



Methane in Lakes: Variability in Stable Carbon Isotopic Composition and the Potential Importance of Groundwater Input

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Methane (CH₄) is an important component of the carbon (C) cycling in lakes. CH₄ production enables carbon in sediments to be either reintroduced to the food web *via* CH₄ oxidation or emitted as a greenhouse gas making lakes one of the largest natural sources of atmospheric CH₄. Large stable carbon isotopic fractionation during CH₄ oxidation makes changes in ¹³C:¹²C ratio (δ¹³C) a powerful and widely used tool to determine the extent to which lake CH₄ is oxidized, rather than emitted. This relies on correct δ¹³C values of original CH₄ sources, the variability of which has rarely been investigated systematically in lakes. In this study, we measured δ¹³C in CH₄ bubbles in littoral sediments and in CH₄ dissolved in the anoxic hypolimnion of six boreal lakes with different characteristics. The results indicate that δ¹³C of CH₄ sources is consistently higher (less ¹³C depletion) in littoral sediments than in deep waters across boreal and subarctic lakes. Variability in organic matter substrates across depths is a potential explanation. In one of the studied lakes available data from nearby soils showed correspondence between δ¹³C-CH₄ in groundwater and deep lake water, and input from the catchment of CH₄ *via* groundwater exceeded atmospheric CH₄ emissions tenfold over a period of 1 month. It indicates that lateral hydrological transport of CH₄ can explain the observed δ¹³C-CH₄ patterns and be important for lake CH₄ cycling. Our results have important consequences for modelling and process assessments relative to lake CH₄ using δ¹³C, including for CH₄ oxidation, which is a key regulator of lake CH₄ emissions.

Keywords: stable carbon isotope, methane, lake, groundwater, endmember

1 INTRODUCTION

Organic matter (OM) transported from catchments is actively processed and transformed in lakes, leading to burial in sediments, carbon (C) gas release to the atmosphere, or downstream transport *via* rivers ultimately to the sea (Cole et al., 2007; Tranvik et al., 2018). Methane (CH₄) production (methanogenesis) plays an important role in the overall processing of OM in lakes by allowing large amounts of OM buried in the sediments to be remineralized, with methanogenesis found to correspond to 20–56% of whole lake C mineralization (Bastviken, 2009). Remineralized OM can

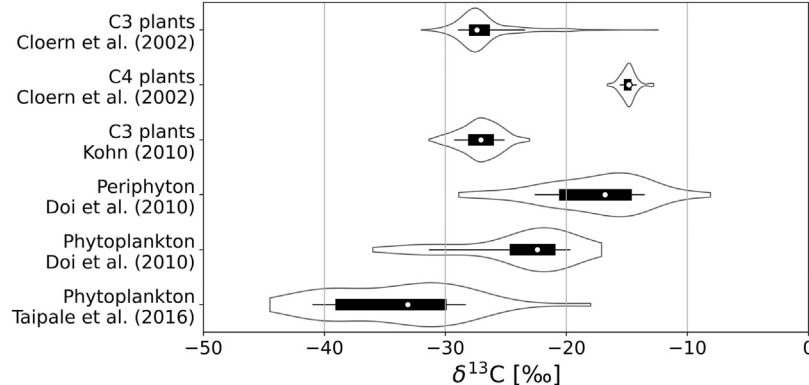


FIGURE 1 | Ranges of $\delta^{13}\text{C}$ values observed in C3 and C4 plants, in phytoplankton and in benthic algae (periphyton) in previous studies. In each distribution, the white dot indicates the median, the thick black bar indicates the interquartile range, the thin black line indicates the range of values between the 10- and 90-percentiles, and the wavy curves indicate the Gaussian kernel density estimate of the distribution of values.

subsequently either be emitted to the atmosphere, directly as CH_4 (Bastviken et al., 2004) or as carbon dioxide (CO_2) after oxidation of CH_4 by methanotrophic bacteria, or it can be reintroduced in lake food webs *via* the methanotrophic biomass (Bastviken et al., 2003; Jones and Grey, 2011; Grey, 2016). With respect to emissions, lakes represent one of the largest natural sources of atmospheric CH_4 (Saunois et al., 2020), yet extensive in-lake CH_4 oxidation (MOX) is believed to mitigate as much as 50% and up to more than 90% of the potential CH_4 emissions, making MOX a key process for the global CH_4 cycling (Bastviken, 2009; Reeburgh, 2014). From the food web perspective, CH_4 -C has been found important to zooplankton during some seasons (Taipale et al., 2009) and has been detected in fish biomass (Sanseverino et al., 2012).

The measurement of the stable isotope ratio of ^{13}C to ^{12}C in different compartments of the C cycle in lakes has been a powerful tool in the past decades to study the fate of C derived from methanogenesis (e.g., Kankaala et al., 2006; Tsuchiya et al., 2020). The $^{13}\text{C}:^{12}\text{C}$ ratio is often expressed relative to a standard (Vienna Pee Dee belemnite with a $^{13}\text{C}:^{12}\text{C}$ ratio of 0.0112372) according to

$$\delta^{13}\text{C} = \left(\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{sample}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{standard}}} - 1 \right) \cdot 1000 (\text{‰}) \quad (1)$$

The quality of $\delta^{13}\text{C}$ as a tracer of C derived from methanogenesis stems from the fact that biogenic CH_4 is highly depleted in $\delta^{13}\text{C}$, such that $\delta^{13}\text{C}$ values in biogenic CH_4 ($\delta^{13}\text{C}\text{-CH}_4$) are low (-110‰ to -50‰ ; Whiticar, 1999) compared to atmospheric CO_2 (-7‰ ; Peterson and Fry, 1987) and primary producers obtaining their C from atmospheric CO_2 , like terrestrial plants (-27‰ in average for C3 plants; Kohn, 2010). Even in algae assimilating C that has been depleted in ^{13}C isotope by prior metabolic processes, $\delta^{13}\text{C}$ values stay above -45‰ in lakes (de Kluijver et al., 2014). **Figure 1** shows the range of $\delta^{13}\text{C}$ values observed in several sources of organic matter to the sediments of lakes. All these values are higher than -45‰ .

Therefore, microbially produced CH_4 is one of the most ^{13}C -depleted C-compounds found in nature, facilitating the study of its fates based on measurements of $\delta^{13}\text{C}$.

An important methodological requirement for studies using $\delta^{13}\text{C}$ to investigate C cycling in lakes is to determine a reference $\delta^{13}\text{C}\text{-CH}_4$ value for the source of CH_4 , sometimes referred to as the endmember $\delta^{13}\text{C}$ signal representative of the CH_4 produced in the sediments. Just as the enzyme systems involved in methanogenesis discriminate strongly against ^{13}C , this is also the case for enzymatic reactions by methanotrophic microorganisms leading to MOX. As a result, the pool of CH_4 that remains after being exposed to MOX gradually gets more enriched in ^{13}C (i.e., $\delta^{13}\text{C}\text{-CH}_4$ becomes less negative) the greater proportion is oxidized while CO_2 and microbial biomass formed by MOX get more ^{13}C depleted. Accordingly, the difference between endmember $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^{13}\text{C}$ of partially oxidized CH_4 pools can be used to estimate the fraction of the original CH_4 being oxidized. In addition, $\delta^{13}\text{C}$ measured in C pools can be compared to the source $\delta^{13}\text{C}\text{-CH}_4$ to estimate the fraction of C that has a methanogenic origin. Therefore, $\delta^{13}\text{C}$ analyses are very useful to quantify complex ecosystem processes involving CH_4 from measurements of limited numbers of samples. However, the success of this approach critically depends on accurate and reliable knowledge of the relevant endmember $\delta^{13}\text{C}\text{-CH}_4$ values.

Several factors can affect the endmember $\delta^{13}\text{C}\text{-CH}_4$ in sediments of lakes. One is the bacterial community composition, and more specifically the share of different methanogenic pathways that are active (Conrad, 2005). The two main methanogenic pathways are based on CO_2 reduction (hydrogenotrophic methanogenesis; HM) and acetate dependent (acetoclastic methanogenesis; AM), and different enzyme systems can fractionate (i.e., discriminate) differently against ^{13}C . A second aspect is the $\delta^{13}\text{C}$ composition of the substrates used by methanogens (Conrad, 2005). This applies to organic substrates, and to the CO_2 or dissolved inorganic C (DIC) used in HM (dissolved CO_2 is connected to the whole DIC pool *via* the carbonic acid chemical equilibrium system). Bacterial communities and substrate composition differ from

TABLE 1 | Characteristics of the lakes investigated in the present study.

