Stable Methane Isotopologues From Northern Lakes Suggest That Ebullition Is Dominated by Sub-Lake Scale Processes

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Abstract Stable isotopes have emerged as popular study targets when investigating emission of methane (CH₄) from lakes. Yet little is known on how isotopic patterns conform to variations in emission magnitudes—a highly relevant question. Here, we present a large multiyear data set on stable isotopes of CH₄ ebullition (bubbling) from three small adjacent subarctic lakes. The δ¹³C-CH₄ and δD-CH₄ range from −78.4‰ to −53.1‰ and from −369.8‰ to −218.8‰, respectively, and vary greatly among the lakes. The signatures suggest dominant hydrogenotrophic methanogenesis, particularly in the deep zones, but there are also signals of seemingly acetoclastic production in some high fluxing shallow areas, possibly fueled by in situ vegetation, but in-sediment anaerobic CH₄ oxidation cannot be ruled out as an alternative cause. The observed patterns, however, are not consistent across the lakes. Neither do they correspond to the spatiotemporal variations in the measured bubble CH₄ fluxes. Patterns of acetoclastic and hydrogenotrophic production plus oxidation demonstrate that gains and losses of sediment CH₄ are dominated by sub-lake scale processes. The δD-CH₄ in the bubbles was significantly different depending on measurement month, likely due to evaporation effects. On a larger scale, our isotopic data, combined with those from other lakes, show a significant difference in bubble δD-CH₄ between postglacial and thermokarst lakes, an important result for emission inventories. Although this characteristic theoretically assists in source partitioning studies, most hypothetical future shifts in δD-CH₄ due to high-latitude lake area or production pathway are too small to lead to atmospheric changes detectable with current technology.

Plain Language Summary Lakes are common in Arctic landscapes, and they are important sources of methane, a powerful greenhouse gas. Most of the lake methane that reaches the atmosphere is released by bubbling, a very erratic process in space and time. All methane is not the same, and the relative amounts of different types (“isotopologues”) can show how and from what source a particular sample of methane was formed. In this study, which focuses on three lakes in northern Sweden, we analyze these different types of methane to determine if various methane-creation and destruction processes can lead to different bubbling rates. We also considered how these processes differed spatially. We found that isotopologues alone do not explain why some parts of lakes bubble more than others. The measurements, however, do suggest that the shallow areas generally produce methane through processes that require more fresh organic material compared to the deeper zones where the sediments are more decomposed. This raises further questions whether certain species of aquatic plants contribute to the methane bubbling we observe and whether loss of methane in the sediments by oxidation is an important process in our lakes. Finally, we compare our results with studies of other types of northern lakes.

1. Introduction

The numerous lakes at high northern latitudes are an important yet uncertain source of methane (CH₄) to the atmosphere. Emission estimates are currently limited by difficulties in scaling when accounting for different types of water bodies and underlying carbon (C) sources, highly heterogeneous release mechanisms, and few long-term measurements (Bastviken et al., 2011; Burke et al., 2019; Wik et al., 2016). Ebullication (bubbling) is often the dominant transport mode of CH₄ from aquatic sediments (DelSontro et al., 2015; Dove et al., 1999; Walter et al., 2007), but it is seldom measured leaving a substantial part of the total flux...
unquantified at many sites (Wik et al., 2016). The contribution from northern lakes to the budget of atmospheric CH$_4$ is not always well separated from that of wetlands (Thornton et al., 2016), the largest natural CH$_4$ source (Kirschke et al., 2013).

Stable isotopes are often included in atmospheric mass-balance calculations aiming to partition bottom-up CH$_4$ source contributions (Bousquet et al., 2006; Ghosh et al., 2015). The common stable isotopologues of methane are $^{13}$CH$_4$ and CH$_3$D. The former is more commonly measured but, unfortunately, surprisingly narrow ranges of $^{13}$C are associated with each specific source, for example, wetlands (Fisher et al., 2017), when actually there are large overlaps, particularly in biogenically produced CH$_4$ due to similar methanogenic pathways. The regional CH$_4$ budgets that rely on isotopic constraints can therefore incorrectly attribute source contributions depending on the initial assumptions of the source signature (Ganesan et al., 2018; Thornton et al., 2016).

Carbon-13 and D are also used to determine the underlying biogenic pathway producing the emitted CH$_4$: acetoclastic or hydrogenotrophic (produced from reduction of carbon dioxide [CO$_2$]), which in turn hint at the C source used by the methanogens (Chanton et al., 2005; Whiticar et al., 1986). The general understanding is that acetoclastic methanogenesis dominates in productive systems that have a faster turnover of recently produced labile C substrate whereas hydrogenotrophic production implies more decomposed or recalcitrant organic material (OM) with much lower Eh (Bouchard et al., 2015; McCalley et al., 2014; Walter et al., 2008). However, the isotopic cutoff point or signature range associated with hydrogenotrophic methanogenesis commonly used as a reference pattern in many studies (e.g., Douglas et al., 2016; Martens et al., 1992) is initially based on marine isotopic data (Whiticar et al., 1986) and may not be directly

Figure 1. Map of Stordalen area, showing our three study lakes (modified from Wik et al., 2014) and its location above the Arctic circle in Sweden. Water depth contours are color coded in blue, and bubble trap locations are indicated by black circles. Red circles show areas where bubble CH$_4$ fluxes are consistently higher than in the other parts of the lakes (Wik et al., 2013).
applicable to freshwater systems. Other questions, which underlie this study, include whether stable isotopes are useful when explaining the heterogeneous patterns of lake CH₄ ebullition and how the signatures found in our lakes compare to water bodies studied elsewhere.

