The impact of seasonal sulfate–methane transition zones on methane cycling in a sulfate-enriched freshwater environment

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Abstract

Lake Willersinnweiher located in south-western Germany is a small eutrophic gravel pit lake fed by sulfate-enriched groundwater. The aim of this study was to investigate the total methane (CH₄) mass balance of Lake Willersinnweiher with a particular focus on the interaction of carbon and sulfur cycling within the lake sediments and the redoxcline of the water column. Our results show that Lake Willersinnweiher permanently releases CH₄ to the atmosphere throughout the whole year 2018 at rates ranging from 5 to 120 mol d⁻¹. Sediment data show the presence of intense anaerobic oxidation of CH₄ in the upper sediment layers during early summer. Here, CH₄ is most likely consumed via sulfate in sulfate–methane transition zones (SMTZs) that have been observed for a few specific freshwater environments only. Seasonal dynamics in biogeochemical processes trigger the non-steady state conditions within the sediments and the CH₄ consumption in the SMTZs. In parallel, CH₄ released from the sediments is completely consumed by aerobic oxidation processes in the redoxcline indicated by minimum CH₄ concentrations with high δ¹³C-CH₄ values. This zone acts as an effective barrier, minimizing CH₄ release into the surface water and the atmosphere and thus CH₄ oversaturation along with near-atmospheric isotopic composition indicate the presence of an additional CH₄ source in the epilimnion of Lake Willersinnweiher.

The emission of the greenhouse gas methane (CH₄) from freshwater lakes has been suggested to play a substantial role in the global methane budget (e.g., Bastviken et al. 2004). Here, significant amounts of CH₄ are emitted, even though CH₄ produced in aquatic systems is largely consumed by anaerobic and aerobic methanotrophs (up to 30–99%; Bastviken et al. 2008). The amount of emitted CH₄ is thereby depending on bioproduction, degradation and mineralization of organic substances in the sediments and the water column of the freshwater lake.

Carbon mineralization in anoxic lake sediments is affected by manganese (Mn) and iron (Fe) reducing bacteria metabolizing competitive substrates, outcompeting CH₄ forming microorganisms (methanogens) within the upper sediment layers (e.g., Whiticar 1999). Sulfate (SO₄²⁻) reduction often has minor implications on organic matter degradation due to low SO₄²⁻ availability in most freshwater environments (Holmer and Storkholm 2001), so that SO₄²⁻ is rapidly depleted with sediment depth and SO₄²⁻ reducing bacteria become inactive or are absent. As a consequence, methanogenesis is the most important process in overall carbon mineralization in anoxic lacustrine sediments (e.g., Rudd and Hamilton 1978).

Competitive metabolisms are not only affecting methanogenesis, but also consumption of CH₄ by bacteria (methanotrophs) in the sediments. The anaerobic oxidation of methane in the sediments of lakes is usually coupled to the reduction of the energetically more favorable electron acceptors, such as nitrate and nitrite (e.g.,raghoebarsing et al. 2006) or Fe(III) and/or Mn(IV) (e.g., Beal et al. 2009). Methanotrophy coupled to SO₄²⁻ reduction is a common feature in marine sediments due to high pore-water SO₄²⁻ concentrations, but was, so far, only observed for a few specific freshwater environments (e.g., Borrel et al. 2011). Those environments are, e.g., groundwater-fed lakes, which may tend to be enriched by SO₄²⁻ originating from the weathering of sulfur-containing rocks in the catchment. This leads to significant SO₄²⁻ reduction, especially in eutrophic lakes, where both the availability of organic matter and SO₄²⁻ concentrations are high. In the sediments, CH₄ is consumed to a great extent by the anaerobic oxidation of methane in
The various pathways of CH₄ formation and degradation can be characterized and distinguished by the determination of the stable carbon isotopic composition of CH₄ (δ¹³C-CH₄ values). The isotopic signature of CH₄ sources is thereby dependent on isotope fractionation during the methanogenic and methanotrophic reactions as well as the δ¹³C values of the used substrates. Fractionation factors for the biogenic methanogenesis vary considerably under different conditions and environments, and differ between the methanogenic pathways (e.g., Conrad 2005). In general, biogenic CH₄ has relatively low δ¹³C-CH₄ values (~40 to ~90‰), due to enrichment of light ¹²C by microbial isotope fractionation (e.g., Rosenfeld and Silverman 1959).

In lakes, biogeochemical processes in shallow sediments are usually controlled by rather fast variations in temperature and redoxcline depth, leading to considerable changes in pore-water chemistry and turnover rates (e.g., Crill and Martens 1987). Thus, reduced products of turnover processes of organic substances are released into pore waters and diffuse into the water column. Here, oxidizing anoxic re-oxidation processes are predominant in the sediments or in the water column, depending on the seasonal stratification of the lake.

The purpose of this study was twofold: (1) to investigate the total methane mass balance of Lake Willersinnweiher and (2) to study the interaction of carbon, sulfur and manganese cycling within the sulfate–methane transition zones in the sediments and at the redoxcline in the water column of Lake Willersinnweiher (SW Germany) in greater detail.

Lake Willersinnweiher has been shown to contain relatively high SO₄²⁻ concentrations (up to 2.4 mM), caused by the inflow of suboxic and SO₄²⁻ enriched groundwater (Schröder 2004). The interplay between groundwater and surface water, as well as high bioproduction in the summer season result in high sulfide (S-II) concentrations in the anoxic hypolimnion of Lake Willersinnweiher. Seasonal and spatial variations of the redoxcline depth and fluxes of the redox sensitive elements manganese and sulfur were observed in the sediments as well as the water column and correlate with the lake depth (Schröder 2004). Schröder (2004) further suggested that CH₄ cycling plays an essential role within the sediments of Lake Willersinnweiher, since the flux balances indicate an imbalance between the cycles of C and S, demonstrating that an additional C source within the sediment is required. Therefore, our study particularly focuses on the extent and seasonal variability of CH₄ production and consumption coupled to sulfur cycling in Lake Willersinnweiher.

**Materials and Methods**

**Study site, hydrological, and hydrogeochemical parameters**

Lake Willersinnweiher is located in the plain of the Upper Rhine Graben near Ludwigshafen, Germany (49.499950°N; 8.397138°E; Fig. 1). It is one of four former gravel pits, which were built for the excavation of gravel and sand from the upper aquifer sediments of Pleistocene age. The fine-grained lake sediments are few centimeters to up to only 50 cm thick and act as a boundary layer to the sandy aquifer material. The lake has a size of 17 ha, is composed of two smaller basins and has a mean depth of about 8 m with a maximum depth of 20 m (Sandler 2000). Lake Willersinnweiher has been routinely monitored for its major pore-water and water-column data as well as groundwater/lake-water interactions for ca. 20 years (e.g., Sandler 2000; Schröder 2004; Wollschläger et al. 2007). The lake is classified as a eutrophic hardwater lake and fed by precipitation and groundwater. The average water residence time was estimated to be 3.7 a (Wollschläger et al. 2007).

In recent years, Lake Willersinnweiher is monomictic with a circulation period in winter from November/December to March/April. During this period, the water column is fully oxic and O₂ reaches the upper few millimeters of the sediments (Schröder 2004). In the upper sediment layer, O₂ is consumed by reduced minerals as, e.g., FeS, and pyrite, which are present in the sediments. These minerals are partly oxidized during the cold season. It was observed that the SO₄²⁻ reduction zone shifts downward in winter, and S(-II) is fully consumed in the oxic sediment layer, whereas it moves upwards during the stratification period, when the reduced products are released into the water column (Schröder 2004).

