An empirical model to predict methane production in inland water sediment from particular organic matter supply and reactivity

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Abstract

The highest CH4 production rates can be found in anoxic inland water surface sediments however no model quantifies CH4 production following fresh particular organic matter (POM) deposition on anoxic sediments. This limits our capability of modeling CH4 emissions from inland waters to the atmosphere. To generate such a model, we quantified how the POM supply rate and POM reactivity control CH4 production in anoxic surface sediment, by amending sediment at different frequencies with different quantities of aquatic and terrestrial POM. From the modeled CH4 production, we derived parameters related to the kinetics and the extent of CH4 production. We show that the extent of CH4 production can be well predicted by the quality (i.e., C/N ratio) and the quantity of POM supplied to an anoxic sediment. In particular, within the range of sedimentation rates that can be found in aquatic systems, we show that CH4 production increases linearly with the quantity of phytoplankton-derived and terrestrially derived POM. A high frequency of POM addition, which is a common situation in natural systems, resulted in higher peaks in CH4 production rates. This suggests that relationships derived from earlier incubation experiments that added POM only once, may result in underestimation of sediment CH4 production. Our results quantitatively couple CH4 production in anoxic surface sediment to POM sedimentation flux, and are therefore useful for the further development of mechanistic models of inland water CH4 emission.

Inland waters, despite their small global surface, have a strong effect on the greenhouse gas concentration in the atmosphere (Cole et al. 2007; Tranvik et al. 2009). In particular, they can emit large quantities of CH4 to the atmosphere (Bastviken et al. 2011), a greenhouse gas with 34 times the global warming potential of CO2 (at 100 yr timescale including climate-carbon feedbacks; Myhre et al. 2013). Most CH4 is emitted from inland waters via the release of CH4 bubbles (ebullition), an emission pathway that is highly variable in space and time (DelSontro et al. 2011; Linkhorst et al. 2020; Siezko et al. 2020). Therefore, the prediction of CH4 emissions at the ecosystem scale, and in particular of CH4 ebullition, is very valuable for quantification of the feedback that inland waters can have on climate (Aben et al. 2017; Schmid et al. 2017).

CH4 ebullition is controlled by physical parameters that regulate the release of CH4 bubbles (e.g., sediment structure, hydrostatic pressure change; Joyce and Jewell 2003; Scandella et al. 2011; Ramirez et al. 2015; Liu et al. 2016) and by biological processes that regulate CH4 production in the sediment. Methanogenesis is the final step of the anoxic decomposition of organic matter, and relies on the supply of organic substrates that are fermented into precursors (e.g., acetate or CO2) for CH4 production (Valentine et al. 1994). When environmental conditions are favorable for methanogenesis (e.g., anoxic conditions, sediment poor in alternative electron acceptors, and pH close to neutral), CH4 production will ultimately depend on temperature, particulate organic matter (POM) supply, and POM reactivity (Valentine et al. 1994; Segers 1998).

In marine sediments, which are rich in alternative electron acceptors and typically feature slow sedimentation rates, favorable conditions for methanogenesis are often met in deep and old sediment layers. However, in inland water sediments, such conditions are frequently met already in the upper cm (Sobek et al. 2009). As POM reactivity decreases exponentially with age (Middelburg 1989), methanogenesis decreases with sediment depth, and the highest CH4 production rates can be found in surface or sub-surface inland water sediments (Clayer et al. 2018;
Isidorova et al. 2019). In particular, in inland waters with anoxic bottom waters, most CH$_4$ will be produced during the first 100 d following POM deposition on the anoxic sediment (Grasset et al. 2018). There are models that correlate CH$_4$ production with the reactivity or stoichiometry of the organic matter that is present in inland water sediments (Clayer et al. 2018; Isidorova et al. 2019), but these models operate at relatively long time scales and over the whole sediment column. Therefore, these models do not include the fresh and often highly reactive POM that is newly added through sedimentation as a predictor, and thus cannot be used to infer the short-term (< 1 yr) and potentially high CH$_4$ production rates resulting from new POM reaching the sediments. Several models of CH$_4$ production at short time-scales exist for peatlands and other wetland ecosystems, but the main components of CH$_4$ production (e.g., plant primary production, water table level; Potter et al. 2014; Gedney et al. 2019; Grant and Roulet 2002) differ from those of inland waters. A mechanistic model predicting CH$_4$ production in inland water surface sediments is currently missing, which limits our capability of modeling CH$_4$ emissions from inland waters to the atmosphere.

