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Application of concentration and 2-dimensional stable isotope measurements of methane to constrain sources and sinks in a seasonally stratified freshwater lake

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Methane (CH₄) emissions from aquatic systems have recently been comprised to account for up to 50% of global CH₄ emissions, with lakes representing one of the largest CH₄ sources within this pool. However, there is large uncertainty associated with CH₄ emissions from freshwater environments to the atmosphere, because of a lack of understanding in the spatial and temporal dynamics of CH₄ sources and sinks, as well as underlying mechanisms and processes. In this study, we investigated the concentrations and stable carbon ($\delta^{13}\text{C}-\text{CH}_4$) and hydrogen ($\delta^2\text{H}-\text{CH}_4$) isotope composition of CH₄ in a small eutrophic lake (Lake Willersinnweiher) with seasonal stratification and its spatial and temporal variation. We found that while supersaturation of CH₄ in the entire water column was present throughout the whole year, the isotopic composition of CH₄ in sediment and water column varied depending on lake stratification, physiochemical conditions, and lake depth. During the stratification period, isotopic characteristics of pelagic surface water CH₄ differed from littoral and sedimentary CH₄, suggesting likely mixing of CH₄ from different sources including vertical and lateral input as well as groundwater input and potentially oxic methane production in the mixed surface water layer. Aerobic CH₄ oxidation indicated by a strong increase in both $\delta^{13}\text{C}-\text{CH}_4$ and $\delta^2\text{H}-\text{CH}_4$ values at the bottom of the oxycline was found to significantly reduce upward migrating CH₄ released at the sediment-water interface. In the sediment, stable isotope characteristics of CH₄ showed an increasing dominance of the acetoclastic CH₄ formation pathway from the pelagic towards the littoral area. Furthermore, the occurrence of sulfate-dependent anaerobic methane oxidation in the sediment was suggested by an increase in $\delta^{13}\text{C}-\text{CH}_4$ and $\delta^2\text{H}-\text{CH}_4$ values. During the mixing period, the isotopic CH₄ composition of the water column was distinctively less negative than during the stratification period potentially resulting from a greater impact of groundwater CH₄ input compared to the stratification period. Our findings implicate that the application of concentrations and dual isotope measurements of CH₄ is a

promising approach for constraining CH₄ sinks and sources in Lake Willersinnweiher and potentially other small lakes to clearly disentangle the complex CH₄ dynamics in lakes both spatially and seasonally.

KEYWORDS

$\delta^{13}\text{C}\text{-CH}_4$ values, $\delta^2\text{H}\text{-CH}_4$ values, methane sources, methane sinks, lake, stable isotopes, stratification

Introduction

Lakes and other freshwater systems cover only a small area of the earth's continental land surface (>3%; Downing et al., 2006), but play an important role in the global carbon cycle and greenhouse gas emissions to the atmosphere (Oswald et al., 2015). Amongst aquatic systems, lakes constitute one of the largest sources of CH₄ emission, releasing 23–142 Tg CH₄ yr⁻¹ to the atmosphere (Rosentreter et al., 2021). Thus, CH₄ cycles in lakes and their fluxes to the atmosphere have been investigated extensively in recent years (e.g. Casper et al., 2000; Bastviken et al., 2004; Natchimuthu et al., 2016; Donis et al., 2017; Peeters et al., 2019) and global lake CH₄ emissions were found to be dominated by small lakes (surface area <1 km²) (Thottathil et al., 2022). Nevertheless, many key factors regarding CH₄ sources and sinks in lakes and their spatial and temporal variability remain unknown (Duc et al., 2010; Loken et al., 2019). Methane dynamics in lacustrine environments are complex and dependent on various biological production and consumption mechanisms as well as different transport pathways, impacting the distribution and accumulation of CH₄ in the water column (Günthel et al., 2019) (Figure 1). In particular, the source of CH₄ supersaturation in the surface water layer has been intensely debated in recent years. Whilst some research groups support

lateral (riverine, littoral) and vertical CH₄ input from anoxic sources as the main mechanisms behind this observation (Fernández et al., 2016; Peeters et al., 2019; Peeters and Hofmann, 2021), others provide evidence for CH₄ production under oxic conditions as a potentially important process in the surface water layer of lakes (Grossart et al., 2011; Tang et al., 2016; Donis et al., 2017; Günthel et al., 2019; Hartmann et al., 2020; Thottathil et al., 2022). However, the large discrepancies in estimations of the contribution of oxic CH₄ production to surface water CH₄ emissions, varying from negligible contributions (Peeters and Hofmann, 2021) to a contribution of oxic methanogenesis to surface water emissions of up to 90% (Donis et al., 2017), reveal a lack of understanding of the processes contributing to surface supersaturation and their spatial and temporal variability. Although the determination of dissolved CH₄ concentrations and CH₄ fluxes is helpful to establish the magnitude of emissions, it cannot resolve the responsible pathways and production mechanisms (Cadieux et al., 2016). Measurement of stable carbon and hydrogen isotopes of CH₄ (expressed as $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values) are known to assist with identifying sources and sinks of CH₄ (e.g., Waldron et al., 1999; Whiticar, 2020; Douglas et al., 2021).

In aquatic systems, biogenic CH₄ production has until recently been assumed to be solely performed by anaerobic methanogenic archaea occurring in the anoxic sediment and water column (e.g. Lessner, 2009). Microbial methanogenesis leads to the formation of ¹³C and ²H depleted CH₄. In anoxic sediment, methanogenesis typically involves two metabolic pathways using competitive substrates: Carbon dioxide (CO₂) reduction *via* H₂ (hydrogenotrophic) and acetate fermentation (acetoclastic). Methane production through CO₂ reduction generates $\delta^{13}\text{C}\text{-CH}_4$ values ranging from -110 to -60‰ and $\delta^2\text{H}\text{-CH}_4$ values from -250 to -150‰, whereas acetoclastic methanogenesis generates $\delta^{13}\text{C}\text{-CH}_4$ values varying from -60 to -40‰ and $\delta^2\text{H}\text{-CH}_4$ values from -400 to -250‰ (Whiticar, 1999; Belle et al., 2015). However, the $\delta^2\text{H}\text{-CH}_4$ values were found not to be exclusively controlled by the methanogenic formation pathway, but other variables such as CH₄ oxidation and $\delta^2\text{H}\text{-H}_2\text{O}$ values also have significant impact on the stable hydrogen isotope composition of CH₄ (e.g., Waldron et al., 1999; Douglas et al., 2021).

The observation of CH₄ oversaturation in the oxic surface mixed water layer of aquatic systems challenges the view of methanogenesis exclusively occurring under anoxic conditions (Donis et al., 2017; Bižić et al., 2020a; Hartmann et al., 2020).

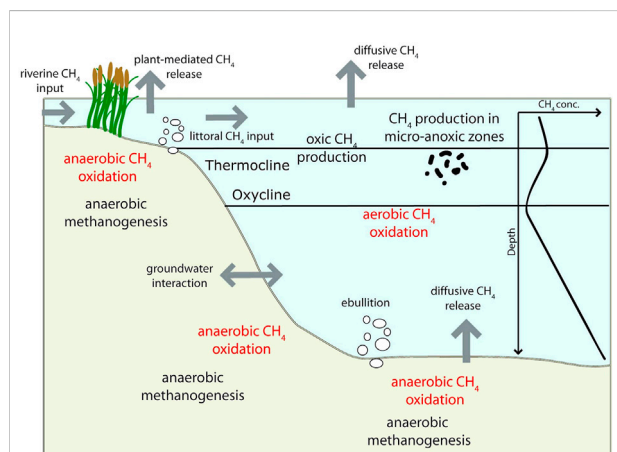


FIGURE 1

Overview of sources and sinks of CH₄ as well as transport processes (transport direction indicated by grey arrows) and a typical concentration profile of CH₄ in the lacustrine environment during thermal stratification. Modified after Tang et al. (2016).

Recent research has shown that CH₄ production is possible under oxic conditions, both in terrestrial environments, e.g. plants and fungi (Kepler et al., 2006; Lenhart et al., 2012), as well as in aquatic environments, e.g. phytoplankton and cyanobacteria (Lenhart et al., 2015; Bižić et al., 2020b). Recently, Ernst et al. (2022) proposed a reaction mechanism for CH₄ formation potentially occurring on a cellular level across all living organisms through the interaction of reactive oxygen species with free iron and methylated sulfur and nitrogen compounds in living cells. Furthermore, the authors found that increased levels of oxidative stress enhanced CH₄ production in all of the investigated organisms, providing a possible explanation not only for CH₄ emissions under oxic conditions but also for the large variability of emission rates observed for many organisms in aquatic and terrestrial environments. However, the related δ¹³C and δ²H isotopic patterns of CH₄ produced in the oxic environment remain yet to be clarified.

Aerobic CH₄ oxidation (MOx) as a counteracting mechanism to CH₄ production is usually observed mainly at the oxic-anoxic interface in aquatic systems where high CH₄ concentrations and dissolved oxygen are present. MOx is generally characterized by an increase in δ¹³C-CH₄ and δ²H-CH₄ values, since methanotrophic bacteria prefer to oxidize the light carbon and hydrogen stable isotopes, leading to a relative enrichment of ¹³C and ²H in the CH₄ pool (Barker and Fritz, 1981; Whiticar, 1999). In the anoxic sediment, anaerobic oxidation of CH₄ (AOM) through electron acceptors other than oxygen can be observed leading to an enrichment in ¹³C and ²H of CH₄, however other isotopic effects discussed later might obscure this typical isotope enrichment. AOM coupled to sulfate reduction is a common and widely described process in the ocean, oxidizing >90% of CH₄ produced in oceanic sediment (Knittel and Boetius, 2009). Due to usually low sulfate concentrations in freshwater systems, sulfate-dependent AOM only occurs in some specific lake environments.

Tsunogai et al. (2020) suggested that the relation between the original carbon and hydrogen isotopic composition of CH₄ can be inferred from the relation between δ¹³C-CH₄ and δ²H-CH₄ values of residual CH₄, irrespective of the isotopic fractionation caused by CH₄ oxidation. Therefore, they introduced a novel stable isotope indicator Δ(2,13), which corrects for the kinetic isotope fractionation effect associated to oxidation by using the ratio of hydrogen to carbon stable isotopes during microbial oxidation and thus can help in characterizing sources of CH₄ in a system. Through the application of dual stable isotopes and flux measurements, model predictions can be complemented and provide a better comprehension of processes occurring in the system and disentangling production and consumption mechanisms.

So far, application of dual isotope and concentration measurements has only found limited use in aquatic environments and an overview of dual stable isotope characterization of limnic CH₄ processes is missing.

Therefore, we analyzed the δ¹³C-CH₄ and δ²H-CH₄ values as well as CH₄ concentrations in Lake Willersinnweiher - a seasonally stratified, eutrophic lake in southwestern Germany - and furthermore applied the novel Δ(2,13) indicator. Detailed profiles of the water column and pore water of the sediments were collected during the stratified and non-stratified lake periods in order to gain a better understanding of the CH₄ sources and sinks and the diffusive and ebullitive processes involved in CH₄ cycling of the lake. We aim to isotopically characterize different sources of CH₄ to the lake and thus to disentangle the contribution of these sources to the complex CH₄ cycling at Lake Willersinnweiher both spatially and temporally.