The groundwater geochemistry in the shallow aquifer of the Upper Rhine Graben near Ludwigshafen is characterized by the consumption of O₂ and NO₃⁻ by pyrite oxidation and calcite equilibration in the aquifer sediments (Isenbeck-Schröter et al. 2016). In previous studies, a dominance of SO₄²⁻ with concentrations up to 2.4 mM was observed in the groundwater (e.g., Schröder 2004). Groundwater infiltrating the lake at the south-eastern shore has further passed at least one of the lakes located upstream of Lake Willersinnweiher (Wollschläger et al. 2007).

Coupling between groundwater and surface water, as well as high bioproduction in the summer season result in high total S(-II) concentrations in the anoxic hypolimnion of Lake Willersinnweiher (Schröder 2004). Further investigations suggested seasonal and spatial variations of the redoxcline depth and fluxes of the redox sensitive elements Mn as well as S-compounds in the sediments as well as the water column, correlating with the lake depth.

Sampling of the water column was carried out at the centre of the lake (profundal site) and sediment samples were taken from three sites (littoral, slope and profundal) with different lake depths (Fig. 1: Supporting Information Table S1). The sediments, lake water, and groundwater inflow and outflow were sampled in May 2017, in January, May, August, and October 2018, as well as in January 2019.

**Sampling and analytical methods**

Pore-water analyses were performed on two parallel cores (core length ~ 20–40 cm) of lake sediments recovered from three locations using a manual gravity corer.
From one core, pore-water was extracted by rhizons (Rhizosphere Research Products, The Netherlands) with a pore size of 0.15 μm according to the procedure described by Seeberg-Elverfeldt et al. (2005) immediately after retrieving the cores from the lake floor. Rhizons were inserted through predrilled holes (sealed with tape while sediment sampling) in the liner used for sediment sampling and connected to the syringes using the leakage-free Luer-Lock adapters. Syringes were then drawn up and locked in this position so that the pore water was sucked into the air-free syringe. In this study, we typically sampled 5–10 mL of porewater, which is sufficient for analysis of all major components. After reaching this volume, the syringe was immediately sealed gastight with a Luer-Lock adapter and stored cold until further analysis. For this purpose, sediment samples were weighed before and after drying for 24 h at 105°C.

In the water column, the field parameters water temperature (°C) and dissolved oxygen (mM) were examined in situ with a multiparameter water probe EXO1 (Xylem Analytics, Norway). Samples for the analysis of dissolved ions in the lake water were collected in a side stream of the membrane-coupled cavity ring down analyzer (M-CRDS system) and filtered through a 0.45 μm cellulose acetate filter. Samples for Fe and Mn were acidified with HNO₃ (6 M). Groundwater wells were sampled using a submersible Grundfos MP1 pump
according to sampling regulations (DVGW 2011). Redox sensitive parameters were constant prior to sampling, indicating access to the flowing aquifer water. Samples were collected following the procedure described for the lake water samples. All pore-water, lake water and groundwater samples were stored dry and cool (4°C) until further analysis.

Lake depth profiles of CH4 concentrations and δ13C–CH4 values in the water column were performed by the M-CRDS system as described in detail by Hartmann et al. (2018). Each depth was measured for 20 min and CH4 and δ13C–CH4 values were averaged over the last 10 min of the measurement interval. For quality control, the working reference gas for CH4 (10 ppmv CH4 in synthetic air) was analyzed prior and at the end of the measurements. The results by the M-CRDS were verified for CH4 concentrations by analyzing subsamples taken from the side stream of the M-CRDS system and analyzed for CH4 concentrations in the laboratory.

Pore-water and water column CH4 concentrations were measured by gas chromatography (GC) with Barrier Ionization Discharge Detector (GC-BID, Shimadzu, Japan) and Flame Ionization Detector (GC-FID, Shimadzu, Japan), respectively. For quality control, working reference gases were analyzed along with GC-BID (1000 ppmv CH4 in synthetic air) and GC-FID measurements (2.192 and 9.872 ppmv in synthetic air). Dissolved CH4 concentrations were determined using Henry’s law and solubility coefficients for CH4 according to Yamamoto et al. (1976). The groundwater δ13C–CH4 values were analyzed with stable isotope ratio mass spectrometry (GC-C-IRMS, Deltaplus XL, Thermo Finnigan, Bremen, Germany) following the procedure described in Hartmann et al. (2018) and values were normalized using two CH4 working standards (isometric instruments, Victoria, Canada) with values of 23.9 ± 0.2 and 54.5 ± 0.2 (in ‰ vs. V-PDB). Please note that pore-water δ13C–CH4 values could not be determined.

Total dissolved concentrations of Na, K, Ca, Fe, and Mn were determined by inductively coupled plasma atomic emission spectroscopy (Agilent ICP-OES 720, USA). For quality control the reference material SPS-SW2 was analyzed along with the samples with a measurement precision for each element <2%. The concentrations of SO42− and nitrate (NO3−) were analyzed by ion chromatography (Dionex ICS 1100, Thermo Fisher Scientific, Waltham, Massachusetts). The measurement precision for each element was <3% and derived from long-term repeated analysis of reference material SPS-NUTR-WW1. Total sulfide (S(II)) was determined photo-electronically (DREL 2800, Hach, Loveland, Colorado) using the Sulfide Test Spectroquart (Merck, Germany) immediately after sampling in the field in an aliquot of the sample, once a sufficient amount of pore water was retrieved during rhizon sampling. Dissolved inorganic carbon (DIC) were measured using the Shimadzu TOC-V CPH (Shimadzu, Japan) with a precision of <2% derived from repeated analysis of an in-house standard water. For quality control of all geochemical analyses, ionic balances were calculated by the sum of major cation and anion concentrations. The deviations were lower than 5%. All laboratory analyses were performed at the Institute of Earth Sciences at Heidelberg University, Germany.

Calculations

Diffusive fluxes for all terminal electron acceptors (TEA) were calculated from concentration gradients assuming steady-state conditions according to Fick’s first law (Eq. 1)

$$J_{TEA} = -\theta * D_S \frac{\partial C}{\partial x}$$

(1)

where J is the diffusive flux of each TEA (mmol m−2 d−1), θ is the porosity of the sediment, C is the TEA concentration (mol m−3), and x is the depth (m). Calculations were based on the diffusion coefficient $D_S$ (m2 s−1) for pore-waters, which is dependent on the tortuosity $\theta$ (dimensionless; Eq. 2).

$$D_S = \frac{D_w}{\theta^2}$$

(2)

$D_w$ is the molecular diffusion coefficient in water (m2 s−1), taken from Broecker and Peng (1974), and the tortuosity θ was calculated using measured porosities and Eq. (3) according to Boudreau (1996)

$$\theta = 1 - \ln\left(\theta^2\right)$$

(3)

Methane release from the sediments into the bottom water layer was calculated based on the concentration gradients given by the pore-water CH4 concentration in the top 3 cm of the sediment. Sedimentary consumption rates in the sulfate methane transition zones were estimated by calculating local transition zone-related fluxes, where both SO42− and CH4 concentrations were decreasing and S(-II) and DIC showed a local maximum. An exemplary illustration of the method used to calculate the various fluxes is provided in the Supporting information (Fig. S1).

Fractionation factors (αf) for CH4 oxidation in the water column were calculated after Claypool and Kaplan (1974) using the closed-system Rayleigh equation (Eq. 4)

$$\delta^{13}C = \left[\delta^{13}C_o + 1000 * f \left(\frac{1}{1000}\right)\right] - 1000$$

(4)

with $\delta^{13}C_o$ as the stable carbon isotope value of CH4 in the bottom-near water layer and $\delta^{13}C$ and as the stable carbon isotope value of CH4 in the zone of CH4 oxidation as well as f as the fraction of oxidized CH4.