The positive relationship between temperature and methanogenesis has been well studied in inland water sediments (Davidson and Janssens 2006; Yvon-Durocher et al. 2014) but the influence of POM supply and reactivity on CH$_4$ production is less straightforward. This is probably because POM sources are diverse and cover a wide range of reactivity (Kankaala et al. 2003; Duc et al. 2010; West et al. 2012), and because the supply rates are highly variable in space and time (Sobek et al. 2009). POM supply rate depends on the frequency of POM supply to the sediment, and on the quantity of POM added to the sediment within a certain amount of time. Recent studies have shown that CH$_4$ production rates strongly increase with the quantity of sediment POM (Rodriguez et al. 2018; Berberich et al. 2019). However, no study has investigated the extent of CH$_4$ production in sediments, although the frequency of POM supply can have a significant effect on OM decomposition via modifying microorganism activity and abundance (Hamer and Marschner 2005; Attermeyer et al. 2014). Recent studies have also shown that POM originating from sources that are considered more reactive (e.g., phytoplankton) can have higher CH$_4$ production rates than POM originating from less reactive sources (e.g., leaves of terrestrial plants; West et al. 2012; Grasset et al. 2018). During aerobic decomposition, POM reactivity decreases exponentially (Boudreau and Ruddick 1991; Koehler and Tranvik 2015), and the time of POM exposure to oxygen before reaching the anoxic sediments is thus also likely to affect anaerobic decomposition rates (Kristensen and Holmer 2001) and CH$_4$ production. The carbon to nitrogen (C/N) ratio or the total nitrogen (TN) content can serve as a proxy of POM reactivity: a low C/N ratio or a high TN content imply a high content in labile N-rich compounds (e.g., proteins) and a low content in recalcitrant C-rich compounds (e.g., structural compounds; Duarte 1992; Enriquez et al. 1993). Accordingly, studies have shown that the CH$_4$ production derived from macrophyte detritus or from inland water sediment correlates negatively to the C/N ratio and positively to the TN content (Duc et al. 2010; Grasset et al. 2019; Isidorova et al. 2019). However, there is at present no quantitative relationship that links CH$_4$ production to POM supply and reactivity across a range of POM sources (e.g., terrestrial and aquatic plants).

Laboratory experiments that measure CH$_4$ production over time in closed systems can derive parameters related to the extent of POM transformation into CH$_4$, i.e., the yield of the reaction in units of % or in g of produced CH$_4$, and to the kinetics of CH$_4$ production, i.e., the time that it takes to produce a certain amount of CH$_4$ (Kankaala et al. 2003; Grasset et al. 2018; Grasset et al. 2019). Those parameters can be used to predict CH$_4$ production rates, i.e., the quantity of CH$_4$ produced per unit of time. Ultimately, such relationships could be used to model CH$_4$ bubble formation in anoxic sediments, which is dependent on both the extent and the kinetics of CH$_4$ production. Indeed, CH$_4$ porewater concentration can exceed solubility, and CH$_4$ can form bubbles in the sediment (Algar and Boudreau 2010). As part of the produced CH$_4$ is lost through oxidation, ebullition or diffusion, CH$_4$ can only accumulate and form bubbles in the sediment if the CH$_4$ production rate is higher than the CH$_4$ loss rate (Schmid et al. 2017; Langenegger et al. 2019).

In this study, we quantified how POM supply rate and POM reactivity control CH$_4$ production in anoxic surface sediment layers, by experimentally amending sediment at different frequencies with different quantities of aquatic and terrestrial POM. From the modeled CH$_4$ production, we derived parameters related to the kinetics and the extent of CH$_4$ production. We hypothesized that in an anoxic sediment, the extent of CH$_4$ production increases with increasing amount of POM supply and decreasing C/N ratio. We also hypothesized that the extent of CH$_4$ production within a certain amount of time would not be dependent on the frequency of POM addition, as long as the total amount of added POM is constant. We further hypothesized that CH$_4$ would be produced more quickly with increasing frequency of POM addition.