2. Methods

The study site and ebullition measurement methodologies are described in detail elsewhere (Wik et al., 2013). In brief, CH₄ bubbles were captured using a total of 40 inverted-funnel bubble traps that were distributed in a depth-stratified sampling scheme across three small (0.01–0.17 km²) and interconnected lakes, Inre Harrsjön, Mellersta Harrsjön, and Villasjön, which border the Stordalen Mire, a subarctic permafrost complex in northern Sweden (Figure 1). Inre and Mellersta Harrsjön are interconnected postglacial features with macrophyte-dense shallow and littoral zones (0- to 2-m water depths) and small deeper profundal areas with maximum depths of 5 and 7 m, respectively. The origin of the much shallower Villasjön (maximum depth of ~1 m) is debatable due to underlying terrestrial peat (Kokfelt et al., 2010; Wik et al., 2018). However, the depression that Villasjön resides in does not appear to have been formed by thermokarst. Although the western part of the lake partly connects to a “flow-through-fen” system that transcends the Stordalen Mire, and there are palsas that hold the water in place, most of Villasjön’s shorelines merges into moraine-covered slopes. Both Inre Harrsjön and Villasjön are also partly spring fed. A previous study (Wik et al., 2013) found that average bubble CH₄ fluxes are highest from Villasjön (22 mg CH₄ m⁻² day⁻¹) and lowest from Inre Harrsjön (6.8 mg CH₄ m⁻² day⁻¹), and they respond rapidly to temperature and decrease with increasing water depth. However, there are areas within the lakes’ littoral zones where the bubbling rates are lower and similar to those measured over the deepest points (Thornton et al., 2015; Wik et al., 2013).

All the bubble traps were sampled frequently, usually every 1–3 days using plastic syringes from June to September. Samples for analysis of ¹³C and D in the CH₄ bubbles were taken at regular intervals (weekly or biweekly) between June and September of 2009–2011. Headspace gas was transferred to 20-ml glass vials (VWR crimp-top headspace vials) filled with saturated sodium chloride (NaCl) solution and capped with thick butyl rubber septas (Apodan, Denmark). All vials were stored upside down allowing for long-term storage without affecting the concentration of the CH₄ (Bastviken et al., 2004).

The analyses of ¹³C and D were made using continuous flow gas chromatography isotope-ratio mass spectrometry (Brass & Rockmann, 2010; Rice et al., 2001) at the Stable Isotope Laboratory (SIL) at Stockholm University. The instruments used were a Trace GC Ultra gas chromatograph and DeltaV plus mass spectrometer connected via a ConFlo IV and GC Isolink interface (Thermo Scientific). The Trace GC was equipped with a split/splitless injector, a 25-m PoraPlot Q capillary column (Varian, Inc.), a combustion oven that oxidizes CH₄ to CO₂, and a high temperature conversion oven (HTC) for quantitative pyrolysis of CH₄ to H₂. We used a helium carrier gas at a flow rate of 1.6 ml min⁻¹ and calibrated reference gases with 100% CO₂ and H₂ concentrations. The flows into the Delta V plus were controlled by the ConFlo IV. The analysis temperatures of the Trace GC oven, the combustion oven, and the HTC were 30°C, 1030°C, and 1420°C, respectively. We used manual direct injections using a 100 μl glass gas-tight syringe (Hamilton) and sample split 60 on the injector. The injection volume varied from 10–80 μl depending on sample CH₄ concentration. Injections of 5 μl standard (100% CH₄) were made every fifth analysis and before the first and after the last sample. Standard delta (δ) notations in per mille of ¹³C and D were calculated relative to Pee Dee Belemnite (PDB) and standard mean ocean water (SMOW). The precision of the analysis was 0.3‰ and 1.8‰ for δ¹³C-CH₄ and δD-CH₄, respectively.

