Organic matter and sediment properties determine in-lake variability of sediment CO₂ and CH₄ production and emissions of a small and shallow lake

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Abstract. Inland waters, particularly small and shallow lakes, are significant sources of carbon dioxide (CO₂) and methane (CH₄) to the atmosphere. However, the spatial in-lake heterogeneity of CO₂ and CH₄ production processes and their drivers in the sediment remain poorly studied. We measured potential CO₂ and CH₄ production in slurry incubations from 12 sites within the small and shallow crater lake Windsborn in Germany, as well as fluxes at the water–atmosphere interface of intact sediment core incubations from four sites. Production rates were highly variable and ranged from 7.2 to 38.5 µmol CO₂ gC⁻¹ d⁻¹ and from 5.4 to 33.5 µmol CH₄ gC⁻¹ d⁻¹. Fluxes ranged from 4.5 to 26.9 mmol CO₂ m⁻² d⁻¹ and from 0 to 9.8 mmol CH₄ m⁻² d⁻¹. Both CO₂ and CH₄ production rates and the CH₄ fluxes exhibited a significant and negative correlation (p<0.05, ρ = ±0.65) with a prevalence of recalcitrant organic matter (OM) compounds in the sediment as identified by Fourier-transformed infrared spectroscopy. The carbon/nitrogen ratio exhibited a significant negative correlation (p<0.01, ρ = −0.88) with CH₄ fluxes but not with production rates or CO₂ fluxes. The availability of inorganic (nitrate, sulfate, ferric iron) and organic (humic acids) electron acceptors failed to explain differences in CH₄ production rates, assuming a competitive suppression, but observed non-methanogenic CO₂ production could be explained up to 91% by prevalent electron acceptors. We did not find clear relationships between OM quality, the thermodynamics of methanogenic pathways (acetoclastic vs. hydrogenotrophic) and electron-accepting capacity of the OM. Differences in CH₄ fluxes were interestingly to a large part explained by grain size distribution (p<0.05, ρ = ±0.65). Surprisingly though, sediment gas storage, potential production rates and water–atmosphere fluxes were decoupled from each other and did not show any correlations. Our results show that within a small lake, sediment CO₂ and CH₄ production shows significant spatial variability which is mainly driven by spatial differences in the degradability of the sediment OM. We highlight that studies on production rates and sediment quality need to be interpreted with care, though, in terms of deducing emission rates and patterns as approaches based on production rates only neglect physical sediment properties and production and oxidation processes in the water column as major controls on actual emissions.
1 Introduction

Inland waters play an important role in the global carbon (C) cycle and contribute significantly to natural emissions of the greenhouse gases carbon dioxide (CO$_2$) and methane (CH$_4$) (Cole et al., 2007; Battin et al., 2009; Bastviken et al., 2011; Raymond et al., 2013; Regnier et al., 2013). Lakes and reservoirs are estimated to emit in total 0.32–0.39 Pg C of CO$_2$ and 0.58 Pg C (CO$_2$ eq.) of CH$_4$ year$^{-1}$ (Cole et al., 2007; Bastviken et al., 2011; Raymond et al., 2013), but especially small lakes (<0.1 km$^2$) have previously been underestimated regarding their spatial expansion and, therefore, their contribution to global greenhouse gas emissions (Downing, 2010). Although small lakes account for ~ 1/3 of the total lake area and cover less than 1% of the global land surface, they contribute 35% of the CO$_2$ and 72% of the CH$_4$ emissions from lakes worldwide (Downing et al., 2006; Holgerson and Raymond, 2016). Further, even though most lakes are found in boreal zones, the largest areas occur in lower latitudes around 50° (Verpoorter et al., 2014). In small lakes, due to their shallowness, shorter water residence times and smaller perimeter to volume ratios, metabolic processes and C turnover are much faster than in larger lakes, making small lakes potentially more susceptible to environmental and climatic changes (Wetzel, 1992; Downing, 2010).

Recently, many studies have shown that both CO$_2$ and CH$_4$ fluxes are highly variable both on spatial and temporal scales, but the majority of these measurements have been performed on larger lakes and are concentrated in boreal regions (Schilder et al., 2013; Wik et al., 2013; Bastviken et al., 2015; Natchimuthu et al., 2016, 2017; Spafford and Risk, 2018). Beyond that, few studies have examined greenhouse gas (GHG) production processes in the sediments or have attempted to link sediment GHG production to emissions. Nevertheless, anoxic sediments are important for whole-lake C cycling as the CO$_2$ and CH$_4$ produced there can be released through the water column to the atmosphere. To understand the spatial patterns of CO$_2$ and CH$_4$ emissions, it is, therefore, of interest to also understand CO$_2$ and CH$_4$ production processes in the sediment, as well as their major controls.

The amount of CO$_2$ and CH$_4$ produced in lake sediments relates to the degradability of the organic matter (OM) present, which depends primarily on its quality, the microbial biomass and enzyme activities (Updegaff et al., 1995; McLatchey and Reddy, 1998; Fenchel et al., 2012). While the origin and the degradation state of OM can be determined using carbon/nitrogen (C/N) ratios, qualitative information about OM components can be determined via Fourier-transformed infrared (FTIR) spectroscopy. The latter technique can, therefore, further provide information about the origin and degree of decomposition of OM (Meyers, 1994; Broder et al., 2012; Biester et al., 2014; Li et al., 2016). The prevalence of different OM compounds leads to specific features in FTIR spectra as functional moieties have wavelength-specific absorption maxima (Niemeyer et al., 1992; Cocozza et al., 2003). Artz et al. (2008) compiled a range of functional moieties that are used to characterize OM in peat soils, which is also applicable to OM in general. While polysaccharides and proteins are preferentially degraded by microorganisms, aliphatic (e.g., waxes) and aromatic compounds (e.g., lignin) are more recalcitrant (due to their molecular structure) and, therefore, residually accumulate in the anoxic sediment (Fenchel et al., 2012; Tfaily et al., 2014).

In anoxic sediments, CO$_2$ and CH$_4$ are produced during the breakdown of OM over a cascade of microbiologically mediated processes. After the cleavage of complex organic polymers, resulting monomers are fermented, which mainly produce hydrogen (H$_2$) and low molecular weight organic compounds (e.g., acetate). The low molecular weight compounds are further oxidized to CO$_2$, and H$_2$ to H$_2$O upon consumption of electron acceptors (EAs; nitrate, sulfate, ferric iron or humic substances). Only subsequently is CH$_4$ production as the final step of terminal electron-accepting processes initiated after all other thermodynamically more favorable EAs are depleted (Achnich et al., 1995; Blodau, 2011). CH$_4$ is mainly produced via two different pathways, the acetoclastic pathway and the hydrogenotrophic pathway, for which either acetate is the substrate (acetoclastic; Reaction R1) or H$_2$ is the substrate and CO$_2$ is the EA (hydrogenotrophic; Reaction R2) (Conrad, 1999; Whiticar, 1999). Values for $\Delta G^0_r$ for these terminal electron-accepting processes and methanogenesis can be calculated from standard formation energies $\Delta G^0_f$ in aqueous state, as listed in Stumm and Morgan (1995) and Nordstrom and Munoz (1994):

\[
\text{CH}_3\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_4 \\
\Delta G^0_f(25\degree\text{C}) = -50.99 \text{kJ mol}^{-1}, \quad (\text{R1})
\]

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow 2\text{H}_2\text{O} + \text{CH}_4 \\
\Delta G^0_f(25\degree\text{C}) = -193.03 \text{kJ mol}^{-1}. \quad (\text{R2})
\]

Several studies have shown that in anoxic wetland and marine sediments rich in labile organic compounds, the acetoclastic pathway dominates. With increasing recalcitrance, the hydrogenotrophic pathway becomes more important as acetate, being the direct precursor of CH$_4$, is depleted (Schoell, 1988; Hornibrook et al., 1997; Miyajima et al., 1997). As suggested by Lojen et al. (1999), the OM quality in lake sediments might thus be responsible for seasonal changes in the dominant CH$_4$ pathway.

Whereas many studies have shown that inorganic EAs can suppress methanogenic activity (e.g., Yao et al., 1999; Fenchel et al., 2012), information on the role of humic substances as organic EAs remains scarce. In one study, Klüpfel et al. (2014) revealed that humic substances can suppress methanogenic activity (e.g., lignin) are more recalcitrant (due to their molecular structure) and, therefore, residually accumulate in the anoxic sediment (Fenchel et al., 2012; Tfaily et al., 2014).
major control on CO₂ and CH₄ production (Gao et al., 2019). As sediments from the lake we investigated are also rich in OM, we wanted to verify whether the EAC and electron-donating capacities (EDC) of humic substances also play a vital role in explaining the spatial variabilities of CO₂ and CH₄ production in lake sediments. Although they are not subject to water table fluctuations, they might, in the upper parts of the sediments, be influenced by oxygen penetration from the water column due to a well-mixed water body, particularly in shallow lakes (Lau et al., 2016).