Whole-lake mass balance was calculated for the littoral, slope and profundal zones based on the diffusive fluxes estimated for the CH4 sinks and sources addressed in this study. The CH4 mass balance accounted for the CH4 consumption in the sediments, diffusive sedimentary CH4 release and CH4.
consumption at the redoxcline, groundwater CH₄ input and loss, as well as the diffusive CH₄ emissions into the atmosphere. The latter was estimated by the wind-mediated gas transfer across the surface water–atmosphere boundary layer after Wiesenburg and Guinasso (1979) and with the gas transfer velocities obtained by calculations based on Cole and Caraco (1998). Wind speeds were obtained from a nearby weather station (49.51°N/8.55°E).

For the whole-lake mass balance, total fluxes per site and season (J_{TEA/site} [mol L⁻¹]) were calculated by multiplying the sedimentary and water column fluxes (J_{TEA}) with the corresponding sediment surface area of the littoral, the slope as well as the profundal site and / or the redoxcline planar area (A_{flux} [m²]), respectively, for each sampling date (Eq. 5).

\[ J_{TEA/site} = \frac{A_{flux}}{d_{season}} J_{TEA} \]  

The sediment surface area as well as the planar lake area were taken from Schröder (2004) (Supporting Information Table S2). Uncertainties in the mass balance were analyzed by considering deviations of 10% each: in diffusion coefficients and pore-water fluxes, average wind speeds as well as in the sediment surface area.

For the integrated annual CH₄ budget, we used a simplified model and assumed the total fluxes per site and season (J_{TEA/site}) to be constant over a distinct period over time, that is characteristic for each stage in the lake stratification period (Supporting Information Table S1). Given that, we obtained the annual flux (J_{Annual} [mol]) by summing the product of J_{TEA/site} with the length of the corresponding period (d_{season} [days]; Eq. 6):

\[ J_{Annual} = \sum_{01.01.2018}^{31.12.2018} J_{TEA/site} d_{season} \]  

All parameters used for the calculation of the whole-lake CH₄ mass balance and the annual budget for Lake Willersinnweiher are presented in the Supporting Information (Table S1). In addition, mass balance uncertainties were analyzed by considering deviations of 10% each: in diffusion coefficients and pore-water fluxes, average wind speeds as well as in the sediment surface area. All Parameters used for the calculation of the whole-lake CH₄ mass balance for Lake Willersinnweiher are presented in the Supporting Information (Table S2).

**Results**

**Characterization of groundwater at Lake Willersinnweiher**

The groundwater feeding Lake Willersinnweiher showed major contents of calcium (Ca), DIC and SO₄²⁻ (Ca–HCO₃–SO₄ type water) with SO₄²⁻ concentrations of up to 2.3 mM (Fig. 2). In comparison the groundwater outflow showed lower SO₄²⁻ but higher DIC concentrations suggesting that during the passage of the lake, SO₄²⁻ is consumed and DIC formed.

The pH values were 8 and 8.3 for groundwater inflow and outflow, respectively, indicating a carbonate equilibrium in aquifer sediments. The groundwater at Lake Willersinnweiher showed anoxic conditions, indicated by redox potentials of −200 mV and O₂ contents below < 0.05 mM (Table 1). Groundwater NO₃⁻ contents were low with a mean value of 8 µM over the year 2018. Dissolved Mn and Fe were observed for all sampling periods with mean concentrations of 14 and 47 µM, respectively. Methane groundwater concentrations upstream of Lake Willersinnweiher varied seasonally, ranging from 0.12 µM in winter to 6.75 µM in summer. Groundwater δ¹³C–CH₄ values varied between −6.3‰ in late summer and −10‰ in winter for the inflow and between −18 and 1.6‰ for the outflow of Lake Willersinnweiher (Table 1; Supporting Information Fig. S2).

**Limnic conditions and aquatic geochemistry**

Lake Willersinnweiher is a monomictic system with circulation following oxic water conditions in winter (Figs. 3 and 4; Supporting Information Figs. S3–S5). In spring, the build-up of the thermal stratification of Lake Willersinnweiher forms the thermocline, separating warm epilimnic from colder hypolimnic water. The redoxcline, where reduced products of

![Fig. 2](image-url)  

**Fig. 2.** STIFF-diagrams of groundwater (a) inflow and (c) outflow as well as surficial Lake water during late-stage thermal stratification in 2018 (b). The groundwater inflow of Lake Willersinnweiher is classified as Ca-HCO₃–SO₄²⁻ type water, the outflow of Lake Willersinnweiher as Ca-HCO₃ type water.
organic turnover processes in the sediments are re-oxidized in the water column, shifts upwards and reaches the thermocline during summer (Fig. 3). This leads to the formation of the well-oxygenated epilimnion separated from the anoxic hypolimnion by a thin transition layer (Fig. 3).

The turnover of organic matter in the sediments of Lake Willersinnweiher is dominated by $\text{SO}_4^{2-}$ reduction and methanogenesis. The reduced products such as Mn or S(-II) showed increasing pore-water concentrations and were released across the sediment–water interface into the bottom water during the year.

Pore-water $\text{SO}_4^{2-}$ decreased within the uppermost 5 cm of the sediments and dissolved S(-II) built up below to maximum concentrations (Fig. 4). Maximum S(-II) concentrations were found around 5–10 cm. Lowest pore-water $\text{SO}_4^{2-}$ concentrations along with highest S(-II) concentrations were found in the sediments in October 2018. This is accompanied by lowest $\text{SO}_4^{2-}$ concentrations and highest S(-II)

Table 1. Mean values of parameters describing redox conditions in the groundwater inflow and outflow of Lake Willersinnweiher in the period of investigation.

| Parameter | EH (mV) | pH | $\text{O}_2$ | Fe (μM) | Mn (μM) | $\text{NO}_3^-$ | CH$_4$ (μM) | $\delta^{13}$C–CH$_4$ (%) |
|-----------|---------|----|-----------|--------|---------|--------------|---------|---------------------|
| GW inflow | $\pm 0^*$ | 8.0 | 0.0 | 46.3 | 12.8 | $<0.01$ | 1.62 | $-4.1$ |
| GW outflow | $+20^*$ | 8.3 | 0.0 | 45.3 | 13.6 | $<0.01$ | 1.92 | $-8.9$ |

*Values were corrected with respect to the electro-chemical reference cell (Ag/AgCl cell/ + 210 mV).

Fig. 3. (a) Water temperature and (b) dissolved oxygen in the water column, as well as (c) DIC and (d) Mn in the sediments and the water column of Lake Willersinnweiher interpolated for the year 2018 based on the sampling in February, May, August, and October 2018 as well as January 2019. Gray dots indicate sampled and analyzed depths and gray hatching indicates no or insufficient data for interpolation. y-axis have different scales for water and sediment depths.
concentrations in the hypolimnion in autumn (Fig. 4). Epilimnic S(-II) concentrations were below the detection limit throughout the year. During winter, $SO_4^{2-}$ concentrations were constant with depth, while S(-II) was not detectable in the entire water column.

Pore-water CH$_4$ generally increased with sediment depth and showed maximum values at the profundal site (Fig. 4). Methane concentrations at the water–sediment interface were up to two orders of magnitude higher than in the hypolimnion (Fig. 4). Maximum CH$_4$ concentrations were found at maximum water depths, whereas minimum CH$_4$ concentrations (up to 60 nM) and highest $\delta^{13}$C–CH$_4$ values of up to $-35\%_o$ were found around the redoxcline. The $\delta^{13}$C–CH$_4$ values in the bottom-near waters decreased from $-65$ to $-79\%_o$ during summer. In general, $\delta^{13}$C–CH$_4$ values showed two distinct zones of the water column: the euxinic hypolimnion with depleted $\delta^{13}$C–CH$_4$ values (up to $-79\%_o$) and the epilimnion with less negative values of $\delta^{13}$C–CH$_4$ (around $-51\%_o$; Fig. 4). Remarkably, CH$_4$ oversaturation (up to 2200 nM) with respect to atmospheric CH$_4$ concentrations (> 3 nM) was found in the entire water column and CH$_4$ accumulated in the surface mixed layer.