**Materials and methods**

**Experimental part**

The experimental part consisted of two anoxic incubation experiments. In experiment I, different amounts of POM from three different sources were added to an anoxic sediment one single time (Fig. 1). Prior to the addition to the sediment, the POM from the different sources was pre-aged at oxic conditions for either 1 or 3 weeks, in order to mimic degradation in the water column prior to deposition onto the sediment. Hence this experiment quantified the effect of POM reactivity (3 POM sources, pre-aged 1 or 3 week[s]) and the amount of POM supply (different quantities of POM added) on CH$_4$ production. In
experiment II, POM from two different sources that was pre-aged for 1 or 3 week(s) was added to an anoxic sediment, adding up to the same total final amount but at different frequencies (POM addition either every 1, 2, or 4 week(s); Fig. 1). POM was added in total eight times for the weekly POM addition, four times for the bi-weekly addition, and twice for the addition every 4 weeks. Hence experiment II quantified the effect of the frequency of POM additions as well as the effect of POM reactivity (2 POM sources, pre-aged 1 or 3 week(s)) on CH₄ production (Fig. 1).

Material sampling and pre-aging

POM sources: Phytoplankton was sampled with a 20 μm net in a small pond in the city of Juiz de Fora (Brazil) during a bloom, and dried at 60°C. The phytoplankton community consisted mainly of cyanobacteria (around 90%) and green algae. A mixture of terrestrial leaves was composed of senescent tree and shrub leaves collected in late autumn in a forest of the city of Uppsala (Sweden) and kept frozen until the start of the experiments. Several individuals of one submerged aquatic plant species (*Elodea canadensis*) were collected at Lake Erken (Sweden) and cultivated in an aquarium until the start of the experiments. The biomass of aquatic plants collected before the start of the experiments was rather low, and limited the material that was available for experiment I (Fig. 1).

CDU sediment: Sediment was sampled with a gravity corer in an oligotrophic drinking water reservoir (Chapéu d’Uvas [CDU], Brazil) in March 2016. The top 5 cm of the sediment were sliced, mixed and stored in a closed bottle in the dark at room temperature, close to in situ temperatures, until the start of the experiments. This sediment was used because previous experiments showed that it provided favorable conditions for methanogenesis (neutral pH, low content in other electron acceptors) and a low TOC content (Grasset et al. 2018; Grasset et al. 2019).

Erken sediment inoculum: Sediment from mesotrophic Lake Erken was also collected shortly (ca. 1–4 weeks) before the start of each incubation experiment (in January 2018 for experiment I and in April 2018 for experiment II) to ensure that an active microbial inoculum would be present in the slurries. The upper 8–10 cm of three sediment cores were mixed together after removing the presumably oxygenated first cm, and kept in a closed bottle at 4°C in the dark until the start of the experiments.

Lake water: Water was also sampled from hard water Lake Erken (mean alkalinity, 1.81 meq L⁻¹), filtered at 0.7 μm using glass fiber filters (Grade GF/F, Whatman, GE Healthcare, UK), and used for dilution of the sediment in the slurries. The water was sampled shortly before the beginning of the experiments (in January 2018) and stored at 4°C.

Chemical analyses of the sampled materials: Sediment and POM sources were dried at 60°C for 24–72 h for total organic carbon (TOC) and TN analysis. Before analysis, samples were manually ground into a fine powder with a mortar and a pestle. About 5 mg of POM, or 30 mg of acidified sediment (with 5% HCl) were encapsulated in tin capsules and analyzed by high-temperature catalytic oxidation with a COSTECH system 4010 elemental analyzer. DOC and TN concentrations of Lake Erken...
water were analyzed with a Shimazu analyzer and total phosphorus (TP) was analyzed on a SEAL AutoAnalyzer 3HR (Seal Analytical; DOC of 10 mg L$^{-1}$, TN of 0.8 mg L$^{-1}$, and TP of 38 μg L$^{-1}$).