We compared the spatial variations in the isotopic signatures to that of the bubble CH₄ fluxes, which have been well established in previous studies, primarily by Wik et al. (2013) with additional data presented by Jansen et al. (2019) and Thornton et al. (2015). In this study, we used 6 years of bubble flux data (2009–2014), an extension of the 4-year data set presented by Wik et al. (2013). We used analysis of variance (ANOVA) and Tukey’s honestly signif Ordinary Least Squares (OLS) were also used to analyze temporal variations, both on monthly and interannual scales. Both the δ¹³C-CH₄ and δD-CH₄ and their residuals followed approximate normal distributions.
3. Results

Our multiyear data set of stable isotopes of CH₄ ebullition shows large spatial variations and substantial differences in isotopic signature patterns among and within the three study lakes. Altogether the bubbles’ δ¹³C-CH₄ ranges from −78.4‰ to −53.1‰ and their δD-CH₄ from −369.8‰ to −218.8‰, with a mean and standard deviation (SD) of −67.8 ± 4.6 and −310.6 ± 21.3, respectively (n = 177 and 251). Bubbles from the overall shallow Villäsjön, the lake with most frequent ebullition, show relatively high δ¹³C-CH₄ and low δD-CH₄ (−62.1 ± 2.1‰ and −327.6 ± 23.8‰, n = 27 and 29; Figures 2a and 3). The isotopic signatures are statistically similar across the transect (P = 0.082 and 0.692), from the high fluctuating littoral zone near the fen toward the southeastern part of the lake where bubble CH₄ fluxes are threefold lower (Figures 1 and 3; P < 0.001), a gradient across which there is also changes in sediment composition (see section 4.1 and Wik et al., 2018). Inre Harrsjön, on the other hand, where ebullition is least frequent, has the largest spatial differences in both δ¹³C-CH₄ and δD-CH₄ (−69.3 ± 5.4‰ and −308.2 ± 21.1‰, n = 76 and 119; Figures 2b and 3). The variation pattern primarily indicates a gradient toward significantly lower δ¹³C-CH₄ and higher δD-CH₄ in the bubbles with increasing water depth (P < 0.001). Apart from relatively high average δ¹³C-CH₄ (−61.9 ± 3.8‰, n = 14) in bubbles emitted from the shallow, macrophyte-dense center of Inre Harrsjön where average ebullition is high (red circle in Figure 1), most of Inre Harrsjön’s littoral and deeper areas show significantly lower δ¹³C-CH₄ (−67.9 ± 4.9 and ≥ −73.0 ± 1.0, n = 13–18) and up to fourfold lower bubble CH₄ fluxes (P < 0.001; Figures 2b and 3). Further, bubbles emitted from Mellersta Harrsjön show similar average δ¹³C-CH₄ and δD-CH₄ (−68.4 ± 2.1‰ and −308.6 ± 18.7‰, n = 74 and 103) as those emitted from Inre Harrsjön (P ≥ 0.208), but lack a clear isotopic shift with depth, particularly in δ¹³C-CH₄ (Figure 3), despite a larger spatial variation in ebullition with up to sevenfold higher bubble CH₄ fluxes in the southern littoral area compared to the profundal zone (Figures 1 and 3).

Although there are large temporal variations in seasonal and annual ebullition rates (Wik et al., 2013, 2014), we found no clear or consistent temporal trend or yearly variation in δ¹³C-CH₄, both when analyzing all data combined (Figure 4a) or binned by lake and water depth (see time series plots in supporting information Figure S1). Further, despite the previously reported strong observed correlation between the lakes’ surface sediment temperature and CH₄ ebullition (Wik et al., 2014), we observed no correlation between seasonal lake warming (measured continuously in vertical profiles using Onset HOBO U22 loggers; see details in Wik et al., 2013) and the bubbles’ δ¹³C-CH₄ (supporting information Figure S2). In contrast to the nonexisting seasonal patterns of δ¹³C-CH₄, there is overall, when combining all the data, significantly lower δD-CH₄ in bubbles emitted in June (−326.0 ± 20.4‰, n = 24) compared to July, August, and September, with the latter showing the highest values on average (−302.8 ± 22.5‰, n = 35; Tukey’s HSD: P < 0.001; Figure 3).

4. Discussion

According to the isotopic interpretation schemes initially established by Whiticar et al. (1986), the biogenic production that fuels CH₄ bubble formation in our lakes appears to be dominated by hydrogenotrophy but with influences of acetoclasty in some shallow and littoral zones where ebullition is most frequent. In the literature, acetoclastic CH₄ production generally is more enriched in ¹³C and depleted in D, resulting in higher δ¹³C-CH₄ (−60% to −50‰) and lower δD-CH₄ (400‰ to −250‰), relative to hydrogenotrophic methanogenesis (δ¹³C-CH₄ = −110‰ to −60‰ and δD-CH₄ = −250‰ to −170‰) (Chanton et al., 2005; Whiticar, 1999). However, the spatial patterns in the isotopic signatures are inconsistent across our lakes, seemingly due to subscale processes related to variations in lake depth, plant growth, and the underlying sediment.