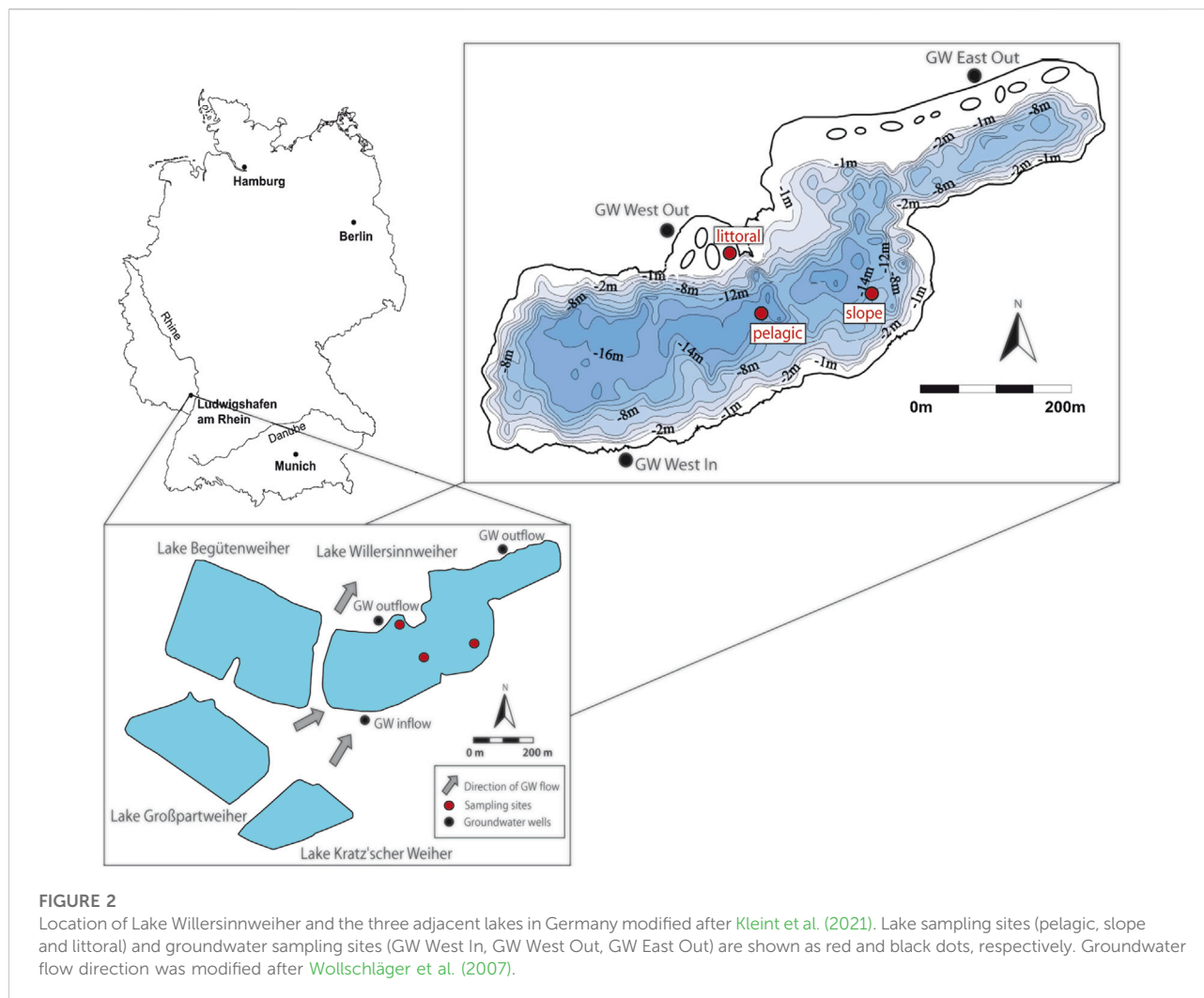
Material and methods

Site description and geochemical characterization

Lake Willersinnweiher is situated in the plain of the Upper Rhine Graben (Germany), northwest of Ludwigshafen (49.499950 °N; 8.397138 °E) and covers an area of 17 ha (Figure 2). It is one of four gravel pit lakes and has neither a surface inflow nor outflow, thereby making inflowing groundwater its main source of water and solutes (Wollschläger et al., 2007). Lake Willersinnweiher is composed of a shallower northeastern basin with a maximum water depth of 14 m and a deeper southwestern basin with a maximum water depth of 20 m. The average water depth in the lake is 8 m (Sandler, 2000).

Geochemically, Lake Willersinnweiher can be characterized as an eutrophic hardwater lake with an average water residence time of 3.7 years and experiences seasonal water stratification during summer (stratification period) (Wollschläger et al., 2007). From November/December to March/April, the water column of Lake Willersinnweiher is fully mixed (mixing period). This leads to fully oxic conditions during the mixing period in the lake with O₂ reaching the upper few millimeters of the sediment (Schröder, 2004). With warming temperatures in spring, thermal stratification starts to build up and reaches a maximum in late summer, leading to the formation of a warm, fully oxygenated epilimnion and the anoxic hypolimnion separated by a thermocline at the upper boundary of the metalimnion and a chemocline/oxycline at the lower boundary of the metalimnion. Chlorophyll-a measurements show a peak in algal activity at the thermocline and at the transition from metalimnion to hypolimnion. As thermal stratification weakens in October due to decreasing temperatures, characteristics of mixing are observed throughout the entire water column reflecting the transition to whole lake circulation prevailing during winter.

Sulfate (SO₄²⁻) concentrations in the lake are unusually high for a freshwater environment (~2 mmol L⁻¹) and are the result of



sulfate-rich groundwater inflow at the southwestern shore of the lake (Schröder, 2004). Hence, due to high SO_4^{2-} concentrations in Lake Willersinnweiher, the potential for sulfate-dependent AOM is high compared to most other limnic environments. The main process of organic matter turnover in Lake Willersinnweiher is degradation *via* sulfate reduction (Kleint et al., 2021). This results in the production of sulfide (S^{2-}) in the lake sediment, causing diffusive release into the bottom water and consequently leading to euxinic conditions in the hypolimnion during the stratification period. Pyrite oxidation in the Quaternary river sediments of the Rhine cause high SO_4^{2-} concentrations in the catchment upstream of the lake (Schröder, 2004; Isenbeck-Schröter et al., 2016). A detailed review of geochemical processes in the lake water and sediment of Lake Willersinnweiher can be found in a recent study by Kleint et al. (2021).

Eutrophic conditions in the lake are caused by agriculture and land use in the vicinity of the lake, resulting in the release of

nutrients into the groundwater, which ultimately reach the lake (Laukenmann, 2002). Groundwater flow in the area of Lake Willersinnweiher is generally directed from southwest to northeast with low flow velocities due to a nearly horizontal groundwater table. Inflowing groundwater at the southwestern shore has already passed at least one of the adjacent smaller lakes upstream of Lake Willersinnweiher (Wollschläger et al., 2007).

Field methods

Sampling of the lake water and sediment was performed at three different sites in the southwestern basin. The pelagic sampling site in the center of the lake has a water depth of 16 m, the slope site is 9 m and the littoral site about 1.5 m deep. Groundwater sampling was carried out at three wells surrounding the lake (see Figure 2). The lake water, groundwater and sediment were sampled in July 2020 during

the stratification period and in March 2021 during the mixing period.

Changes in lake water parameters (temperature, pH, dissolved oxygen and chlorophyll-a) with depth were recorded using an Exo1 multiparameter probe (*Xylem Analytics*, Norway). The probe was calibrated for each of the monitored parameters prior to each sampling session.

Samples of the water column were taken at different depth levels using a submersible pump (COMET-Pumpen Systemtechnik GmbH & Co. KG, Germany) and prepared for measurement of CH₄ concentration and isotopic composition using a headspace technique (Kampbell et al., 1989). Samples collected for dissolved ion and dissolved inorganic carbon (DIC) analysis were filtered through a 0.2 µm filter into falcon tubes (Corning, United States). Samples for cation analysis were acidified with 150 µl of 6 M nitric acid.

For pore-water analysis, two sediment cores (core length ~21–29 cm) were taken at each site with a manually operated gravity corer. Pore-water was extracted from one of the cores immediately after sampling in defined intervals using rhizons (*Rhizosphere Research Products*, Netherlands) with a pore-size of 0.15 µm. For measurement of sedimentary CH₄ concentration and its isotopic composition, the sediment of the second core was subsampled in the same intervals as the pore-water by transferring 3 ml of lake sediment with a cut-off plastic syringe into glass vials. The sediment was treated with 5 ml of sodium hydroxide (1 M NaOH) and subsequently the vials were crimped with a lid containing a butyl rubber septum and shaken vigorously to impede any further microbial activity. In the laboratory, the sediment samples were shaken for ~10 min to equilibrate porewater CH₄ and the gas headspace. Afterwards, the headspace was extracted for further analysis. The sediment samples were dried in an oven at 105°C for several days to determine their water content and porosity.

Groundwater wells were sampled using a submersible pump (MP1, Grundfos GMBH, Germany) after pumping until groundwater parameters showed steady values (~30 min). Groundwater samples were prepared in the same way as lake water samples.

Ebullitive CH₄ was sampled for its isotopic composition in November 2020 and September 2021 by deploying an in-house built bubble trap consisting of inverted plastic funnels. A gravity corer was dropped into the sediment in order to release gas bubbles trapped in the sediment. Released gas bubbles were collected with the bubble traps directly under the lake surface and the accumulated gas was analyzed for CH₄ concentration as well as its carbon and hydrogen isotopic composition. The collection of ebullitive CH₄ via this method was only performed in November 2020 and September 2021 at the littoral and slope sites, whereas at the pelagic site, it was not possible to collect gas bubbles at the surface after dropping the weight into the sediment.

Gas samples for measurement of δ¹³C-CH₄ and δ²H-CH₄ values released to the atmosphere via diffusion were taken between March and September 2021 using a floating chamber. The floating chamber consisted of a plastic body with a volume of 8.6 L, two tubes equipped with three-way valves in order to take samples and a floatable ring made from polyethylene that kept the chamber afloat and the edges of the chamber in the water at a depth between 2 and 3 cm. Samples were collected when the chamber was placed in the water and after 15 and 30 min. All gas samples collected from the water column, sediment, groundwater and via the floating chamber were analyzed for their CH₄ concentrations, δ¹³C-CH₄ and δ²H-CH₄ values in the laboratory.

Laboratory methods

All laboratory analyses were performed at the Institute of Earth Sciences at Heidelberg University, Germany.

δ¹³C and δ²H stable isotope analysis of CH₄ and DIC

The natural stable isotopic composition of CH₄ is expressed in the conventional δ-notation in permil (‰) versus Vienna Peedee Blemnite (V-PDB) for carbon and Vienna Standard Mean Ocean Water (V-SMOW) for hydrogen. The δ-notation is defined as the relative difference of isotope ratios of a sample compared to the standard substance, hence the ¹³C/¹²C ratio of a sample compared to V-PDB (δ¹³C value) and the ²H/¹H ratio of a sample compared to V-SMOW (δ²H value) (Eq. 1):

$$\delta_S = \frac{R_S - R_{Std}}{R_{Std}} = \frac{R_S}{R_{Std}} - 1 \quad (1)$$

A Delta^{PLUS} XL IRMS (*Thermo Fisher Scientific*, Bremen, Germany) was used to analyze δ¹³C-CH₄, δ²H-CH₄ and δ¹³C-DIC values in the headspace samples. For ¹³C-DIC analysis, the water sample was first acidified with a few drops of hydrochloric acid (≥25% HCl) in order to transform all DIC to CO₂ prior to sampling the headspace gas. The IRMS was coupled to a HP 6890N GC (*Agilent Technologies*, United States) via a GC Combustion III Interface (GC-C; *ThermoFisher Scientific*, United States) with an oxidation reactor at 960°C and a thermo conversion reactor (GC-TC) at 1450°C for carbon and hydrogen stable isotopic analysis, respectively. The GC was equipped with a CP-PoraPLOT Q capillary column (length: 27.5 m; inner diameter 0.25 mm; film thickness: 8 µm; *Varian*, United States). For δ¹³C-CH₄ and δ²H-CH₄ measurements, the GC-C/TC-IRMS was linked to a cryogenic pre-concentration unit, whereas for δ¹³C-DIC an A200S autosampler was applied (*CTC Analytics*, Switzerland).

For CH₄ measurements with the pre-concentration unit, the headspace gas samples were transferred to an evacuated 40 ml sample loop. Methane was trapped on HayeSep D at -125°C, separated from other remaining compounds by GC, and then

introduced into the IRMS system *via* the interface described above. All $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values were corrected using two CH_4 reference standards (*Isometric instruments*, Canada) with $\delta^{13}\text{C}\text{-CH}_4$ values of -42.32‰ and -66.35‰ and $\delta^2\text{H}\text{-CH}_4$ values of -190.6‰ and -149.9‰ , respectively, that were calibrated against International Atomic Energy Agency (IAEA) and National Institute of Standards and Technology (NIST) reference substances. Sample values were normalized according to Paul et al. (2007).

Analysis of CH_4 *via* gas chromatography

Pore-water, water column and groundwater samples were analyzed using a gas chromatograph (14B GC-FID, Shimadzu, Japan) equipped with a flame ionization detector for CH_4 concentrations ranging from 100 ppbv to 50 ppmv and a gas chromatograph (GC-2010 BID, Shimadzu, Japan) equipped with a barrier discharge ionization detector for CH_4 concentrations higher than 50 ppmv. The 14B GC-FID was fitted with a stainless-steel column (length: 2 m; inner diameter: 1/8 inches) filled with a molecular sieve 5A (60–80 mesh; pore-size: 5 Å diameter). The GC oven temperature was isothermal at 125°C. Two reference standards (2.192 ppmv and 9.655 ppmv) were also analyzed for quality control. The GC-2010 BID was fitted with a stainless-steel ShinCarbon ST packed column (80/100 mesh; length: 2 m; diameter: 0.53 mm). The GC oven temperature was programmed to hold at 30°C for 6.5 min, rise to 75°C at a rate of 10°C/min and then to 180°C at a rate of 30°C/min. The GC-2010 BID was calibrated using several standards ranging from 50 ppmv to 97% CH_4 . A 1000 ppmv CH_4 standard was analyzed for quality control.

DIC, SO_4^{2-} and S^{2-} analysis

For determination of the dissolved inorganic carbon (DIC) concentration, a TOC-V CPH (*Shimadzu*, Japan) was used. The instrument was calibrated by repeated analysis of an in-house standard solution prior to each measurement. The SO_4^{2-} concentration was analyzed by a Dionex™ ICS-1100 Ion Chromatography System (ThermoFisher Scientific, United States). The measurement precision for each element was <3% and derived from long-term repeated analysis of reference material SPS-NUTR-WW1. The S^{2-} concentration within the water column, pore-water and groundwater samples was determined photometrically (DREL 2800, Hach, United States) immediately after returning to the laboratory from sampling in the field. The samples were prepared using the Spectroquant® Sulfide Reagent Test (Merck, Germany) and measured at a wavelength of 665 nm. The concentrations of cations in the water samples were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES 720, Agilent Technologies, United States). For quality control, the reference material SPS-SW2 was analyzed along with the samples with a measurement precision for each element <2%.