Lake		Stortjärn (STJ)	Stora galten (SGA)	Nästjärn (NAS)	Ljusvattentjärnen (LJR)	Parsen (PAR)	Venasjön (VEN)
Location		64.262° N 19.763° E	58.245° N 12.040° E	64.150° N 18.800° E	64.093° N 18.929° E	58.341° N 16.204° E	58.456° N 16.189° E
Maximum depth (m)		6.7	22	10	9	7.5	11
Area (ha)		4	31	1	1	14	68
DOC (mg/L)	Surface	21 (±3)	4.5 (±0.1)	6.4 (±0.7)	9.0 (±1.3)	16 (±1)	15 (±1)
	Bottom	25 (±2)	3.8 (±0.2)	11.5 (±0.7)	13.5 (±1.5)	19 (±1)	18 (±3)
TN (mg/L)	Surface	0.34 (±0.01)	0.26 (±0.02)	0.27 (±0.02)	0.26 (±0.04)	0.64 (±0.04)	0.99 (±0.02)
	Bottom	0.45 (±0.00)	0.51 (±0.15)	1.0 (±0.1)	0.69 (±0.15)	0.85 (±0.10)	2.3 (±0.2)
TP (µg/L)	Surface	25 ^a (±9)	6.2 (±1.7)	11 (±5)	8.9 (±1.3)	18 (±5)	43 (±2)
	Bottom	45 ^a (±18)	8.2 (±2.9)	33 (±9)	95 (±43)	29 (±22)	330 (±280)
Chl a (µg/L)	Surface	—	3.0 (±0.9)	3.2 (±1.2)	0.93 (±0.19)	5.6 (±2.7)	38 (±7)
Δ($\delta^{13}\text{C-CH}_4$) (‰)		16.0	13.4	11.8	10.0	7.2	3.5

^aTotal phosphorus in Stortjärn was not measured on the same days as $\delta^{13}\text{C-CH}_4$.

Concentrations are averages (±1 standard deviation) from monthly sampling over the summer stratification period of each lake. Abbreviations: DOC, dissolved organic carbon; TN, total nitrogen; TP, total phosphorus; Chl a, Chlorophyll a; Δ($\delta^{13}\text{C-CH}_4$), $\delta^{13}\text{C-CH}_4$ difference between bubbles in littoral sediments and deep, anoxic hypolimnion water in August.

one lake to another, leading to varying $\delta^{13}\text{C-CH}_4$ endmember values in different systems (Rinta et al., 2015).

Recent results from a few subarctic lakes also indicate pronounced differences in endmember $\delta^{13}\text{C-CH}_4$ within lakes, e.g., between littoral and profundal regions (Cadieux et al., 2016; Thompson et al., 2016; Wik et al., 2020). Such variability would have important implications for the applicability of the $\delta^{13}\text{C}$ approach to trace CH_4 -related C cycling but has rarely been investigated systematically across different lake types and it is not clear if the observed patterns are widespread and consistent features of lakes or specific to the few subarctic systems studied so far. In this study, we compare $\delta^{13}\text{C-CH}_4$ values in littoral sediments, where the water was approximately 1 m deep, and in the deep, anoxic hypolimnion in the profundal zone, representing commonly used endmembers for estimation of MOX near the oxyclines of sediments or water columns, of six boreal lakes with different characteristics. We also carry out more detailed studies of one of the lakes where information about CH_4 input *via* groundwater (GW) from an adjacent mire could be generated. We hypothesize that $\delta^{13}\text{C-CH}_4$ values in deep, anoxic water are more negative than in littoral sediments, as previous observations mentioned above have shown, but that the magnitude of the difference increases in lakes with higher contrasts in the composition of OM reaching littoral and profundal sediments.

2 MATERIALS AND METHODS

2.1 Study Sites

Samples were collected in six lakes across Sweden during the ice-free season in 2017, 2018, and 2019 (see Table 1 for their characteristics). STJ, LJR, and NAS are small lakes, located in the same region in the north of Sweden, that cover a range of dissolved organic C (DOC) levels. They are surrounded by coniferous forests (Scots pine and Norwegian spruce) with some presence of birch. Human activities around these lakes are connected to forestry. PAR and VEN are located in the

southeastern part of Sweden. PAR is a small humic lake surrounded by a forest where Scots pine and Norwegian spruce dominate, and birch is also present. VEN is a relatively big and eutrophic lake. It is surrounded by some forested areas and agricultural land. SGA is a middle-size, deep, and clear lake located in the southwestern part of Sweden. It is surrounded by forests with a majority of Scots pines and Norwegian spruces and some birches.

All lakes were sampled monthly during the summer stratification period (except STJ, where sampling was conducted several times but only in August) after some CH_4 had accumulated in anoxic parts of the water column close to the bottom to ensure a minimum influence of MOX on the deep water CH_4 sampled.

2.2 Sampling and Analyses Common to All Lakes

2.2.1 $\delta^{13}\text{C}$ Values of CH_4

The $\delta^{13}\text{C-CH}_4$ at the bottom of the lakes was measured in anoxic water less than 1 m above the sediments, near the deepest point of each lake. A possibility exists that $\delta^{13}\text{C}$ values measured in dissolved CH_4 in the water column above the sediments differ from $\delta^{13}\text{C-CH}_4$ values in the sediment bubbles if anaerobic oxidation of CH_4 (AOM) occurs at the interface between sediments and water column. However, the net effect of AOM would be to increase $\delta^{13}\text{C-CH}_4$ values in the water overlaying the sediments compared to $\delta^{13}\text{C-CH}_4$ values in the sediments themselves (Rinta et al., 2015; Marcek et al., 2021). Therefore, using our initial assumption that $\delta^{13}\text{C-CH}_4$ values are lower in the deep water of the profundal zone than in littoral sediment bubbles, differences in $\delta^{13}\text{C-CH}_4$ values between littoral sediment bubbles and dissolved CH_4 at the bottom of the water column provide a conservative estimate of the difference in $\delta^{13}\text{C-CH}_4$ values between profundal and littoral sediment bubbles.

Headspace extraction was used to collect $\delta^{13}\text{C-CH}_4$ samples from the deep water. Three methods were applied for the extraction. A first method consisted of sampling water at a

given depth using a plastic Ruttner-type water sampler equipped with a tubing at the bottom. The water was then transferred to a 1.2 L plastic bottle by inserting the tube of the water sampler down to the bottom of the bottle. To prevent the build-up of air bubbles in the bottle and the exchange of gas between the water and the air during the transfer, the bottle was overflowed with 2 L of water. The bottle was then closed with a rubber stopper equipped with two tubes attached to three-way luer-lock valves on the outside. A headspace was created in the bottle by injecting 60 ml of synthetic air containing only molecular nitrogen (N_2), O_2 and argon, and by simultaneously retrieving 60 ml of water through the tubes. The synthetic air used for the headspace was the same as the gas used as carrier by the instrument on which these samples were analyzed (see below). The bottle was then shaken manually for 3 min to equilibrate the gas in the water with the newly created headspace. Finally, the equilibrated gas in the headspace was retrieved from the bottle using a 60 ml syringe (Becton Dickinson, Franklin Lakes, New Jersey, United States) and transferred to a glass vial capped with a 10 mm butyl rubber stopper. The vial was manually evacuated using another 60 ml syringe right before transferring the sample. The residual CH_4 concentration in the vial after evacuation did not affect the measured $\delta^{13}C-CH_4$ because of the high CH_4 concentration in samples stored in manually evacuated vials (concentrations in the vials ranged between 76 ppm and 137000 ppm, and the residual gas only contained atmospheric CH_4 concentration). Also, the presence of O_2 in the vial did not cause CH_4 to be oxidized because only gas and no water was injected. Upon sample injection in the vial, an overpressure was created in the vial by injecting a larger volume of gas than the volume of the vial. A second extraction method consisted of sampling 60 ml of water at a given depth either through a long tube attached to a weight and connected to a 60 ml syringe at the surface, or by transferring water collected with the Ruttner sampler to a 60 ml syringe. Water was then transferred from the syringe to a 118 ml vial that had been flushed, filled with N_2 , and amended with 0.2 ml of 85% H_3PO_4 prior to sampling. The acid was added because the vials were also used to collect samples for DIC, and to prevent biological production and oxidation of CH_4 in the vials. When prefilling the vials with N_2 an overpressure was created, which was released just before injecting the sample. Thereby, the total pressure of the vial headspace after sample injection could be calculated from the total barometric pressure when sampling, the sample volume, and temperature, and the equilibrated headspace constituted the gas sample analyzed. The third extraction method consisted in collecting 30 ml of water and 30 ml of air in a 60 ml syringe. CH_4 was extracted from the water to the headspace by shaking the syringe for at least 1 min and the headspace air was transferred to a manually pre-evacuated vial. When using this method, a sample of atmospheric air was taken separately to correct for the effect of atmospheric CH_4 on the concentration and $\delta^{13}C-CH_4$ in the sample extracted from the water.

Bubbles from littoral sediments were collected on the same days as deep-water samples. Locations where bubble samples were taken were not always similar among sampling occasions in each lake. The sampling procedure consisted in holding a plastic

funnel connected to a 60 ml syringe upside down under water, physically disturbing the sediment under the funnel, and collecting released bubbles in the funnel syringe as they rose towards the surface. The collected gas was injected to a manually pre-evacuated vial (12–30 ml). Enough gas was transferred to the vial to create a minimum of 10 ml overpressure.