Fig. 4. Concentrations of (a) $SO_4^{2-}$, (b) S(-II), (c) CH$_4$, and (d) $\delta^{13}$C–CH$_4$ values in the sediments and the water column of Lake Willersinnweiher interpolated for the year 2018 based on the sampling in February, May, August, and October 2018 as well as January 2019. Gray dots indicate sampled and analyzed depths and gray hatching indicates no or insufficient data for interpolation. CH$_4$ concentration data are shown in log scale and y-axis have different scales for water and sediment depths.
during the summer season (Fig. 4 and Supporting Information Fig. S6).

Pore-water DIC concentrations significantly increased with depth with local maxima at 5–10 cm depth. The DIC concentrations were lower during winter than during late summer, with less pronounced seasonal impact for profundal sediments compared to littoral sediments. Maximum pore-water DIC concentrations of > 10 mM were found in late summer, coinciding with highest DIC concentrations of 3.1 mM in the hypolimnion. In the epilimnion, DIC concentrations decreased during the year and epilimnic DIC concentrations in summer (range from 1.08 to 1.4 mM) were substantially lower than in winter (range from 2.0 to 2.1 mM).

Pore-water Mn concentrations peaked within the uppermost 8 cm of the sediments, with maximum concentrations (60 μM) found in October 2018 (Fig. 3). Dissolved Mn concentrations were highest in the bottom water and below detection limit in the epilimnion. Maximum water column Mn concentrations (up to 40 μM) were found in the redoxcline. Pore-water Fe and NO$_3^-$ concentrations are in the very low μM-range (up to 3 μM; Supporting Information Tables S3–S5).

**Sulfate methane transition zones**

The pore-water profiles of the redox sensitive parameters CH$_4$, SO$_4^{2-}$ and S(-II) in the sediments showed temporal dynamics at the slope site over the year. Maximum pore-water CH$_4$ concentrations increased from < 0.1 to > 1 mM over the summer months and pore water S(-II) concentrations built up with time to up to 6 mM in October 2018. The depth of CH$_4$ maxima and SO$_4^{2-}$ minima, here classified as “sulfate-methane transition zones” in Fig. 5, were determined at all sites in spring 2017. In addition, the slope site showed two distinct transition zones in 5 and 10 cm depth. Over the year 2018, sulfate–methane transition zones were detected at the slope site only. Here, the depth of the zone decreased from May to October 2018 from below 22 to 8 cm (Fig. 5).

**Quantification of CH$_4$ consumption and release**

At Lake Willersinnweiher, sediment fluxes of CH$_4$ and SO$_4^{2-}$ showed maximum values of 0.66 and 4.56 mmol m$^{-2}$ d$^{-1}$, respectively, whereas maximum Mn reduction rates were substantially lower (Supporting Information Table S6). Pore-water fluxes of Mn, DIC, SO$_4^{2-}$ and CH$_4$ were generally increasing during summer and with increasing bathymetric depth of the sampled site. Sulfate reduction is the main pathway of the conversion of organic matter in the lake sediments (Supporting Information Tables S6; Fig. 6). The flux of electrons, calculated for the reduction (CH$_4$ and SO$_4^{2-}$) and the corresponding product (DIC and S(-II)) in the transition zone also follow a seasonal pattern at all sites (Fig. 6). The calculated fluxes for CH$_4$ and SO$_4^{2-}$ in this zone are in the same range, whereas the DIC and S(-II) fluxes are all slightly higher than the corresponding CH$_4$ and SO$_4^{2-}$ consumption rates. The DIC fluxes are higher than the corresponding CH$_4$ fluxes in the littoral zone, but lower in the profundal zone. The slope site follows the littoral pattern in spring and the profundal pattern in summer and autumn. The fluxes of the sulfur compounds show slightly different seasonal patterns which is apparent at all sites.

In addition, the opposing DIC and SO$_4^{2-}$ fluxes (normalized to the electrons transferred) show that slightly more SO$_4^{2-}$ was consumed than DIC was formed in the sulfate–methane transition zone (considering the 1:1 stoichiometry of CH$_4$ oxidation via SO$_4^{2-}$; Fig. 7e). The S(-II) fluxes are significantly lower than the corresponding SO$_4^{2-}$ fluxes in spring, but exceed these fluxes by an order of 2–3 in autumn.
The littoral sediments showed lowest CH$_4$ (< 0.01 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and DIC (~ 0.8 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) fluxes in the transition zone during winter and highest fluxes of 1.4 mmol e$^{-}$ m$^{-2}$ d$^{-1}$ and ~ 3 mmol e$^{-}$ m$^{-2}$ d$^{-1}$, respectively, during spring. Both rates showed an identical seasonal pattern and were descending during the remaining year. Maximum SO$_4^{2-}$ and S(-II) fluxes in the transition zones were timely offset and showed highest rates in August (2.8 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and in October 2018 (4.3 mmol e$^{-}$ m$^{-2}$ d$^{-1}$), respectively. Both sulfur compound fluxes were considerably lower in winter (both ~ 0.5 mmol e$^{-}$ m$^{-2}$ d$^{-1}$; Fig. 6).

At the slope site, all fluxes follow the same seasonal pattern with constantly increasing CH$_4$ (up to 2.0 mmol e$^{-}$ m$^{-2}$ d$^{-1}$), DIC (up to 2.4 mmol e$^{-}$ m$^{-2}$ d$^{-1}$), S(-II) (up to 8.8 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and SO$_4^{2-}$ fluxes (up to 2.2 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) rates during the summer months.

The profundal sediments showed a less pronounced seasonal impact on the sedimentary fluxes within the sulfate-methane transition zone (Fig. 6). The DIC flux remained rather constant through the year with ~ 1.1 mmol e$^{-}$ m$^{-2}$ d$^{-1}$. The CH$_4$ fluxes were highest in August (2.1 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and delayed to the SO$_4^{2-}$ flux maxima in spring 2018 (1.6 mmol e$^{-}$ m$^{-2}$ d$^{-1}$). Minimum CH$_4$ and SO$_4^{2-}$ fluxes were both found in spring (0.8 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and 1.2 mmol e$^{-}$ m$^{-2}$ d$^{-1}$, respectively. The S(-II) fluxes followed the seasonal CH$_4$ flux pattern and were lowest in spring (0.7 mmol e$^{-}$ m$^{-2}$ d$^{-1}$) and highest in summer 2018 (4.2 mmol e$^{-}$ m$^{-2}$ d$^{-1}$; Fig. 6).

Comparing the diffusional fluxes in the sulfate-methane transition zone in relation to their total values in the sediments of Lake Willersinnweiher, shows that sulfate reduction in this zone generally accounts for between 10 and 45% of the total sulfate reduction (Fig. 7). The extent of the sulfate reduction in the transition zone is strongly dependent on the site. Profundal sediments generally show lower proportions of sulfate reduction in this zone (10–15%) than the littoral and slope sediments. The percentage of sulfate reduction in the transition zone of profundal sediments decreases during the summer months to minimum values of 10% and increases to maximum values (15%) in spring. In contrast, littoral sediments show minimum percentages in spring (28%) and constantly increasing ratios throughout the year to up to 45% in winter.
The percentages of DIC turnover rates in the sulfate–methane transition zones are around 65% and are temporally and spatially relatively homogeneous (Fig. 7). In the profundal zone, DICSMTZ/DICtot show minimum values during the winter months, whereas the littoral zone shows minimum percentages in summer and constantly increasing ratios over the remaining year. The largest variations in DICSMTZ/DICtot ratios are found in the slope zone, where the percentage is halved in autumn, compared to the spring and summer levels.