The ionic balance was determined by the sum of major cation and anion concentrations in order to qualitatively control the results of total dissolved ion composition analysis. Results of ionic balance calculations showing a deviation less than 5% displayed good quality control of the measurements.

Application of stable isotopes of methane

Methane formation and consumption processes are associated with a kinetic isotope effect leading to a change in its isotopic composition. The magnitude of this kinetic isotope effect is expressed in the isotope fractionation factor α . Processes of CH_4 formation can be distinguished by determining the apparent carbon isotopic fractionation factor $\alpha_{\text{CH}_4\text{-CO}_2}$ between CH_4 and CO_2 according to Eq. 2:

$$\alpha_{\text{CH}_4\text{-CO}_2} = \frac{\delta^{13}\text{C}\text{-CO}_2 + 1000}{\delta^{13}\text{C}\text{-CH}_4 + 1000} \quad (2)$$

In order to calculate the $\delta^{13}\text{C}\text{-CO}_2$ values from $\delta^{13}\text{C}\text{-DIC}$ values the methodology as described by Gonzalez Moguel et al. (2021) was used. Shortly, in a first step the DIC concentrations as well as the pH values in the respective depth were used to calculate the distribution of CO_3^{2-} , HCO_3^- and dissolved CO_2 . Using this species distribution and the isotope fractionation factors between gaseous CO_2 and dissolved CO_2 , HCO_3^- and CO_3^{2-} as reported by Zhang et al. (1995), dissolved $\delta^{13}\text{C}\text{-CO}_2$ values were calculated. An $\alpha_{\text{CH}_4\text{-CO}_2}$ between 1.050 and 1.060 indicates acetoclastic methanogenesis whilst between 1.060 and 1.090 shows predominance of hydrogenotrophic methanogenesis (Thottathil and Prairie, 2021).

Isotopic fractionation factors $^{13}\alpha$ for carbon and $^2\alpha$ for hydrogen during CH_4 oxidation were determined using the Rayleigh model for closed systems according to Bastviken et al. (2002) (Eq. 3):

$$\ln(1 - f) = \frac{[\ln(\delta_P + 1000) - \ln(\delta_{Ox} + 1000)]}{[\alpha - 1]} \quad (3)$$

where f is the fraction of CH_4 being oxidized, δ_P the carbon/hydrogen isotopic value of CH_4 in the near-bottom water and the zone of CH_4 production in the sediment, respectively, and δ_{Ox} the carbon/hydrogen isotopic value of CH_4 in the oxidation zone in the aerobic water column or the anoxic sediment, respectively. During CH_4 oxidation, a gradual enrichment of the heavier isotope in the residual CH_4 compared to initial $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values occurs due to kinetic fractionation. The magnitude of this enrichment is expressed by the isotope fractionation ε (Eq. 4).

$$\varepsilon = \alpha - 1 \quad (4)$$

The Keeling plot method was used to determine the $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values of CH_4 released from the

water column to the atmosphere (Keeling, 1958). For a detailed description of the Keeling plot method, we refer the reader to Pataki et al. (2003) and Keppler et al. (2016). Briefly, a graphical approach based on the Keeling plot method was used to estimate stable isotope source values of CH₄. Here, the inverse CH₄ mixing ratios of the three individual measurements during sampling of diffusion (*x*-axis) are plotted against their respective δ¹³C-CH₄ or δ²H-CH₄ values measured *via* GC-C-IRMS (*y*-axis). Then a linear regression was employed and the intercept of this linear regression with the *y*-axis reflects the δ¹³C-CH₄ or δ²H-CH₄ source value. Reported errors are based on the standard error of the linear regression.

A novel stable isotope indicator Δ(2, 13) was applied, which was introduced by Tsunogai et al. (2020), to characterize sources of CH₄ by correcting for changes in the isotopic composition of CH₄ due to the progression of oxidation (Eq. 5).

$$\Delta(2, 13) = \delta^2H - \Lambda x \delta^{13}C \quad (5)$$

The Λ value thereby corresponds to the ratio of stable hydrogen vs. stable carbon isotope fractionation ϵ_H and ϵ_C , respectively, during microbial CH₄ oxidation (Eq. 6), and thus corrects for the kinetic isotope effect associated with oxidation.

$$\Lambda = \frac{\epsilon_H}{\epsilon_C} \quad (6)$$

To calculate the Δ(2, 13) values, the Λ value for Lake Willersinnweiher needs to be known. Hence, a MOx incubation experiment was performed in the month of July 2021 with water from a depth of 6 m (Supplementary Figure S1). Lake water was filled into acid washed and autoclaved vials and incubated without any further treatment for 20 days in the dark at the lake temperature (15°C). Temporal sampling of CH₄ was performed three times in triplicate during the incubation period *via* the headspace technique (Kampbell et al., 1989). The δ¹³C-CH₄ and δ²H-CH₄ was subsequently analyzed and Λ was then estimated from the slope of the linear regression between the changes in δ¹³C-CH₄ and δ²H-CH₄ values analyzed at the different intervals during the incubation yielding a result of $\Lambda = 9.3 \pm 0.3$ (Supplementary Figure S1). In order to calculate Δ(2, 13), the concentration-weighted mean isotopic value of the lake water samples was used.

Results

Dissolved CH₄ concentrations and isotopic composition of CH₄ in the sediment

Sediment porewater profiles of CH₄ concentrations at the pelagic site showed an increase with greater sediment depth during the stratification period with maximum concentrations

ranging around 1 mmol L⁻¹, which were accompanied by δ¹³C-CH₄ values of -80 to -75‰ and δ²H-CH₄ values of -314 to -328‰ (Figure 3D). A brief shift towards more positive isotopic values of CH₄ within a few centimeters in the upper sediment occurred, where δ¹³C-CH₄ values showed a maximum increase of 11%, however δ²H-CH₄ data is missing for this depth interval due to problems in collecting samples. The upper sediment core at the pelagic site was characterized by decreasing CH₄ concentrations towards the sediment surface. Sulfate concentrations decreased with depth in the upper few centimeters (3–5 cm) of the sediment while simultaneously a rise in S²⁻ concentrations was recorded in the same depth interval (Figure 3E). Also, DIC concentrations in the pore-water increased with depth and showed lowest δ¹³C-DIC values of around -14‰ in the upper part and an enrichment in ¹³C with increasing sediment depth to -9‰ (Figure 3F).

At the slope site an increase in CH₄ concentrations with depth was observed accompanied by δ¹³C-CH₄ values varying from -77 to -69‰ and δ²H-CH₄ values ranging from -338 to -271‰ (Figure 4D). Similarly to the pelagic site, a decrease in SO₄²⁻ and an increase in S²⁻ concentration with depth was recorded (Figure 4E), whereas DIC concentrations increased towards the bottom of the sediment core coinciding with an overall decrease in δ¹³C-DIC values (Figure 4F).

At the littoral site, profiles of the sediment porewater showed CH₄ concentrations of up to 1.3 mmol L⁻¹ (Figure 5D). ¹³C-enriched CH₄ values compared to the pelagic and slope sites around -58 to -52‰ were found during the stratification period along with δ²H-CH₄ values ranging from -333 to -256‰ (Figure 5D). Similar to the deeper sites, a shift towards a more positive isotopic signal particularly in δ²H-CH₄ values was recorded in the upper sediment layers, whereas δ¹³C-CH₄ values showed a gradual increase from -57‰ at 3 cm to -51‰ at 9 cm sediment depth.

During the mixing period, porewater CH₄ at the pelagic site showed highly variable δ¹³C-CH₄ values throughout the entire sediment core varying from -39 to -75‰, whereas δ²H-CH₄ showed a rather uniform distribution ranging between -234 and -266‰, respectively (Figures 3J–L). Methane concentrations were at a much higher level than during the stratification period at the pelagic site, increasing to 3.1 mmol L⁻¹ in the lower part of the core. In contrast, the littoral sediment recorded significantly lower CH₄ concentrations around 0.3 mmol L⁻¹ during the mixing period (Figure 5). For the slope site no CH₄ data is available for the mixing period. Similar to the stratification period, SO₄²⁻ concentrations decreased in the upper part of the pelagic sediment and stayed at very low levels in the lower part of the core (Figure 3K). No increase in S²⁻ concentration was observed in the sediment of the three investigated sites during the mixing period. DIC concentrations showed a similar trend as during the stratification period and increased with sediment

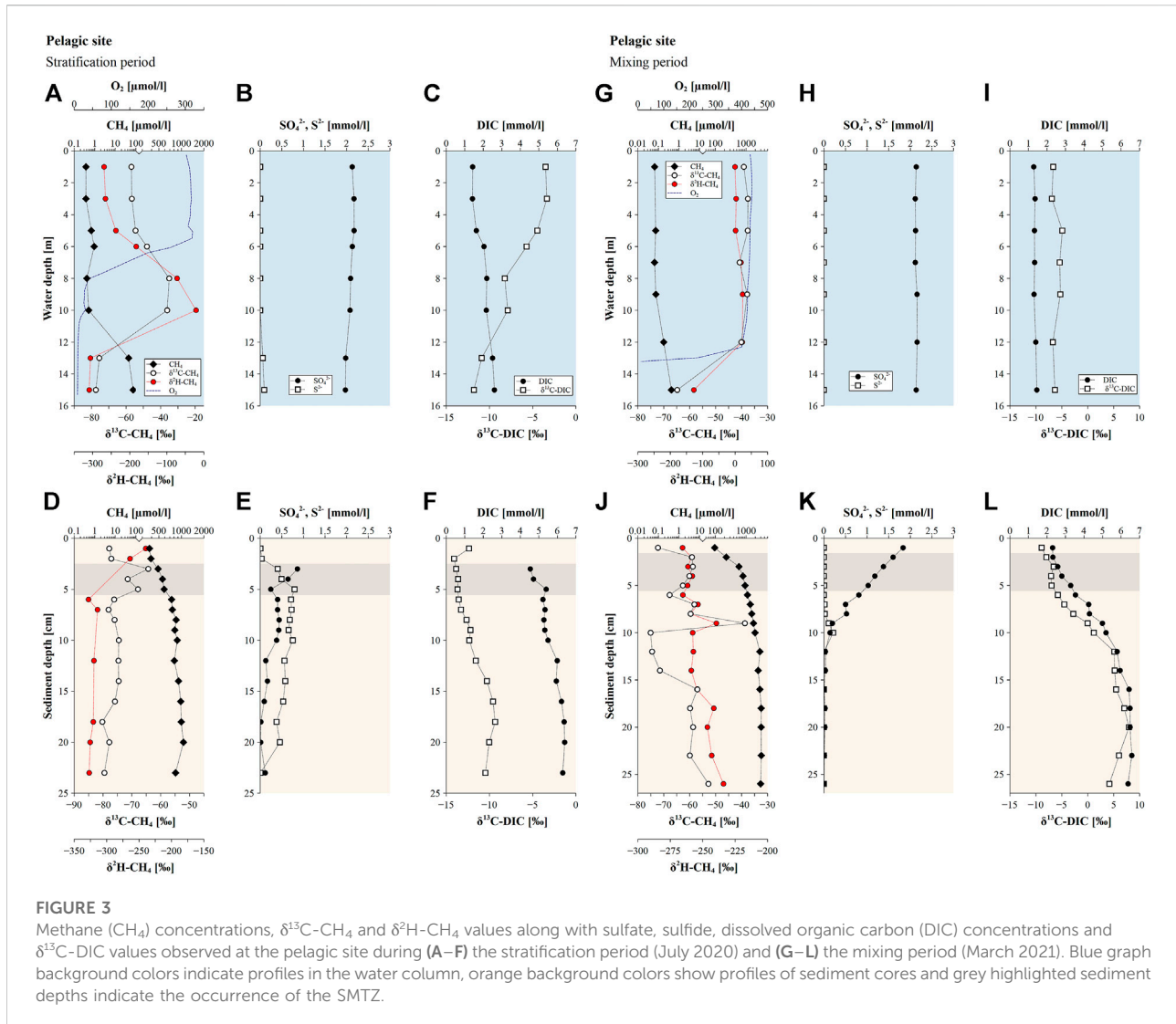


FIGURE 3

Methane (CH₄) concentrations, δ¹³C-CH₄ and δ²H-CH₄ values along with sulfate, sulfide, dissolved organic carbon (DIC) concentrations and δ¹³C-DIC values observed at the pelagic site during (A–F) the stratification period (July 2020) and (G–L) the mixing period (March 2021). Blue graph background colors indicate profiles in the water column, orange background colors show profiles of sediment cores and grey highlighted sediment depths indicate the occurrence of the SMTZ.

depth accompanied by increasing δ¹³C-DIC values (Figure 3L).