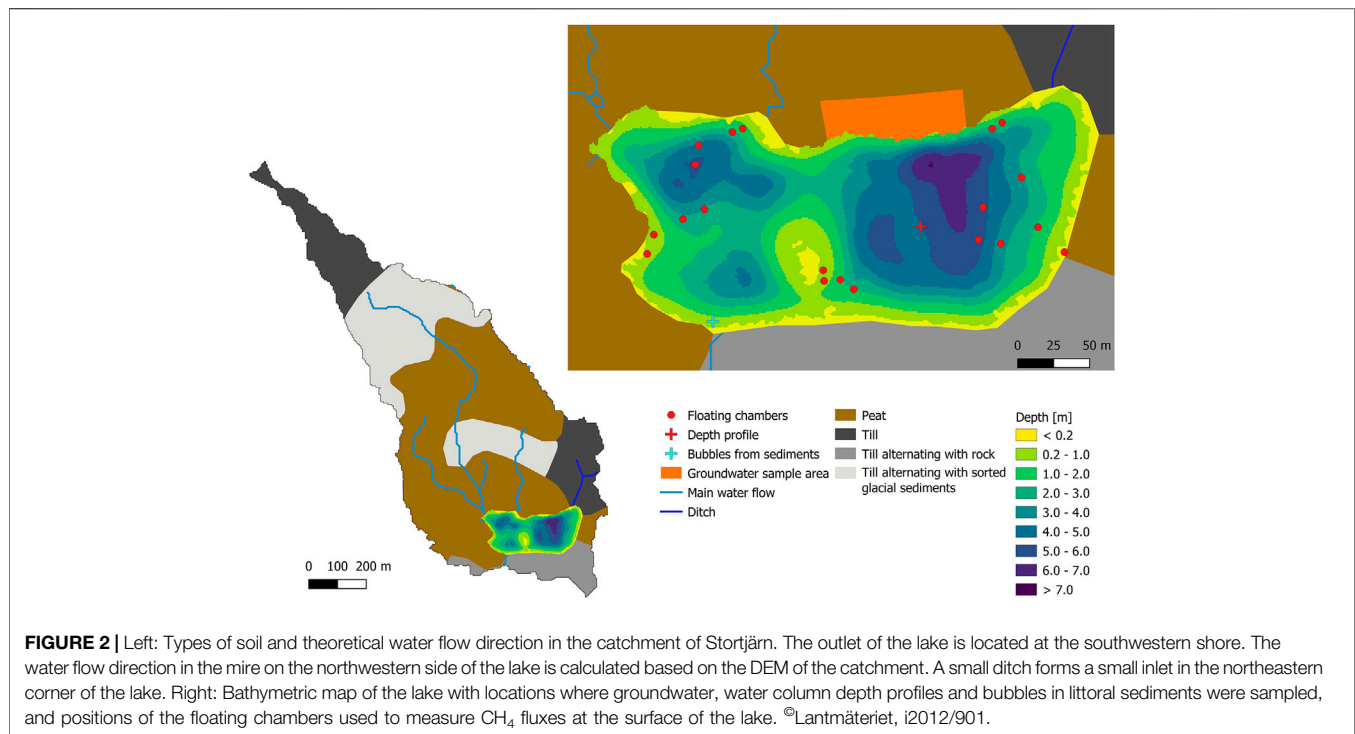
$\delta^{13}C-CH_4$ samples were analyzed on a cavity ring-down spectrometer (G2201-I Isotopic Analyzer, Picarro, Santa Clara, California, United States). Samples with high concentration of CH_4 were diluted with high purity synthetic air (80% N_2 and 20% O_2) to fall in the optimal range of concentrations for the instrument. To correct for non-linearity and guarantee the accuracy of the measurements in this range of concentrations, calibration curves were derived from the analysis of two standards with $\delta^{13}C-CH_4$ values of $-66.5 \pm 0.2\text{‰}$ and $-23.9 \pm 0.2\text{‰}$ (Isometric Instruments, Victoria, British Columbia, Canada), that were diluted to 20–25 different concentrations ranging logarithmically from 1 to 200 ppm CH_4 . All values obtained after the analysis were corrected according to the calibration curves.

2.2.2 Water Chemistry

In all lakes except for STJ, where water chemistry is routinely monitored by the research station managing the site, water samples for DOC, DIC, total nitrogen (TN), total phosphorus (TP), and Chlorophyll a (Chl a) analysis were collected on the same days as the $\delta^{13}C-CH_4$ samples. Water was collected near the deepest point of each lake at 1 m depth and 1 m above the sediments using a Ruttner sampler equipped with a tubing at the bottom. For each depth, water was transferred to one 4 L cubitainer for DOC, DIC, TN, and Chl a analysis and two 125 ml HDPE bottles for TP analysis. The samples were kept in cooling boxes in the field. Within the same day as collection, a part of the water from the cubitainers was directly transferred to 50 ml PP tubes (Sarstedt, Nümbrecht, Germany) and stored at 4°C for subsequent TOC and TN analysis. Another part of the water from the cubitainers was filtered using pre-combusted 0.7 μm pore size grade GF/F filters (Whatman, Maidstone, United Kingdom) and the filtrate was stored in 50 ml PP tubes at 4°C for subsequent DOC and DIC analysis. The remaining water was filtered using 1.2 μm nominal pore size grade GF/C (Whatman) filters that were frozen immediately after filtration for storing them until subsequent Chl a analysis.

DOC, DIC, and TN samples were analyzed with a TOC/TN analyzer (Shimadzu, Kyoto, Japan). Samples containing more than 20 mg TOC/L were diluted with deionized water before analysis. Standard samples containing 10 mg TOC/L and 1, 2 or 5 mg TN/L were also analyzed and were used to correct the results from the lake samples. TP samples were analyzed on a continuous flow analyzer (AutoAnalyzer II, Bran + Luebbe) according to the AutoAnalyzer method no. G-175-96 Rev. 16 based on Murphy and Riley (1962). Chl a was extracted from the GF/C filters in methanol and measured by spectrophotometry following the Swedish Standard SS 28170, which is based on Richards and Thompson (1952).

Based on the measured concentrations of DIC, a value expressing the relative contribution of respired C to the DIC



pool in the hypolimnion, the hypolimnetic excess DIC, was calculated as:

$$\text{Hypolimnetic excess DIC} = \frac{DIC_{\text{bottom}} - DIC_{\text{surface}}}{DIC_{\text{surface}}} \quad (2)$$

where DIC_{bottom} and DIC_{surface} are the concentrations of DIC at the bottom and surface of the lake, respectively.

2.3 Extended Sampling and Analyses in Stortjärn

At STJ extended sampling was carried out warranting additional description. STJ is located in the Krycklan Catchment Study area in northern Sweden (Laudon et al., 2013). The lake has a maximum and mean depth of 6.7 and 2.7 m, respectively (Denfeld et al., 2018). It is separated in two main basins along a north-south axis, the western basin being about half the size of the eastern basin (Figure 2). More than half of the shoreline of the lake is covered by a Sphagnum mire (Figure 2). The lake catchment has as surface area of 65 ha and is composed at 39.5% of wetland and at 54% of forest (Figure 2; Laudon et al., 2013). The main types of soils in the catchment are till deposits covered locally by thin forest soil and peat. Average yearly precipitation in the catchment is 614 mm (Laudon et al., 2013).

The additional mapping of lake CH₄ fluxes of relevance carried out in STJ included water samples taken in the mire to measure δ¹³C-CH₄ in the GW, measurements of daily CH₄ emissions at the water-atmosphere interface of the lake using floating

chambers, and estimation of daily input of CH₄ from the mire using a water balance of the lake.

2.3.1 CH₄ Concentration in the Water

Water samples for CH₄ concentration were collected using a 10 ml plastic syringe (Becton Dickinson). After rinsing the syringe, 5 ml of water were collected at 10 cm depth making sure that no air bubble was introduced in the syringe. The water was transferred to a 22 ml vial capped with a butyl rubber stopper that had been amended with 0.1 ml of 85% H₃PO₄ for sample preservation and then flushed and filled with N₂ prior to sampling. The vials were prepared with a N₂ overpressure that was released just prior to injecting the water sample to equilibrate the pressure towards the present barometric pressure and ensure that vials did not leak before used for samples. The concentration of dissolved CH₄ in the water was calculated from the sum of 1) the CH₄ content in the vial headspace measured with gas chromatography (GC System 7890A with a 1.8 m × 3.175 mm Porapak Q 80/100 column from Supelco, a methanizer and a flame ionization detector, Agilent Technologies, Santa Clara, California, United States) and determined using the common gas law, and 2) remaining CH₄ dissolved in the water in the vial determined from headspace concentration using Henry's law and the temperature adjusted Henry's solubility constant for CH₄ in freshwater.