4.1. Apparent Acetoclastic Production in Villäsjön

At first glance, the relatively high δ¹³C-CH₄ and low δD-CH₄ in the Villäsjön bubbles suggest dominant and consistent acetoclastic production (Figure 2a; Chanton et al., 2005). The δ¹³C-CH₄ alone is higher, and thus possibly a stronger indicator of acetoclasty, than the signatures of the Eriophorum spp.-dominated sites of the nearby fen (δ¹³C-CH₄ of −66.3 ± 1.6‰) where acetoclastic methanogens are known to be abundant (McCauley et al., 2014). Further, Villäsjön appears similar to many shallow thermokarst water bodies in its ebullition isotopic signature, reminiscent of the Yakutsk alasses where there is little evidence of hydrogenotrophy (Nakagawa et al., 2002).
Judging by the consistencies in the isotopic signatures (Figure 2a), the large internal variability in Villasjön’s bubble CH₄ fluxes (Figure 3) is seemingly not a result of shifting production pathways, despite differences in the underlying source material. Villasjön’s high fluxing littoral zone close to the fen (51.5 mg CH₄ m⁻² day⁻¹ on average, n = 912) is underlain by a layer of mixed peat (*Sphagnum* and *Carex* spp.), which eroded from the mire around 2,100 years BP (Kokfelt et al., 2010), on top of coarse detritus gyttja, as opposed to other shallow areas of lower average flux (17.3 mg CH₄ m⁻² day⁻¹, n = 1,297) where only gyttja is present (Wik et al., 2018). Overall, mixed peats can be more effective in producing CH₄ than those only containing *Sphagnum* spp., but they are not an obvious source of abundant acetate (Hines et al., 2008). In comparison to the Stordalen fen, and the other two lakes (see below), Villasjön has little growth of submerged vegetation (macrophytes) that could provide acetate to the methanogens (Wik et al., 2018).

**Figure 2.** Relationships of δ¹³C-CH₄ and δD-CH₄ in bubbles from (a) the overall shallow Villasjön, (b) Inre Harrsjön, and (c) the deepest but smallest Mellersta Harrsjön. Data are color coded by lake zone; shallow and littoral (0–2 m), intermediately deep (2–4 m), and profundal (4–7 m). The arrows emphasize an apparent production shift in Figure 2b and a possible oxidation effect in Figure 2c.

| Lake         | Zone                | Flux (mg CH₄ m⁻² d⁻¹) | δ¹³C-CH₄ (%) | δD-CH₄ (%) |
|--------------|---------------------|-----------------------|-------------|-----------|
| Inre Harrsjön| Shallow center      | 525                   | 14          | 22        |
|              | Littoral            | 1556                  | 18          | 29        |
|              | Intermed. deep      | 1885                  | 30          | 50        |
|              | Profundal           | 549                   | 13          | 18        |
| Mellersta Harrsjön | Littoral (southern)   | 807                   | 18          | 28        |
|              | Other littoral      | 802                   | 26          | 36        |
|              | Intermed. deep      | 1084                  | 14          | 20        |
|              | Profundal           | 810                   | 14          | 19        |
| Villasjön    | Littoral (near fen) | 912                   | 12          | 13        |
|              | Other shallow       | 1297                  | 15          | 16        |

**Figure 3.** Boxplots of the bubbles’ CH₄ flux, δ¹³C-CH₄, and δD-CH₄ binned by lake and depth zone. Whiskers indicate 10th and 90th percentiles, and gray circles outliers. Black circles and horizontal lines denote means and medians, respectively. Different letters among categories indicate statistical variation at a significance level of 5% (Tukey’s HSD: P < 0.05). The table to the right shows number of samples (n) in each category. Data are color coded by lake zone; shallow and littoral (0–2 m), intermediately deep (2–4 m), and profundal (4–7 m). A table version of this figure also showing whole lake totals is available in the supporting information (Table S1).
Without an obvious source for the acetate, it is possible that the apparent acetoclastic signature in Villasjön is in fact a result of CH4 oxidation, in the sediment before the bubbles are released. Oxidation in the water column of our study lakes has recently been confirmed to remove 30–100% of dissolved CH4 (Jansen et al., 2019), shifting the δ13C-CH4 by up to ~+20‰ from sediment bubble CH4 to dissolved CH4. If some of that oxidation occurs prior to bubble release, it could explain much of apparent spread toward acetoclasty in our bubble CH4 data. Anaerobic oxidation of CH4 (AOM), possibly with sulfate, is also a potential process in our lake sediments (Wik et al., 2018). AOM has been found to occur in high-latitude shallow lakes; a recent study presents isotopic and microbial evidence for this type of oxidation in the sediments of a shallow thermo-karst lake (Winkel et al., 2019).

However, in Villasjön a clear oxidation signal is not present, which in theory would show as enrichments of both 13C and D (Chanton et al., 2005). Previously, such isotopic patterns have been found in dissolved CH4 (Cadieux et al., 2016; Jansen et al., 2019) but not yet in bubbles, possibly because oxidation requires exchange of CH4 into the dissolved phase prior to oxidation by methanotrophs. On the other hand, because the isotopic fractionation due to AOM is not fully understood (Norði et al., 2013; Schubert et al., 2011; Winkel et al., 2019) there is a possibility that it might shift the isotopic signature differently.