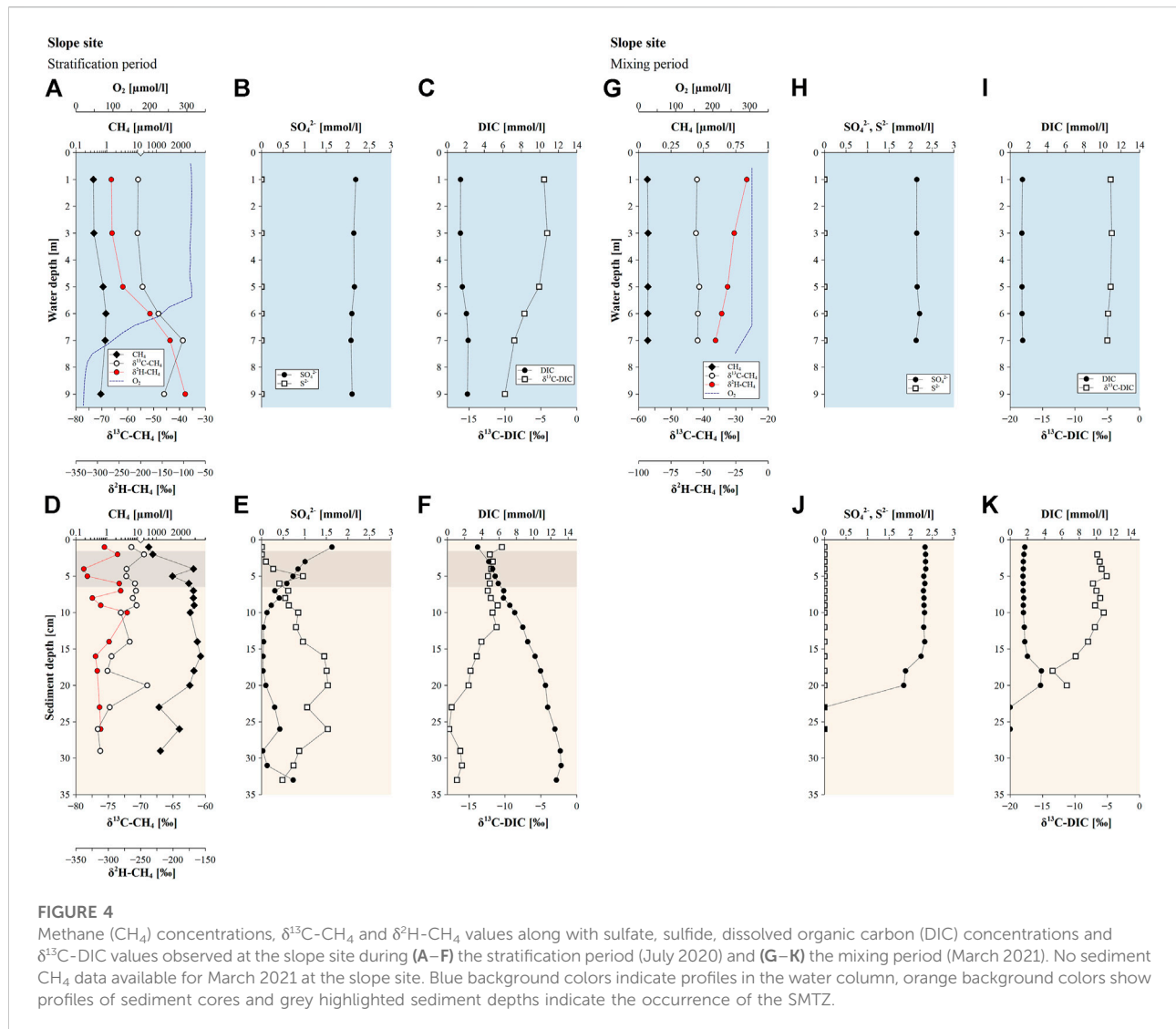
Dissolved CH₄ concentrations and isotopic composition of CH₄ in the water column

In the lake water column, CH₄ concentrations showed a decrease from the hypolimnion towards the surface water accompanied by an enrichment in ¹³C-CH₄ and ²H-CH₄ during the stratification period (Figure 3A). Methane in the bottom water of Lake Willersinnweiher showed δ¹³C-CH₄ and δ²H-CH₄ values of -77‰ and -309‰, respectively, and increased to δ¹³C-CH₄ values of -56‰ and -269‰, respectively, in the surface water layer during the stratification period. Methane concentrations in the

surface water ranged from 0.37 to 0.67 μmol L⁻¹ with highest concentrations found at the littoral site (Figure 5A). DIC concentrations rose from 1.4 mmol L⁻¹ in the surface water to 2.6 mmol L⁻¹ in the bottom water, while δ¹³C-DIC showed a decrease from -4 to -12‰ throughout the water column from top to bottom (Figure 3C).

A pronounced zone of low CH₄ concentrations was observed at the pelagic site during the stratification period in the lower metalimnion, showing a maximum decline in CH₄ of 0.52 μmol L⁻¹ from 6 to 8 m water depth (Figure 3A). This minimum in CH₄ concentrations coincided with maximum δ¹³C-CH₄ and δ²H-CH₄ values in the water column.

A local peak in CH₄ concentrations in the oxic upper water column was found at a water depth of 6 m during the stratification period at the pelagic site, showing a maximum increase of 0.56 μmol L⁻¹ compared to surface water CH₄



concentrations during the stratification period (Figure 3A). This was accompanied by an increase in the $\delta^{13}\text{C}-\text{CH}_4$ and $\delta^2\text{H}-\text{CH}_4$ values of about 9‰ and 83‰, respectively, compared to surface water values, resulting in $\delta^{13}\text{C}-\text{CH}_4$ values of -48 ‰ and $\delta^2\text{H}-\text{CH}_4$ values of -83 ‰ at 6 m water depth.

The slope site showed similar surface water CH_4 concentrations ($0.37\text{--}0.39\ \mu\text{mol L}^{-1}$) and isotopic compositions ($\delta^{13}\text{C}-\text{CH}_4$ of -56 ‰ and $\delta^2\text{H}-\text{CH}_4$ of -268 ‰) as the pelagic site and recorded an increase in the concentration with depth up to $0.94\ \mu\text{mol L}^{-1}$, which was accompanied by an increase in $\delta^{13}\text{C}-\text{CH}_4$ and $\delta^2\text{H}-\text{CH}_4$ values of 17‰ and 136‰, respectively (Figure 4A). The littoral site displayed CH_4 enriched in $^{13}\text{C}-\text{CH}_4$ and $^2\text{H}-\text{CH}_4$ ($\delta^{13}\text{C}-\text{CH}_4$ and $\delta^2\text{H}-\text{CH}_4$

values of -51 and -263 ‰) in the surface water (Figure 5A) compared to the pelagic and slope sites.

During the mixing period, distinctively lower CH_4 concentrations around $0.07\ \mu\text{mol L}^{-1}$ at the pelagic site throughout almost the entire water column were characterized by $\delta^{13}\text{C}-\text{CH}_4$ values of -39 ‰ and $+11$ ‰ for $\delta^2\text{H}-\text{CH}_4$ values (Figure 3G). The bottom water showing CH_4 concentrations of $0.4\ \mu\text{mol L}^{-1}$ recorded more negative isotopic values of -65 ‰ and -127 ‰. The concentration of DIC in the water column stayed at the same level throughout the water column and the carbon isotopic pattern recorded a minor shift towards slightly more positive values from -7 to -5 ‰ at intermediate water depths (Figure 3I). While CH_4 concentrations and $\delta^{13}\text{C}-\text{CH}_4$ values at the slope and littoral site were similar to those found at

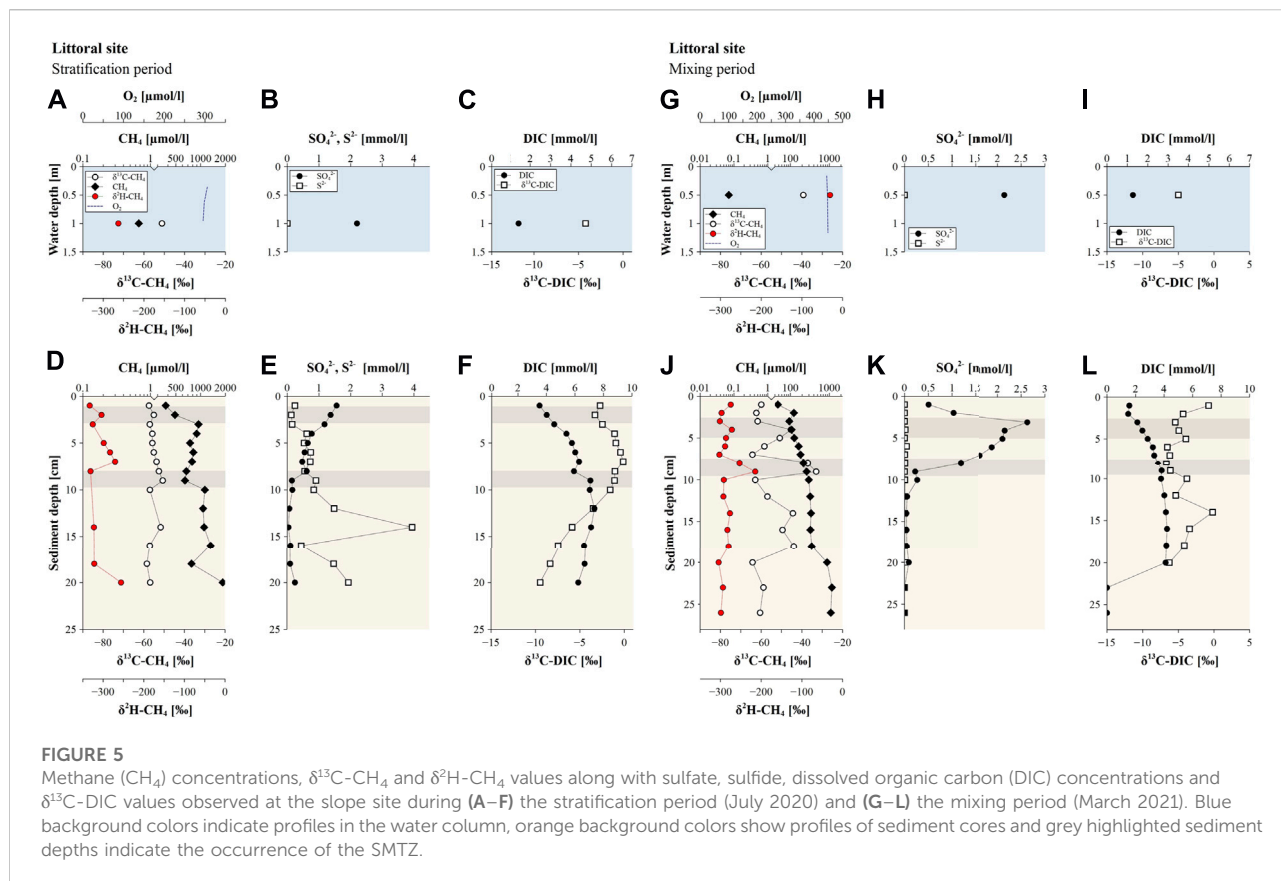


FIGURE 5

Methane (CH₄) concentrations, δ¹³C-CH₄ and δ²H-CH₄ values along with sulfate, sulfide, dissolved organic carbon (DIC) concentrations and δ¹³C-DIC values observed at the slope site during (A–F) the stratification period (July 2020) and (G–L) the mixing period (March 2021). Blue background colors indicate profiles in the water column, orange background colors show profiles of sediment cores and grey highlighted sediment depths indicate the occurrence of the SMTZ.

the pelagic site, δ²H-CH₄ values were more negative varying around -30‰ (Figures 4G, 5G).

Isotopic fractionation factors in the sediment and water column

The apparent carbon isotopic fractionation factor α_{CH₄-CO₂} between CH₄ and CO₂ (Eq. 2) in the sediment was determined for sediment depths where CH₄ concentrations were highest, therefore most likely indicating the occurrence of methanogenesis in these depths. The α_{CH₄-CO₂} was found to be lowest at the littoral site (1.047) and rose with increasing lake depth (1.058 at the slope site and 1.066 at the pelagic site) during the stratification period (Supplementary Table S1). During the mixing period, the pelagic and littoral site exhibited a α_{CH₄-CO₂} of 1.070 and 1.047, respectively.

Isotopic fractionation factors associated with CH₄ oxidation (Eq. 3) were determined in the upper sediment layers of the three sampled locations where a zone of decreasing CH₄ was observed. During the stratification period, isotopic fractionation in these zones was found to range from 1.005 to 1.031 for ¹³α and from 1.042 to 1.124 for ²α (Table 1). During the mixing period, ¹³α varied from 1.051 to 1.087 and ²α from 1.015 to 1.169. In the

TABLE 1 Isotopic fractionation factors for carbon (¹³α) and hydrogen (²α) calculated for the for zones of decreasing CH₄ concentrations in the water column (bottom water to lower metalimnion) and in the upper sediment during the stratification period (July 2020) and for the upper sediment during the mixing period (March 2021).

		¹³ α	² α
July 2020	Pelagic water column	1.009	1.057
	Pelagic sediment	1.031	—
	Slope sediment	1.005	1.042
	Littoral sediment	1.012	1.124
March 2021	Pelagic sediment	1.051	1.015
	Slope sediment	—	—
	Littoral sediment	1.087	1.169

water column, an isotopic fractionation factor was determined for the zone between the upper and the lower metalimnion at the pelagic site in which a decrease in CH₄ concentrations was apparent for the stratification period, where an ¹³α of 1.009 and an ²α of 1.057 was calculated. An isotopic fractionation factor for the mixing period could not be calculated since no changes in the isotopic composition of

TABLE 2 $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values of diffusively released CH_4 from the lake surface water into the atmosphere.