Overall, the amount of CO₂ and CH₄ produced, therefore, depends on either the availability and degradability of the OM itself, the presence of EAs, or the levels of H₂ and acetate as substrates for methanogenesis (Segers, 1998; Conrad, 1999; Megonigal et al., 2003; Blodau, 2011; Fenichel et al., 2012).

Within lakes, the spatial distribution of OM and other sediment properties vary considerably in terms of their origin (terrestrial vs. aquatic), degradability, elemental geochemistry and grain size (Muri and Wakeham, 2006; Ostrovsky and Tęgowski, 2010; Tolu et al., 2017). Further, sediment grain size is an important factor for the evolution of CH₄ bubbles in sediments (Ostrovsky and Tęgowski, 2010; Liu et al., 2018); for example, Liu et al. (2016) revealed that CH₄ ebullition decreases with increasing shares of sand in lake sediments. Overall though, CH₄ ebullition is thought to account for a large proportion (75 %) of CH₄ emissions from lakes (Bastviken et al., 2011).

Until now, laboratory incubations of lake sediments were mostly conducted with samples from one or a few sites within one lake with a focus on comparing different lakes with each other rather than covering a high in-lake variability of production rates. Further, these studies emphasize temperature effects on production rates (Duc et al., 2010; Gudasz et al., 2010, 2015; Fuchs et al., 2016). Unlike in peat soils, where a broad range of process-based controls on CO₂ and CH₄ production has been studied, in small lakes, controls such as OM quality, the occurrence of alternative EAs, thermodynamic processes and sediment grain size have not or have only individually been systematically surveyed. To close this knowledge gap, we determined the magnitude and spatial variability of sediment CO₂ and CH₄ production in a small and shallow temperate lake in order to relate observed production patterns to measured OM and sediment characteristics, thermodynamics of methanogenesis, and water-atmosphere fluxes. To this end, we conducted slurry and intact sediment core incubations with sediment from the crater lake Windsborn in Germany. This site was chosen as a model system because it has a high sediment OM content (∼30 %), a very small catchment area, and no surficial in- or outflows, meaning minimal influence from the surrounding catchment.

We hypothesized (I) that CO₂ and CH₄ production vary spatially in the sediment, (II) that the variability in the production rates is reflected in the flux patterns; and (III) that the variation in production rates, methanogenic pathways and flux patterns can be explained by factors of OM degradability, availability of organic and inorganic EAs, and grain size distribution, or a combination of these factors.

2 Materials and methods

2.1 Study site

The studied Lake Windsborn is a polymictic, small, shallow crater lake in the Volcanic Eifel, Rhineland Palatinate, southwest Germany, and is part of the Mosenberg volcano group (see Fig. 1) (LfU, 2013). The climate is temperate with a mean annual temperature of 8.3 °C and 931 mm precipitation (multiannual mean 1981–2010; DWD, 2019). Lake Windsborn is the only genuine crater lake north of the Alps. It emerged approx. 29 000 years ago after a volcanic eruption when the top of the volcano had been blasted away and the newly formed crater was subsequently filled with water (LfU, 2013). The present lake formed around 1850, after drainage of the lake and the partial removal of the peat from the lake bottom (Kappes and Sinsch, 2005). The lake is part of a conservation area that was established in 1927 (Kappes and Sinsch, 2005). From 1950 until the 1990s, the lake was used as a fishing ground and was, therefore, stocked with fish and limed (LfU, 2013). The lake is nearly circular and surrounded by a 20 to 30 m high rampart which consists of an alternation of red-brown ashes, slag and lapilli from the eruption. Therefore, it has a very small catchment of only about 8 ha compared to the lake surface of 1.41 ha, and it has no surficial in- and outflows and is only fed by groundwater and precipitation (LfU, 2013; Meyer, 2013). The maximum lake depth varies between 1.3 and 1.7 m (Kappes and Sinsch, 2005). The area is underlain by Devonian quartzite (Kampf, 2005).

The lake’s shoreline is vegetated with Carex rostrata, Comarum palustre and Menyanthes trifoliata, all indicating poor nitrogen (N) supply (Ellenberg et al., 2001). At the northwestern riparian zone, there is a quaking bog mainly dominated by Sphagnum spec. whose expansion will slowly lead to the silting up of the lake. Lake Windsborn was previously considered humic-oligotrophic, but, in the early 1990s, it transitioned to a eutrophic lake, and now it is slowly recovering from human impacts and nutrient input (Kappes et al., 2000). During our measurement campaigns in 2017 and 2018, the lake exhibited partly meso- and eutrophic features as shown in Table 1.

2.2 Slurry incubations

2.2.1 Sampling and preparation of slurry incubations

For the slurry incubation experiment, samples were taken on three occasions (in March, April and May 2018) from 12 of the 13 sampling sites within the lake from three transects covering multiple water depths (<50, <100, <125 and
Table 1. Selected lake water characteristics measured in Lake Windsborn in 2017 and 2018: pH, conductivity (cond.), dissolved oxygen (O$_2$), chlorophyll α (Chl. α), dissolved organic carbon (DOC), total dissolved nitrogen (TN), chloride (Cl), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorous (P) and sulfur (S).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Average</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
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<td>pH</td>
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<td>6.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Cond.</td>
<td>387</td>
<td>19.4</td>
<td>1.7</td>
</tr>
<tr>
<td>O$_2$</td>
<td>397</td>
<td>9.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Chl. α</td>
<td>163</td>
<td>27.7</td>
<td>18.7</td>
</tr>
<tr>
<td>DOC</td>
<td>419</td>
<td>13.7</td>
<td>2.3</td>
</tr>
<tr>
<td>TN</td>
<td>419</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Cl</td>
<td>361</td>
<td>2.9</td>
<td>3.4</td>
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<table>
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<tr>
<th>Parameter</th>
<th>n</th>
<th>Average</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
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<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Fe</td>
<td>378</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>K</td>
<td>379</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Mg</td>
<td>379</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Na</td>
<td>379</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>P</td>
<td>329</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>S</td>
<td>379</td>
<td>0.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<150 cm) (see Fig. 1). On each sampling date, 4 of the 12 sampling sites were chosen randomly as it was not possible to set up the experiment with all samples at once. At the sampling dates, measured air, water and sediment temperatures at the sampling dates were 7.7, 5.6 and 5.2 °C (March), 13.8, 15.1 and 10.1 °C (April), and 23.9, 23.6 and 13.8 °C (May), respectively. Sediment samples were taken in duplicates with a gravity corer (UWITEC, Mondsee, Austria) from a boat in 60 cm long PVC tubes and transported in an insulated box at ∼5 °C. The next day, sediment cores were cut with a core cutter in the laboratory in segments of 5 cm thickness (0–5 and 5–10 cm sediment depth). Duplicate samples were homogenized, and then 20 g of each sediment was filled into 120 mL crimp vials containing 20 mL lake water, after which the vials were closed with a butyl rubber stopper and aluminum crimp cap. Samples were flushed with N$_2$ for 30 min in order to remove any remaining oxygen from the water and headspace, and then they were precultured for one week so they were fully anoxic. They were again flushed with N$_2$ prior to the actual incubation. Slurry incubations were set up in triplicates and maintained at 25 °C (corresponding to maximum measured in situ sediment temperatures in summer 2018) in the dark. During each run, one set of parallel samples was incubated at 10 °C in order to determine a $Q_{10}$ value for CO$_2$ and CH$_4$ production rates.

The remaining sample material was freeze-dried (Alpha 1–4 LDplus, Christ, Osterode, Germany), ground with a ball mill (Mixer Mill MM 400, Retsch, Haan, Germany) and used for solid-phase analyses, as outlined below.

2.2.2 Sediment OM quality

Freeze-dried and ground sediment samples were analyzed for total C and N concentrations and stable isotopes using isotope-ratio mass spectrometry (IRMS; EuroVector EA3000 coupled with Nu Instruments Nu Horizon, HEKAtech, Weggberg, Germany) and for OM components using FTIR spectroscopy (Cary 670 FTIR Spectrometer, Agilent, Santa Clara, USA).

For IRMS, 5 mg of sample was weighed out into a tin cup, together with 4 mg of vanadium pentoxide (V2O5). The combustion and reduction furnaces were set to 1000 and 650 °C, respectively, and the resultant gaseous compounds were quantified by IRMS. Results are provided in percent (%) by mass for C and N contents (precision <1% for C and <0.1% for N) and in per mill (‰) vs. VPDB/AIR (Vienna Pee Dee Belemnite; atmospheric N$_2$) for C and N isotopic signatures (precision <0.05‰ for $^{13}$C and <0.5‰ for $^{15}$N). For isotope analyses, appropriate certified reference materials were used: IAEA 600 ($\delta^{13}$C = −27.77‰; $\delta^{15}$N = 1.0‰) and BBOT (2.5-Bis-(5-
tert.-butyl-2-benzo-oxazol-2-yl)thiophen; HEKAtech, Weggberg, Germany), birch leaf, wheat flour and sorghum flour standards (IVA Analysetechnik e. K., Meerbusch, Germany) as working standards covering a range of −27.5 % to −13.68 % for $^{13}$C and −0.6 % to 2.12 % for $^{15}$N.