Figure 7 shows that about ~ 20–80% of the upwards diffusing CH4 is oxidized in the sulfate–methane transition zones before reaching the water column. Profundal sediments thereby generally show substantially lower proportions of anerobic oxidation of methane (< 40%) than the littoral and slope sites (Fig. 7). The percentages of diffusive CH4 fluxes into the transition zone show a strong seasonality, notably in the shallower zones of Lake Willersinnweiher. In the littoral zone, around 75% of total diffusive sedimentary CH4 flux occur within the sulfate–methane transition zone in winter, whereas summer fluxes only make up <25% of total CH4 values.

At all sites, CH4 was considerably released across the sediment–water interface into the bottom water, ranging from 0.02 to 0.41 mmol m⁻² d⁻¹ at the littoral sites and up to 0.66 mmol m⁻² d⁻¹ at the profundal sites, respectively (Fig. 7e & Table 2). The maximum CH4 release rates were found in early summer for littoral sediments and in late summer for slope and profundal sediments. The rates for CH4 release from the sediments are thereby substantially dependent on the sulfate reduction within the transition zone: the higher the sulfate reduction rates in this zone, the more CH4 is oxidized and the less CH4 is released via diffusion from the sediments into the water column of Lake Willersinnweiher.
Methane mass balance of Lake Willersinnweiher

The CH₄ budget of Lake Willersinnweiher bases on the diffusive fluxes in the sediments and the water column as well as groundwater contribution and calculated atmospheric emission rates. Mass balance uncertainties were analyzed by considering deviations in diffusion coefficients, pore-water fluxes, average wind speeds as well as in the sediment surface area.

In general, sedimentary and water column conversion and emission rates of CH₄ increased during summer stratification and show maximum rates in summer and minimum rates in winter (Supporting Information Fig. S7). In addition, their relative shares shifted gradually over the course of the year (Supporting Information Table S6). In winter and spring, considerably more CH₄ is consumed in the sediments than released into the water column at all sites. During summer this trend reverses and a larger proportion of CH₄ was released than consumed in the sediments of Lake Willersinnweiher in the late summer. At the redoxcline, CH₄ consumption rates (73 ± 11 mol d⁻¹) are significantly higher than the rates for diffusive sedimentary release below (54 ± 8 mol d⁻¹) in autumn. Thus, considerably larger quantities of CH₄ were consumed in the water column than released from sub-redoxcline sediments, based on the diffuse CH₄ fluxes. Data on CH₄ ebullition from profundal and littoral sediments was not available for the year 2018 and presented data was estimated as the difference between calculated rates for diffusional release and emission to the atmosphere. Methane emissions from the profundal zones were highest in summer (120 ± 9 mol d⁻¹) and more than 20 times higher than in winter (5 ± 1 mol d⁻¹). In the littoral zone, CH₄ oxidation and release rates were highest in summer and the difference between the calculated rates based on sedimentary diffusive fluxes and the surface water CH₄ concentration and emission persisted throughout the year. The main difference was observed in January, when the release rates were at a minimum (0.04 ± 0.01 mol d⁻¹), but the measured littoral surface CH₄ concentrations were up to 1 μM, resulting in an emission of 110 ± 5 mol d⁻¹ CH₄.

The integrated annual CH₄ budget of Lake Willersinnweiher is presented in Fig. 8. Groundwater added 0.5 ± 0.0 kmol and removed 0.5 ± 1.1 kmol CH₄ to both the hypolimnion and the epilimnion of Lake Willersinnweiher in 2018. The estimated rates for CH₄ consumption within the sulfate-methane transition zone in the sediments were lower for the littoral zone (1.9 ± 0.3 kmol) than for the slope (2.0 ± 0.3 kmol) and for the profundal zone (2.5 ± 1.3 kmol).

The diffusive release from the sediments into the bottom water layer was highest in the profoundal zone (8.4 ± 1.3 kmol). The littoral zone has released less than half (2.7 ± 0.4 kmol) and the slope zone less than a quarter (1.8 ± 0.3 kmol) of the profundal zone. The rates for CH₄ released from the sub-redoxcline sediments (profundal and slope zone) as well as CH₄ consumption at the redoxcline are consistent and CH₄ was consumed almost completely at the redoxcline (9.7 ± 1.5 kmol).

Nevertheless, CH₄ oversaturation was present in the surface water layer and the profoundal zone emitted 19 ± 1.4 kmol into the atmosphere over the year 2018. In the littoral zone the calculated diffusive CH₄ release from the sediments (2.7 ± 0.4 kmol) was almost 10 times lower than the surface CH₄ concentrations or calculated CH₄ emissions into the atmosphere (25 ± 1.8 kmol). Thus, more CH₄ was emitted from the surface water in littoral zones than released from the sediments by diffusive CH₄ flux.

Discussion

Groundwater feeding Lake Willersinnweiher is part of the groundwater system within the Upper Rhine Graben and

Table 2. Calculated seasonal fluxes for CH₄ from the sediments into the bottom water layer of Lake Willersinnweiher. Fluxes for CH₄ oxidation at the redoxcline and its fractionation factors (αc) for in the water column of Lake Willersinnweiher were calculated for May, August, and October 2018.

|                        | February 2018 | May 2018 | August 2018 | October 2018 | January 2019 |
|------------------------|--------------|----------|-------------|--------------|--------------|
| **Sedimentary CH₄ release (F(CH₄, released)** |               |          |             |              |              |
| Profundal sediments    | 0.52         | 0.36     | 0.46        | 0.66         | 0.32         |
| Slope sediments        | –            | 0.00     | 0.03        | 0.39         | –            |
| Littoral sediments     | 0.00?        | 0.10     | 0.41        | 0.01         | 0.00         |
| **Redoxcline (aerobic CH₄ oxidation)** |               |          |             |              |              |
| F(CH₄) (mmol m⁻² d⁻¹) | –            | 0.16     | 0.51        | 1.04         | –            |
| αc                     | –            | 1.014    | 1.025       | 1.041        | –            |
significantly enriched in SO$_4^{2-}$ (> 2 mM) due to the oxidation of pyrite in the quaternary river Rhine sediments (Isenbeck-Schröter et al. 2016). As a consequence, biogeochemical reactions and carbon mineralization in the sediments of Lake Willersinnweiher are most likely controlled by sulfate reduction and sulfur cycling, which is uncommon for lake environments (e.g., Holmer and Storkholm 2001). High contents of Ca and HCO$_3^-$ result from buffer reactions indicated by a Ca: HCO$_3^-$ relation near 1, and, hence, groundwater Fe$^{2+}$ and Mn$^{2+}$ contents are limited by carbonate equilibria (Isenbeck-Schröter et al. 2016).

In Lake Willersinnweiher, the cycling of both redox sensitive elements is more complex. In general, Fe is limited since Fe is bound as Fe(OH)$_3$ and FeS$_x$ in the oxic and euxinic water layer, respectively. The latter is partly oxidized during the winter circulation, when O$_2$ reaches the sediment surface. Under these conditions, sedimentary Mn(IV) minerals are also formed (Schröder 2004).

With the build-up of the lakes’ stratification, the sediment–water interface becomes anoxic in early spring. By this, e.g., sedimentary Mn-oxides are reduced, and Mn(II) is released from the sediments into the water column. Manganese is thus significantly enriched below the redoxcline and contents are controlled by the sedimentary Mn(II) release and carbonate equilibria via the formation of, e.g., rhodochrosite (Schröder 2004). In addition, Mn diffuses towards the redoxcline, where it is re-oxidized and Mn(IV)-minerals are formed (Schröder 2004).