Month	Site	$\delta^{13}\text{C}\text{-CH}_4$ diffusion [‰]	$\delta^2\text{H}\text{-CH}_4$ diffusion [‰]
March 2021	pelagic	-37 ± 1	1 ± 34
May 2021		-56 ± 1	—
June 2021		-59 ± 1	-310 ± 8
July 2021		-52 ± 1	-208 ± 3
August 2021		-54 ± 1	-250 ± 25
September 2021	slope	-53 ± 1	-271 ± 4
March 2021		-42 ± 1	26
May 2021		-53 ± 2	—
June 2021		-58 ± 1	-306 ± 13
July 2021		-53 ± 2	-205 ± 14
August 2021	littoral	-53 ± 1	—
September 2021		-52 ± 1	-220 ± 11
March 2021		-36 ± 8	—
May 2021		—	—
June 2021		—	—
July 2021		-53 ± 1	-250 ± 14
August 2021		-51 ± 1	-195 ± 22
September 2021		-55 ± 2	-224 ± 4

SD corresponds to the slope uncertainty in the linear regression of the Keeling plot.

CH_4 was observed in the water column and similar CH_4 concentrations throughout the entire water column were recorded (Figure 3G).

Concentration and isotopic composition of diffusive and ebullitive CH_4

Methane released *via* diffusion from the lake surface water into the atmosphere during the stratification period (May to September) recorded similar $\delta^{13}\text{C}\text{-CH}_4$ values at each of the three sampled sites, varying between -52 and -58‰ (Table 2). $\delta^2\text{H}\text{-CH}_4$ values were more variable ranging from -195 to -310‰ at the different sites, whereby the pelagic and slope site showed in July the most positive values during the stratification period (-208 ± 3 and $-205 \pm 14\text{‰}$, respectively) and the littoral site in August ($-195 \pm 22\text{‰}$). During the mixing period, CH_4 emitted from the lake was considerably enriched in the heavier isotopes at all investigated sites compared to the stratification period. $\delta^{13}\text{C}\text{-CH}_4$ values ranged from -36 to -42‰ and $\delta^2\text{H}\text{-CH}_4$ values from 1 to 26‰.

The concentrations and isotopic composition of ebullitive CH_4 were analyzed for the littoral and slope site of the lake in November 2020 and September 2021 (Supplementary Table S2). Ebullitive CH_4 was not collected for analysis in July 2020 and March 2021 due to logistical reasons. Nevertheless, the stratified lake period is represented by the data from September 2021, whereas the mixing period is represented by the data from November 2020.

In September during thermal stratification, CH_4 concentrations in gas bubbles in the littoral area reached $80.8 \pm 4.5\%$. In November, CH_4 concentrations of ebullitive CH_4 were in the range of $62.1 \pm 7.4\%$ and $66.8 \pm 3.2\%$ at the littoral and slope site, respectively. $\delta^{13}\text{C}\text{-CH}_4$ values at the littoral site were more negative in September compared to November with $-58 \pm 1\text{‰}$ and $-50 \pm 12\text{‰}$, respectively. In contrast, $\delta^2\text{H}\text{-CH}_4$ values in September and November at the littoral site were very similar with $-323 \pm 3\text{‰}$ and $-326 \pm 4\text{‰}$. At the slope site, $\delta^{13}\text{C}\text{-CH}_4$ values were more negative compared to the littoral site with $-64 \pm 11\text{‰}$, but $\delta^2\text{H}\text{-CH}_4$ values were in a similar range compared to the littoral site with values of $-318 \pm 1\text{‰}$.

Groundwater methane

Groundwater in the area of Lake Willersinnweiher showed highly variable CH_4 concentrations, $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values and was strongly enriched in $^2\text{H}\text{-CH}_4$ and $^{13}\text{C}\text{-CH}_4$ (Table 3). During the stratification period, inflowing groundwater yielded a CH_4 concentration of $1.01 \mu\text{mol L}^{-1}$ with an isotopic composition enriched in ^{13}C and ^2H ($\delta^{13}\text{C}\text{-CH}_4$ value of -24‰ and $\delta^2\text{H}\text{-CH}_4$ value of $+106\text{‰}$). Outflowing groundwater of the southwestern basin showed slightly lower CH_4 concentrations of $0.95 \mu\text{mol L}^{-1}$ along with $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values of $+18\text{‰}$ and $+582\text{‰}$, respectively. CH_4 concentrations of groundwater flowing out of the northeastern basin were $0.77 \mu\text{mol L}^{-1}$ during the

TABLE 3 Methane concentration and its stable isotopic composition of the three different groundwater wells during the stratification period (July 2020) and the mixing period (March 2021).

	CH ₄ [μmol l ⁻¹]	δ ¹³ C [‰]	δ ² H [‰]
July 2020			
GW inflow SW Basin	1.01	-24	+106
GW outflow SW Basin	0.95	+18	+582
GW outflow NE Basin	0.77	-50	-187
March 2021			
GW inflow SW Basin	1.97	-19	—
GW outflow SW Basin	2.71	-37	+24
GW outflow NE Basin	30.89	-58	-245

stratification period accompanied by δ¹³C-CH₄ values of -50‰ and δ²H-CH₄ values of -187‰. Between the two outflowing groundwater wells in the western and eastern part of Lake Willersinnweiher a disparity in CH₄ concentrations and stable isotope values is worth noticing. This observation is probably caused as outflowing groundwater in the western part consists of a mixture of outflowing groundwater from the lake and groundwater with properties similar to inflowing groundwater, that also passed subjacent lakes, due to its location and groundwater flow direction (see Figure 2). During the mixing period, groundwater at the three wells showed CH₄ concentrations of 1.97 and 2.71 μmol L⁻¹ for inflowing and outflowing groundwater of the southwestern basin, respectively, and even 30.89 μmol L⁻¹ for groundwater flowing out of the northeastern basin. Groundwater outflow of the southwestern basin showed a δ¹³C-CH₄ value of -37‰ and a δ²H-CH₄ value of +24‰. The isotopic composition of groundwater at the other two groundwater wells was in the range of values reported during the stratification period (Table 3).

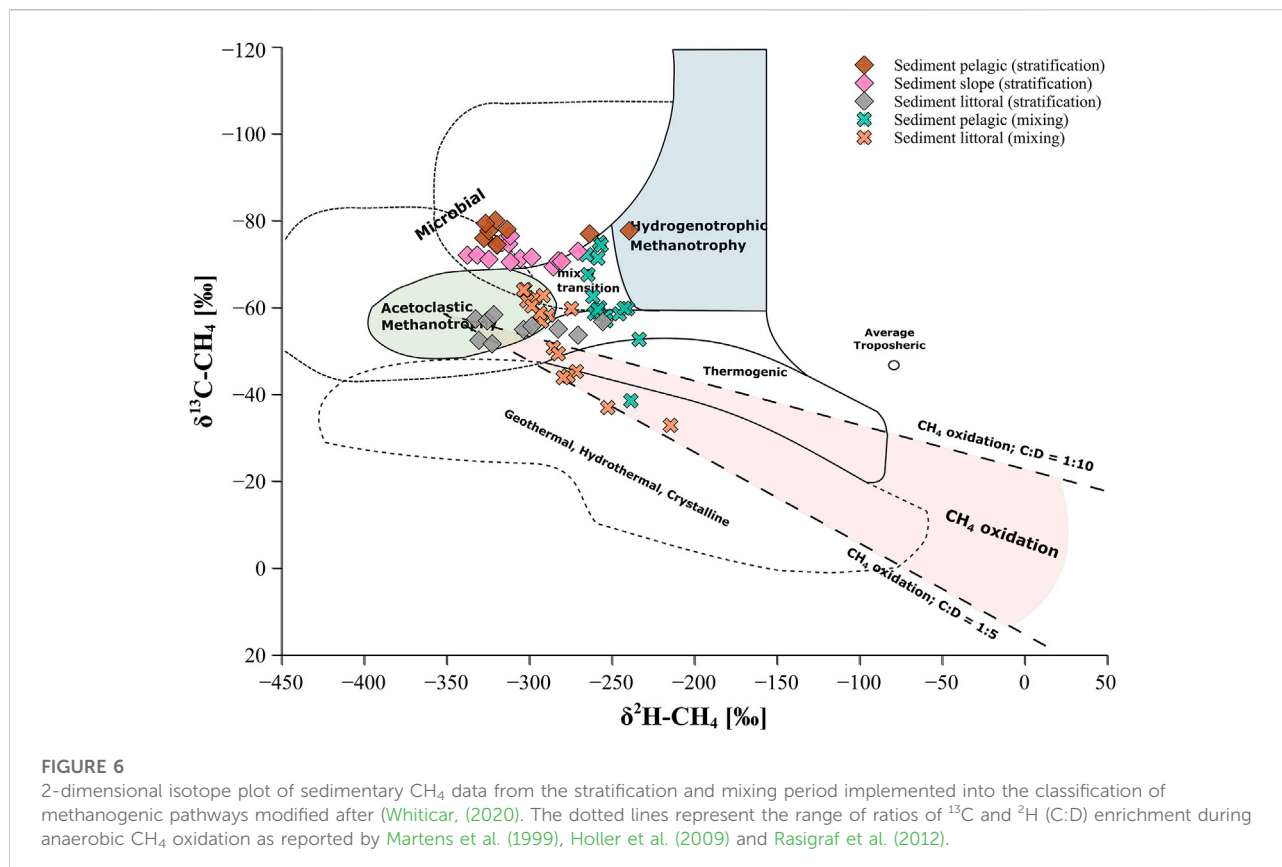
Discussion

Methanogenesis in the sediment

Methanogenesis is the last step in the microbial degradation of organic matter and is performed by methanogenic archaea in the anaerobic environment (Conrad, 2005). However, in presence of other electron donors (such as SO₄²⁻), organic matter degradation *via* sulfate reduction is favored over methanogenesis. This is most likely the case for Lake Willersinnweiher, where sulfate-reducing bacteria are believed to thermodynamically outcompete methanogenic archaea for available carbon compounds and H₂ in the upper sediment due to high SO₄²⁻ concentrations in the lake water (Kleint et al., 2021). In aquatic systems with high SO₄²⁻ concentrations, anaerobic methanogenesis is therefore

restricted to greater sediment depths, where SO₄²⁻ concentrations are low (Holmer and Storkholm, 2001; Reeburgh, 2007; Schubert et al., 2011). Methanogenesis in Lake Willersinnweiher is suggested to occur in zones of the deeper sediment where CH₄ in the sediment accumulated (Figures 3D, 4D, 5D). Hydrogenotrophic methanogenesis as the dominant process of CH₄ production in the sediment during the stratification period was indicated by relatively negative δ¹³C-CH₄ values (ranging from around -80 to -75‰) observed in the lower sediment of the pelagic and slope site, where maximum CH₄ concentrations were recorded (Figure 3D). Whereas the associated δ²H-CH₄ values of around -321 to -246‰ were rather negative for this type of methanogenesis (Whiticar, 1999). However, δ²H-CH₄ values in this environment are likely mostly linked to the δ²H value of the source water (Waldron et al., 1999; Douglas et al., 2021) and seem to be less indicative of the methanogenic pathway compared to δ¹³C-CH₄ values as δ²H-CH₄ values are less studied and influenced by more factors. Above that a mixing of the hydrogenotrophic and acetoclastic pathway is possible (Sugimoto and Wada, 1995; Walter et al., 2008). During methanogenesis, hydrogen originating from ambient water is incorporated into the CH₄ molecule, thus δ²H-CH₄ values are mostly dependent on the hydrogen isotopic composition of the water (Sugimoto and Wada, 1995). Plotting sedimentary δ¹³C-CH₄ values against δ²H-CH₄ values and classifying them after Whiticar (2020) indicated an overlap of the two different methanogenic pathways at the pelagic and slope site (Figure 6). For the littoral site, a dominance of acetoclastic methanogenesis seems to be implied due to less negative δ¹³C-CH₄ values compared to the pelagic and slope site.

Furthermore, based on the carbon isotopic shift between CH₄ and CO₂, we determined apparent carbon isotopic fractionation factors associated to anaerobic methanogenesis, which is an indicator to distinguish between the hydrogenotrophic and acetoclastic pathway. At the pelagic site, an α_{CH₄-CO₂} of 1.066 supports the dominance of hydrogenotrophic methanogenesis during the stratification period (Supplementary Table S1). The α_{CH₄-CO₂} decreased towards the littoral site, indicating an increasing contribution of the acetoclastic pathway. Overlapping characteristics were hence especially visible at the slope site, where δ¹³C-CH₄ values around -75‰ point to hydrogenotrophic methanogenesis whereas the calculated α_{CH₄-CO₂} of 1.058 is representative for acetoclastic CH₄ formation. The proposed increasing dominance of acetoclastic methanogenesis from the pelagic towards the littoral area is based on the isotopic values of sedimentary CH₄ observed at Lake Willersinnweiher and might be coupled to changes in sediment temperatures and differences in organic matter availability with varying lake depth (Thottathil and Prairie, 2021). However, further investigations are needed to gain more insight into the occurrence and distribution of



the different methanogenic pathways in the sediment of Lake Willersinnweiher.