Aerobic pre-aging of POM: The POM from the different sources was pre-aged through incubation at oxic conditions in the dark at 20°C in 2 L bottles filled with lake water and a diluted sediment inoculum (ca. 0.5 g from CDU and Erken sediment each). The bottles were open and continuously shaken on a rotating table at 50 rpm to ensure oxic conditions, during 1 week for half of them, and during 3 weeks for the other half. After 1 or 3 week(s) of incubation, water was removed from the bottles by centrifugation, and the POM was kept frozen at –20°C for experiment II, or directly used for experiment I. The relatively short oxic decomposition times of 1 and 3 week(s) were chosen to mimic the time that it can take for detrital POM to be deposited on the sediment surface. The sinking rate of phytoplankton has been estimated on average as 0.64 ± 0.31 m d$^{-1}$ (Bienfang 1981). The sinking time of aquatic plants is expected to be shorter since aquatic plants are mainly found in littoral areas and are denser than phytoplankton. The time for terrestrial POM to reach the sediment is more complex to estimate (longer in case the POM particles resemble large organic flocs, shorter if the POM is attached to mineral particles). In the rest of the manuscript, we refer to POM that has been aerobically decomposed for 1 or 3 week(s) as 1 or 3 week(s) pre-aged.

**Anaerobic decomposition of POM**

Experiment I: This was a fully factorial experiment that tested the effect of the three different factors (quantity of POM addition, POM source, and POM pre-aging) on CH$_4$ production. The POM from the three different sources, pre-aged 1 or 3 weeks, was added once in triplicates in three to five different quantities (6–274 mg C), to 160 mL glass bottles with 15 g CDU sediment (corresponding to a volume of 12 mL, and an OC content of 2.9%), 40 mL lake water and 0.3 g Erken sediment (corresponding to a volume of 12 mL), 40 mL lake water and 0.3 g Erken sediment inoculum (Table S1; Fig. 1). After addition of POM, the slurry OC content varied from 3% to 8%, which is in the same order of magnitude as an average lake sediment OC content (range, 0.5–30%; mean, 5%; (Sobek et al. 2009). If we consider that these single additions correspond to what can be deposited on a sediment over 1 month and that POM is mixed in the upper cm layer, the POM additions correspond to a deposition rate of 5–228 g C m$^{-2}$ per month. This rate is within the same range as the sediment deposition rates given in Sobek et al. (2009; 0.6–110 g C m$^{-2}$ per month). At day 0 of the incubation, the bottles were flushed with N$_2$, closed with 10 mm thick bromo-butyl septa and aluminum crimps, and flushed again with N$_2$ for 10 min. The bottles were then flushed with N$_2$ every week to remove gases or other volatile compounds that could inhibit methanogenesis (Guérin et al. 2008; Magnnusson 1993). The bottles remained closed, at 20°C and in the dark until the end of the experiment, which lasted between 52 and 94 d, depending on the time that was needed to observe a plateau in CH$_4$ production (January 2018–April 2018).

Experiment II: This was a fully factorial experiment that tested the effect of three different factors (frequency of POM addition, POM source, and POM pre-aging) on CH$_4$ production. The POM from two different sources, pre-aged 1 or 3 week(s), was added several times (3–3.7, 6–7.1, and 12–14.3 mg C of POM added in triplicates every 1, 2, and 4 week(s), respectively) to 160 mL glass bottles with 13 g CDU sediment (corresponding to a volume of 11 mL), 40 mL lake water and 0.3 g Erken sediment inoculum (Table S1; Fig. 1). Thus, the total final amount of POM added in experiment II (between 24 and 28 mg C) corresponded to the second level of POM added in experiment I (POM 2 between 19 and 32 mg C, Table S1). At day 0 of the incubation, the bottles were flushed with N$_2$, closed and flushed again with N$_2$ for 10 min. The bottles were then opened in an anaerobic glove box (Belle Technology) for POM additions every 1, 2, or 4 week(s). POM additions were performed during 8 weeks, and the bottles then remained closed, at 20°C and in the dark until the end of the experiment, which lasted ca. 220 d (April 2018–November 2018). All bottles were flushed with N$_2$ for 10 min every week during the first 8 weeks of the experiment (directly after POM addition), and then three times over the remaining time of the experiment.