In the following months, full stratification of Lake Willersinnweiher is reached from July/August to November/December and the redoxcline decouples the oxic surface water from the euxinic hypolimnion. The rising sedimentary conversion rates of organic substances during summer, indicated by increasing DIC rates, result in an enhanced release and diffusion of further reduced species, such as Mn(II) and S(-II), into the water column and towards the redoxcline. Thus S(-II) might fuel a local Mn-shuttle by the reduction of Mn(IV)-minerals via sulfide below the redoxcline (e.g., Havig et al. 2015).

Due to the intense reduction of SO$_4^{2-}$ and sulfur cycling, the lake sediments act as a sink for sulfur, whereas the sediments are the major source of DIC and CH$_4$ found in the euxinic hypolimnion of Lake Willersinnweiher. Calculated sedimentary SO$_4^{2-}$ reduction rates of Lake Willersinnweiher (0.2–4.6 mmol m$^{-2}$ d$^{-1}$) are in a similar range as those values reported for the few SO$_4^{2-}$-enriched lakes (0.4–50 mmol m$^{-2}$ d$^{-1}$, Holmer and Storkholm 2001; Schubert et al. 2011; Norði et al. 2013) and marine sediments (0.5–27 mmol m$^{-2}$ d$^{-1}$, Jorgensen and Kasten 2006). By this, redox processes in the sedimentary layer are intensively influenced by the interaction of both, the sulfur and the carbon cycling processes.

The presence of SO$_4^{2-}$ generally limits sedimentary CH$_4$ formation as carbon compounds are favorably metabolized by non-methanogens. In SO$_4^{2-}$-rich zones, sulfate reducing bacteria and methanogens compete for available H$_2$ and acetate.
(Cappenberg 1975). As considerable pore-water concentrations of free sulfide were found, sulfate reducing bacteria might out-compete the methanogens for available H₂ in the sediments of Lake Willersinnweiher. Methane is consequently produced via the hydrogenotrophic pathway (carbonate reduction) at greater sediment depths, where SO₄²⁻ is depleted and sulfate reducing bacteria are inactive.

However, sulfate–methane-interaction is not only affecting CH₄ formation, but also CH₄ consumption in the sediments. At Lake Willersinnweiher, sedimentary SO₄²⁻ minima coincide with the depth of CH₄ minima, along with local maxima of pore-water S(−II) and DIC concentration, indicating the presence of sulfate–methane transition zones (SMTZ) at all sites. Sulfate–methane transition zones are a common feature first described for marine sediments due to high pore-water SO₄²⁻ concentrations (Martens and Berner 1974), whereas pronounced SMTZ have so far only been observed for a few specific freshwater environments (Borrel et al. 2011; Schubert et al. 2011; Timmers et al. 2016). In the sediments, CH₄ is most likely oxidized with SO₄²⁻ as electron acceptor by methane-oxidizing archaea (anaerobic methanotrophs) and sulfate reducing bacteria consuming upward migrating CH₄ (Hinrichs et al. 1999; Boetius et al. 2000).

The anaerobic CH₄ oxidation in freshwater environments is usually coupled to the reduction of the energetically more favorable electron acceptors, such as nitrate and nitrite (e.g., Raghoebarsing et al. 2006) or Fe(III) and/or Mn(IV) (e.g., Beal et al. 2009). In Lake Willersinnweiher, NO₃⁻ and Fe concentrations are negligible in the pore-water and dissolved Fe is trapped as sulfides in the sulfur-dominated sediments (Schröder 2004). Therefore, we assume that NO₃⁻ and Fe do not play any role in sedimentary anaerobic CH₄ oxidation in Lake Willersinnweiher.

The anaerobic CH₄ oxidation by the oxidation of Mn-oxides might be an additional pathway in the sediments of Lake Willersinnweiher since Mn-oxides as electron acceptors are energetically more favorable than SO₄²⁻. Intense Mn-cycling and the formation of Mn(IV) minerals in the sediments was reported for Lake Willersinnweiher during winter, when the uppermost 2–3 mm of the sediment become oxic (Schröder 2004). The formation of the anoxic hypolimnion in spring then leads to the lake internal Mn cycling and a re-reduction of the Mn-oxides via sulfide in the sediments. This phenomenon has been described in the past for various eutrophic lakes (e.g., Davison and Tipping 1984). At Lake Willersinnweiher, dissolved Mn was found in the pore-water (up to 60 μM), potentially indicating anaerobic CH₄ oxidation via Mn-oxides. Direct anaerobic CH₄ oxidation via Mn-oxides as electron acceptors might be quantitatively significant but subordinated to the anaerobic CH₄ oxidation via SO₄²⁻ due to the dominance of SO₄²⁻ reduction in the sediments of Lake Willersinnweiher. However, it is more likely that Mn supports sedimentary anaerobic CH₄ oxidation via SO₄²⁻ through re-oxidation of reduced S species, as it was also recently described for Lake Cadagno (Su et al. 2019).

The interaction of methanogenesis, sulfur cycling and the recycling of DIC produced by anaerobic CH₄ oxidation leads to a sedimentary carbon (re-)cycling at the top of the methanogenic zone. Our results indicate, that 50%–70% of sedimentary DIC produced results from anaerobic CH₄ oxidation within the transition zone, which in turn might fuel a secondary methanogenesis via CO₂ reduction (see Fig. 9). This phenomenon might explain the imbalance between the normalized CH₄, DIC, S(−II) and SO₄²⁻ fluxes in the sulfate-methane transition zone of Lake Willersinnweiher. The SMTZ-associated secondary methanogenesis or “cryptic methane cycling” was previously reported to account for up to 60% of the organoclastic sulfate reduction within marine SMTZ (Beulig et al. 2019).

These processes and interactions between carbon and sulfur as well as Mn cycling might be recorded in the sulfur (δ⁳⁴S values of SO₄²⁻ and S(−II)) and carbon (δ¹³C values of DIC and CH₄) isotope composition of the specific compounds involved in the sediments of Lake Willersinnweiher. Future investigations of sedimentary δ¹³C and δ³⁴S values are therefore desirable and would provide further evidence of anaerobic CH₄ oxidation in the sediments of Lake Willersinnweiher by covering all major constituents in carbon cycling.

At Lake Willersinnweiher, the extent of the interaction of carbon and sulfur cycling in the sediments is mainly driven by seasonal variations of the thermocline and redoxcline depth. The seasonal changes in temperature thereby lead to considerable changes in pore-water chemistry and turnover rates, controlling the biogeochemical processes in sediments (e.g., Crill and Martens 1987).

In a short period, apparently occurring in spring, sulfate-methane transition zones are clearly identifiable at all
sampling sites. This period is characterized by rather moderate sedimentary organic matter degradation and mineralization—indicated by moderate DIC fluxes—and moderate sulfate reduction rates (below 1.5 mmol m$^{-2}$ d$^{-1}$), as well as a deeper transition zone depth within the sediments. When the thermal stratification of the lake builds up, the redoxcline shifts upwards in the water column resulting in a vertically shifting of the sedimentary transition zones during the summer months.

Secondary methanogenesis and intense sulfur cycling within the sediments also trigger non-steady state conditions in the settings of anaerobic CH$_4$ oxidation (e.g., Dale et al. 2008). These variable conditions also result in occasionally more than one transition zone within the slope sediments, where maximum temperature gradients are present. Seasonal displacement of the transition zone depths have also been previously reported for costal marine environments (e.g., Dale et al. 2008).

As the redoxcline zone shifts further up from the sediments into the water column, due to the seasonal evolution of the sedimentary organic matter degradation and mineralization, pore-water fluxes increase considerably at all sites. Total DIC concentrations and fluxes increase during the summer months, while the SMTZ-associated DIC formation in littoral and profundal sediments were highest in spring and decreased during the remaining year.