During the mixing period, the observed $\alpha_{\text{CH}_4\text{-CO}_2}$ of 1.070 for the pelagic site (Supplementary Table S1) was higher than during the stratification period suggesting a higher dominance of hydrogenotrophic CH₄ formation. This might possibly reflect the limited availability of fresh organic material in the lake. However, higher sedimentary CH₄ concentrations found at the pelagic site during the mixing period compared to the stratification period (Figures 3D,J) seemed to contradict the expected decrease of CH₄ concentrations due to lower primary production in winter and therefore reduced organic matter availability. This indicated that processes other than microbial activity in the sediment must have contributed to the high observed sedimentary CH₄ concentrations and isotopic pattern in the sediment at the pelagic site and highlights the complex interaction of the occurring processes influencing the sedimentary CH₄ cycle. However, we suggest that infiltrating groundwater as a potential CH₄ source might also play an important role at Lake Willersinnweiher since it yielded very high CH₄ concentrations characterized by extremely positive isotopic $\delta^{13}\text{C-CH}_4$ and $\delta^2\text{H-CH}_4$ values both for inflowing and outflowing groundwater (Table 3). Mixing of groundwater and lake sediment CH₄ might potentially skew

the calculated fractionation factors for the mixing period, hence these have to be considered with caution and can only be used as a preliminary indicator for the methanogenic pathway.

Anaerobic methane oxidation in the sediment

The shift to more positive $\delta^{13}\text{C-CH}_4$ and $\delta^2\text{H-CH}_4$ values partially observed in the upper sediment layers during the stratification period was most likely caused by the anaerobic oxidation of CH₄ coupled to SO₄²⁻ reduction. The occurrence of sulfate-dependent AOM in Lake Willersinnweiher was described in a recent study by Kleint et al. (2021) and we therefore refer the reader to this study for more detailed discussion on this process at Lake Willersinnweiher. Here we want to give a brief summary on sulfate-dependent AOM at Lake Willersinnweiher and discuss the associated isotopic effects of this process. In the sediment, upward rising CH₄ and downward diffusing SO₄²⁻ meet in the so-called sulfate-methane transition zone (SMTZ), where anaerobic methanotrophic archaea in syntrophic association with sulfate-reducing bacteria are able to reverse methanogenesis and oxidize CH₄ using SO₄²⁻ (Boetius et al., 2000). In most freshwater environments, low SO₄²⁻

concentrations usually preclude sulfate-dependent AOM, whereas in the oceans, sulfate-dependent CH₄ oxidation is a widely described and common process due to high SO₄²⁻ concentrations in the ocean water (Knittel and Boetius, 2009). Since Lake Willersinnweiher shows high SO₄²⁻ concentrations, compared to other lakes, as a result of sulfate-rich groundwater input, sulfate-dependent AOM is possible.

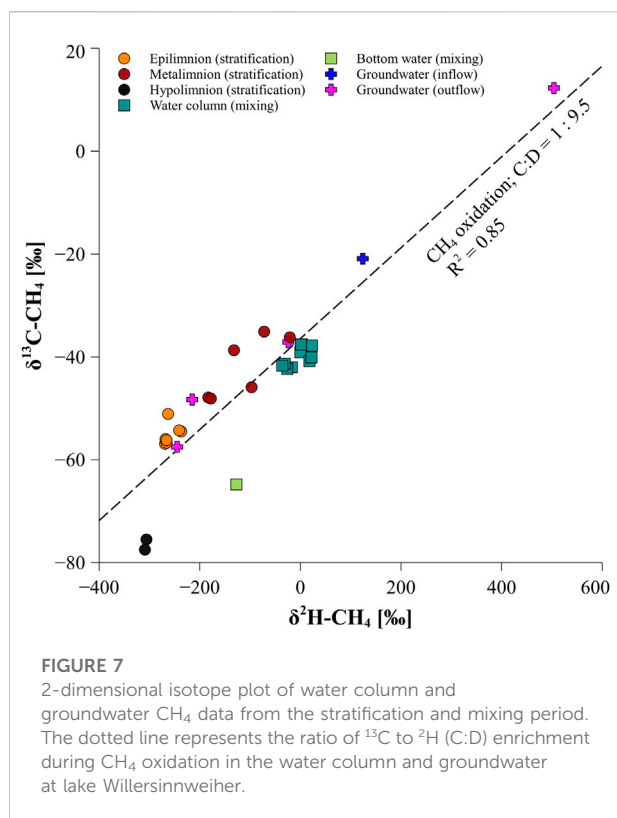
Indicative of AOM might furthermore be the observation of DIC enriched in ¹²C in the upper lake sediment compared to the lower sediment during the stratification period since ¹²C-CH₄ is preferably oxidized by the microorganisms, thereby providing HCO₃⁻ relatively enriched in ¹²C to the sedimentary DIC pool (Schubert et al., 2011). With increasing sediment depth, microbial hydrogenotrophic CH₄ production caused a depletion in isotopically light DIC, thus leading to DIC enriched in ¹³C-DIC in the sediment pore-water.

Zones in the lake sediment where sulfate-dependent AOM was presumed to take place were not always characterized by a clear shift towards less negative δ¹³C-CH₄ and δ²H-CH₄ values (Figures 3J, 5D), as one might typically expect for CH₄ oxidation or restrained to a few centimeters within the upper sediment at the investigated sites of Lake Willersinnweiher. Plotting sedimentary δ¹³C-CH₄ values against δ²H-CH₄ values shows a progression of the littoral sediment data of the mixing period within a predicted CH₄ oxidation line between a ratio of C:D of 1:5 and 1:10 (e.g., Wang et al., 2016; Whiticar, 2020), referring to an enrichment of CH₄ in hydrogen isotopes 5–10 times more than in carbon isotopes during AOM (Figure 6). This observation is in good accordance with previously reported C:D ratios during AOM ranging between 6.4 and 10 (Martens et al., 1999; Holler et al., 2009; Feisthauer et al., 2011; Rasigraf et al., 2012). However, this trend was less pronounced at the other two sites, limiting the use of carbon and hydrogen stable isotopes of CH₄ to characterize AOM in the lake sediment. Nevertheless, carbon and hydrogen isotopic fractionation factors ¹³α and ²α were also determined to identify the occurrence of potential AOM in the respective zones of the sediment. The calculated carbon isotopic fractionation factors ¹³α values for the observed enrichment in ¹³C-CH₄ and ²H-CH₄ and associated with AOM in the sediment (1.002–1.019, Table 1) were on the lower end of ¹³α values determined for AOM in marine and brackish environments and in laboratory studies (1.009–1.039, Whiticar and Faber, 1986; Holler et al., 2009; Knittel and Boetius, 2009). The hydrogen isotopic fractionation factors ²α (1.033–1.057) determined for Lake Willersinnweiher were substantially lower than ²α modelled for aquatic environments and found in *in vitro* experiments (1.109–1.315, Whiticar and Faber, 1986; Alperin et al., 1988; Holler et al., 2009). It is important to mention that natural systems might differ significantly from modelled conditions and *in vitro* experiments, thus leading to discrepancies in isotopic fractionation factors. Furthermore, it must be noted that isotopic fractionation factors of AOM determined

from *in situ* isotopic signatures, as in the case of Lake Willersinnweiher, have to be considered with caution since AOM and methanogenesis might overlap in the sediment and carbon isotope equilibrium effects caused by forward and backward AOM influence the isotopic composition of the CH₄ pool in opposite ways (Holler et al., 2009; Yoshinaga et al., 2014; Chang et al., 2019). A study by Wegener et al. (2021) furthermore showed, that the accumulation of ¹³C and ²H-depleted CH₄ in zones of AOM was driven under low sulfate conditions (similar to SO₄²⁻ concentrations found in lake Willersinnweiher) because of intracellular reactions associated with the enzymatic AOM mechanism. They found that the reactions in the enzymatic AOM pathway became more reversible and thus closer to equilibrium at low SO₄²⁻ conditions driving the formation of ¹³C and ²H depleted CH₄. The lack of a clear enrichment in the heavier isotopes of CH₄ in sediment zones where AOM is presumed to occur in Lake Willersinnweiher might therefore be resulting from the above-mentioned isotopic effects.

Due to AOM in the lake sediment, the diffusive release of CH₄ at the sediment-water interface is reduced in Lake Willersinnweiher (Kleint et al., 2021). Our concentration depth profiles of CH₄ in the sediment show a decreasing gradient towards the sediment water interface, caused by CH₄ diffusion into the bottom water and most likely AOM. Significant decreases in sedimentary CH₄ concentrations observed particularly at the slope and littoral site were suggested to be related to AOM as they coincided with an enrichment in ¹³C-CH₄ and ²H-CH₄ (Figures 4D, 5D). Methane diffusion from the sediment into the bottom water significantly elevated hypolimnic CH₄ concentrations (up to 74 μmol L⁻¹ at the pelagic site). The negative isotope values of hypolimnic CH₄ of -77‰ and -309‰ for δ¹³C-CH₄ and δ²H-CH₄ values, respectively, during the stratification period (Figure 3A) fell well within the range of δ¹³C-CH₄ and δ²H-CH₄ values found in sediment depths where anaerobic methanogenesis is presumed to occur, hence suggesting anaerobic CH₄ production in the sediment as its origin.

During the mixing period, shifts towards more positive δ¹³C-CH₄ and δ²H-CH₄ values in the upper sediment at the pelagic and littoral site suggested the occurrence of AOM in the sediment (Figures 3J, 5J). Furthermore, decreasing CH₄ concentrations and increasing SO₄²⁻ concentrations towards the sediment surface might be indicative of the occurrence of sulfate-dependent AOM (Figures 3J,K, 5J,K). The apparent lack of S²⁻ accumulation in the sediment might be caused by re-oxidation of sulfide to sulfate *via* manganese oxides in the sediment pore-water as described by Kleint et al. (2021). The determined fractionation factors associated to AOM during the mixing period (1.051–1.087 for ¹³α and 1.015–1.169 for ²α, Table 1) do not reflect typical fractionation factors for AOM, however as discussed above, these fractionation factors have to be considered cautiously as different isotopic effects can influence the isotopic composition of CH₄ in the SMTZ. Furthermore, a



mixture with other CH₄ sources, e.g., groundwater CH₄ input, might affect the carbon and hydrogen isotopic composition of sedimentary CH₄ since mixing of groundwater and sedimentary CH₄ might lead to an increase in sedimentary δ¹³C-CH₄ and δ²H-CH₄ values due to the very enriched ¹³C and ²H isotopic composition of groundwater CH₄. AOM most likely reduced the release of CH₄ at the sediment-water interface, however diffusion from the uppermost sediment layers into the bottom water layer caused elevated CH₄ concentrations in the bottom water.

Aerobic methane oxidation in the water column

During the stratification period, a strong increase in the isotopic composition of CH₄ (δ¹³C-CH₄ values of -35‰ and δ²H-CH₄ values of -72‰, Figure 3A) along with a decrease in CH₄ concentrations at the bottom of the oxycline in the water column compared to surface water CH₄ suggested MOx of sedimentary released CH₄. The enrichment in the δ¹³C-CH₄ and δ²H-CH₄ signal is caused by the preference of methanotrophic organisms to metabolize light atoms ¹²C and ¹H of CH₄, leading to an enrichment in ¹³C-CH₄ and ²H-CH₄ of the remaining CH₄ (Silverman and Oyama, 1968; Barker and Fritz, 1981; Coleman et al., 1981). From the 2-dimensional isotope plot it can be inferred that MOx occurs in the

metalimnion during the stratification period since the metalimnic stable isotope data are distributed along a gradient showing a stronger enrichment of ²H compared to ¹³C during MOx with a C:D ratio of 9.5 (Figure 7). This observation fits well within similar reported C:D ratios during MOx ranging between 5.9 and 14.9 (Coleman et al., 1988; Kinnaman et al., 2007; Powelson et al., 2007; Feisthauer et al., 2011; Wang et al., 2016). In comparison, epilimnic water samples plot did not show this progression, hence implying that δ¹³C-CH₄ and δ²H-CH₄ values there are most likely not or less affected by MOx.

The carbon isotopic fractionation factor ¹³α of 1.009 determined for the observed enrichment in Lake Willersinnweiher furthermore suggested the occurrence of MOx at this depth as it is in the range of ¹³α determined for MOx in experimental studies (1.003–1.039; Templeton et al., 2006). The estimated ²α of 1.057 on the other hand is substantially lower than values determined for MOx in closed cultures (1.103–1.325; Alperin et al., 1988). However, a higher ²α than ¹³α (factor of ~9.5) at Lake Willersinnweiher confirms the larger isotopic fractionation generally observed for stable hydrogen isotopes compared to stable carbon isotopes during microbial oxidation. The isotopic partitioning between hydrogen isotopes is greater than between carbon isotopes because of a larger mass difference between ²H and ¹H than between ¹³C and ¹²C (e.g., Wang et al., 2016). This leads to a stronger enrichment in hydrogen isotopes than in carbon isotopes during oxidation.