2.3.2 CH₄ Emissions to the Atmosphere

The rate of CH₄ emissions at the lake's surface was measured at different locations using flux chambers (Bastviken et al., 2004). These flux chambers were made of plastic buckets (6 L, 800 cm² opening area) equipped with floats and placed at the surface of the lake. The

flux of CH₄ between the surface water and the atmosphere was determined by measuring the change in CH₄ concentration in the chamber over time. Each chamber was attached to a separate float connected to an anchor to allow the chambers to move freely with waves and water level changes. When chambers were deployed, atmospheric air samples and surface water samples were collected near some of the chambers. Atmospheric air samples taken 20 cm above the surface of the water were used to represent initial concentration in the chambers. Surface water samples were collected as described previously. After a deployment period, each chamber headspace was sampled through a polyurethane tube (5 and 3 mm outer and inner diameter, respectively) equipped with a three-way luer-lock valve. These samples were collected prior to redeploying the chambers to start a new measurement cycle. The deployment period of the chambers ranged from 4 to 48 h during the sampling period. Note that CH₄ was highly supersaturated in the studied lakes and that equilibration with chambers therefore took much longer than 48 h—in contrast to CO₂ which typically equilibrates in flux chambers within a few hours and therefore demands much shorter chamber deployments than CH₄. Samples of gas from the chambers and of ambient air were collected using three 60 ml syringes for having a total volume of 180 ml of gas. They were transferred to 22 ml vials with butyl rubber stoppers. Each vial was flushed using 165 ml of the collected gas. The remaining 15 ml of gas were added after removing the gas outlet needle from the vial stopper, creating an overpressure in the vials. All CH₄ gas samples were analyzed with gas chromatography as above. The overpressure in the vials containing the samples was checked as a leakage indicator and was released to equilibrate vials with atmospheric pressure just before the analysis.

2.3.3 $\delta^{13}\text{C-CH}_4$ in Groundwater

GW samples for $\delta^{13}\text{C-CH}_4$ analyses were collected in the mire on the north side of STJ with the use of piezometers (Figure 2). GW samples were collected at different distances from the lake and at 1 m depth in the mire. After discarding the water that was trapped in the tubing, 60 ml of water were collected using a 60 ml syringe. This water was transferred to a 118 ml vial prepared as explained in the description of the second method used to collect $\delta^{13}\text{C-CH}_4$ water samples.

2.3.4 Weather Variables and Depth Profiles

Air temperature, relative humidity, barometric pressure, and wind speed were measured by a weather station located on a platform in the center of the lake. Data were averaged and logged every 10 min. Daily precipitation data were provided by a rain gauge located 2 km away from the lake. Water temperature was logged every 10 min by sensors positioned every 25 cm from the surface to the bottom of the lake at the platform where weather parameters were measured. The water level in the lake was averaged and logged every 10 min. Daily discharge in the outlet of the lake was measured at a V-notch weir located 30 m downstream of the lake.

2.3.5 Flux Calculation

CH₄ fluxes at the surface of the lake were measured using the method described in Bastviken et al. (2004). The calculation of diffusive flux relies on Fick's law of diffusion:

$$F = k(C_w - C_{fc}) \quad (3)$$

where F is the flux of CH₄ across the water-air interface (mmol m⁻² d⁻¹), k is the piston velocity (m d⁻¹), C_w is the concentration of CH₄ in the surface water (μmol L⁻¹) and C_{fc} is the calculated equilibrium concentration of CH₄ in water corresponding to the flux chamber headspace concentration (μmol L⁻¹). Using this equation and measurements of C_w and C_{fc} after some time, k is solved for by accounting for the gradual change in flux due to the increasing C_{fc} over time, i.e., using a non-linear regression over the measurement cycle as described in Bastviken et al. (2004). Then, k for chambers receiving only diffusive flux (see below) was used with C_w to estimate instantaneous diffusive flux, setting C_{fc} to the initial air background CH₄ level.

Floating chambers accumulate CH₄ emitted by both diffusion and ebullition. Their relative contribution to the measured total flux is estimated based on the method given in Bastviken et al. (2004). The values of k derived for different chambers from the measured flux and water concentrations during the same period were divided by the minimum k value observed for this period. Chambers for which this k ratio was below a certain threshold [we used a threshold of 2 in this study based on independent findings in Bastviken et al. (2004); Schilder et al. (2013)] were assumed to receive CH₄ only by diffusion. Therefore, it was assumed that the diffusive part of the flux is rather homogeneous across the lake surface (within the 2-fold variability on k combined with variability on C_w) and that not all chambers collect CH₄ bubbles. The contribution of ebullition in the chambers where the k ratio was above the threshold was calculated by subtracting the average flux from the chambers collecting only diffusive flux from the total flux of each chamber.

2.3.6 Water Balance and Lateral CH₄ Input

The water balance is used here to estimate the potential lateral input of CH₄ to STJ, which can be approximated by the volume of water flowing to the lake multiplied by the concentration of CH₄ in this water. The water balance of the lake expressed for the inflow of water to the lake is:

$$Inflow = Outflow + E + A\Delta h - P_{lake} \quad (4)$$

where *Inflow* is the total amount of water entering the lake from the catchment, *Outflow* is the water leaving the lake in the outlet, E is the evaporation at the lake surface, A is the surface area of the lake, Δh is the water level change in the lake and P_{lake} is the precipitation falling directly in the lake. The surface area of the lake was assumed to be constant over the range of water level changes. Evaporation was calculated according to Sartori (2000). All terms on the right-hand side of the equation could be calculated from parameters measured routinely in or around the lake.

To estimate the lateral CH₄ input to the lake, we assume that the water input is primarily in the form of GW flowing through the mire. This assumption is supported by the catchment distribution (Figure 2), the absence of any stream inlet to STJ, except for a small ditch in the northeastern corner of the lake, and the negligible input of CH₄ with GW from the forested shoreline, confirmed by previous measurements of GW CH₄ (Denfeld et al., 2020).

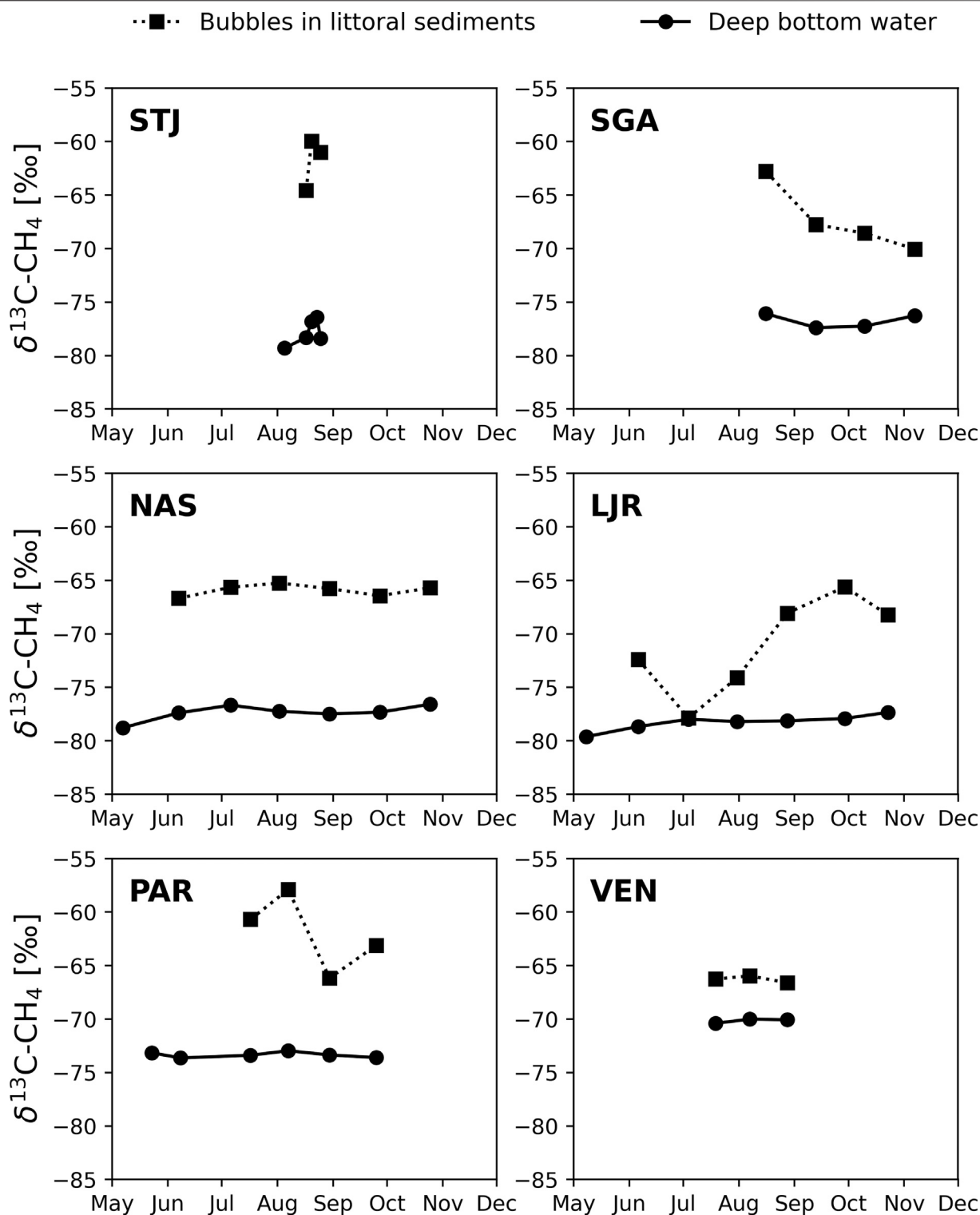
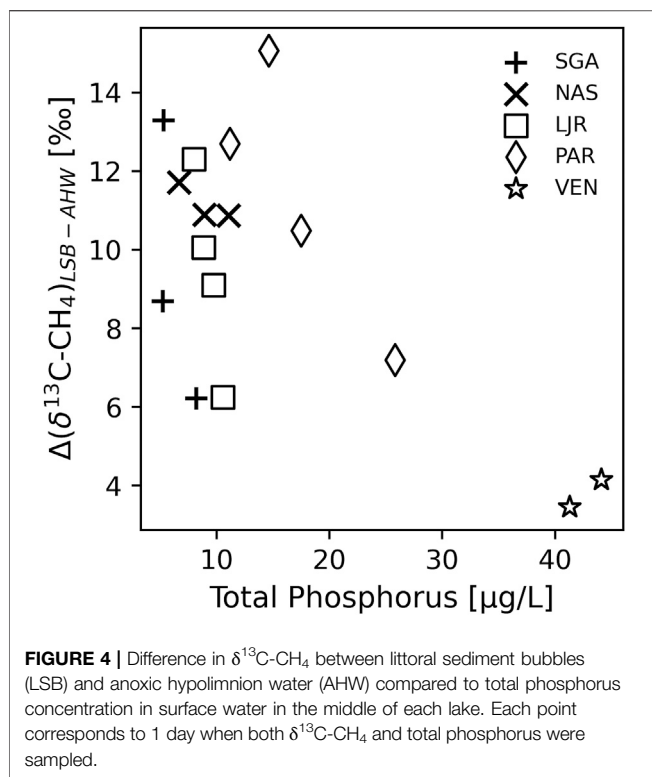