Functional groups of OM compounds were identified by FTIR spectroscopy. For this purpose, 2 mg of freeze-dried sample was ground together in a mortar with 200 mg KBr (potassium bromide), pressed into 13 mm pellets and analyzed. Each sample was scanned from 599 to 4000 cm$^{-1}$ with a resolution of 2 cm$^{-1}$ and was baseline corrected. Distinct peaks at specific wavelengths were assigned to functional groups according to Artz et al. (2008) and normalized to the peak intensity at 1031–1035 cm$^{-1}$ (indicative of polysaccharides) in order to obtain intercomparable peak ratios of functional moieties in all samples, as FTIR spectra only provide information about the relative abundance of certain functional moieties in one sample.

### 2.2.3 CO$_2$ and CH$_4$ production rates from slurry incubations

Potential CO$_2$ and CH$_4$ production rates were determined by measuring the increase in concentration of CO$_2$ and CH$_4$ in the incubation vials over time. Concentrations were obtained by analyzing the headspace at the beginning of the experiment ($t_0$) and after 1, 3, 8, 11, 14 and 18 d ($t_1$–$t_6$). Samples were taken from the vial with a 10 mL polypropylene syringe equipped with a three-way stopcock and a 0.6 mm needle. Before each sampling, the pressure inside the vial was determined with a pressure sensor (GMH 3110, Greisinger, Regensburg, Germany), and the syringe was flushed three times with N$_2$. Then 2 mL of N$_2$ was left in the syringe before the needle was stabbed through the vial’s stopper, whereby the N$_2$ was added to the headspace and mixed, and, subsequently, 2 mL of the sample was taken from the vial so that the volume inside the vial remained constant. The gas samples were analyzed for CO$_2$ and CH$_4$ concentrations with a gas chromatograph (8610 GC-TCD/FID, SRI Instruments, Torrance, USA) equipped with a flame ionization detector (FID) and methanizer to simultaneously measure CO$_2$ and CH$_4$. Before every sampling day, the gas chromatograph was calibrated with standard gas mixtures of known concentrations (CO$_2$: 385, 5000 and 50 000 ppmV; CH$_4$: 5, 1000 and 50 000 ppmV).

First, measured concentrations (in ppmV) were pressure corrected and converted using the ideal gas law:

$$n = (p \cdot V) / (R \cdot T),$$

(1)

where $n$ is the amount of substance (in mol), $p$ is the gas partial pressure (in atm), $V$ is the headspace volume (in L), $R$ is the ideal gas constant ($0.082 \text{ L atm mol}^{-1} \text{ K}^{-1}$) and $T$ is the laboratory temperature (in K).

Total CO$_2$ and CH$_4$ concentrations in the gas and water phase in the incubation vials were calculated from headspace concentrations using Henry’s law:

$$c = K_h \cdot p,$$

(2)

where $c$ is the concentration in the water phase (in mol L$^{-1}$), $K_h$ is the temperature-dependent Henry constant (CO$_2$: $25^\circ \text{C} = 0.0339 \text{ mol L}^{-1} \text{ atm}^{-1}$; CH$_4$: $25^\circ \text{C} = 0.00129 \text{ mol L}^{-1} \text{ atm}^{-1}$; Sander, 2015) and $p$ is the gas partial pressure (in atm).

Moreover, CO$_2$ concentrations were pH corrected in order to obtain pH-independent values for total CO$_2$ concentrations using the Henderson–Hasselbalch equation and equilibrium constants according to Stumm and Morgan (1995):

$$n_{\text{ΣCO}_2} = n_{\text{water}} \cdot 10^{pH-6.4} + (n_{\text{water}} \cdot 10^{pH-6.4} \cdot 10^{pH-10.25}),$$

(3)

where $n_{\text{ΣCO}_2}$ is the pH-corrected CO$_2$ amount (in mol) and $n_{\text{water}}$ is the calculated amount of CO$_2$ in the water phase (in mol).

Finally, production rates were calculated by linear regression ($R^2 > 0.8$ for CO$_2$ and $R^2 > 0.9$ for CH$_4$) from concentration change in gas and solute phase over time. To evaluate the effect of temperature on CO$_2$ and CH$_4$ production, we calculated $Q_{10}$ values, describing the relative increase of production rates with an increase in temperature of 10 K (Fenchel et al., 2012):

$$Q_{10} = (R_2 / R_1)^{10/(T_2-T_1)},$$

(4)

where $R_2$ is the production rate at $T_2$ ($25^\circ \text{C}$) and $R_1$ is the production rate at $T_1$ ($10^\circ \text{C}$).

### 2.2.4 Thermodynamics and methanogenic pathways

In order to calculate the thermodynamic energy yield for hydrogenotrophic and acetoclastic methanogenesis, we measured H$_2$ concentrations at the beginning, after 8 d ($t_5$) and at the end of the experiment, and we measured acetate concentrations at the beginning and at the end of the incubation. The thermodynamic energy yield, expressed as Gibbs free energy, was calculated using the Nernst equation and total dissolved and gaseous concentrations of educts and products in the incubation vials as described in Beer and Blodau (2007):

$$\Delta G_i = \Delta G_i^0 + R \cdot T \cdot \ln(\Pi_i(\text{products})^{vi}/\Pi_i(\text{educts})^{vi}).$$

(5)

By calculating the Gibbs free energy ($\Delta G_i$), it is possible to evaluate whether these processes are feasible under given conditions. In order for each reaction to occur, a theoretical threshold of $\Delta G_i = -20$ to $-25 \text{ kJ mol}^{-1}$ had to be exceeded (Schink, 1997; Conrad, 1999; Blodau, 2011).

For H$_2$ concentration measurements, 2 mL of sample was taken from the incubation headspace with a syringe and needle and replaced with the same amount of N$_2$. Samples were analyzed with a reduction gas detector (RGD) hydrogen and carbon monoxide analyzer (ta3000R Gas Analyzer, Ametek, Pittsburgh, USA) that was calibrated with
gas standards of 5, 25 and 50 ppmV H₂. Measured H₂ concentrations were corrected for pressure and converted into dissolved concentrations using Henry’s law (\(K_h(H_2, 25^\circ C) = 0.00078 \text{ mol L}^{-1} \text{ atm}^{-1}\); Sander, 2015) analogous to CO₂ and CH₄.

Acetate concentrations were determined through ion chromatography with chemical suppression (883 Basic IC plus, Metrohm, Filderstadt, Germany; A Supp 5 column, Metrohm, Filderstadt, Germany). Aqueous samples were filtered with 0.45 µm nylon plus glass microfiber syringe filters (Simplepure, BGB Analytik, Rheinfelden, Germany) and kept frozen at −21°C until analysis.

2.2.5 Alternative EAs

To quantify alternative EAs that could support anaerobic respiration and potentially suppress methanogenesis in anoxic incubations, we measured nitrate (NO₃⁻), sulfate (SO₄²⁻), ferric iron (Fe³⁺), and the EAC and EDC of the OM (EACOM and EDCOM) at the beginning and the end of the slurry incubation.

As the analysis of EACOM and EDCOM is only possible on finely ground materials, thus providing potential capacities rather than true in situ capacities, we set up a second set of slurry incubations for a sediment depth of 0–5 cm with samples from 10 sites (all except 3.50 and 3.100) using 0.4 g of the freeze-dried sediment material and 100 mL of Milli-Q® water. Slurry incubations were set up in six replicates, flushed with N₂, preincubated and stored analogously to the first set of slurry incubations. Samples were analyzed at the beginning and at the end of the experiment since at every sampling occasion, three of the replicates had to be sacrificed (destructive sampling). Prior to analysis, samples were transferred into a glovebox (O₂ <1 ppm; Innovative Technology, Amesbury, USA) to avoid alteration of the samples’ redox state.

EACOM and EDCOM were measured using chronoamperometry (CHI1000C, CH Instruments, Austin, USA) by mediated electrochemical reduction (MER) and oxidation (MEO) (Aeschbacher et al., 2010; Klüpfel et al., 2014). The cell consisted of a cylindrical glassy C working electrode, a platinum wire counter electrode in a ceramic glass frit and an Ag/AgCl reference electrode. Cells were filled with 10 mL of 0.01 M MOPS and 0.1 M KCl buffer to stabilize the pH at 7 and were continuously stirred during measurement. To facilitate electron transfer, organic mediators were added to the buffer: 180µL DQ (diaquat dibromide monohydrate; Sigma-Aldrich) for MER and 180µL ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-sulfonic acid), Sigma-Aldrich) for MEO at a potential of \(E_h = -0.69 \text{ V}\) and \(E_h = +0.41 \text{ V}\), respectively (reported vs. the standard H₂ electrode but experimentally measured vs. the Ag/AgCl reference electrode). To determine the electron transfer, 100 µL of suspended samples were added to the buffer solution (Lau et al., 2015) which resulted in an increase of the current, recorded as a peak in the analysis software. After ~ 30 min, when the baseline was reached again, the next sample was added to the cells. Samples were analyzed in duplicate. The electron transfer was calculated with the Nernst equation and normalized to the C content in the samples (Lau et al., 2015):

\[
\text{EACOM}(\text{EDCOM}) = \text{peak area} / (V \cdot F \cdot C),
\]

where \(V\) is the sample volume (in µL), \(F\) is the Faraday constant (96,485 A sec/mol e⁻) and \(C\) is the C content (in mg L⁻¹) (EACOM and EDCOM in µmol e⁻ gC⁻¹; peak area in µA seconds).