The zone of MOx at the oxic-anoxic interface in Lake Willersinnweiher during the stratification period acts as a barrier and effectively limits the release of sedimentary produced CH₄ into the surface mixed water layer to minimum concentrations (Kleint et al., 2021). The occurrence of MOx at the bottom of the oxycline indicated by maximum δ¹³C-CH₄ and δ²H-CH₄ values suggests that CH₄ oxidizing bacteria are most active at low O₂ levels and their presence is restricted as O₂ concentrations rise in the upper water body (Schubert et al., 2010; Thottathil et al., 2019). At the littoral site, the isotopic composition of the water column agrees well with sedimentary δ¹³C-CH₄ and δ²H-CH₄ values, suggesting that MOx did not occur here. The apparent absence of MOx at the littoral sampling site might be caused by fully oxygenated conditions in the shallow water column. Another controlling factor of MOx might be the availability of light in the photic zone of the water column. Murase and Sugimoto (2005) showed that MOx was inhibited under light conditions, whereas a linkage between a light driven supply of O₂ through photosynthesis and MOx was found in a study by Oswald et al. (2015), implying that MOx is dependent on light availability.

MOx leads to the formation of CO₂, which is converted to HCO₃⁻ according to the bicarbonate buffer system due to a prevailing pH of 8.5–7.3 in the water column of the lake. This contributed to an increase in DIC concentrations in the metalimnion, which was accompanied by decreasing δ¹³C-DIC

values (Figure 3C) caused by CH₄ oxidizing organisms and their preference for ¹²CH₄ consumption leading to the production of ¹²C enriched DIC. However, increased DIC concentrations might also originate from the degradation of organic matter. Organic material is generally characterized by relatively negative δ¹³C values, consequently causing the trend to more negative δ¹³C-DIC values with depth as degradation proceeds and inorganic carbon is released from the sediment into the water column.

During the mixing period, constant CH₄ concentrations as well as similar δ¹³C-CH₄ and δ²H-CH₄ values in the water column, except between a depth of 12 and 15 m, where stable isotope values of CH₄ became more enriched, did not suggest MOx (Figure 3G). Since almost the entire water column was fully oxygenated, this might have prevented efficient MOx as methane oxidizing bacteria were found to be sensitive to high O₂ concentrations (Thottathil et al., 2019). However, MOx could still have occurred in the lake water column in the absence of O₂, δ¹³C-CH₄ and δ²H-CH₄ gradients due to mixing and thus might contribute to CH₄ enriched in ¹³C and ²H during the mixing period compared to the stratification period. Plotting δ¹³C-CH₄ values against δ²H-CH₄ values further suggests the occurrence of MOx during the mixing period as the data points show a distribution along the gradient of isotope enrichment (Figure 7). Beyond this the enrichment in ¹³C and ²H of lake water CH₄ during the mixing period (δ¹³C-CH₄ values of -39‰ and δ²H-CH₄ values of +11‰) compared to lake water values from the stratification period, the enrichment might also have originated from the input of groundwater CH₄ since inflowing and outflowing groundwater recorded extremely positive δ¹³C-CH₄ and δ²H-CH₄ values (Table 3). Groundwater CH₄ might therefore have constituted an important source to lake water CH₄ during the mixing period and contributed to the shift towards more positive δ¹³C-CH₄ and δ²H-CH₄ values of lake water during the mixing period, when CH₄ concentrations in the water column were much lower compared to the stratification period and the relative importance of groundwater on lake water CH₄ thus increases.

Methane supersaturation in the surface water layer

During the stratification period, fully oxygenated conditions in the epilimnion of Lake Willersinnweiher likely prevented efficient MOx in the upper water column, thus leading to the accumulation of CH₄ in the surface mixed water layer. The surface water of Lake Willersinnweiher with CH₄ concentrations ranging from 0.3 to 0.6 μmol L⁻¹ during the stratification period was oversaturated compared to the atmosphere, since water in equilibrium with the atmosphere

shows CH₄ concentrations of ~3 nmol L⁻¹ (Wiesenburg and Guinasso, 1979).

During the stratification period, the δ¹³C-CH₄ and δ²H-CH₄ values of CH₄ in the pelagic surface water were around -56‰ and -270‰, respectively. These values were significantly different from hypolimnetic δ¹³C-CH₄ values of -77‰ and δ²H-CH₄ values -323‰. This difference raises the question for the source of CH₄ supersaturation in the surface water layer (Figure 8A). The isotopic signatures of surface water CH₄ is likely altered by the mixing of different CH₄ pools, e.g., through littoral and vertical input, ebullition, groundwater interactions and internal oxic CH₄ production (e.g., Tang et al., 2016; Hartmann et al., 2020).

Vertical input of CH₄ from the sediment is strongly reduced in the lower metalimnion through MOx (Figure 3A). However, sedimentary CH₄ might still migrate into the upper water layer and become enriched in ¹³C and ²H through MOx, resulting in less negative surface water δ¹³C-CH₄ and δ²H-CH₄ values compared to hypolimnetic values. Transport of CH₄ from the littoral sediment to pelagic lake areas comprises an important source especially in small lakes with organic rich littoral zones, where CH₄ deriving from the sediment can be distributed in the surface water layer of the entire lake through turbulences, e.g., induced by wind activity (Tang et al., 2014). As thermal stratification builds up in the Lake Willersinnweiher, around 0.3 μmol L⁻¹ higher CH₄ concentrations can be observed in the littoral area of Lake Willersinnweiher than in the epilimnion of the pelagic and slope sites during the stratification period. Sediments in the littoral zone are usually not isolated through thermal stratification from the overlying water column compared to pelagic sites, thus often resulting in higher CH₄ concentrations in littoral waters (Loken et al., 2019). Higher sediment temperatures and disruption by waves fuel CH₄ production and release in the littoral zone during summer (Hofmann et al., 2010). A similar pattern of spatiotemporal CH₄ distribution was observed in other lakes and highlights the importance of the littoral area considering CH₄ emission to the atmosphere (Wang et al., 2006; Tsunogai et al., 2020). During maximum stratification, the isotopic CH₄ pattern of littoral surface water showed a δ¹³C-CH₄ value of -51‰ which is different from the values observed for the pelagic and slope sites (δ¹³C-CH₄ value of -56‰), whereas δ²H-CH₄ values were rather similar around -265‰, suggesting that sources of CH₄ in the surface water might differ between the littoral and deeper sites (Figure 8A). However, it is possible that mixing of littoral and vertical CH₄ input caused the observed more negative isotopic values of surface water CH₄ in the pelagic zone of the lake compared to the littoral area. A further potential source of CH₄ in the surface water layer of aquatic system is oxic CH₄ production despite the long-standing paradigm defining methanogenesis as a process occurring only under anoxic conditions (Grossart et al., 2011; Tang et al., 2014, 2016; Donis et al., 2017; Günthel et al., 2019; Hartmann et al., 2020;

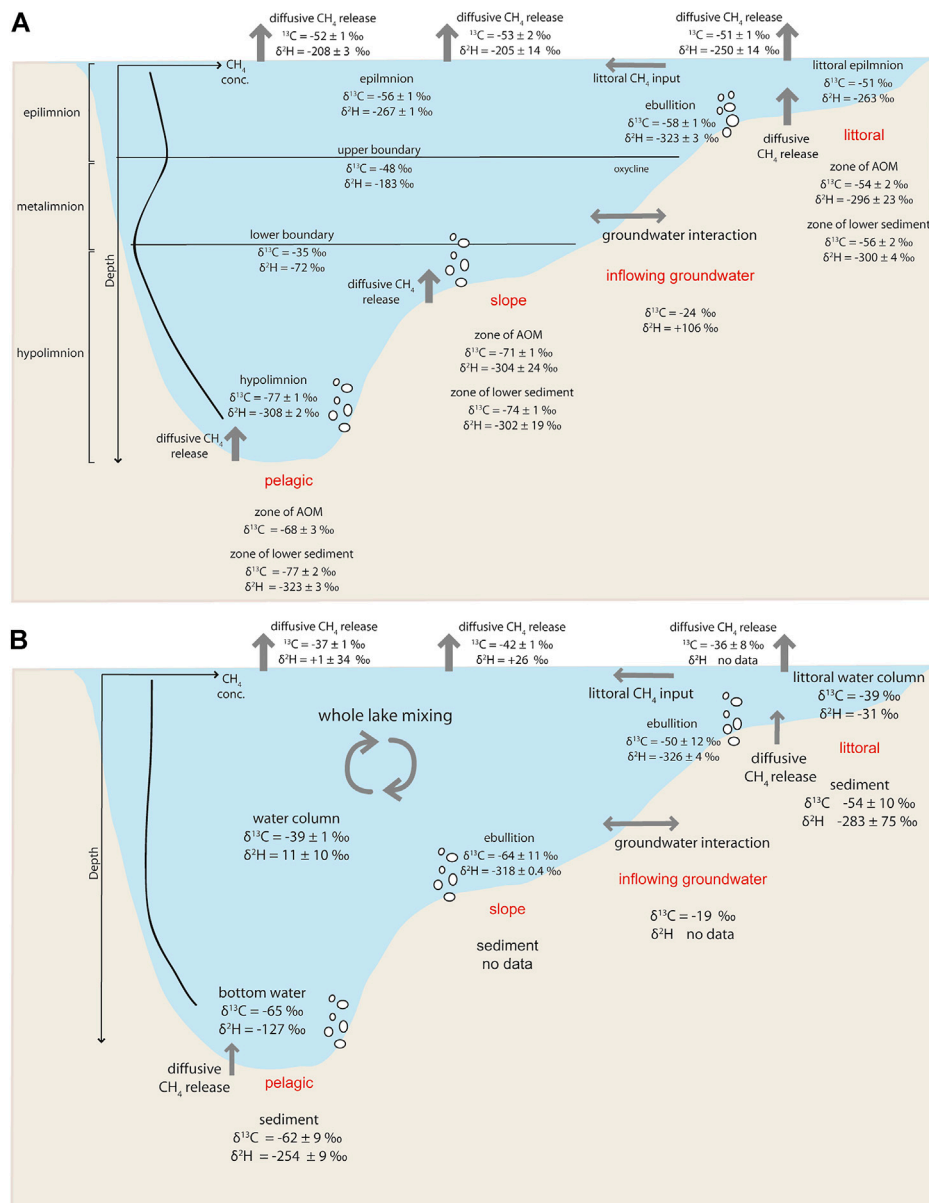


FIGURE 8 Methane cycling in Lake Willersinnweiher showing CH₄ sources and sinks with ^{δ13}C-CH₄ and ^{δ2}H-CH₄ values (where available) as well as transport mechanisms (grey) of different water layers (black) observed at the three investigated sites during (A) the stratification period (July 2020, for diffusive release at water-air interface data from July 2021 is shown and for ebullition data from September 2021) and (B) the mixing period (March 2021 and for ebullition data from November 2020 is shown).

Thottathil et al., 2022). In Lake Willersinnweiher, CH₄ in the oxic upper water column peaked at a water depth of 6 m during the stratification period, which was accompanied by an increase in the ^{δ13}C-CH₄ and ^{δ2}H-CH₄ values to -48‰ and -183‰, respectively, compared to surface water ^{δ13}C-CH₄ values of -57‰ and ^{δ2}H-CH₄ values of -269‰ (Figure 3A). Although the isotopic signature of CH₄ produced in the oxic water column is hitherto unknown, Hartmann et al. (2020)

proposed that oxic CH₄ production might cause an enrichment in ¹³C in the CH₄ pool in the lake surface layer. More recently, ^{δ13}C mass balance estimates by Thottathil et al. (2022) supported this finding by stating that CH₄ produced from oxic sources is relatively enriched in ¹³C compared to anoxic sources with ^{δ13}C-CH₄ values ranging between -64 and -38‰. More positive ^{δ13}C-CH₄ values in the surface mixed layer compared to sedimentary ^{δ13}C-CH₄ values have also been

found in other oxic lakes such as Lake Stechlin (-50% ; Tang et al., 2014), Lake Cromwell (-40% ; Bogard et al., 2014) and Lake Lugano (-55% ; Blees et al., 2014). These isotopic values are in line with the observed increase in $\delta^{13}\text{C}\text{-CH}_4$ values to around -48% in the upper metalimnion during the stratification period in Lake Willersinnweiher. The slight enrichment in ^{13}C might be due to different precursors and pathways of *in situ* oxic CH_4 production compared to anoxic methanogenesis and/or an interplay of co-occurring MOx. (Donis et al., 2017). Beyond $\delta^{13}\text{C}\text{-CH}_4$ values, better constraints on $\delta^2\text{H}\text{-CH}_4$ values of oxic CH_4 production would assist in the characterization of *in situ* produced CH_4 in the oxic environment as well as the possible influence of MOx in the observation of enriched stable isotope values of CH_4 .