FIGURE 3 | $\delta^{13}\text{C-CH}_4$ in the anoxic water close to the bottom at the deepest point (dots with continuous lines) and in the bubbles in littoral sediments (squares with dotted lines) in six lakes.

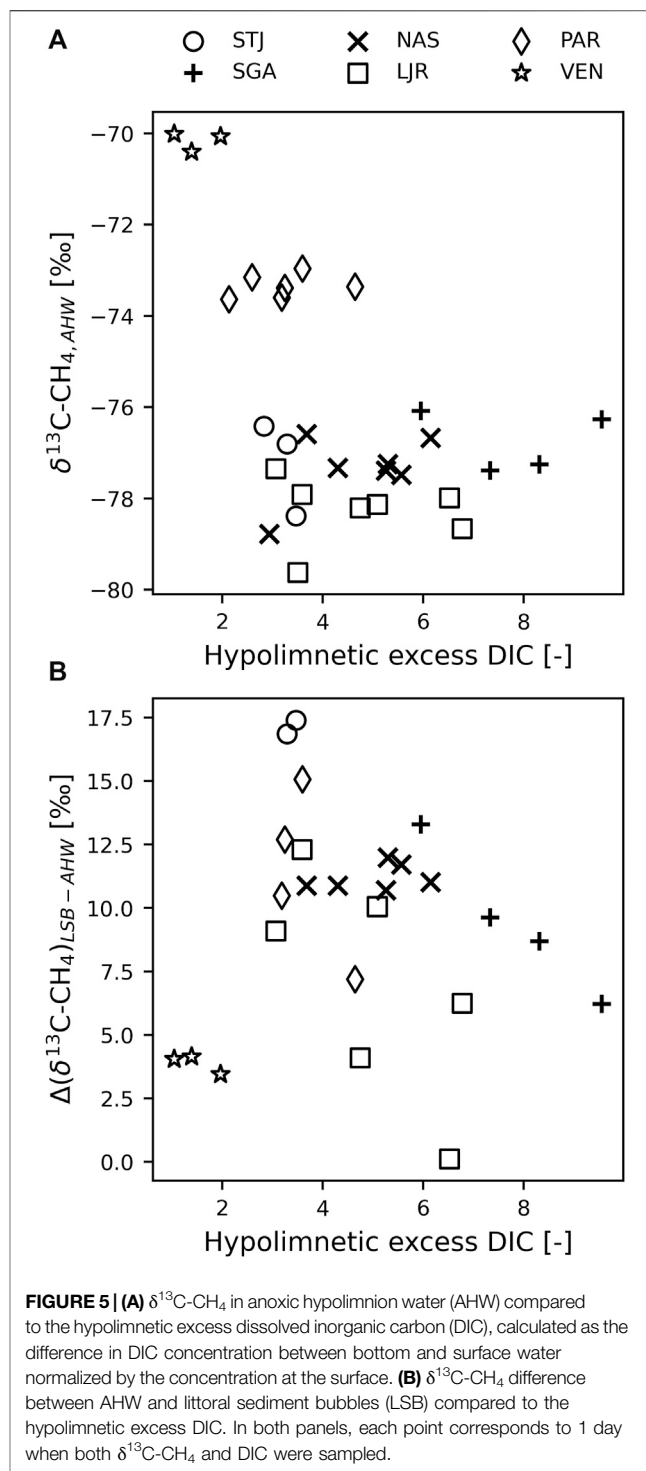


3 RESULTS

3.1 $\delta^{13}\text{C-CH}_4$ in Anoxic Hypolimnion Water Versus Near-Shore Sediment Bubbles

In four lakes (STJ, SGA, NAS, LJR), the $\delta^{13}\text{C-CH}_4$ in the anoxic hypolimnion ranged from -80‰ to -76‰ during the measurement period (Figure 3). SGA is deep and oligotrophic, whereas the other three lakes are small and moderately to highly humic. By contrast, in PAR, which is also a humic lake located farther south but with higher TN concentrations and potential influence from more nutrient rich soils, $\delta^{13}\text{C-CH}_4$ at the bottom ranged between -75‰ and -73‰ . In VEN, a relatively large and eutrophic lake, the corresponding values ranged between -71‰ and -69‰ . In all lakes, $\delta^{13}\text{C-CH}_4$ values from littoral sediments ranged between -71‰ and -58‰ , except for LJR where lower values of -78‰ to -72‰ were observed between June and August. In the second half of August, when lakes were likely to have been stratified for an extended period and the only time when data was available for all lakes, the difference between $\delta^{13}\text{C-CH}_4$ measured in the littoral sediment bubbles and in the anoxic hypolimnion water ($\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$) was 16.0‰ in STJ, 13.4‰ in SGA, 11.8‰ in NAS, 10.0‰ in LJR, 7.2‰ in PAR, and 3.5‰ in VEN (Table 1). $\delta^{13}\text{C-CH}_4$ was lower in the anoxic hypolimnion than in the littoral sediments in all six lakes investigated (Figure 3).

$\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$ showed a tendency to decrease with increasing surface water concentration of TP if considering all times where both these variables were available (Figure 4). This tendency is partly a result of clearly lowest $\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$ in the most nutrient rich lake VEN. In addition, some



lakes showed decreasing $\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$ with increasing TP when comparing different sampling times within the same lake (Figure 4). More generally, TP may not be an optimal indicator of lake productivity, e.g., as TP can be bound to DOC in humic boreal lakes, but we also observe that the two lakes with the smallest $\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$ in August (VEN and PAR), presumably reflecting the integrated result of the

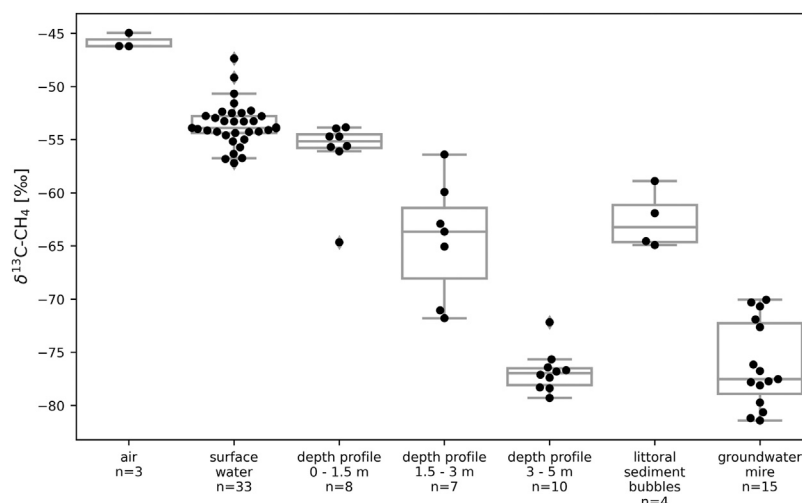


FIGURE 6 | Boxplots and individual measurements of $\delta^{13}\text{C-CH}_4$ values in the air, in lake water (surface and depth profiles) and littoral sediments, and in the mire groundwater in Stortjärn in August. “Surface water” data represents spatial variability at the surface of the lake while “depth profile” data shows short term variability at different depths in the central part of the lake. **Figure 2** shows a map with all sampling locations. Boxes extend from the lower quartile to the upper quartile. Whiskers extend to the nearest measurements within bounds corresponding to one and a half time the width of the boxes.

summer productivity, had highest TP, TN, and Chl *a* levels (**Table 1**).