EACOM and EDCOM had to be corrected for either ferric iron (Fe³⁺) or ferrous iron (Fe²⁺) and sulfide (S²⁻) concentrations, respectively, since with the applied potential, Fe³⁺ would be reduced and Fe²⁺ and S²⁻ would be oxidized (Lau et al., 2015; Agethen et al., 2018).

Fe²⁺, Fe³⁺ and S²⁻ were determined colorimetrically (Gilboa-Garber, 1971; Tamura et al., 1974) with a spectrophotometer (Cary 100 UV-Vis, Agilent, Santa Clara, USA). Because 1,10-phenanthroline can only detect Fe³⁺, the Fe³⁺ in the samples was reduced to Fe²⁺ with 10 % ascorbic acid. Ferric iron was thus determined as the difference of total Fe and ferrous iron in the samples.

NO₃⁻ and SO₄²⁻ concentrations were determined with ion chromatography (IC), as described above. For this purpose, samples were filtered with a 0.45 µm syringe filter, added into micro-centrifuge tubes, retrieved from the glovebox and stored frozen at −21°C until analysis.

Total EAC (EAC_{tot}) was calculated as the sum of EACOM and EAC_{inorg} (EAC from nitrate, sulfate, ferric iron) considering the respective amounts of electrons transferred during the main pathways of dissimilatory reduction, i.e., assuming a reduction of NO₃⁻ to N₂, of SO₄²⁻ to S²⁻ and of Fe³⁺ to Fe²⁺ (Konhauser, 2009):

\[
\text{EAC}_{\text{tot}} = \text{EACOM} + \text{NO}_3^- \cdot 5e^- + \text{SO}_4^{2-} \cdot 8e^- + \text{Fe}^{3+} \cdot 1e^-.
\]

Assuming reversibility of electron transfer to and from OM, EACOM and EDCOM correlate with each other. If EACOM decreases, EDCOM increases equivalently as quinones are reduced to hydroquinones; in practice, values of EDCOM are potentially biased as MEO does not only capture EDCOM but may also irreversibly oxidize phenolic moieties, which are sensitive to the slightest changes in pH and potentials (Aeschbacher et al., 2011; Walpen et al., 2016, 2018). The discussion will, therefore, focus on EACOM data.

2.3 Intact sediment core incubations

2.3.1 CO₂ and CH₄ fluxes

To obtain ex situ CO₂ and CH₄ gas fluxes and estimate changes in sediment CO₂ and CH₄ stocks, intact sediment cores (PVC tubes, 60 cm length, 5.8 cm diameter) were taken in triplicate from 4 out of the 12 sites in November 2017.
CH$_4$ fluxes were determined by closing the cores with the stopper for 24 h and taking a gas sample right after closing and then again after 24 h with a syringe from the headspace. CH$_4$ fluxes were calculated according to Bastviken et al. (2004):

$$F (\text{CH}_4) = k \cdot (C_w - C_{fc}),$$  

where $F$ is the CH$_4$ flux (in mmol m$^{-2}$ d$^{-1}$), $k$ is the piston velocity (in m d$^{-1}$), $C_w$ is the measured CH$_4$ concentration in the water phase (in mmol m$^{-3}$) and $C_{fc}$ is the CH$_4$ equilibrium concentration in the headspace at the given CH$_4$ water concentration.

The piston velocity $k$ was determined as follows:

$$k = (-\ln((c_{sat} - c_{end}) / (c_{sat} - c_{start})) / \Delta t \cdot V) / (A \cdot K_h \cdot R \cdot T),$$  

where $c_{sat}$ is the saturation concentration in the chamber headspace at the measured CH$_4$ water concentration, $c_{end}$ is the measured CH$_4$ concentration in the chamber headspace at the end of the flux measurement and $c_{start}$ is the measured CH$_4$ concentration in the chamber headspace at the beginning of the flux measurement (all in µatm).

The flux was corrected for the nonlinear increase in CH$_4$ concentration in the headspace over time due to saturation and was divided into diffusive and ebullitive proportions based on the piston velocity ($k < 2 = $ diffusion, $k > 2 = $ ebullition).

2.3.2 Sediment gas stock change

CH$_4$ and CO$_2$ concentrations in the sediment were obtained from gas-permeable silicon tubes, determined by gas chromatography as described above (Sect. 2.2.3) and calculated by Henry’s law using temperature-corrected Henry’s constants (see Eq. 4). Measured CO$_2$ concentrations were corrected for pH (Eq. 5).

The storage change of CO$_2$ and CH$_4$ in the sediment was calculated for each depth segment between two sampling ports as the difference of CO$_2$ and CH$_4$ concentrations obtained from silicon gas samples at the beginning and at the end of gas flux measurements:

$$\Delta \text{CO}_2 = \left( (c(\text{CO}_2)_{\text{end}} \cdot V_{\text{seg}}) - (c(\text{CO}_2)_{\text{start}} \cdot V_{\text{seg}}) \right) / \Delta t,$$

$$\Delta \text{CH}_4 = \left( (c(\text{CH}_4)_{\text{end}} \cdot V_{\text{seg}}) - (c(\text{CH}_4)_{\text{start}} \cdot V_{\text{seg}}) \right) / \Delta t,$$

where $\Delta$CO$_2$ ($\Delta$CH$_4$) is the storage change (in mmol d$^{-1}$), $c(\text{CO}_2)_{\text{end/start}}$ ($c(\text{CH}_4)_{\text{end/start}}$) is the CO$_2$ or CH$_4$ sediment pore gas concentration at the end/beginning of the flux measurement (in mmol m$^{-3}$), and $V_{\text{seg}}$ is the volume of the sediment core segment between two samplers (in m$^3$).

After the completion of flux measurements, intact sediment core incubations were eventually cut into 10 cm slices,
freeze-dried and ground for solid-phase analyses, as described above.

2.4 Other chemical and physical parameters in sediment and lake water samples

Total phosphorus (P), sulfur (S), manganese (Mn) and iron (Fe) in the sediment were determined by wavelength dispersive X-ray fluorescence (WD-XRF; ZSX Primus II, Rigaku, Tokyo, Japan). To this end, 500 mg of freeze-dried and ground sample was pressed into pellets together with 50 mg of wax (Hoechst wax C, Merck, Darmstadt, Germany) as a pelleting agent. Calibration of the instrument was done using a set of 22 certified reference materials.

Grain size distribution was determined after Austrian standards (OENorm B 4412, 1974; OENorm L 1050, 2016; OENorm L 1061-2, 2019) of the Physio-geographic Lab of the Institute of Geography and Regional Research of the University of Vienna. To this end, the organic matter was removed from the samples with hydrogen peroxide (H2O2) prior to analyses, and mineral fine sediment was divided into clay (<2 µm), silt (fine: 2–6 µm; medium: 6–20 µm; coarse 20–63 µm) and sand (fine: 63–200 µm; medium: 200–630 µm; coarse: 630–2000 µm).

Values of pH, conductivity and dissolved O2 concentration were determined in situ with a multi-probe (WTW Multi 3420 + IDS sensor, WTW GmbH, Weilheim, Germany). DOC and TN in lake water samples were determined by catalytic oxidation and subsequent NDIR (nondispersive infrared) detection with a total C and N analyzer (TOC-L/TNM-L, Shimadzu, Kyoto, Japan). Total Cl, Ca, Fe, K, Mg, Na, P and S concentrations in lake water samples were determined by inductive-coupled plasma optical emission spectroscopy (ICP-OES; Spectroblue, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

2.5 Statistics

All statistical analyses were conducted with R Studio, Version 3.5.2 (R Core Team, 2018). Data were tested for normal distribution and homoscedasticity with the Shapiro–Wilk and the Levene tests (Fox and Weisberg, 2011), respectively. For non-normally distributed data, significant differences between groups were identified using the Kruskal–Wallis test and the post hoc Dunn’s test (Dinno, 2017) for comparing two groups. If the condition of homoscedasticity was not fulfilled, groups were compared with Mood’s median test. Correlations and regressions between production rates and sediment parameters were calculated by Spearman’s rank correlation and by linear regression models, respectively. All data were tested on a 95 % confidence interval and a significance level of α = 0.05.