**Gas concentration measurements**

The O$_2$ concentration was checked in one of the three replicates of each treatment during the anaerobic incubation with an optical sensor system and noninvasive oxygen sensor spots placed in the headspace (Fibox 3 and PS3, PreSens–Precision Sensing GmbH, Regensburg, Germany). Anoxic conditions (O$_2$ not detected by the sensor, LOD < 0.03%) were reached after the N$_2$ flushing at day 0, and maintained during the entire experiments.

CH$_4$ and CO$_2$ production were measured bi-weekly in experiment I during the entire incubation, and in experiment II during the first 8 weeks, and then seven times until the end of the experiment. CH$_4$ and CO$_2$ production over time were calculated from their respective concentration measured before flushing the headspace with N$_2$. For CH$_4$ and CO$_2$ concentration measurements, 2 mL of the bottle headspace were sampled with a 2.5 mL plastic syringe equipped with a three-way valve and injected into an Ultra-Portable Greenhouse Gas Analyzer (Los Gatos Research Inc., Mountain View, CA) according to Grasset et al. (2018). The gas analyzer was equipped with a gas-tight custom-made sample inlet and a water filter. Ambient air was used as carrier gas, with a CO$_2$ absorber containing soda lime connected upstream of the inlet, which decreased the CO$_2$ baseline to ca. 1 ppm. The peaks (concentration in ppm over time) were integrated with the R software, and calibrated against a calibration curve established from dilution series of certified gas standards. The concentrations of the dilution series were cross-checked with a gas chromatograph equipped with a flame ionization detector (FID; Agilent Technologies, 7890 A GC system). The bottles were gently stirred manually while flushing, and we estimated that flushing removed on average 95% of CH$_4$ and
describes well the three phases of CH₄ production for relatively short time scales (incubation time < 50–100 d; Grasset et al. 2019; Kankaala et al. 2003; Vavilin et al. 2008):

\[
\text{CH}_4(t) = \frac{\text{Asym}}{1 + \exp[(xmid - t)/\text{scal}]}
\]  

(1)

CH₄ (t): total CH₄ production in g C and t: time since the beginning of the incubation in d.

Asym, xmid, and scal are the model parameters, returned by fitting Eq. 1 to the measured total CH₄ production (Fig. 2):

Asym: the horizontal asymptote at t∞, in g C. Asym thus refers to the extent of CH₄ production (i.e., the yield of the reaction).

xmid: the t value at which CH₄ (t) equals Asym/2 and corresponds to the length of the lag phase plus the exponential growth phase in d.

scal: the distance on the x-axis between xmid and the point where CH₄ (t) equals Asym/(1 + e⁻¹) in d. scal relates to how quickly Asym, the asymptote, is reached. xmid and scal thus refer to the kinetics of CH₄ production, and both are given in d.

We fitted Eq. 1 to the measured data using the nlsList function in the R software (R core Team 2016) to determine the non-linear model parameters for each combination of factors (23 levels: 3 POM quantities, 1 POM ages and 3 POM sources). The nlsList function was implemented with the logistic self-starting object “SSlogis” that determines the starting estimates for the model parameters of Eq. 1; (nlme package; Pinheiro et al. 2020). A reactivity continuum model was also fitted to the fraction of remaining POM and total CH₄ production and is described in the supplementary material. The logistic model was preferred to the reactivity continuum model because the latter was not able to adequately describe the two
initial phases of CH₄ production (lag time and exponential growth) and was thus fitted to the total CH₄ production only after removing the first ca. 6–20 d (Figs. S1, S2).

**Relationships between CH₄ production, the amount, and the reactivity of POM supply (experiment I)**

In order to test if the extent of CH₄ production (Asym) can be predicted from different amounts of supplied POM (added POM) and POM with different reactivities (C/N ratio), linear regression models were tested using the lm function in R. To this analysis, we added published data from a similar experiment on CH₄ production from 10 different macrophyte species decomposing in an anoxic reservoir sediment at 23°C (Grasset et al. 2019), including POM quantity and C/N ratio. We assumed that the slight difference in temperature of 3°C between the two experiments would not significantly affect the extent of CH₄ production compared to the effect of the experimental treatments.