Although both the presence of CH4 oxidation and acetate and its effects on ebullition remain to be investigated in Villasjön, this lake might be an example of why previously established isotopic signature patterns, partly based on marine samples, for example, those presented by Whiticar et al. (1986), are not straightforward or ideal as reference schemes when investigating CH4 production pathways in shallow freshwater lakes. Currently, isotopologues of the emitted CH4 provide few clues to explain Villasjön’s heterogeneous ebullition patterns.

4.2. Depth-Dependent Isotopic Shifts in Inre Harrsjön

Compared to Villasjön, the large spread in isotopic signatures in the bubbles from Inre Harrsjön (Figure 2b) suggest an overall shift from apparent acetoclastic to hydrogenotrophic methanogenesis with increasing water depth (Chanton et al., 2005; Whiticar et al., 1986). Rooted macrophytes are abundant in some of Inre Harrsjön’s shallow and littoral zones (Horruitiner, 2018) and would provide acetate to the methanogenic communities (Hines et al., 2008). Inre Harrsjön’s high fluxing shallow center where the bubbles’ δ13C-CH4 is highest and δD-CH4 is the lowest (Figures 2b and 3), thus seemingly dominated by acetoclastic production (Figure 2b), has abundant growth of Myriophyllum alterniflorum (commonly known as alternateflower watermilfoil; Horruitiner, 2018). Interestingly, most of Inre Harrsjön’s other shallow and littoral zones which bubble less frequently and isotopically appear to be more influenced by hydrogenotrophy (Figures 2b and 3) are dominated by growth of Potamogeton alpinus (alpine pondweed; Horruitiner, 2018). Perhaps the spatial differences in plant species play a much larger role in contributing to CH4 production and bubble formation through both microbial communities and C sources in this lake than what has been considered previously, to the extent of acting as proxies for ebullition potential.

The observed isotopic shift further implies that the source C is gradually depleted of acetate moving toward the deeper parts of the lake and therefore less suitable to sustain acetoclastic methanogenesis (Hodgkins et al., 2014; Whiticar et al., 1986) and CH4 production to sustain frequent ebullition. Already at 3.5-m water depth the sediments consists mainly of fine-grained, highly decomposed OM (Kokfelt et al., 2009) as opposed to more coarse OM at shallower depths (Wik et al., 2018). Inre Harrsjön also lacks a permanent surface water
inlet and is partly spring fed (Nilsson, 2006), suggesting limited C input and slow lateral transport of OM across the lake.

Apart from the shallow center, overall recalcitrant OM in Inre Harrsjön might explain why it consistently bubbles the least of our three study lakes (Figure 3). It is noteworthy that the lowest average bubble CH$_4$ fluxes (0.4–4 mg m$^{-2}$ day$^{-1}$) are measured in the eastern littoral zone of Inre Harrsjön where plant growth is almost absent. The few bubbles that are released here also carry relatively little CH$_4$ (average concentrations of 7–16%) (Wik et al., 2013), again suggesting that certain species of macrophytes might play a role in fueling littoral zone ebullition through deposition of high quality C. Again, another possible explanation for the low bubble CH$_4$ fluxes and concentrations in Inre Harrsjön could be oxidation prior to bubble release, as explained above (see section 4.1). However, similar to Villasjön a clear oxidation signal is not present in Inre Harrsjön’s isotopic data (Figure 2b).

### 4.3. Clustered Signatures in Mellersta Harrsjön

As opposed to Inre Harrsjön, the clustered signature pattern in Mellersta Harrsjön (Figure 2c) suggests a homogenous production pathway despite this lake’s steeper hypsographic curve and a larger spatial variation in bubble CH$_4$ flux (Figure 3). Mellersta Harrsjön also has abundant rooted macrophytes, mainly *Myriophyllum alterniflorum* and *Sparganium angustifolium* (floating Bur-reed) and coarse plant debris across its littoral zone sediments and its deep hole has merely fine-grained, highly decomposed OM (Horruitiner, 2018; Wik et al., 2018). However, Mellersta Harrsjön is different from Inre Harrsjön in many ways. The main difference is arguably the permanent water inlet via the catchment’s main stream and a similar size outlet nearby (Figure 1). The stream transports terrestrial particulate organic carbon (POC), which would settle when reaching the deeper lake water depths (Lundin et al., 2013; Olefeldt & Roulet, 2012). Apart from being deeper, Mellersta Harrsjön is also much smaller and has greater potential for lateral transport of both suspended and deposited OM.