### Table 2. C, N, P, S, Mn and Fe contents, C/N and FTIR peak ratios (peak maximum for polysaccharides) of identified OM compounds, and their minimum and maximum values, n = 16–22.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Min</th>
<th>Max</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>2.2</td>
<td>33.2</td>
<td>26.5</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.2</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>11.0</td>
<td>19.1</td>
<td>13.8</td>
</tr>
<tr>
<td>δ13C (%)</td>
<td>−27.8</td>
<td>−27.3</td>
<td>−27.6</td>
</tr>
<tr>
<td>δ15N (%)</td>
<td>−6.3</td>
<td>1.3</td>
<td>−0.9</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.1</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>370</td>
<td>1160</td>
<td>490</td>
</tr>
<tr>
<td>Fe (%)</td>
<td>2.1</td>
<td>7.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Polysaccharides (1033–1035 cm−1)</td>
<td>0.58</td>
<td>1.24</td>
<td>0.81</td>
</tr>
<tr>
<td>Lignin (1220–1234 cm−1)</td>
<td>0</td>
<td>0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>Humic acids (1417–1419 cm−1)</td>
<td>0.04</td>
<td>0.49</td>
<td>0.33</td>
</tr>
<tr>
<td>Phenols &amp; aliphatics (1456 cm−1)</td>
<td>0.02</td>
<td>0.48</td>
<td>0.32</td>
</tr>
<tr>
<td>Other aromatics (1623–1646 cm−1)</td>
<td>0.21</td>
<td>0.83</td>
<td>0.63</td>
</tr>
<tr>
<td>Fats, waxes, lipids (2850–2856 cm−1)</td>
<td>0.18</td>
<td>0.49</td>
<td>0.35</td>
</tr>
</tbody>
</table>

3 Results

3.1 Slurry incubations

3.1.1 Sediment OM quality

The C content in the samples was between 2.2 % and 33.2 % with the lowest values at site 3.50 and the highest at site 1.50. C/N ratios ranged from 11.0 at site 1.150 to 19.1 at site 3.100. Neither the C content nor the C/N ratio showed significant changes with sediment or lake depth, but the C/N ratio was significantly higher in samples taken close to the shore (50) than in samples from the lake center (150) (p < 0.01) (see Fig. 2a). C and N isotopic values did not vary much between sites and were on average −27.3 ‰ and −0.9 ‰, respectively.

OM quality as identified by FTIR analysis was dominated by strong absorption features of polysaccharides, lignin, humic acids, phenolic and aliphatic structures, aromatic compounds, and fats, waxes and lipids (see Fig. S1 in the Supplement). Except for lignin, which was not identified at sites 2.50, 2.100, 2.125, 3.50 and 3.100, all components were abundant at all sites. Ranges of peak ratios for each component class can be found in Table 2. Overall, the lowest FTIR peak ratios were present at site 3.50 and the highest at site 3.125, corresponding to the highest and lowest CH4 production rates (see below). All peak ratios correlated with each other. They tended to increase (i.e., indicate more decomposed material) with sediment depth and toward the lake center, but this change was not significant (see Fig. 2b).

3.1.2 CO2 and CH4 production rates

Overall, production rates decreased from the shore to the center of the lake. Rates for potential CO2 production at
0–5 cm depth ranged from 10.3 ± 0.0 µmol gC⁻¹ d⁻¹ at site 3.125 to 38.5 ± 2.5 µmol gC⁻¹ d⁻¹ at site 2.100. Potential CH₄ production was between 7.3 ± 0.1 µmol gC⁻¹ d⁻¹ at site 3.125 and 33.5 ± 7.2 µmol gC⁻¹ d⁻¹ at site 3.50. Production rates at 5–10 cm depth were always lower compared to the upper sediment layer and were between 7.2 ± 0.1 and 14.7 ± 0.4 µmol gC⁻¹ d⁻¹ for CO₂ and between 5.4 ± 0.6 and 14.3 ± 0.3 gC⁻¹ d⁻¹ for CH₄. Both CO₂ and CH₄ production rates showed significant differences between sites (Kruskal–Wallis, p < 0.001) and were significantly (Dunn’s test, p < 0.05) higher at the shore (50 + 100) than in the center of the lake (125 + 150) (see Figs. 3, S2 and S3).

CO₂ and CH₄ production rates decreased with time. CO₂ production was highest at the beginning, while CH₄ production was impeded and highest only after 3 to 8 d of incubation (see Fig. 4, Table 3). The CO₂/CH₄ amount ratio constantly decreased during the incubation with a maximum value of 62.1 ± 58.4 at the beginning of the incubation at site 1.125 and minimum values of 1.2 ± 0.0 at the end of the incubation at site 2.50, approaching ratios of 1 as expected under strictly methanogenic conditions (see also Table 3).

Q₁₀ values were between 1.6 ± 0.1 and 2.2 ± 0.1 for CO₂ production rates and between 2.7 ± 0.5 and 11.4 ± 1.0 for CH₄ production rates.

Sampling dates did not have any impact on production rates, as confirmed by a Kruskal–Wallis test (CH₄: p = 0.17; CO₂: p = 0.76).

Neither CO₂ nor CH₄ production rates exhibited significant correlations with C content, the C/N ratio, δ¹³C or δ¹⁵N, but we found significant negative correlations (p < 0.01; ρ < −0.6) between all FTIR peak ratios and CO₂ and CH₄ production rates, as well as of FTIR peak ratios and Q₁₀ values of CH₄ production (p < 0.05, ρ = −0.82) (see Fig. 5; for test statistics, see Table S1 in the Supplement).

### 3.1.3 Methanogenic pathways

Both H₂ and acetate concentrations increased during the incubation. H₂ concentrations were between 0 and 1587.4 ± 170.1 nmol L⁻¹, and acetate concentrations ranged from 219.7 ± 104.3 to 1212.7 ± 11.4 µmol L⁻¹ (see Fig. 6). Gibbs free energy for acetoclastic methanogenesis was between −96.3 ± 0.8 and −66.4 ± 0.3 kJ mol⁻¹, and for hydrogenotrophic methanogenesis it was between −62.4 ± 0.9 and −7.4 ± 0.0 kJ mol⁻¹. Energy yields decreased for the acetoclastic pathway and significantly increased (Mood’s median; t₀–t₆: p < 0.05, t₅–t₆: p < 0.001) for the hydrogenotrophic pathway throughout the incubation (see Fig. 6). Energy yields did not differ significantly between lake depths except for the acetoclastic pathway at the end of the incubation (p < 0.001) when the energy yield was highest in samples from the center of the lake. Significant differences between all sites were found for the acetoclastic and hydrogenotrophic pathways at the beginning (Kruskal–Wallis, p < 0.01) and at the end of the incubation for acetoclastic methanogenesis only (Kruskal–Wallis, p < 0.01). H₂ concentrations at the end of the experiment exhibited a significant positive correlation with average CO₂ (p < 0.001, ρ = +0.51) and CH₄ (p < 0.001, ρ = +0.45) production rates (for test statistics see Table S2). Further, Gibbs free energy of acetoclastic methanogenesis exhibited a significant positive correlation with the C/N ratio (p < 0.05, ρ = +0.45) at the end of the experiment. Acetate concentration at the end of the incubation exhibited significant negative correlations with FTIR peak ratios and the C/N ratio (p < 0.05, ρ < −0.44). Gibbs free energy of hydrogenotrophic methanogenesis exhibited significant positive correlations with FTIR peak ratios of fats, humic acids, phenolics and C content (p < 0.05, ρ > 0.17); i.e., a high energy yield was associated with low FTIR peak ratios and C content. Correspondingly,
H$_2$ concentrations exhibited significant negative correlations with all FTIR peak ratios and C content ($p<0.05$, $\rho<-0.15$) (for test statistics see Table S2).

### 3.1.4 Alternative organic and inorganic EAs

EAC$_{OM}$ was between 218.7 ± 97.2 and 545.7 ± 60.3 μmol e$^-$/gC$^-1$ at the beginning and decreased on average by 44.9 μmol e$^-$/gC$^-1$ until the end of the slurry incubation. The highest values for EAC$_{OM}$ were found at site 3.125 corresponding to the lowest measured CH$_4$ production rates at that site. EAC$_{inorg}$ was, with values between 2.9 ± 1.0 and 39.2 ± 4.4 μmol e$^-$/gC$^-1$, much lower than EAC$_{OM}$ and also decreased during the incubation by a mean of 6.4 μmol e$^-$/gC$^-1$. EAC$_{tot}$ ranged from 250.8 ± 93.9 μmol e$^-$/gC$^-1$ at site 3.150 to 551.4 ± 60.3 μmol e$^-$/gC$^-1$ at site 3.125 and decreased from the beginning to the end of the incubation by, on average, 51.2 μmol e$^-$/gC$^-1$. EDC$_{OM}$ was between 149.5 ± 26.7 and 462.7 ± 18.6 μmol e$^-$/gC$^-1$ at the beginning and between
Table 3. CO₂ and CH₄ production rates and the CO₂ / CH₄ amount ratio over time (rates in µmol gC⁻¹ d⁻¹; ratios in µmol). Values in brackets are standard deviations.