The exact pathways of CH_4 formation in the oxic water column are not fully understood to date. However recently, Ernst et al. (2022) suggested a reaction mechanism for CH_4 formation associated to the interaction of reaction oxygen species with free iron and methylated sulfur and nitrogen compounds occurring on a cellular level across all living organisms and that increasing the level of oxidative stress enhanced the production of CH_4 . Moreover, it has been discovered that cyanobacteria produce CH_4 (Bižić et al., 2020b) and that light as well as temperature yield a significant control over oxic CH_4 production rates by influencing phytoplankton communities (Klitzsch et al., 2020). Furthermore, the role of submerged macrophytes for oxic methane production has so far been ignored, but might also contribute significantly (Hilt et al., 2022). Hence, oxic CH_4 production is most likely closely coupled to primary production, potentially contributing to an increase in CH_4 concentrations in the surface water layer during the stratification period as it was also observed at Lake Willersinnweiher. A peak in chlorophyll- α co-occurring with maximum CH_4 concentrations in the upper metalimnion at Lake Willersinnweiher might support a linkage between CH_4 production and algal activity as proposed already in other studies (Grossart et al., 2011; Bogard et al., 2014; Tang et al., 2016; Hartmann et al., 2020; Thottathil et al., 2022). Nevertheless, Donis et al. (2017) suggested that the accumulation of CH_4 at the thermocline can be caused by physical processes in the water column and the highest production rates of CH_4 can be found in the surface water layer.

Beyond the 2-dimensional stable isotope approach, we applied a novel stable isotopic indicator $\Delta(2,13)$ introduced by Tsunogai et al. (2020) which corrects for fractionation effects caused by CH_4 oxidation, to isotopically characterize different sources of CH_4 to the lake and disentangle the sources of CH_4 to the lake surface water layer. Since the $\Delta(2,13)$ indicator has been introduced only recently more research is necessary to reliably assess its strengths and weaknesses to constrain CH_4 sources and sinks in aquatic systems. Hence, we only briefly discuss the application of this parameter in this section (for a more detailed description see

Supplementary Annotations A1). $\Delta(2,13)$ values of the surface water layer showed good agreement at the pelagic and slope site ($266 \pm 4\%$ and $260 \pm 5\%$, respectively) but differed distinctively from the littoral water column and sediment ($214 \pm 2\%$ and $219 \pm 33\%$) (Supplementary Figure S2A). This might imply a different CH_4 source to the pelagic and slope surface water layer compared to the littoral site, making littoral input as the main CH_4 source in the entire lake surface water layer unlikely and potentially indicating internal oxic CH_4 production as an important source during the stratification period. However, it is important to note that $\Delta(2,13)$ only corrects for the fractionation effects caused by CH_4 oxidation and does not account for fractionation due to mixing of different CH_4 sources. Hence, mixing of vertically migrating sedimentary CH_4 and littoral CH_4 input might cause the observed shift in the epilimnic $\Delta(2,13)$ values of the pelagic and slope site compared to the littoral site.

Infiltrating groundwater might further contribute to CH_4 supersaturation in the surface water layer of Lake Willersinnweiher and most likely influenced lake water CH_4 during the entire year due to the lake's direct accessibility to the upper aquifer and high groundwater CH_4 concentrations around $1 \mu\text{mol L}^{-1}$ (Wollschläger et al., 2007). Possibly, groundwater CH_4 yielding less negative $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values could also have contributed to the observed enrichment in ^{13}C and ^2H at the upper thermocline. So far the origin of the groundwater CH_4 remains unclear and different possibilities have been proposed to explain the source of CH_4 in the inflowing groundwater and its unusually positive isotopic signature including CH_4 production in a contamination site through oxidative biodegradation of hydrocarbons such as volatile chlorinated organic compounds, in organic-rich sediments from old branches of the Rhine river or in aquifers located upstream of Lake Willersinnweiher (Wollschläger et al., 2007; Kleint et al., 2021). Only limited data about groundwater CH_4 concentrations and $\delta^{13}\text{C}\text{-CH}_4$ values are available to date and groundwater that enriched in $^{13}\text{C}\text{-CH}_4$ and $^2\text{H}\text{-CH}_4$ seems to be only a rare occurrence (Schloemer et al., 2016). The highly enriched isotopic values of infiltrating groundwater might point to intense AOM since the groundwater is suboxic. The oxidation might therefore be coupled to the reduction of other electron acceptors such as nitrate, nitrite, manganese and iron (Kleint et al., 2021). Methane oxidation causing the highly enriched isotopic composition of groundwater CH_4 might also be supported by the 2-dimensional isotope plot, revealing a distribution of inflowing and outflowing groundwater along the gradient of CH_4 oxidation at Lake Willersinnweiher (Figure 7).

Mixing of lake and groundwater might lead to a change towards more positive isotope values in the lake water column, however the impact of groundwater CH_4 on lake water CH_4 is hard to estimate based on the current dataset and more detailed research concerning groundwater CH_4 in the area of Lake

Willersinnweiher is desirable. Due to lower primary productivity and lower CH₄ concentrations in the lake during the mixing period we suggest that groundwater input might be an important source of CH₄ to the lake especially during the mixing period. This might also contribute to the less negative isotopic composition of lake water CH₄ observed during the mixing period (Figures 3G, 4G, 5G).

Supersaturation of CH₄ in the surface water leads to CH₄ release into the atmosphere during the stratification period. Additionally, the surface water was also oversaturated with CH₄ during the mixing period (70 nmol L⁻¹, Figures 3G, 4G, 5G), highlighting that Lake Willersinnweiher emits CH₄ all year round. Methane can be emitted *via* plant-mediated processes, diffusion or ebullitive transport (Bastviken et al., 2004), however since plant-coverage at Lake Willersinnweiher is negligible, plant-mediated release of CH₄ to the atmosphere is considered to be of minor importance. Methane emitted *via* diffusion from the surface water into the atmosphere showed a great range in the isotopic composition over 1 year with most positive values found at the three investigated sites during the mixing period ($\delta^{13}\text{C-CH}_4$ of -42 to -37‰ and $\delta^2\text{H-CH}_4$ of $+1$ to $+26\text{‰}$) and distinctively lower values observed during the stratification period ($\delta^{13}\text{C-CH}_4$ of -59 to -51‰ and $\delta^2\text{H-CH}_4$ of -310 to -195‰ , Table 2). These isotopic values reflect the isotopic composition of lake surface water, which is enriched in the heavier isotopes during the mixing period. Especially during the mixing period, $^{13}\text{C-CH}_4$ and $^2\text{H-CH}_4$ were much more enriched compared to previously reported values (e.g., from Thottathil and Prairie, 2021 ranging from -58.8‰ to -43.5‰), highlighting the potential impact and importance of diffusive CH₄ emissions from lakes with similar properties, such as high sulfate concentrations, for regional and/or global isotope source apportionment studies.

We found that in Lake Willersinnweiher, $\delta^{13}\text{C-CH}_4$ and $\delta^2\text{H-CH}_4$ values of CH₄ released *via* ebullition displayed temporal and spatial disparities (Figure 8). At the deeper sampling site, CH₄ transported *via* ebullition yielded more negative $\delta^{13}\text{C-CH}_4$ values ($-64 \pm 11\text{‰}$) when compared to $\delta^{13}\text{C-CH}_4$ values at the littoral site ($-50 \pm 12\text{‰}$ during the mixing period in November 2021). These differences in the isotopic composition were most likely induced by CH₄ formation taking place mainly *via* the hydrogenotrophic and acetoclastic pathways at the slope and littoral sites, respectively, as discussed above. Ebullition plays a particularly important role in the littoral area of aquatic systems since the potential for CH₄ transported *via* gas bubbles rises to reach the surface water with decreasing water depth. In particular, CH₄ release to the atmosphere *via* ebullition likely represents an important pathway and has been found to vary significantly spatially and temporally (West et al., 2016; Thottathil and Prairie, 2021). Interestingly, $\delta^2\text{H-CH}_4$ values only show small differences between the littoral and slope site at Lake Willersinnweiher ($326 \pm 4\text{‰}$ for the littoral site and $318 \pm 1\text{‰}$ for the slope

site, Figure 8B). In previous studies, changes in the $\delta^2\text{H-CH}_4$ values of CH₄ emitted *via* ebullition were attributed to changes in the $\delta^2\text{H}$ values of water (e.g. due to evaporation in shallow lakes), which is a hydrogen source for methanogens (Wik et al., 2020). As Lake Willersinnweiher is a distinctively deeper lake (average depth of 8 m), this effect might only play a minor role and therefore have a smaller influence on $\delta^2\text{H-CH}_4$ values. However, Douglas et al. (2021) showed that even though the $\delta^2\text{H-H}_2\text{O}$ values highly influenced $\delta^2\text{H-CH}_4$ values, other processes such as methane oxidation and the type of the methanogenic pathway might also contribute to the isotopic composition of CH₄. Furthermore, sulfate-dependent AOM in the sediments of lake Willersinnweiher might play an important role regarding the isotopic composition of CH₄ released *via* ebullition and therefore might affect $\delta^{13}\text{C-CH}_4$ values and to a lesser extent $\delta^2\text{H-CH}_4$ values (Wegener et al., 2021). However, the influence of AOM is hard to estimate as AOM most probably takes place heterogeneously throughout the sediment. In November, when there is less organic matter input than in September (which is also indicated by the lower CH₄ concentrations within the gas bubbles, Supplementary Table S2), pockets of CH₄ within the sediment might be subject to heterogeneously occurring AOM, which might contribute to the relatively high variability of $\delta^{13}\text{C-CH}_4$ values. During the uprise of gas bubbles in the water column, CH₄ partly dissolves into the water (e.g. McGinnis et al., 2006). However, isotope fractionation associated with dissolution of CH₄ was found to be small and most likely lower than the variability of the measured isotope values (Fuex, 1980; Bergamaschi, 1997). Additionally, the surface water layer showed isotopic values enriched in ^{13}C and ^2H compared to the gas bubbles ($\delta^{13}\text{C-CH}_4$ of -56‰ and $\delta^2\text{H-CH}_4$ of -270‰ for lake surface water and $\delta^{13}\text{C-CH}_4$ of $-64 \pm 11\text{‰}$ and $\delta^2\text{H-CH}_4$ of $-318 \pm 1\text{‰}$ for ebullitive CH₄). We therefore conclude that ebullition is not the main source of surface water CH₄ supersaturation in the pelagic area of the lake.

Conclusion

We investigated the sources and sinks of CH₄ in a seasonally stratified freshwater lake using concentration measurements of CH₄ and dual isotope analysis. Our main findings are presented in Figures 8A,B for the stratification period and the mixing period:

- $\delta^{13}\text{C-CH}_4$ and $\delta^2\text{H-CH}_4$ values of sedimentary CH₄, anaerobic methanogenesis at the three investigated sites indicated a mix of the hydrogenotrophic and acetoclastic pathway at the pelagic and slope site and an increasing dominance of the acetoclastic methanogenesis towards the shallower littoral site. During the mixing period, the dominance of hydrogenotrophic methanogenesis

potentially increased at the pelagic and slope sites. Throughout the whole year, sulfate-dependent AOM caused shifts towards heavier $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values in the upper sediment and reduced the diffusive release of CH_4 at the sediment-water interface.

- MOx efficiently consumed upward migrating CH_4 in the water column during thermal stratification of the lake, indicated by a strong enrichment of $^{13}\text{C}\text{-CH}_4$ and $^2\text{H}\text{-CH}_4$ values.
- Supersaturation of CH_4 in the surface water was present both during the stratification period and the mixing period, whereas supersaturation during the stratification period was significantly higher than supersaturation during the mixing period. Supersaturation of CH_4 in the pelagic area of the lake possibly originated from the vertical and lateral input of sedimentary and littoral CH_4 , however oxic CH_4 production might also supply CH_4 to the surface water layer of the lake and might contribute to a local peak in concentrations at the upper thermocline during the stratification period. On the contrary, CH_4 input from groundwater with unusually positive $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values and CH_4 diffusion from the littoral sediment were most likely the main source of lake water CH_4 during the mixing period, leading to rather positive isotopic $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values in the water column.
- Methane released from the water into the atmosphere *via* diffusion covered a wide range from -59 to -36% for $\delta^{13}\text{C}\text{-CH}_4$ and -310 to $+26\%$ for $\delta^2\text{H}\text{-CH}_4$ values during the stratification period and mixing of the water column during the mixing period, highlighting the potential importance of diffusive CH_4 emissions from lakes with similar properties for regional and/or global isotope source apportionment studies.

Our study provides an overview of the isotopic characteristics of CH_4 sources and sinks in a seasonally stratified lake and gives new insight into the sources contributing to CH_4 supersaturation in the surface water layer of lacustrine systems. It furthermore outlines the potential for disentangling different sources and sinks both spatially and temporally based on their isotopic signature, especially considering the still highly debated contribution of oxic CH_4 production to lake water CH_4 concentrations. This clearly highlights the need for a multiparameter approach including the investigation of stable isotopes when researching the complex CH_4 dynamics in an aquatic system since only then can a complete understanding of the involved processes be achieved.

Data availability statement

The experimental data used in this study are available from *heiDATA*, which is an institutional repository for research data

of the Heidelberg University (<https://doi.org/10.11588/data/KLFDVF>). Requests to access the datasets should be directed to teresa.einzmann@unibas.ch.

Author contributions

FK, TE, and MS conceived the study. TE and MS performed the field measurements and experiments under supervision of FK. TE and MS analyzed the data and visualized the results. MG validated stable isotope measurements of methane and analyzed stable carbon isotope measurements of dissolved inorganic carbon. TE, MS, FK, and JK discussed the results. The manuscript was written under the lead of TE and MS, with contribution of all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.865862/full#supplementary-material>

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