All statistical analyses and models were carried out using the R software. The models were assessed by visualizing predicted vs. measured values and after examination of the residuals. For the linear relationships between the model parameters total CH₄ production and the amount and reactivity of POM supply, all variables except scal were logged (natural logarithm) to normalize distributions.

**Calibration of the model describing CH₄ production for POM added at different frequencies (experiment II)**

The logistic model as defined above for experiment I was developed from a single POM addition to an anoxic sediment. However, in a natural system, POM is deposited on a sediment at different frequencies and is more likely to continuously fuel CH₄ production. We first assumed that the parameter related to the extent of CH₄ production (Asym) was not affected by the frequency of POM addition. To check this assumption, we compared the total CH₄ production at day 219 between the different frequencies of POM addition with the non-parametric Kruskal-Wallis test.

Second, the parameters related to the kinetics of CH₄ production (xmid and scal) were assumed to change significantly after the second POM addition. We expected a faster CH₄ production due to the suppression of the lag phase (depletion of alternative electron acceptors), and a stimulation of microbial activity and abundance for high frequency additions. We consequently optimized the model derived from experiment I for POM added at different frequencies in experiment II by adjusting the parameters related to the kinetics of CH₄ production (xmid and scal). We applied the optimization to the first 8 weeks (56 d) when POM was added to the sediment every 7 to 28 d depending on the treatment. Here, CH₄ production rates rather than total CH₄ production were used to optimize the parameters because the production rates are more sensitive to changes in the kinetics of CH₄ production. The predicted CH₄ production rate \( P(\text{CH}_4)_{\text{tot}}(t) \) in g C d⁻¹ for multiple POM additions in experiment II was calculated as the sum of the CH₄ production rates derived from single POM additions as predicted from experiment I. For example, for the treatment with weekly POM additions (POM added at \( t = 0, 7, 14, 21, 28, 35, 42, 49 \)) between day 0 and day 6:

\[
P(\text{CH}_4)_{\text{tot}}(t) = P(\text{CH}_4)(t) + P(\text{CH}_4)(t-7)
\]

between day 7 and day 13:

\[
P(\text{CH}_4)_{\text{tot}}(t) = P(\text{CH}_4)(t) + P(\text{CH}_4)(t-7) + \ldots + P(\text{CH}_4)(t-49)
\]

with \( P(\text{CH}_4)(t) \) being the predicted CH₄ production rate of a single POM addition at time \( t \) in g C d⁻¹, calculated according to the logistic model developed in experiment I. \( P(\text{CH}_4)(t) \) was obtained from Eq. 1 after derivation of total CH₄ production according to time:

\[
P(\text{CH}_4)(t) = \frac{\text{CH}_4(t)}{\text{scal}} \times \left(1 - \frac{\text{CH}_4(t)}{\text{Asym}}\right)
\]

The model parameter related to the extent of CH₄ production (Asym) was predicted according to their linear relationships with added POM (Table 1). The parameters related to the kinetics of CH₄ production (xmid and scal) were given the value obtained for the second level of POM added in experiment I before optimization (POM 2, Tables S1, S2).

| Treatment | Equation | \( R^2 \) of the model (p value) |
|-----------|----------|-------------------------------|
| All pooled* | \( \ln \text{Asym} = -1.68 + 0.97 \times \ln \text{added POM} + 0.19 \times \ln \text{C/N} \) | 0.97 (≤ 0.0001) |
| Phytoplankton 1 week aged | \( \ln \text{Asym} = -2.26 + 0.87 \times \ln \text{added POM} \) | 0.99 (0.0002) |
| Terrestrial leaves 1 week aged | \( \ln \text{Asym} = -2.48 + 0.93 \times \ln \text{added POM} \) | 1.0 (≤ 0.0001) |
| Phytoplankton 3 weeks aged | \( \ln \text{Asym} = -1.96 + 0.99 \times \ln \text{added POM} \) | 1.0 (≤ 0.0001) |
| Terrestrial leaves 3 weeks aged | \( \ln \text{Asym} = -2.74 + 0.93 \times \ln \text{added POM} \) | 1.0 (≤ 0.0001) |

*This model also include 10 macrophytes that were decomposed in an anoxic reservoir sediment (Grasset et al. 2019).