<table>
<thead>
<tr>
<th>Sediment depth</th>
<th>Day of incubation</th>
<th>CO₂ production rate</th>
<th>CH₄ production rate</th>
<th>CO₂ / CH₄ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>0–5 cm</td>
<td></td>
<td>91.5 (40.3)</td>
<td>44.7 (16.8)</td>
<td>26.5 (9.7)</td>
</tr>
<tr>
<td>5–10 cm</td>
<td></td>
<td>39.9 (14.2)</td>
<td>19.6 (6.0)</td>
<td>11.0 (2.7)</td>
</tr>
<tr>
<td>0–5 cm</td>
<td></td>
<td>14.4 (7.4)</td>
<td>22.8 (11.3)</td>
<td>23.9 (11.5)</td>
</tr>
<tr>
<td>5–10 cm</td>
<td></td>
<td>9.4 (2.7)</td>
<td>11.7 (2.9)</td>
<td>11.6 (3.1)</td>
</tr>
<tr>
<td>CO₂ / CH₄ ratio</td>
<td>0–5 cm</td>
<td>14.6 (6.7)</td>
<td>8.7 (2.3)</td>
<td>3.8 (1.3)</td>
</tr>
<tr>
<td></td>
<td>5–10 cm</td>
<td>16.2 (24.9)</td>
<td>5.6 (1.6)</td>
<td>2.9 (0.9)</td>
</tr>
</tbody>
</table>

Figure 5. Correlations between CO₂ and CH₄ production rates and OM quality parameters as determined by elemental analysis or FTIR spectroscopy. Different colors denote different depth categories (see Fig. 4).

152.9 ± 53.8 and 370.7 ± 196.2 µmol e⁻ gC⁻¹ at the end of the incubation, showing a slight decrease by, on average, 31.4 µmol e⁻ gC⁻¹. The lowest EDC was at both the beginning and the end of the incubation was found at site 2.100, corresponding to the highest CO₂ production rates there (see Fig. 7).

We further found significant differences for EACOM and EACtot (ANOVA, p < 0.001) between all sites with the highest average values at site 3.125 and the lowest at sites 1.125 (t₁₀) and 3.150 (t₁₂) (see Fig. S4). Average CO₂ and CH₄ production rates exhibited a significant negative correlation with initial EDCOM (p < 0.05, ρ = -0.7), whereas we did not find any significant correlation between the production rates and EAC, electron exchange capacity (EEC; sum of EAC and EDC), or the EAC / EDC ratio, although low CH₄ production rates were associated with high EAC. Acetate concentration exhibited significant negative correlations with EACOM and EACtot (p < 0.01, < 0.001, ρ < -0.38). Gibbs free energy of hydrogenotrophic methanogenesis exhibited significant positive correlations with EACOM and EACtot (p < 0.05, ρ = 0.43). We did not find any significant correlations between EAC or EDC and OM quality parameters except for EDCOM and the FTIR peak ratio indicative of fats, waxes and lipids (p < 0.05, ρ = -0.76). Of the inorganic EAs, total S content in the sediment exhibited a significant negative correlation with CO₂ production rates (p < 0.05, ρ = -0.45) (for test statistics see Table S1).

Calculated potential CO₂ production from prevalent organic and inorganic EAs was always lower than the measured CO₂ production in slurry incubations and was between 110.5
and 586.4 μmol CO₂ eq. gC⁻¹ (see Fig. 8), which could explain 38 % to 91 % of measured CO₂ production, whereof 4 % to 51 % were explained by EACOM alone.

3.2 Intact sediment core incubations: fluxes, sediment storage change and grain size

CO₂ and CH₄ fluxes measured from intact sediment core incubations ranged from 10.8 ± 4.4 to 17.9 ± 2.0 and 0.02 ± 0.01 to 1.5 ± 2.6 mmol m⁻² d⁻¹, respectively. CH₄ ebullition played a major role in cores from sites 2.100 and S.150 and accounted for up to 100 % of the fluxes there. CO₂ fluxes at site 3.125 were significantly lower (t-test, p<0.05) than at the other sites, while total CH₄ fluxes at site 2.100 were significantly higher (Kruskal–Wallis, p<0.01) compared to all other sites. Fluxes were within the same range as potential production rates in slurry incubations but only partly followed the pattern observed in slurry incubations (see Fig. 9a, b).

Concentrations of dissolved CH₄ and CO₂ – Σ(CO₂, HCO₃⁻, CO₃²⁻) – in the sediment ranged from 4.0 to 129.5 mmol m⁻³ (CH₄) and from 322.9 to 3811.4 mmol m⁻³ (CO₂), with the lowest values found at site 3.125 and the highest values at S.150. We did not see significant changes in CO₂ and CH₄ concentrations in the depth profile (see Fig. S5).

Observed changes of CH₄ and CO₂ concentrations in the sediment of intact cores were overall very low and were between 0.1 and 2.5 mmol d⁻¹ (CH₄) and 0.6 and 57.1 mmol d⁻¹ (CO₂) in the upper 5 cm of the sediment. These changes were on the same order of magnitude as measured fluxes but did not correlate with them.

Grain sizes distribution differed between the four sites, with site 3.125 having the highest share of sand (21.6 %) and the lowest shares of silt and clay (35.7 % and 42.7 %), whereas the other sites were dominated by finer material and were comprised of less than 5 % of sandy components (see Fig. 9c). CH₄ and CO₂ fluxes exhibited significant correlations with clay and sand content but not with silt (see Table 4).

Similar to slurry incubations, CH₄ fluxes exhibited significant negative correlations with some FTIR peak ratios indicative of recalcitrant OM compounds and with the C/N ratio but not with C content. CO₂ fluxes did not show any significant correlation with OM quality parameters at all (see Table 4).

4 Discussion

4.1 Slurry incubations

4.1.1 Variability of CO₂ and CH₄ production rates and OM quality

Results from the slurry incubation experiment showed that both CO₂ and CH₄ production rates were – despite its small size - highly variable within the examined lake. CH₄ production rates were within the range of previously reported values from lakes in central Sweden, a reservoir in Brazil and in sediments from lakes Stechlin and Geneva in Germany.
and Switzerland, respectively (Duc et al., 2010; Fuchs et al., 2016; Grasset et al., 2018). CO₂ production rates were high compared to rates found in Lake Kinneret in Israel and in the Pantanal and Amazon regions in Brazil, exceeding reported values by a factor of 7 to 100 (Schwarz et al., 2008; Conrad et al., 2011) and supporting the assumption that small lakes generally have higher C turnover rates compared to larger ones.

In all samples, production rates were higher at the beginning of the experiment than toward the end. While CO₂ production rates were highest right after the start and then constantly decreased until reaching a plateau around day 8, CH₄ production rates peaked after 3 to 8 d and then slowly decreased afterwards, approaching a 1 : 1 CO₂ : CH₄ production ratio. This typically observed three-phase pattern (Yao et al., 1999) might be among other things due to thermo-dynamic constraints, such as end-product accumulation in the sample vials and subsequent inhibition of respiration and methanogenesis (Beer and Blodau, 2007; Blodau et al., 2011; Boniauitt et al., 2017). The observed delay in notable CH₄ production was expected as prevalent alternative EAs (NO₃⁻, Fe³⁺, SO₄²⁻, humic substances) likely suppressed methanogenic activity (Lovley et al., 1996; Yao et al., 1999; Fenchel et al., 2012).

Both CO₂ and CH₄ production rates were higher in the topmost sediment layer compared to the 5–10 cm sediment depth, which suggests that the first centimeters of the sediment play a major role in GHG production as a consequence not only of temperature but also of microbial community distribution and changes in OM quality (Falz et al., 1999; Sobek et al., 2009; Wilkinson et al., 2015). This finding corresponded with decreasing C/N ratios and increasing FTIR
peak ratios with sediment depth. While sediment age typically increases with depth, the OM quality and, thus, reactivity usually decreases with sediment depth as recalcitrant compounds are not being completely mineralized but are instead residually enriched, buried and preserved in the sediment (Avnimelech et al., 1984; Burdige, 2007; Sobek et al., 2009). Similar to den Heyer and Kalff (1998), we found higher production rates in samples close to the lake shore in contrast to lower production rates in the center, which suggests either that the input of OM (quantity) in the sediments is higher at the shore sites or that the OM quality is more labile and, therefore, more easily degradable. As we did not find any correlations between CO₂ or CH₄ production rates and sediment C content as proposed, for example, by Conrad et al. (2011) and Romeijn et al. (2019), we instead suggest that the OM quality rather than quantity might be the determining factor for CO₂ and CH₄ production.