From sediment cores taken in Mellersta Harrsjön (Wik et al., 2018), we can assume that this lake’s fine-grained deep zone sediments lack abundant acetate, and that the CH$_4$ emitted from here is hydrogenotrophic. Because of this and the clustered signature pattern (Figure 2c) we also assume that hydrogenotrophic methanogenesis is dominant throughout Mellersta Harrsjön’s littoral zones. Further, about 50% of the isotopic data in this lake show signs of CH$_4$ oxidation, particularly in bubbles released from the deep zone (Figure 2c). Due to frequent sampling (most often daily) and an elongated and narrow funnel tip, forming a small area to volume ratio for the headspace in each trap, oxidation, and diffusive resorption of accumulated CH$_4$ can be considered negligible (Wik et al., 2013). Whether CH$_4$ oxidation (possibly AOM) is truly present in the sediment before the bubbles are released and what effect it could have on ebullition and whether acetoclastic methanogenesis plays a role in Mellersta Harrsjön remains to be investigated, potentially by investigating the microbial communities. Once again the stable CH$_4$ isotopes provide few direct clues of the ebullition. As for now, the ~30 mg m$^{-2}$ day$^{-1}$ difference in average bubble CH$_4$ flux from the littoral area in the southern part of the lake to the profundal zone (Figure 3) seems to be largely physically controlled, that is, by variations in energy transfer and temperature (Jansen et al., 2019; Wik et al., 2014), instead of differences in production pathway related to varying qualities of the underlying sediment (possibly related to OM input from the stream and burial of aquatic plant debris), unless of course the abundance and activity of microbial communities are inconsistent across the lakes.

### 4.4. Seasonal Isotopic Variations

The apparent nonexistent temporal patterns in the bubbles’ $\delta^{13}$C-CH$_4$ (Figure 4a and supporting information Figure S1) suggest that there are no seasonal shifts in the CH$_4$ production pathways or short-term changes in the OM used by the methanogens. This is consistent with the recent observation that gas bubbles’ $\delta^{13}$C-CH$_4$ in the sediment of the same lakes was similar in summer and winter; $–70 \pm 8\%e$ in winter and $–68 \pm 6\%e$ in summer (Jansen et al., 2019). Gas pockets from which bubbles are released requires an overlying sediment burden to form (Fendinger et al., 1992). The OM, either terrestrially derived or autochthonous, for example, from in situ macrophytes, must therefore undergo diagenesis before contributing to ebullition. Sedimentation rates in the Stordalen lakes are slow (1.5 mm year$^{-1}$ in the intermediate depth zone of Inre Harrsjön; Kokfelt et al., 2009) and the potential effect of annual rooting from plant growth on ebullition is likely not observable over a few years only.
The seasonal shift in the bubbles’ ΔD-CH₄ with a significantly overall lower signal in the beginning of the summer (Figure 4b) is likely driven by evaporation. In sediment gas pockets, the summertime ΔD-CH₄ has been measured to −308 ± 43‰; essentially the same as the winter −301 ± 12‰ (Jansen et al., 2019), suggesting that the difference is not due to changes in CH₄ production pathways. When the lakes heat up over summer evaporation can cause hydrogen isotope fractionation of the water due to evaporation from the surface, which becomes enriched in D (Cappa et al., 2003). The lakes are often mixed down to 3 m (Wik et al., 2013), and because water is a H source for methanogens (Ferry, 1992), this may explain the temporal shift in monthly ΔD-CH₄. Over winter, the Stordalen lake waters has ΔD-H₂O = −93.1 ± 1.1‰ (−93.5 ± 7.7‰ before ice out; Jansen et al., 2019); as this value becomes less depleted due to evaporation over the course of the summer, the contribution of H from this lake water to the CH₄ production would tend to produce CH₄ slightly more enriched in D as the summer progresses, in agreement with our observations. The fractionation effect of the water may also drive the overall spread in ΔD-CH₄ observed in the littoral, shallow and intermittently deep zones (2–4 m) of all three lakes (Figure 2).

4.5. Intersite Comparisons

4.5.1. Large Overlaps in Δ¹³C-CH₄ Among Lake Types

The few existing lake studies that report stable isotopes of CH₄ bubbles hint that there are distinct signature differences among lake types, related to differences in production pathway and underlying OM. For example, Walter et al. (2008) report persistent CH₄ bubbling from thermokarst lake seeps as hydrogenotrophic and lower more erratic fluxes as acetoclastic. Such findings are contrasting to the more inconsistent isotopic patterns in relation to ebullition variation shown and discussed in this paper. Cadieux et al. (2016) and Thompson et al. (2016) also found somewhat clearer separation between seemingly hydrogenotrophic and acetoclastic production of CH₄ from profundal (5- to 8-m depth in that study) versus littoral sediments of glacially carved, hydrologically closed lakes in western Greenland. In contrast to our study, it seems possible that the limited vegetation and hydrological linkages of the Greenland lakes contribute to more clear distinguished zones of isotopic variation there and thus also apparent differences between acetoclastic and hydrogenotrophic. Nonetheless, the general isotopic trend suggesting more influences of hydrogenotrophic production from profundal zones and acetoclastic from littorals matches our observations in Inre and Mellersta Harrsjón (Figure 2).