**Empirical model of CH₄ production**
The predicted CH$_4$ production rates were optimized using the Nelder and Mead algorithm (Nelder and Mead 1965). The optimization adjusted the parameters related to the kinetics of CH$_4$ production ($x_{mid}$ and $scal$) to obtain the closest fit between predicted and measured CH$_4$ production rates (i.e., resulting in the lowest residual sum of squares). The best parameters were selected after 100 iterations with random starting parameters (“multiStartoptim” function, mcGlobaloptim package; Moudiki 2013). Furthermore, to visually assess how the optimized parameter sets compare between treatments, the average predicted CH$_4$ production rates after optimization were calculated with the average of $x_{mid}$ and $scal$ for the 12 treatments.

**Results**

**Prediction of CH$_4$ production according to POM reactivity and supply (experiment I)**

The logistic model provided a very good fit to the measured total CH$_4$ production after a single POM addition (experiment I) ($R^2 = 0.99$; Fig. 3). The extent of CH$_4$ production (model parameter $Asym$) was strongly and positively correlated with the amount of POM supply and negatively correlated to the C/N ratio of the POM ($p$ value $< 0.0001$ for both predictors; Fig. 4a and Table 1). The extent of CH$_4$ production, normalized by the quantity of added POM ($Asym$/added POM in %) decreased significantly and linearly with C/N for POM derived from different sources (phytoplankton, terrestrial and macrophyte POM; Fig. 4b). Pre-aging of POM prior to addition to the sediment did not visibly affect the extent of CH$_4$ production for phytoplankton, and decreased the extent of CH$_4$ production for terrestrially derived POM (Fig. 4b). Additionally, when comparing the extent of CH$_4$ production only between 1 and 3-week (s) pre-aged phytoplankton or only between 1 and 3 week (s) pre-aged terrestrial POM, the extent of CH$_4$ production did not decrease with the C/N ratio (Fig. 4b).

**Calibration of the model describing CH$_4$ production for POM added at different frequencies (experiment II)**

The measured total CH$_4$ production at day 219 in experiment II was very similar for the different frequencies of POM

![Fig 3. Measured (dots) total CH$_4$ production during experiment I and fitted logistic model (lines). The legend describes the different amounts of POM added to the sediment, POM 1 being the lowest amount, and POM 5 being the highest (Table S1).](image-url)
addition (Table 2, p value of the Kruskal–Wallis test > 0.5). The measured rates of CH₄ production with different frequencies of POM addition (experiment II) were poorly predicted by the model derived from a single POM addition (experiment I) (gray lines in Figs. 5, 6). The apparent mismatch between the predicted and measured CH₄ production rates indicated that the model parameters related to the kinetics of CH₄ production (xmid and scal) strongly differed between experiments I and II (gray lines, Figs. 5, 6). The optimization of xmid and scal returned better fits (i.e., lower residual sum of squares) between the predicted and measured CH₄ production rates (black lines on Figs. 5, 6). The optimization consistently decreased xmid and scal (except for 1 out of 12 treatments, Figs. 5, 6). The maximum CH₄ production rate following POM addition was always reached more quickly and was higher than predicted from experiment I. The average predicted CH₄ production rates calculated from the average optimized xmid and scal for all 12 treatments were also relatively close to the measured CH₄ production rates (pink dashed lines in Figs. 5, 6).

**Discussion**

Effect of the amount and the reactivity of POM supply on CH₄ production

We propose here the first predictive relationships between the extent of CH₄ production in inland water sediment and the amount and reactivity of POM supplied to the sediment (Fig. 4; Table 1). These relationships may be used for modeling CH₄ production in surface sediments of inland waters. In contrast to other models that predict CH₄ production from properties of already existing sediment (Gebert et al. 2006; Isidorova et al. 2019), the relationships reported here quantify

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**Table 2.** Measured total CH₄ production at day 219 in experiment II.