C/N ratios are frequently used to characterize the degradation state of OM, but we did not find correlations between C/N ratios and CO₂ and CH₄ production rates in the slurry incubations. Although OM of autochthonous origin was found to fuel higher degradation rates than allochthonous OM (West et al., 2012; Grasset et al., 2018), we found evidence of predominant inputs of allochthonous (terrestrial) material at sites with higher production rates close to the shore (higher C/N ratios), whereas sites with lower production rates in the lake center received mainly autochthonous (aquatic) OM, as indicated by lower C/N ratios (Meyers, 1994). On the other hand, high C/N ratios also indicate a lower degradation state and, therefore, higher degradation potential, whereas low C/N ratios are usually typical of highly decomposed OM having a lower CO₂ and CH₄ production potential (Malmer and Holm, 1984; Kuhry and Vitt, 1996). These two possibilities of interpreting C/N ratios might be the reason for apparently contradictory findings and the missing relationship between C/N ratios and CO₂ and CH₄ production rates.

The abundance of recalcitrant OM compounds (lignin, aromatics, humics, phenols and lipids), as indicated by FTIR peak ratios, proved to be a more suitable explanation for observed CO₂ and CH₄ production patterns, as indicated by strong significant negative relationships between peak ratios and production rates. All FTIR peak ratios indicative of more refractory components increased toward the lake center, which indicated an increasing predominance of more recalcitrant OM with greater distance from the lake shore and corresponded with decreasing production rates from lake shore to center. At first sight, this was unexpected as allochthonous material is known to be richer in recalcitrant compounds like lignin or aromatics compared to autochthonous biomass and, therefore, is effectively buried in lake sediments (Sobek et al., 2009). However, before reaching the sediment, the internally produced material in the lake center must pass through a deeper water column while undergoing degradation processes such that more decomposed OM reaches the sediment (Meyers, 1994; Torres et al., 2011). As this process might not be of the same importance in shallow lakes compared to deeper lakes, we additionally suggest that the more decomposed OM in the lake center might have undergone degradation processes during resuspension and focusing of small particles as a result of wind-induced bed shearing (Mackay et al., 2011). The less decomposed OM close to the shore might further originate from labile aquatic plant substrates growing at the shoreline (Wetzel, 1992; Cole et al., 2007).

To summarize, the OM degradability does not necessarily depend on its origin, and the OM of terrestrial origin is not necessarily more recalcitrant to degradation by aquatic microorganisms in this study. Sites closer to the shore probably received higher rates of terrestrial OM matter that was less decomposed, whereas autochthonous production in the lake center was overall low due to the low nutrient status of the lake and was already undergoing degradation during sedimentation; this likely led to more recalcitrant OM in the sediment. Earlier studies by West et al. (2012) and Grasset et al. (2018) that found allochthonous material to be less decomposable compared to autochthonous matter were conducted in larger lakes where the influence of the watershed was overall much lower than in our case because larger lakes have a lower perimeter to volume ratio. We hypothesize that in small and nutrient-poor lakes, the pattern might be reversed as the system adapts to overall low productivity and a simultaneously high input of terrestrially produced OM.

In accordance with previous studies (den Heyer and Kalff, 1998; Sobek et al., 2009; Gudasz et al., 2015), we found that with a temperature increase of 10 °C, production rates
of CO₂ doubled, and those of CH₄ were even 2 to 11 times higher. $Q_{10}$ values for CO₂ were within the range of earlier reported values by Liikanen et al. (2002) and Bergström et al. (2010), whereas $Q_{10}$ values for CH₄ production were slightly higher than values found by Duc et al. (2010). The large observed range of $Q_{10}$ values, especially for CH₄, implies that responses to temperature increases might not be homogeneously distributed within a lake. The observed negative correlation between $Q_{10}$ values and FTIR peak ratios further suggests that sites with more labile OM are more susceptible to increasing temperatures in terms of CH₄ production, whereas at sites with more recalcitrant OM, the inherent quality of this recalcitrant OM may limit the degradation processes. We, therefore, assume that sediment GHG production – particularly CH₄ production – in small and shallow lakes might in the course of global warming increase to a larger extent than in deeper lakes with respect to the fact that shallow waters, as opposed to deeper lakes, do not develop a stable stratification in summer, and thus shallow sediments warm much faster (Jankowski et al., 2006).

The high measured variability in production rates proves (1) that it is necessary to sample lake sediments from different locations in one lake in order to obtain potentials of CO₂ and CH₄ production representative of the whole lake and (2) that upscaling production rates based on single point measurements may be highly biased. Considering only sediment production rates and based on findings from other studies, it seems that the observed spatial variability of CO₂ and CH₄ emissions from lakes might to a large extent be controlled by variability in sediment GHG production (Schilder et al., 2013; Bastviken et al., 2015; Natchimuthu et al., 2016; Natchimuthu et al., 2017; Spafford and Risk, 2018). Yet still, as sediment production and emission patterns have so far only been considered separately, other factors driving actual emissions might have been neglected. Therefore, in Sect. 4.2, we further discuss the relationships between CO₂ and CH₄ production and actual emissions, as assessed in the intact sediment core incubation, and relate them to respective sediment properties.

### 4.1.2 Methanogenic pathways

To make methanogenesis a profitable reaction for microorganisms, theoretical thresholds of −10.6 kJ mol⁻¹ for the hydrogenotrophic pathway and −12.8 kJ mol⁻¹ for the acetoclastic pathway must be exceeded (Hoehler et al., 2001). Except for a few samples at the beginning of the incubation for the hydrogenotrophic pathway, the theoretical thresholds were always exceeded, suggesting that both pathways could potentially contribute to CH₄ production during the whole experiment. Still, this approach does not allow us to evaluate which of the pathways is actually dominant.

Against our expectations, Gibbs free energy of acetoclastic methanogenesis at the end of the incubation was significantly lower (i.e., higher energy yield) in the center of the lake, and $\Delta G_r$ exhibited a significant positive correlation with the C/N ratio. Considering that we found the most decomposed material (following peak ratios from FTIR analyses and low C/N ratios) in the lake center and that low C/N ratios in this case indicate OM of high recalcitrance, whereas the acetoclastic pathway is thought to predominate with high-quality substrates, we would have expected a reverse pattern (Liu et al., 2017; Berberich et al., 2019). Concomitantly, Gibbs free energy of hydrogenotrophic methanogenesis exhibited significant positive correlations with some FTIR peak ratios indicative of higher contributions of recalcitrant OM.
compounds, although we expected that a high abundance of recalcitrant OM compounds would make hydrogenotrophic methanogenesis more feasible (Liu et al., 2017). Acetate and H₂ concentrations, on the other hand, both exhibited significant negative correlations with some FTIR peak ratios. While this seemed reasonable for acetate concentrations (less acetate available in strongly decomposed OM), this result again proved to be against our expectations in terms of H₂ concentrations. This suggests that more recalcitrant material in general slows fermentative processes, delivering both acetate and H₂ as a rate-determining step, and all fermentation products are thus kept at low levels.

4.1.3 Alternative EAs

The average EACOM measured in our experiment (302.8 ± 124.6 µmol e⁻ g⁻¹) was slightly lower but on the same order of magnitude compared to values found by Lau et al. (2015) and Gao et al. (2019) in other lake sediments or peat soils, respectively. Given that C contents in the study by Gao et al. (2019) were much higher than in our study (46.2 %–49.4 %; Agethen and Knorr, 2018) and that Lau et al. (2015) fully oxidized their samples prior to analyses (C content 11.0 %–27.4 %), our measured capacities seem reasonable.

We found that both organic and inorganic EAs decreased during the experiment, indicating that all EAs become similarly depleted with time as they are being reduced by microorganisms. Nevertheless, both absolute EACOM and EACinorg values and relative changes were rather low, which might have been caused by the 1-week preincubation, during which a majority of the reducible organic and inorganic compounds might have already been depleted. Klüpfel et al. (2014) showed that the EAC of oxidized humic acids already strongly declined after 1 d of anoxic incubation and did not change much afterwards. Inorganic EAs were also shown to decrease exponentially during anoxic incubation (Yao et al., 1999). The high CO₂ production rates (91.5 µmol g⁻¹ d⁻¹) at the beginning of the slurry incubation are an indication of these processes.

Sites with higher EACtot had lower CH₄ production rates and vice versa as an increased concentration of oxidized humic substances would suppress methanogenic activity on longer timescales, supporting the findings of Agethen et al. (2018) and Gao et al. (2019). Although the relationship between EAC and CH₄ production was not significant – probably due to the low number of samples and the sensitivity of the measuring method – a trend was observable.