We also observed that $\delta^{13}\text{C-CH}_4$ in the anoxic, hypolimnetic water was lower in lakes with higher hypolimnetic excess DIC (defined in Eq. 2; **Figure 5A**). In VEN, where DIC concentrations were relatively homogeneous over the water column (hypolimnetic excess DIC = 1–2), the $\delta^{13}\text{C-CH}_4$ values at the bottom were around -70‰ (**Figure 5A**). PAR was an intermediate case with hypolimnetic excess DIC of 2–4 and bottom $\delta^{13}\text{C-CH}_4$ values between -73‰ and -74‰ (**Figure 5A**). The other lakes all had hypolimnetic excess DIC of 3–10 and bottom $\delta^{13}\text{C-CH}_4$ values between -76‰ and -80‰ (**Figure 5A**). However, no particular relation between $\Delta(\delta^{13}\text{C-CH}_4)_{\text{LSB-AHW}}$ and hypolimnetic excess DIC could be observed (**Figure 5B**).

Overall, it was clear and consistent across all lakes that bottom anoxic water $\delta^{13}\text{C-CH}_4$ was more negative (more depleted in ^{13}C) than littoral CH_4 bubbles, with indications of possible links to lake productivity (here estimated *via* proxies), and a proxy for in lake respiration (hypolimnetic excess DIC).

3.2 Possible Groundwater Influence on CH_4 in Lake Stortjärn

The $\delta^{13}\text{C-CH}_4$ values measured in the surface water of STJ ranged between -57.2‰ and -47.4‰ with an average of -53.6‰ (**Figure 6**). In the bubbles collected in the littoral sediments, $\delta^{13}\text{C-CH}_4$ ranged between -64.9‰ and -58.9‰ with an average of -62.6‰ (**Figure 6**). In the GW, $\delta^{13}\text{C-CH}_4$ ranged between -81.4‰ and -70.1‰ with an average of -76.2‰ (**Figure 6**). The water samples taken at different depths at the deepest point of the lake show that $\delta^{13}\text{C-CH}_4$ decreased with depth. The average value was -56.2‰ between 0 and 1.5 m and went down to -76.8‰ at depths below 3 m (**Figure 6**).

Precipitation measurements in the catchment of the lake and the water budget of the lake show that three major rain events occurred during the sampling period, each lasting between one and 2 days and releasing decreasing amounts of water to the lake over several days after each rain event. The daily input of CH_4 from the mire to the lake was calculated by multiplying the daily water inflow from the mire with the average concentration of CH_4 that was measured in the GW of the mire ($178\text{ }\mu\text{M}$; very close to the average concentration measured in the same mire over the entire ice-free period in another study; Denfeld et al., 2020). The daily input of CH_4 from the mire ranged from 0.14 to 6.8 kg CH_4 with an average of 1.6 kg CH_4 (**Figure 7**).

Fluxes of CH_4 measured at the surface of the lake ranged between 0.01 and 64 mg $\text{CH}_4\text{ m}^{-2}\text{d}^{-1}$, with mean and median values of 2.9 and 1.4 mg $\text{CH}_4\text{ m}^{-2}\text{d}^{-1}$, respectively. Daily whole-lake emissions of CH_4 , calculated as the average of all chamber measurements for each day multiplied by the surface area of the lake, ranged from 24 g CH_4 to 200 g CH_4 with an average of 90 g CH_4 . Flux rates were one to two orders of magnitude smaller than the calculated daily CH_4 input from the mire (**Figure 7**).

4 DISCUSSION

4.1 $\delta^{13}\text{C-CH}_4$ in Anoxic Hypolimnion Water Versus Near-Shore Sediment Bubbles

The thermal stratification in the lakes resulted in typical depth profiles of CH_4 concentrations and $\delta^{13}\text{C-CH}_4$ values, with low CH_4 concentrations and high $\delta^{13}\text{C-CH}_4$ values in the oxic epilimnion, and high CH_4 concentrations and low $\delta^{13}\text{C-CH}_4$ values in the anoxic hypolimnion. The $\delta^{13}\text{C-CH}_4$ values we observed in the anoxic hypolimnion of all six lakes are in the same range as observations previously reported in other lakes (Rinta et al., 2015; Thompson et al., 2016). Our results also show

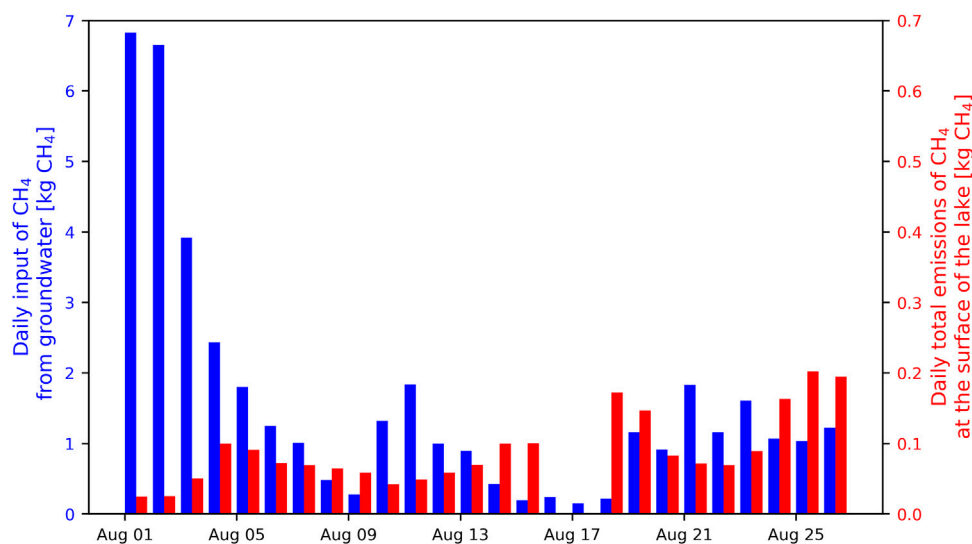


FIGURE 7 | Comparison between the estimated daily input of CH_4 from the mire to Stortjärn (blue bars) and the total amount of CH_4 emitted each day at the surface of the lake (red bars; note the tenfold difference between left and right y-axis scales). Emissions of CH_4 at the surface of the lake were not measured on 16–17 August.

that CH_4 was more ^{13}C enriched in littoral sediment bubbles of all six lakes than in anoxic water in the hypolimnion (Figure 3). Considering that CH_4 in profundal sediment bubbles would most likely be more $\delta^{13}\text{C}$ depleted than in the overlying water column (Rinta et al., 2015; Marcek et al., 2021), particularly in cases where significant AOM occurs, it implies that even larger differences in $\delta^{13}\text{C}$ values than the ones we observed can be expected between CH_4 in profundal and littoral sediment bubbles. Lower $\delta^{13}\text{C}$ - CH_4 values in the profundal compared to the littoral zones have also been observed in sediments of three small subarctic lakes in western Greenland (Cadieux et al., 2016; Thompson et al., 2016) and in one small subarctic lake located on the border of a permafrost complex in northern Sweden (Wik et al., 2020). However, as such studies were made on a limited number of lakes with similar properties, it was unclear whether such differences in endmember $\delta^{13}\text{C}$ - CH_4 are common across lakes. Our results indicate that patterns of highly variable endmember $\delta^{13}\text{C}$ - CH_4 values, but with a consistent difference between littoral and profundal zones is a common property of boreal and subarctic lakes. This observation is critical for how to use and sample $\delta^{13}\text{C}$ - CH_4 values to assess CH_4 cycling, MOX, and other related lake ecosystem processes or food web interactions.

It is also remarkable that $\delta^{13}\text{C}$ - CH_4 values in the anoxic hypolimnion water were relatively similar among sampling occasions in all lakes while littoral sediment $\delta^{13}\text{C}$ - CH_4 values were more variable. The variability was observed as variability over time but could in fact also represent variability in space because sediment bubbles were not collected at identical locations every time and the temporal variability was asynchronous between lakes, even in similar lakes like NAS and LJR. This means that local conditions in the littoral sediments, for example sources of organic matter, benthic processes, and vegetation cover, can vary in ways that could influence endmember $\delta^{13}\text{C}$ - CH_4 signals substantially.

4.2 Possible Explanations for Observed Patterns

We discuss below a range of potential explanations for the difference in $\delta^{13}\text{C}$ - CH_4 we observed between littoral sediment bubbles and the anoxic hypolimnion.