If there are lake type-specific signatures that represent underlying CH₄ sources, they are not distinguishable within the large overlaps, particularly in Δ¹³C-CH₄ that exist in the published data (Figure 5). Our observations of Δ¹³C-CH₄ from the Stordalen lakes alone (ranging from −78.4‰ to −53.1‰), where seeps are absent (Wik et al., 2013), overlap most signatures of bubbles from other high-latitude water bodies, including thermokarst lakes in Alaska and Siberia of which some are underlain by Pleistocene C and have persistent seep ebullition (−79.7‰ to −53.7‰; Walter et al., 2008), and smaller ponds in Arctic Canada (−67.6‰ to 52.1‰; Bouchard et al., 2015). They also overlap the Δ¹³C-CH₄ of total wetlands emissions, both locally from the Stordalen Mire (−79.6 to −66.3; McCalley et al., 2014) and on regional scales across the Arctic and boreal zone (−76.8‰ to −55.0‰; Fisher et al., 2017; Quay et al., 1988; Sriskantharajah et al., 2012; Thornton et al., 2016). Fisher et al. (2017) have pointed out that air appears to mix quickly above northern wetland sources, such that CH₄ emissions on larger scales can be assigned very precise signatures. Wetland sites as analyzed in Fisher et al. (2017) included embedded lakes, and it seems not possible at this point to distinguish the wetland and lake sources within the isotopic signatures reported in that work. Additionally, ebullition is not the only pathway for lakes to emit CH₄ to the atmosphere. Diffusive flux from dissolved CH₄, which is subject to more in-lake oxidation than bubble CH₄, represent a substantial fraction of the net emissions (Bastviken et al., 2011; DelSontro et al., 2018; Wik et al., 2016).

Although the general understanding is that most lakes receive much of their organic loading from the surrounding landscape and from autochthonous algae and phytoplankton production (Tranvik et al., 2009), and thermokarst lakes via erosional margins (Sannel & Kuhry, 2011), in situ submerged vegetation should not be overlooked as an important source of labile C. Given the intense growth of macrophytes in the Stordalen lakes (Horruitiner, 2018; Wik et al., 2018), which we consider representative for many other shallow northern water bodies, there is a possibility that autochthonous plant debris contributes to the similarities in Δ¹³C-CH₄ among lake types. Indeed, for isotopic characterization of northern lakes, it appears that local water depth is a large factor in determining the isotopic signature of CH₄ in bubbles. However, that
signature varies internally across lakes, with littoral shallows differing greatly from profundal areas in studies which distinguished the sampling depth (Bouchard et al., 2015). Here also, it is interesting that the shallowest of our study lakes, Villasjön, produces bubbles with \( \delta^{13}C-\text{CH}_4 \) consistently closest to that emitted from the nearby flooded fen (~\(-56\%\); Singleton et al., 2018). In contrast, the profundal areas of Inre and Mellersta Harrsjön produce \( \text{CH}_4 \) with more depleted \( \delta^{13}C-\text{CH}_4 \) values; this is consistent with less oxidation in profundal zones compared to littoral portions of the lakes.

Despite the fact that \( \delta^{13}C-\text{CH}_4 \) are overlapping in the available data, potential isotopic differences among lake types cannot be ruled out. Measurements of \( ^{13}C-\text{CH}_4 \) from northern lakes are still rare in comparison

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Relationships of \( \delta^{13}C-\text{CH}_4 \) and \( \delta D-\text{CH}_4 \) in bubbles emitted from different types of northern lakes. The symbols are site-specific means, and error bars denote local SDs reported in the studies or calculated from published data. The upper and lower horizontal dashed lines indicate the overall mean \( \delta D-\text{CH}_4 \) of postglacial (including the Manitoba beaver pond) and thermokarst lake sites, respectively. The underlying ellipses are schematic and highlight the ranges of in SDs for each of the two lake types.
to reported fluxes. This mainly concerns postglacial water bodies, particularly those in the boreal region, of which many are deeper and different in terms of sediment composition compared to the more commonly studied thermokarst lakes (Bouchard et al., 2015; Matveev et al., 2018; Walter et al., 2008).

### 4.5.2. Higher δD-CH₄ From Postglacial Lake Eubullition

There are also visually large overlaps in eubullition δD-CH₄ among lake types (Figure 5), but there are differences in the current data. Variations in δD-CH₄ have been found previously in bubbles emitted from different generations of thermokarst lakes (Brosius et al., 2012). Further, the bubbles from our lakes at Stordalen show relatively high average δD-CH₄ (~327.6 ± 23.8 to –308.2 ± 21.1) compared to other northern lake sites (Figure 5). This comparison excludes some Alaskan lakes that are in a coastal or thermogenic setting underlain by coal seams (Anthony et al., 2012; Douglas et al., 2016). In general, when looking at the published isotopic numbers, including the data from this study, bubbles from postglacial lakes have significantly higher average δD-CH₄ (~340.5 ± 24.3‰, n = 15) compared to the thermokarst water bodies (~361.0 ± 28.4‰, n = 25; p = 0.026; Figure 5). We might explain part of this difference as the general shallower nature of thermokarst water bodies compared to postglacial lakes; the deep zone sediments in our study and in both Cadieux et al. (2016) and Thompson et al. (2016) emitted bubbles with higher δD-CH₄ than the littoral sediments. Thermokarst lakes, being shallower, receive substantial OM inputs from the surrounding shorelines, and in their eubullition isotopic signature patterns are similar to the littoral zone of postglacial lakes with their greater seasonal organic input from macrophytes. Further, we speculate that the high productivity and OM content and relatively shallow nature of another water body type, the beaver pond, causes it to isotopeically appear more acetoclastic dominated, and have a much lower δD-CH₄ (Dove et al., 1999), similar to thermokarst lakes than postglacial lakes overall.

