserved in the sediment and contribute to $DMSP_{ex\text{-}HCl}$ and DMS_{sed} production in the sediment. The high concentration of $DMSP_{bound}$, an order of magnitude higher than $DMSP_{ex\text{-}HCl}$ (Fig. 5d), supports this suggestion and it may be the source of this ongoing supply of $DMSP_{ex\text{-}HCl}$. Furthermore, the $\delta^{34}S$

values of $DMSP_{bound}$ are similar to the $\delta^{34}S$ values of $DMSP_{ex\text{-}HCl}$, indicating that particulate organic matter is a source for HCl-extracted DMSP. It should be noted though that $DMSP_{bound}$ could also include sulfonium compounds such as gonyol, that could be produced by *Peridinium gatunense*,[22] or other base-released DMS complexes.

DMS concentration profiles in sediments showed a decrease with depth, indicating consumption processes. DMS can be degraded by methanogens and sulfate reducers in freshwater, estuarine and sludge sediment.[38,45,46] Methanogenesis is known to be a very intensive process in the deep sediment[47] and DMS may be one of its substrates.

In-situ profiles of all species in the sediments, $\delta^{34}S_{DMS}$, $\delta^{34}S_{DMSPex\text{-}HCl}$ and $\delta^{34}S_{DMSPbound}$, were conducted only in March 2014. It seems that during the mixed period (March 2014), DMS isotopic values in the sediments were slightly ^{34}S depleted compared to $\delta^{34}S_{DMSPex\text{-}HCl}$, but ^{34}S enriched relative to $\delta^{34}S_{DMSwc}$ measured in the hypolimnion. This finding indicates that DMS originates in the sediment from a combination of $DMSP_{ex\text{-}HCl}$ enzymatic cleavage and H_2S methylation. However, based on the $\delta^{34}S$ of DMS_{sed} and $DMSP_{ex\text{-}HCl}$, it is clear that the dominant contribution for DMS in the sediment is DMSP cleavage (Fig. 8d). This conclusion is also supported by the much higher concentration of DMSP (including $DMSP_{bound}$) relative to DMS_{sed}, which likely overwhelmed any contribution from H_2S methylation.

Conclusions

Lake Kinneret is a dynamic system with two main pathways of DMS production: (1) DMSP cleavage and (2) methylation of sulfide species. The $\delta^{34}S_{DMSP}$ values in the water column are similar to $\delta^{34}S_{SO_4}$, which suggests a sulfur source from assimilatory sulfate reduction. The $\delta^{34}S_{DMSwc}$ values in the mixed oxic water column and upper surface at late stratification were 1.1–4.8 ‰ ^{34}S enriched relative to $\delta^{34}S_{DMSPwc}$, a higher enrichment than observed in seawater (~1 ‰). This may indicate a different enzymatic cleavage operating in fresh water relative to sea water or different fractionation during DMS consumption processes. Below the thermocline, in the anoxic water, $\delta^{34}S_{DMS}$ values were highly ^{34}S depleted (−2.7 to −7.0 ‰) and slightly enriched compared with $\delta^{34}S_{H_2S}$, which suggests a H_2S methylation mechanism for the formation of DMS. This DMS diffused upward during stratification periods and mixed with the DMS that came from DMSP cleavage in the epilimnion. In the sediment $\delta^{34}S_{DMS}$ values were slightly lower than $\delta^{34}S_{DMSP}$. This intermediate value between $\delta^{34}S_{DMSP}$ and $\delta^{34}S_{H_2S}$ indicates DMS production in the upper sediment is a combination of both pathways, with the DMSP cleavage as the dominant one, likely because H_2S and its first methylation product, MT, have numerous alternative fates. Base addition to Lake Kinneret sediments released large amounts of DMS ('bound' DMSP) but its origin is not known and may be related to intercellular or absorbed DMSP or other sulfonium compounds. The 'bound' DMSP is probably the on-going source for $DMSP_{ex\text{-}HCl}$ in Lake Kinneret sediments based on their similar $\delta^{34}S$ values and the fact that 'bound' DMSP is 20–40 fold more abundant than HCl-extracted DMSP. In summary, the natural abundance sulfur isotope ratio of the DMSP–DMS system in Lake Kinneret provides insights into their sulfur origin. Additional data, with better temporal resolution and specific chemical identification of the 'DMSP' pools, as well as other analysis of other volatile sulfur compounds (e.g. MeSH, COS) will improve understanding of sulfur cycling in this and other freshwater lakes.

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