4.2.1 Substrates for Methanogenesis

$\Delta(\delta^{13}\text{C}-\text{CH}_4)_{\text{LSB-AHW}}$ can be related to differences in $\delta^{13}\text{C}$ of the substrates used in the anoxic degradation of OM in sediments of lakes. $\delta^{13}\text{C}$ - CH_4 is determined by the initial $\delta^{13}\text{C}$ of the substrate and by the fractionation imposed by metabolic reactions, such that $\delta^{13}\text{C}$ of the substrate plays an important role in setting $\delta^{13}\text{C}$ of CH_4 released in the water column (Conrad, 2005). The dominant sources of OM to the sediments of lakes include autochthonous primary production by phytoplankton, benthic algae, or littoral vascular plants on one hand, and allochthonous terrestrial OM on the other hand. Terrestrial OM is derived from the vegetation growing in the catchment of the lake, which is predominantly constituted of C3 plants in boreal regions (Cerling et al., 1997). $\delta^{13}\text{C}$ for C3 plants are typically between -37‰ and -20‰ and most commonly near -27‰ (Kohn, 2010). In general, values of -27‰ or -28‰ are commonly used for allochthonous C in boreal lakes (Meili et al., 1993; Pace et al., 2004). $\delta^{13}\text{C}$ values of littoral vascular plants are also predominantly in the range between -30‰ and -20‰ (Cloern et al., 2002; Lammers et al., 2017; Figure 1). On the other hand, $\delta^{13}\text{C}$ values for different phytoplankton species have been reported in the range -44.5‰ to -18‰ in several boreal lakes, most of them being in the range -39‰ to -30‰ (Taipale et al., 2016), and measurements in several lentic systems across a broad range of latitudes have revealed $\delta^{13}\text{C}$ values ranging between -36‰ and -17‰ in phytoplankton and between -29‰ and -8‰ in benthic algae, with values usually 2‰–11‰ lower (6‰ in average) in

phytoplankton than in benthic algae for a given system (Doi et al., 2010), as illustrated in **Figure 1**. Therefore, benthic algae seem usually enriched in ^{13}C compared to phytoplankton and allochthonous OM. There are indications that methanogens preferentially use autochthonous freshly produced OM when possible (West et al., 2012).

Hence, in low-nutrient lakes, the littoral where light is available may represent a zone with concentrated production of fresh, autochthonous OM (Karlsson et al., 2009). Nutrients released from sediments may be the main nutrient source in such low-nutrient lakes, favoring benthic algae relative to phytoplankton. If so, littoral $\delta^{13}\text{C}\text{-CH}_4$ in low-nutrient lakes may be influenced by benthic algae, while profundal CH_4 production is reflecting $\delta^{13}\text{C}$ from allochthonous OM (or respired DIC as discussed further below), which given this scenario could lead to a large $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$. In more nutrient rich lakes, settling phytoplankton may contribute labile autochthonous OM fueling methanogenesis everywhere in the lake (in both littoral and profundal sediments) leading to lower $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$. The wide range of $\delta^{13}\text{C}$ values in phytoplankton reported in previous studies (Doi et al., 2010; Taipale et al., 2016) and illustrated in **Figure 1** does not necessarily contradict this explanation, as measured $\delta^{13}\text{C}\text{-CH}_4$ values reflect average $\delta^{13}\text{C}$ values in the sediments, and the mean $\delta^{13}\text{C}$ value in phytoplankton is significantly lower than the mean $\delta^{13}\text{C}$ value in benthic algae (Wilcoxon signed-rank test on the data from Doi et al. (2010); $N = 49$, $W = 1.0$, $p < 0.001$). However, the wider range of phytoplankton $\delta^{13}\text{C}$ values can add to the variability among lakes. Although the substrate explanation is a speculation at this stage it is realistic given available scientific evidence and could explain our observations of lower $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$ in lakes with highest TP, TN, and Chl *a* values (**Table 1**).

In addition, DIC is the C substrate for HM, and it is possible that DIC in profundal sediments under isolated hypolimnion is primarily generated by OM respiration which causes depletion in ^{13}C compared to DIC in littoral parts of lakes where continuous exchange with atmospheric CO_2 is possible (Miyajima et al., 1997; Karlsson et al., 2007). A few measurements in the lakes studied here confirm this tendency and indicate that $\delta^{13}\text{C}\text{-CO}_2$ at the bottom of the water column is often between 2‰ and 10‰ lower than in surface water (data not shown). Therefore, $\delta^{13}\text{C}$ values of CH_4 produced by HM should decrease at the bottom of lakes with larger differences between bottom and surface DIC concentrations (expressed as hypolimnetic excess DIC in the Results) being a sign of a larger share of accumulated respired C in deep waters. Accordingly, we observed the most positive hypolimnion $\delta^{13}\text{C}\text{-CH}_4$ values in VEN, which also had smallest DIC accumulation in deeper water. Comparing lakes with distinctively different $\delta^{13}\text{C}\text{-CH}_4$ values in the hypolimnion, we also noted that increasing hypolimnetic DIC accumulation was linked with lower $\delta^{13}\text{C}\text{-CH}_4$ values in the hypolimnion (**Figure 5A**). This indicates that some CH_4 was produced by HM in the hypolimnion of the studied lakes.

The subsequent question is if a depletion of $\delta^{13}\text{C}\text{-CH}_4$ from HM using respired C could explain the observed $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$. If this was the case, we would expect

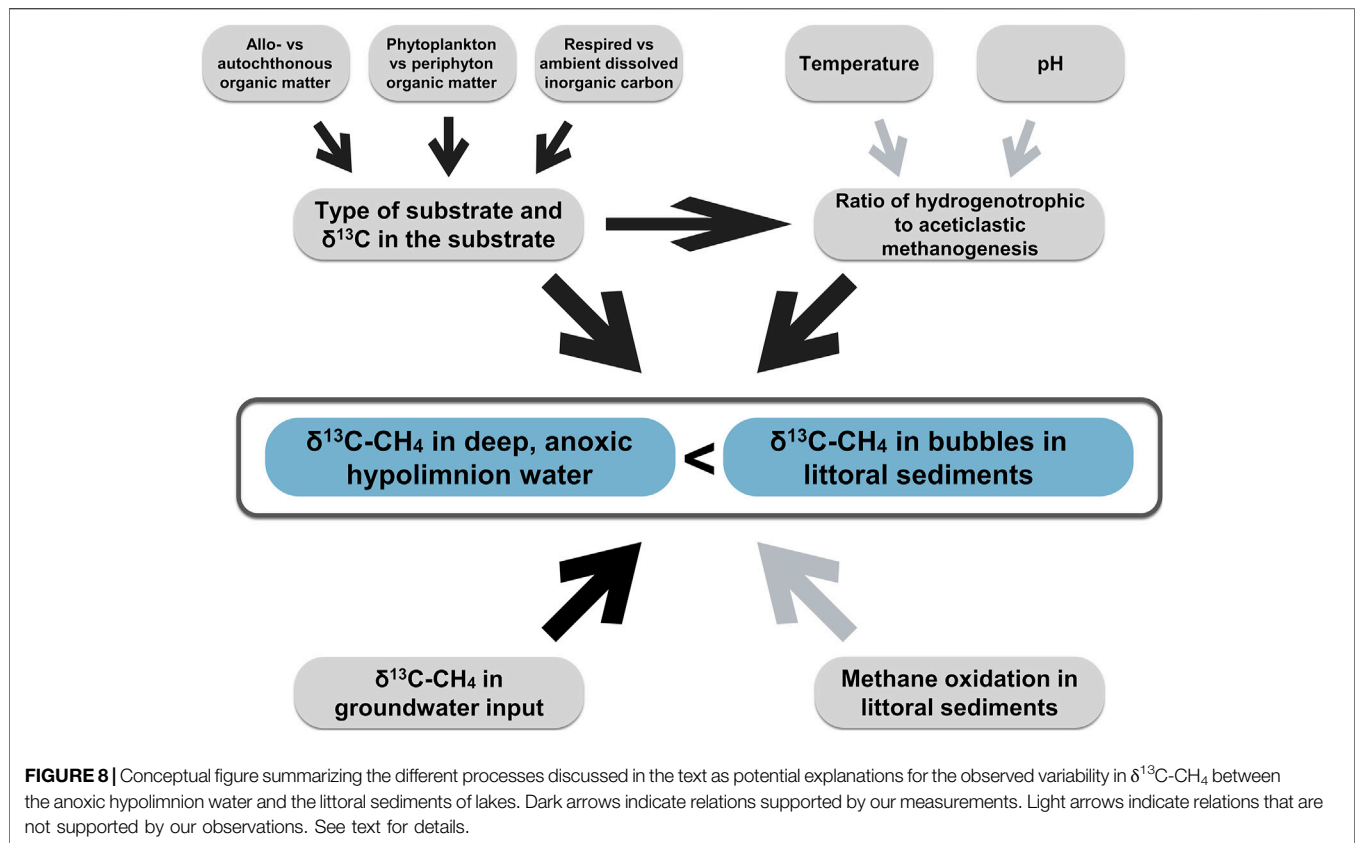
larger $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$ when a greater share of respired C were present in the hypolimnion, i.e., we would expect a positive relationship between $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$ and the hypolimnion excess DIC. However, no such strong pattern could be observed (**Figure 5B**). Therefore, we conclude that while differences in types of OM substrates (terrestrial/macrophytes vs. phytoplankton vs. periphyton) could provide explanations, CH_4 production from respired C does not seem to be a main direct driver for the observed $\Delta(\delta^{13}\text{C}\text{-CH}_4)_{\text{LSB-AHW}}$.