The difference in δD-CH₄ among lake types may also be partly caused by latitudinal and regional variations in the isotopic composition of the precipitation (Dansgaard, 1964) and possibly also by evaporation, depending on when the measurements have been made. Note that the shift in δD-CH₄ over the season in the Stordalen lakes (discussed in section 4.4 and Figure 3) equals the overall difference between the postglacial and thermokarst water bodies illustrated in Figure 5. Interestingly, if we would have made measurements in June only, our δD-CH₄ would be similar to the thermokarst lakes. Hence, more whole-season measurements are needed and may resolve whether the difference in δD-CH₄ among lake types truly reflects natural variability.

Even though our data compilation suggests that lake type is more distinguishable in δD-CH₄ than δ¹³C-CH₄, the practical usefulness of this is currently limited. Northern lakes emit globally significant amounts of CH₄ (Wik et al., 2016), but compared to the total annual CH₄ inputs to the atmosphere it is relatively small (~3–4%; Saunois et al., 2016). Shifts in the δD-CH₄ on this single emission type would produce proportionately smaller shifts in atmospheric δD-CH₄, making attribution of emitters via δD-CH₄ observations extremely difficult (to say nothing of the present relative rarity of atmospheric δD-CH₄ measurements). Nonetheless, this is a factor that should be included in comprehensive assessments of future atmospheric CH₄ isotopic composition. For current understanding of lake and landscape CH₄ emissions and currently available instrumentation for δD-CH₄ measurements, the overlap of wetland and lake emissions currently prohibits distinguishing lakes and wetlands in such studies, though this may change in the future. Although postglacial and thermokarst lakes appear to have significantly different δD-CH₄, future changes in high-latitude lake abundance seem unlikely to produce a shift in atmospheric δD-CH₄ that is globally significant, in contrast to the potential shift in δ¹³C-CH₄ due to thawing permafrost (McCalley et al., 2014).

## 5. Summary and Concluding Remarks

Using a substantial data set collected over several years, we have shown that the stable isotopic signatures of CH₄ bubbles vary persistently and significantly across three small interconnected subarctic lakes. Most of the variability is in the δ¹³C-CH₄, which overlap the majority of signatures reported from other northern sites, including wetlands. The spread in the data seems to be driven by differences in organic loading due to the lakes’ varying morphometrics, and possibly in some zones the burial of in situ macrophytes controlling the δ¹³C-CH₄, and evaporation-driven isotopic fractionation of the source water causing temporal variations in δD-CH₄. There does appear to be a trend in our data and other studies that the signatures of bubbles emitted from profundal portions of lakes predominantly suggest hydrogenotrophy and from littoral zones
acetoaclastic CH$_4$ production; due to the general shallowness of thermokarst lakes, they appear more aceto-
clastic, like the shallowest lake in our study. Nonetheless, in the Stordalen lakes, the isotopic patterns do not
correspond to the variation in bubble CH$_4$ flux, at least not consistently. At best the isotopic signatures
currently hint at methanogenic pathway and whether CH$_4$ oxidation is present but overall they provide
few clues on our lakes’ heterogeneous ebullition patterns. Possible heterogeneity of AOM within the sedi-
ments of our study lakes, though still unconfirmed, could also lead to the observed spread in the $\delta^{13}$C-CH$_4$ values. The same considerations likely apply for lakes across regional scales. Local isotopic data
appear highly arbitrary and although they contain useful information it is erratic across our studied lakes.
Without previous knowledge of the underlying sediment and water depth regimes the isotopic interpretation
of lake CH$_4$ bubbles is a shot in the dark. Currently, it seems nearly impossible to distinguish CH$_4$ emissions
of different lake types or sources using stable isotopes; the overlaps are simply too large. Further, the inter-
pretation schemes commonly used for linking stable isotopic signatures to methanogenic pathway might be
misleading or at least not as straightforward as believed. Perhaps this is due to yet large unknowns surrounding
AOM and possible effects it can have on the isotopic patterns; diagnostics for AOM and methanotrophy
are needed to inform interpretation of the isotopic patterns. There is still a relative lack of isotopic observa-
tions in lake CH$_4$ flux research. Further studies, linking both $\delta^{13}$C-CH$_4$ and $\delta^{2}$D-CH$_4$ to sediment C quality,
microbial communities, plant species, and to AOM, should narrow down the variations to more precise
source-specific signatures from northern lakes.
This could differentiate them from other high-latitude terres-
trial sources, which would benefit models aiming to partition atmospheric CH$_4$ inputs.

Data Availability Statement
All the data used in the analyses are available for download at the Bolin Centre Database (https://bolin.su.
se/data/stordalen-lakes-ch4-ebul-3).

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