The extent of sulfate reduction within the sulfate–methane transition zones also substantially controls the extent of anaerobic CH$_4$ oxidation in the sediments of Lake Willersinnweiher: higher sulfate reduction rates in this zone results in higher rates of anaerobic CH$_4$ oxidation. In the profundal zone, sulfate reduction rates in the transition zone is generally less significant for total sulfate reduction rates than in the shallower areas, resulting in substantially higher diffusional CH$_4$ release from the sediments than in the littoral and slope zones. Hence, diffusional CH$_4$ release into the water column is linked to the anaerobic CH$_4$ oxidation efficiency in the transition zone, which might be controlled by the different nature of OM degradation and the substrate availability for sulfate reducing bacteria changing with lake depth (Boetius et al. 2000). Substantially increasing anaerobic CH$_4$ oxidation rates were observed for the slope and littoral site, most likely due to the fast seasonal temperature changes (Figs. 6, 7), which was reported to be the main factor behind the seasonality in rates in the sulfate–methane transition zones (Dale et al. 2008). At Lake Willersinnweiher, the availability of substrates is also an important factor controlling sedimentary processes and overall benthic metabolism (e.g., Westrich and Berner 1984).

The proportion of transition zone-related sulfate reduction rates in the total sulfate reduction rates has considerably changed and halved at all sites in autumn 2018, indicating seasonal variations in dissolved substrates for sulfate reducing bacteria. Potentially, the Mn(IV) minerals formed in winter are used up and are therefore unavailable as oxidants for the sedimentary sulfur (re-)cycling in late summer. In parallel, CH$_4$ release into the water column increased fivefold and thereby considerably more compared to the anaerobic CH$_4$ oxidation rates within the sediment. This might indicate that oxidation activity in the sedimentary transition zone could not keep up with increasing methanogenesis during summer and, hence, the relative impact of transition zone-related anaerobic CH$_4$ oxidation on CH$_4$ release into the water column decreased considerably.

However, it must be noted that this work does not include studies on sedimentary microbial activities and it remains speculative whether the activity of anaerobic CH$_4$ oxidation (I) has decreased, (II) has partially shifted into the euxinic bottom water layer, as it was suggested for other freshwater or marine environments (e.g., Zigah et al. 2015), or whether (III) these dynamics are also overprinted by other processes, such as a more dynamic methanogenesis or CH$_4$ diffusion within the sediments. These questions cannot be answered within the frame of this study, as they would require further detailed geochemical and microbial investigations of the sediment and the water column.

The extent of anaerobic CH$_4$ oxidation in the water column would only be subordinate to sedimentary anaerobic and aerobic CH$_4$ oxidation. The dominant CH$_4$ oxidation pathway in the water column of Lake Willersinnweiher is the oxidation via O$_2$ at the redoxcline (see Fig. 8), where more than 98% of the aquatic CH$_4$ is consumed through aerobic methanotrophs. The $\delta^{13}$C–CH$_4$ values increase towards the redoxcline, indicating aerobic microbial oxidation within the water column of Lake Willersinnweiher (e.g., Barker and Fritz 1981). The calculated $\alpha_r$ for redoxcline CH$_4$ oxidation at Lake Willersinnweiher ranges from 1.014 to 1.041 for spring and late summer, respectively, and is consistent with reported fractionation factors of aerobic oxidation ranging from 1.003 to 1.039 (e.g., Zigah et al. 2015). As a consequence, the interaction of anaerobic (sediment) and aerobic (water column) oxidation of CH$_4$ acts as an effective barrier to minimize CH$_4$ release into the surface water and the atmosphere during summer stratification.

Emissions during the summer stratification mainly result from CH$_4$ oversaturation (up to 2200 nM) compared to the atmospheric CH$_4$ equilibrium concentration of ~3 nM, which was present in the surface water of Lake Willersinnweiher all year. In 2018, littoral surface water CH$_4$ concentrations were considerably higher than profundal, which is in good agreement with previous studies on the spatiotemporal distribution pattern of CH$_4$ at other lakes (e.g., Hofmann 2013). The high surface water CH$_4$ concentrations of the littoral zones and, hence, CH$_4$ emissions into the atmosphere, cannot be solely explained by the diffusional fluxes from the sediment (Fig. 8; Supporting Information Fig. S7). Our CH$_4$ mass balance indicates that only about 10–30% of the littoral CH$_4$ emissions result from diffusive release from the littoral sediments, which is in very good agreement with previous studies.
(e.g., Bastviken et al. 2004). Since anaerobic CH₄ oxidation in the sulfate–methane transition zone effectively limits the diffusive CH₄ release from the sediments, littoral CH₄ release at Lake Willersinnweiher most likely originates from “direct” release pathways such as plant-mediated and ebullitive transfer. Plant ventilation might occur as the littoral areas at Lake Willersinnweiher are mostly overgrown by submerged plants that might lead to intense exchange between sediment, water, and atmosphere. These root-associated methane emissions have been reported as the most important pathway of CH₄ emissions in wetlands (e.g., Schütz et al. 1989). Ebullition is the transport of gas bubbles super-saturated with CH₄ from sediments into the water column and the atmosphere and occurs in marine and lacustrine environments (e.g., Bastviken et al. 2004). Methane bubbles released from the sediment were identified as a main pathway for CH₄ emissions from lakes and are known to show significant spatial and temporal heterogeneity.

At Lake Willersinnweiher, ebullition might explain the discrepancy in the CH₄-budget for the littoral zones especially during winter, when surface water CH₄ concentrations are high, but both sedimentary aerobic and anaerobic CH₄ oxidation reduces diffusional CH₄ release to a minimum. Although ebullition might be generally less intense during winter and spring due to low (pore-)water temperatures and therefore slower organic matter decomposition, but was shown to be the main pathway for CH₄ emission from Lake Kinneret during winter (Schmid et al. 2017).

Due to the dominance of “direct” CH₄ release pathways in the littoral zone, our mass balance might underestimate the total CH₄ emission rates for Lake Willersinnweiher as CH₄ gas bubbles are known to be directly emitted to the atmosphere (McGinnis et al. 2006). Thus, future research should focus on the quantification of ebullition volume and fluxes derived from sediments and its spatial variability, as this seems to be the important CH₄ transport pathway at least for littoral sediments at Lake Willersinnweiher.

The rates for diffusive CH₄ release from the sub-redoxcline sediments (profundal and slope zone) and CH₄ consumption at the redoxcline are consistent, indicating that rather diffusional than ebullitive or plant-mediated release is driving the profound CH₄ release into the deeper water layer of Lake Willersinnweiher. By this, the probability that CH₄ reaches the surface water via bubbles transport from profundal sediments seems to be rather negligible and sedimentary released CH₄ is mediated by the extent of anaerobic CH₄ oxidation within the sulfate–methane transition zones.

In the profundal zone, surface water CH₄ concentrations are higher and δ¹³C–CH₄ values shifted towards lighter values in the epilimnion compared to the metalimnion, during the stratification period. This indicates that the redoxcline at Lake Willersinnweiher decouples the surface water from the deep water reservoirs (Fig. 4; Supporting Information Figs. S6 and S8). Although the sulfate–methane transition zone acts as an effective barrier by minimizing CH₄ release into the water column, CH₄ accumulates in the anoxic, deep water layer during summer. Stored CH₄ is then emitted during the autumn overturn period by vertical water mass mixing (“storage flux”). The extent of the sudden release of CH₄ previously sealed off from the atmosphere is unknown to date, but will result in temporarily high emission rates of CH₄ into the atmosphere (e.g., Bastviken et al. 2004).