4.2.2 Pathways of Methanogenesis

If the balance between methanogenic pathways differs between littoral and profundal sediments, $\delta^{13}\text{C}\text{-CH}_4$ values could be affected because HM has been suggested to generate more depleted $\delta^{13}\text{C}\text{-CH}_4$ than AM (Whiticar, 1999). The lower $\delta^{13}\text{C}\text{-CH}_4$ values that we report in the deep hypolimnion compared to littoral sediments could then be explained if a greater share of HM is taking place in profundal sediments compared to littoral ones. Our data on $\delta^{13}\text{C}\text{-CH}_4$ and DIC measurements discussed above indicates that HM occurs and consumes respired CO_2 in the deep hypolimnion. While we do not have direct estimates of AM and HM, several factors influencing the share of CH_4 produced by HM and AM have recently been reviewed by Conrad (2020). They include temperature, pH, and the quality of the available OM. More specifically, previous observations have shown that the HM:AM ratio decreases at lower temperature (Schulz and Conrad, 1996; Nozhevnikova et al., 2007) while low pH values can limit the availability of H_2 resulting in a smaller contribution from HM (Goodwin et al., 1988). In addition, Liu et al. (2017) have shown that the HM:AM ratio increased in deeper layers of sediment cores from a mountain lake, which was explained by the decreasing quality of the OM in older sediments. Given present literature information, our data is not clearly compatible with conditions that would favor HM in profundal zones and AM in the littoral, as some variables such as temperature conditions could be expected to yield the opposite result. Hence, although this HM vs. AM hypothesis cannot be excluded as a potential explanation for our results, we find it unlikely that it is of main importance.

4.2.3 Partial MOX

A difference in rates of MOX in sediments across depths needs to be considered as a potential explanation for the spatial variability of $\delta^{13}\text{C}\text{-CH}_4$ because MOX leaves ^{13}C enriched CH_4 by preferentially consuming lighter $^{12}\text{C}\text{-CH}_4$. More MOX is expected to happen in littoral sediments than in deep, anoxic hypolimnia as MOX in littoral sediments can be supported by the transport of suitable electron acceptors into the sediments *via* plant roots or *via* diffusion or percolation of O_2 -rich water. However, if the collected bubbles in the littoral sediments were released from sediment layers below the zone where suitable electron acceptors for MOX or AOM were depleted, $\delta^{13}\text{C}\text{-CH}_4$ values measured in these bubbles would not have been affected by oxidation. Unfortunately, in the absence of measurements of $\delta^{13}\text{C}\text{-CH}_4$ profiles in the pore water of the sediments, it is not possible to confirm if this was the case in the present study.



Measurements in a small, eutrophic kettle lake in central Europe, which included collection of sediment bubbles using a similar approach as the one described here, as well as profiles of pore water $\delta^{13}\text{C-CH}_4$, showed that bubbles released by physically disturbing the sediments originated from sediment layers where CH_4 was not or marginally affected by oxidation (Langenegger et al., 2019). If the same is true in the littoral and profundal sediments of the lakes studied here, it would indicate that MOX is not a likely explanation for the higher $\delta^{13}\text{C-CH}_4$ values observed in littoral sediment bubbles compared to in anoxic, deep water in the profundal zone of the lakes. Direct measurements of CH_4 concentrations and $\delta^{13}\text{C-CH}_4$ values along pore water profiles in the sediments in littoral and profundal zones could provide more conclusive results by allowing a more detailed analysis of the active processes in different sediment layers and should be included in future studies.

4.2.4 Groundwater Influence on CH_4 in Lake Stortjärn

GW often contains high concentration of CH_4 compared to lake water due to anoxic conditions prevailing in soils (Bugna et al., 1996). Moreover, GW can also be a significant source of CH_4 to some lakes (Einarsdottir et al., 2017; Lecher et al., 2017; Dabrowski et al., 2020). A previous study indicates that large amounts of CH_4 might be transported to STJ *via* GW inputs (Denfeld et al., 2020). The GW flowing into STJ passes through a mire and CH_4 emitted from wetlands and mires often displays $\delta^{13}\text{C-CH}_4$ values lower than -60‰ (Quay et al., 1988; Lansdown

et al., 1992), especially in Scandinavian wetlands where average values are as low as -75‰ (Ganesan et al., 2018). Our measurements show that $\delta^{13}\text{C-CH}_4$ in GW from the mire (-82‰ to -70‰) matches $\delta^{13}\text{C}$ in the dissolved CH_4 (-79‰ to -72‰) under the oxycline in the middle of the lake near the deepest point (Figure 6). The deep water $\delta^{13}\text{C-CH}_4$ in the profundal zone is at the same time different from littoral sediment bubble $\delta^{13}\text{C-CH}_4$ values that were measured on the opposite side of the lake (-65‰ to -59‰ ; Figure 6). Together with the fact that the GW is expected to enter the lake in the deeper layer due to its low temperature (higher density) compared to the epilimnion, it points to a possible mire GW origin of CH_4 in the deeper water layer of the lake. On the other hand, most of the littoral sediment bubbles CH_4 is probably derived from sediment CH_4 production and less influenced by the mire CH_4 . The GW explanation might also apply to other lakes than STJ. Unfortunately, the absence of GW measurements near the five other studied lakes prevents us to establish any link between GW inputs of CH_4 depleted in ^{13}C and the lower $\delta^{13}\text{C-CH}_4$ values observed in the anoxic hypolimnion of these lakes. Alternatively, the low $\delta^{13}\text{C-CH}_4$ values observed in the anoxic hypolimnion of STJ might also arise for other reasons described previously, like the variability in the composition of the substrate available in different zones of lakes. It is likely that the suggested mechanisms have a combined effect on the difference in $\delta^{13}\text{C}$ values in endmember CH_4 between littoral and profundal zones of lakes.

Furthermore, the amount of CH_4 transported by GW to the lake was estimated to be much larger than the amount of CH_4 emitted by the lake to the atmosphere (Figure 7). It implies that large quantities of CH_4 can be transported from the mire to the lake and most of this CH_4 is oxidized in the lake before reaching the surface. It suggests that the importance of GW CH_4 contributions to lakes CH_4 cycling may have been underestimated. In fact, these observations indicate that GW CH_4 inputs could be high enough to sustain most dissolved CH_4 in some lakes—meaning that hypolimnetic CH_4 and episodic fluxes of dissolved CH_4 upon lake mixing would be attributed to GW CH_4 , while ebullition is derived from intrinsically produced CH_4 from the lake sediments. These findings deserve further studies and illustrate that catchment processes and hydrological transport should be considered in assessments of factors controlling CH_4 cycling in lakes. Accurate and relevant estimates of the volume of various sources and sinks of water to and from a lake, and of the CH_4 concentration in each of these sources and sinks are important requirements for this method to be successful in assessing the contribution of external inputs of CH_4 to the lake. Also, the temperature of the groundwater entering a lake is critical in determining in which layer of the lake the groundwater will end up.

5 CONCLUSION

The main result of our study is that there seems to be a systematic difference in endmember $\delta^{13}\text{C}-\text{CH}_4$ between littoral sediments and anoxic profundal zones of subarctic and boreal lakes and that it is important to consider which parts of the lake CH_4 cycling can be represented by different source $\delta^{13}\text{C}-\text{CH}_4$ values. We also discuss potential explanations to the observed pattern (Figure 8) and argue that several mechanisms are likely to contribute at different degrees to the offset in endmember $\delta^{13}\text{C}-\text{CH}_4$ between littoral and profundal zones of lakes, with no conclusive evidence for one specific mechanism against all others based on our observations.

It has been shown that stratified lakes in essence have two separated CH_4 cycles—one epilimnetic and one hypolimnetic (Bastviken et al., 2008). In the epilimnetic CH_4 cycle, CH_4 produced in littoral sediments is released to the atmosphere by bubbling or diffuses into the epilimnion where it can be oxidized or emitted. The epilimnetic CH_4 cycle contributes most of the CH_4 emission during open water periods and the best source $\delta^{13}\text{C}-\text{CH}_4$ value there may be measured in littoral sediment bubbles. In the meta-hypolimnetic CH_4 cycle, CH_4 primarily leaves the profundal

sediment as dissolved CH_4 , and a concentration gradient develops between the surface of the sediments and the water layer where suitable electron acceptors for MOX are available and where most of the CH_4 is oxidized. For estimating MOX in this part of the lake, dissolved CH_4 in the anoxic bottom water layer of the lake or CH_4 contained in profundal sediments may be the best source $\delta^{13}\text{C}-\text{CH}_4$ value. Distinguishing these two CH_4 cycles would represent an important progress and contribute to increased knowledge about the regulation and feedbacks of lake CH_4 and C cycling and emissions.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: DiVA (<https://doi.org/10.48360/5PPN-C440>).

AUTHOR CONTRIBUTIONS

The study was designed by HS, JS, and DB. Field work was performed by JS, HS, AS, GP, DR, EH, and KF. Sample analyses were performed by JS, HS, AS, GP, DR, EH, and KF. Funding for this study was provided through projects led by DB, HL, and JK. The article was drafted by JS with contributions from all authors.

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