Non-methanogenic CO₂ production could by 38 % to 91 % be explained by prevalent organic and inorganic EAs, whereas organic EAs alone were able to explain up to 52 % of measured CO₂ production in slurry incubations. Similar contributions were observed in previous studies from freshwater systems and peatlands, where up to 70 % and 95 % of non-methanogenic CO₂ production was explained by total EAC and 25 % to 55 % by decreases in EACOM alone (Lau et al., 2015; Agethen et al., 2018; Gao et al., 2019). The authors suggest several possible reasons for the gap between calculated and experimentally measured CO₂ production. Fermentation processes could explain excess CO₂, but in this case reduced equivalents of fermentation products such as H₂ would be expected to accumulate. Moreover, decarboxylation processes that occur in oxygen-rich, labile OM might occur. Further, the assumption of a nominal oxidation state of C of zero – as assumed in our approach – might be wrong so that when assuming a higher oxidation state, measured changes in EACOM might explain a higher share of CO₂ production (Gao et al., 2019). As we did not observe the accumulation of fermentation products and as the sediment under study was not strongly oxidized, we assume these processes are of minor importance and instead suggest that the proportion of unexplained CO₂ production can be explained by unknown consumed inorganic EAs during the slurry incubation. In this case, this was most likely solid-phase iron which we found to comprise 2 %–3 % (±53–137 mg Fe g⁻¹) of our samples, but we were not able to draw conclusions about its speciation. We suppose this concentration is high enough to reach a closed budget of measured CO₂ production and calculated EA turnover; if, on average, 7.8 % (4 %–18.7 %) of solid-phase iron were present as ferric iron, this would suffice to close the CO₂ budget.

The large range (4 % to 52 %) of the contribution of organic EAs to CO₂ production shows that a distinct small-scale in-lake variability of terminal electron-accepting processes and their contribution to CO₂ formation exists in the sediment of Lake Windsborn. Interestingly, the contribution of EACOM was highest at site 3.125 where we observed the lowest CO₂ production rates and highest OM recalcitrance. Given that both the absolute C content in the incubation vials and the C : Fe ratio were highest at this site, this supports the findings of Lau et al. (2015), who stated that the contributions of EECOM to total EEC increase with the OC : Fe ratio, indicating more peat-like material.

4.2 Comparison of production rates, fluxes and sediment parameters

Comparing the order of magnitude of potential production rates from slurry incubations and fluxes from intact sediment cores, the results were in good accordance with each other. While CO₂ production rates were overall somewhat below measured fluxes, CH₄ production rates were considerably higher than actual CH₄ fluxes. The differences in CO₂ production rates and fluxes can be explained by the influence of the water column; CO₂ is produced not only in the sediment but also in the water column, for example, by the degradation of OM or zooplankton respiration (Kling et al., 1992) and can, therefore, contribute to water–atmosphere fluxes. The differences concerning CH₄ could be explained by several factors. First, preparing slurry incubations leads to the ho-
mogenization of the sediment and higher surface area and, therefore, an even distribution of substrates and microorganisms such that the substrates are more readily available to the microorganisms (Hoehler et al., 2001). Second, sediment fluxes from intact sediment cores may be low compared to homogenized slurry incubations as maximum sediment concentrations were already reached there in shallow depths, suggesting that strong changes in sediment gas concentrations that drive fluxes are likely to occur only in the upper millimeters to centimeters. Additionally, the preparation of slurries reduces the thermodynamic constraints that usually exist in intact profiles due to end-product accumulation (Blochau et al., 2011; Bonaiti et al., 2017). Third, not all CH$_4$ produced in and emitted by the sediment will reach the water–atmosphere interface because some of the CH$_4$ will be consumed in the oxygenated water column on the way to the surface; through its oxidation in the water column, the amount of CH$_4$ emitted from lakes to the atmosphere can be substantially reduced by 51% to 100% (Bastviken et al., 2002, 2008).

Interestingly, spatial patterns of CO$_2$ and CH$_4$ emissions could not be well reproduced from slurry incubations. Although the highest CO$_2$ emissions were observed at site S.150, we found the lowest CO$_2$ production rates there. Additionally, we did not see any correlations between sediment OM quality and CO$_2$ fluxes, suggesting that CO$_2$ production processes in the water column overlying the sediment play a much more important role than in the sediment itself. Moreover, CH$_4$ production rates at sites 1.50 and 3.125 differed significantly from each other, while this was not the case when comparing fluxes. We assume that the different patterns of CH$_4$ fluxes and production can be explained by grain size distribution and, thus, physical sediment properties. Sediment grain size determines sediment pore size, which is essential for the evolution of CH$_4$ bubbles in the sediment (Boudreau et al., 2005). Ebullication is the major component of CH$_4$ fluxes in shallow waters as it can efficiently bypass oxidation in the water column (Bastviken et al., 2004; Wik et al., 2013; Natchimuthu et al., 2016). We found that ebullition was responsible for most of the CH$_4$ fluxes in two of our four intact sediment core incubations, whereas sites with higher shares of sand exhibited less ebullitive fluxes, confirming the findings of Liu et al. (2016, 2018). The authors justify their findings with the dominant pathway of bubble formation in the sediment, which is by displacing surrounding sediment particles. As this mechanism is more efficient in soft silty sediments compared to sandy material, CH$_4$ bubbles likely accumulate more easily in silt, creating a network of macropores and, therefore, conduits for subsequent bubble release. We further found that OM quality partly exhibited significant negative correlations with actual CH$_4$ fluxes but to a lesser extent than with sediment CH$_4$ production. As discussed, when preparing slurry incubations, the physical sediment structure is destroyed so that OM quality likely becomes the major controlling factor for GHG production. These findings suggest that besides OM quality, grain size distribution is a main driver of spatial CH$_4$ flux patterns in intact sediment core incubations and that only a combination of the sediment’s physical characteristics and its OM quality could sufficiently explain CH$_4$ emission patterns of lakes.

The missing link between sediment gas storage and both diffusive and ebullitive emissions can potentially be explained by our sampling procedure. While fluxes were partly dominated by bubbles, these bubbles cannot be captured in the sediment by silicon samplers. Silicon samplers rely on the slow diffusion of gas between the pore gas and the sampler, whereas bubbles may form on shorter timescales and can quickly be released to the water column. Thus, concentrations in the bulk sediment stay consistent, while the released bubbles cause high fluxes. Moreover, the resolution of our gas samplers was probably not high enough as they could not cover the uppermost centimeters of the sediment, which we assume to be crucial for diffusive fluxes.

5 Conclusions

Our results show that there exists a significant spatial variability of CO$_2$ and CH$_4$ production in the sediment of a small and shallow lake and, therefore, that it is not possible to upscale sediment production rates from single point measurements. We further proved that CH$_4$ production strongly depends on temperature and that the extent of temperature dependence might differ within a lake, especially between littoral and central parts due to differences in OM quality. Small lakes seem to be very sensitive to temperature increases in terms of accelerated C cycling, which implies they might become larger sources of CO$_2$ and particularly of CH$_4$ emissions under climate change scenarios. Although such lakes might not react homogeneously, we would expect that sediments in shallower parts might react more sensitively due to shallow water depths and the input of reactive allochthonous OM, which is particularly important for oligotrophic lakes without significant contributions of autochthonous primary production. The strong negative correlations we found between recalcitrant OM compounds and CO$_2$ and CH$_4$ production rates provide evidence that OM quality plays a major role in controlling the mineralization of anoxic lake sediment and can be used to explain and predict patterns of production rates within lakes. Both hydrogenotrophic and acetoclastic methanogenesis were feasible during slurry incubations, but we could not clearly attribute observed patterns to OM quality or EEC; therefore, we suggest that fermentation was the rate-determining step of OM mineralization and that the subsequent consumption of acetate and H$_2$ by electron-accepting processes or methanogenesis kept the levels of these fermentation products low, as expected. We did not find clear patterns between EAC and CH$_4$ production, but organic and inorganic EAs could explain up to 91% of the observed non-methanogenic CO$_2$ production in slurry incubations.
In sum, measured production rates were only partly reflected in CO_2 and CH_4 flux patterns obtained from intact sediment core incubations. We suppose that this was because the physical sediment structure (e.g., pore size) was destroyed in the slurry incubations, but these parameters are crucial for the evolution of CH_4 bubbles in the sediment. We also suppose that this was because in the slurry incubations, thermodynamic and gas transport constraints that exist in intact anoxic sediment profiles were removed. Further, the interpretation of production rates only would neglect the importance of the water column as a sink of sediment generated CH_4 through oxidation and a source of CO_2 through degradation and respiration processes, which might then cause discrepancies between observed production and flux rates.

So far, direct flux measurements of CO_2 and CH_4 between water and the atmosphere seem to be the most accurate way to determine the magnitude and variability of emissions from lakes, but still our study contributes substantially to the understanding of controls underlying potential CO_2 and CH_4 production in lake sediments and sediment-inherent factors determining emission.

Data availability. The underlying research data of this study can be found in the Supplement.

Supplement. The supplement related to this article is available online at: https://doi.org/10.5194/bg-17-5057-2020-supplement.

Author contributions. LP, MS, SS, NP and KHK designed the study. NP and SS carried out the experiments and sample analyses with the help of LP and MS. LP performed data analyses and wrote the paper with the help of NP, SS, KHK and GB.

Competing interests. The authors declare that they have no conflict of interest.

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