This contrasts with the permanent CH₄ emission due to the CH₄ oversaturation of the profundal surface water layer, which occurs throughout the year and therefore requires a source located in the upper oxic water layer. Methane oversaturation in the oxic water column has been consistently reported for various stratified lakes and might result from transport of CH₄ from the littoral zones (Murase et al. 2005; Peeters and Hofmann 2015) as profound CH₄ concentrations in Lake Willersinnweiher were higher in the littoral than profundal. Based on the higher CH₄ concentrations in the littoral areas, a littoral origin of the CH₄ oversaturation in Lake Willersinnweiher cannot be excluded. However, the data of this study cannot provide any evidence for this assumption, as no systematic transect of water column profiles over lake width was conducted. Epilimnic CH₄ oversaturation was also attributed to internal CH₄ production (e.g., Grossart et al. 2011). In this context some recent studies have unambiguously shown, applying stable isotope techniques, that CH₄ is produced by organisms such as freshwater and marine algae (e.g., Klintsch et al. 2019; Hartmann et al. 2020) and cyanobacteria (Bižić et al. 2020) and even in the presence of oxygen (Keppler et al. 2009). These alternative processes might also account for the observed elevated CH₄ concentrations at the profundal surface water of Lake Willersinnweiher, since at least its surrounding lakes are facing growing eutrophication and intense cyanobacterial growth in the last years (https://badeseen.rlp-umwelt.de/servlet/is/1137/, Accessed 11 November 2020). Detailed investigations of this newly discovered oxic CH₄ production are currently ongoing with the aim to decipher CH₄ formation in the epilimnion of the lake Willersinnweiher.

At Lake Willersinnweiher, groundwater inflow might represent an additional, and so far, overlooked source of surface water CH₄ in lakes, as the feeding groundwater is significantly enriched in CH₄ (up to 2.4 μM) compared to the lake’s surface water. By this, groundwater would not only drive the extensive anaerobic CH₄ oxidation by providing SO₄²⁻ to the sediments of Lake Willersinnweiher, it also affects the total CH₄ budget and thus also the emission of Lake Willersinnweiher, as the surface water CH₄ oversaturation occurred all year (Fig. 8). Groundwater δ¹³C–CH₄ values ranged between –18 and 1.6‰ and are rather unusual for natural environments (e.g., Scholem et al. 2016). Background data of CH₄ concentrations and δ¹³C–CH₄ values in shallow aquifers are barely reported for (southern) Germany thus far.
The origin of groundwater CH4 remains still unanswered to date. Groundwater CH4 could originate from various sources ranging from CH4 as a product of (I) oxidative biodegradation of (highly volatile chlorinated) hydrocarbons in a contamination site to (II) organic-rich sediments from old branches of the river Rhine or (III) from lakes upstream (Wollschläger et al. 2007; Supporting Information Table S3). In general, CH4 formation via oxidative biodegradation of (highly volatile chlorinated) hydrocarbons in contamination sites might result in naturally uncommon δ13C–CH4 values (e.g., Hunkeler et al. 2005). The isotopic composition of CH4 is thereby controlled by the δ13C-ratio of the original substrate. Contamination-driven biodegradation and methanogenesis is likely but, however, contamination sites are not documented for the nearby environment. Potentially, these substrates might derive from the organic-rich sediments from old branches of the river Rhine which span over a large area upstream of Lake Willersinnweiher. On the other hand, aquifer-related methanogenesis is substantially limited in the presence of SO4\(^{2-}\) and CH4 originating from these sediments might also have been fully oxidized within the lakes upstream before reaching Lake Willersinnweiher as Wollschläger et al. (2007) have shown that groundwater infiltrating the lake has passed at least one of the lakes located in the catchment of Lake Willersinnweiher. Here, lake water infiltrates into the aquifer through the porous littoral sediments that are significantly enriched in CH4 before entering the aquifer and Lake Willersinnweiher. The resulting groundwater CH4 with δ13C–CH4 values between 1.6 and –18‰ would then imply extreme fractionation factors for CH4 oxidation processes in the groundwater, which might be coupled to the reduction of NO3\(^{-}\), NO2\(^{-}\), Fe, and Mn as O2 is absent or limited within the groundwater.

Further investigations of the lake waters and sediments upstream as well as along the groundwater flow path are therefore crucial for the understanding of the complex interactions of groundwater, surface water and sediments within Lake Willersinnweiher and comparable lakes in the Upper Rhine Graben.

**Conclusion**

The calculated total CH4 mass balance of Lake Willersinnweiher indicates that several important processes including formation, consumption and transport/diffusion are controlling the fluxes to the atmosphere. In this study, we focused on the total methane mass balance of Lake Willersinnweiher and the interaction of carbon, sulfur and manganese cycling within the sulfate–methane transition zones in the sediments and at the redoxcline in the water column of Lake Willersinnweiher (SW Germany). Groundwater feeding the lake with SO4\(^{2-}\) leads to anaerobic CH4 oxidation in the upper sediment layers. Upward migrating CH4 is thereby most likely consumed via SO4\(^{2-}\) in the transition zone, which are uncommon in freshwater environments, to date. In contrast to its marine analogues, the transition zones in freshwater lakes are subject to significant seasonal dynamics in lake conditions and biogeochemical processes. These non-steady state conditions within the sediments and the water column result in a seasonally dependent impact of sulfate–methane transition zones on methane cycling in freshwater environments.

The interaction of anaerobic CH4 oxidation in the sulfate–methane transition zones and aerobic CH4 oxidation in the water column thereby acts as an effective barrier, minimizing CH4 release into the surface water and the atmosphere. Therefore, we postulate that most CH4 released from the profundal water surface to the atmosphere might stem from transport of CH4 produced in the littoral sediments or originate from in situ production of aerobic organisms in the epilimnion. Significant CH4 concentrations in the groundwater inflow might represent an additional source for year-round CH4 oversaturation in the surface water of Lake Willersinnweiher. By this, groundwater is not only providing SO4\(^{2-}\) for anaerobic CH4 oxidation in the sediments, but also affecting CH4 emissions of Lake Willersinnweiher.

Groundwater-lake interactions are generally known to have severe implications on biogeochemical processes in the lakes. Often, groundwater-fed lakes tend to be enriched by SO4\(^{2-}\) originating from the weathering of sulfur-containing rocks in the catchment, leading to significant SO4\(^{2-}\) reduction, especially in eutrophic lakes, where both the availability of organic matter and SO4\(^{2-}\) concentrations are high. Lake Willersinnweiher is therefore exemplary for the many monomictic or dimictic lakes in the Upper Rhine Graben in Germany and probably also in other sedimentary basins that at least temporarily develop an anoxic hypolimnion. Hence, anaerobic CH4 oxidation via sulphate as an electron acceptor might play a more significant role in controlling methane fluxes from limnic systems than previously estimated.

**Author Contributions**

Jan F. Kleint and Yannic Wellach developed the hypotheses supported by Margot Isenbeck-Schröter. Jan F. Kleint visualized the data and prepared the original draft, Margot Isenbeck-Schröter supervised. Sampling was planned and conducted by Jan F. Kleint and Yannic Wellach. Instrumentation and methodology were provided by Margot Isenbeck-Schröter for hydrogeochemical and in-field methane analyses and Frank Keppler for gas and stable carbon isotope analysis in the laboratory. Jan F. Kleint, Yannic Wellach, and Moritz Schroll collected the data and Margot Isenbeck-Schröter, Frank Keppler validated it. Jan F. Kleint, Yannic Wellach, Moritz Schroll, Frank Keppler, and Margot Isenbeck-Schröter interpreted the results. The manuscript was written under the lead of Jan F. Kleint, with contributions of all authors.
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Conflict of Interest

None declared.