| Treatment                                           | Measured total CH₄ production at day 219 (%)* |
|-----------------------------------------------------|----------------------------------------------|
| Phytoplankton 1 week pre-aged, every week           | 26 ± 1                                       |
| Phytoplankton 1 week pre-aged, every 2 weeks        | 27 ± 0                                       |
| Phytoplankton 1 week pre-aged, every 4 weeks        | 27 ± 1                                       |
| Phytoplankton 3 weeks pre-aged, every week          | 30 ± 1                                       |
| Phytoplankton 3 weeks pre-aged, every 2 weeks       | 31 ± 2                                       |
| Phytoplankton 3 weeks pre-aged, every 4 weeks       | 34 ± 1                                       |
| Terrestrial leaves 1 week pre-aged, every week      | 22 ± 1                                       |
| Terrestrial leaves 1 week pre-aged, every 2 weeks   | 20 ± 2                                       |
| Terrestrial leaves 1 week pre-aged, every 4 weeks   | 20 ± 5                                       |
| Terrestrial leaves 3 weeks pre-aged, every 2 weeks  | 18 ± 1                                       |
| Terrestrial leaves 3 weeks pre-aged, every 4 weeks  | 16 ± 2                                       |

*The measured total CH₄ production is normalized by added POM and given in % (i.e., calculated in g C g⁻¹ × 100).
the response of CH4 production to new supply of POM from different sources. These relationships can thus be used in biogeochemical inland water models to directly couple CH4 and sedimentation fluxes. We further note that the data derived from previously published decomposition experiments of 10 different macrophyte species (Grasset et al. 2019) well fitted the data measured in the present experiments with respect to relationships between the C/N ratio, the amount of added POM and the extent of CH4 production (Fig. 4). This is remarkable, since Grasset et al. (2019) studied tropical macrophytes and used a sediment inoculum from tropical lagoons, while here, we used POM from boreal terrestrial and aquatic plants and used sediment inoculum from a boreal lake. This indicates that our relationship might apply to a wide range of different inland waters, but additional studies are nevertheless needed to assess its applicability to different systems with different sediments and POM sources.

As hypothesized, the extent of CH4 production linearly increased with the amount of supplied POM (Fig. 4a; Table 1). This result suggests that within the range of aquatic and terrestrial OC sedimentation rates that can be found in inland waters, the total CH4 production increases linearly with the POM deposition rate. However, POM from certain plant species may contain or form compounds (e.g., polyphenols or fatty acids) during its decomposition that can inhibit CH4 production (Emilson et al. 2018; Grasset et al. 2019). Hence, the relationship that we propose here might not be valid for POM derived from some floating-leaved or emergent species that are rich in polyphenolic compounds (Smolders et al. 2000), or for forest litter rich in phenols (Emilson et al. 2018).

The extent of CH4 production also linearly decreased with increasing C/N ratio of the POM (Fig. 4b; Table 1), as hypothesized. This confirms similar findings of previous studies (Gebert et al. 2006; Duc et al. 2010; Gebert et al. 2019;...
Isidorova et al. 2019). We show here that the C/N ratio can be used to compare CH4 production between POM derived from a broad range of different sources (terrestrial leaves, aquatic plant leaves, phytoplankton). However, pre-aging of POM prior to addition to the sediment did not strongly affect the extent of CH4 production for phytoplankton, despite a higher C/N ratio (and thus a presumably lower reactivity) of the 3 weeks pre-aged phytoplankton-derived POM (Fig. 4b). Furthermore, the POM derived from 3 weeks pre-aged terrestrial leaves had a lower C/N ratio (and thus a presumably higher reactivity), but also a lower extent of CH4 production than the 1 week pre-aged terrestrial-leave-derived POM (Fig. 4b). Our results consequently suggest that the C/N ratio might not be an accurate proxy of POM reactivity and the extent of CH4 production at the relatively small-scale changes potentially occurring to the POM within such short time (i.e., a couple of weeks).

**Effect of POM supply frequency on CH4 production**

While the frequency of POM addition did not significantly affect the extent of CH4 production (Table 2), it strongly affected the kinetics of CH4 production (Figs. 5, 6). In particular, the maximum CH4 production rate was reached more quickly and was higher than that predicted with the model derived from only one POM addition (Figs. 5, 6). The highest CH4 production rates (ca. 0.1 – 0.15 mg C d\(^{-1}\) corresponding to 46 – 80 μmol d\(^{-1}\) per g C of slurry) were in the same order of magnitude as the highest production rates from other freshwater sediments incubated without POM addition (e.g., ca. 30 μmol d\(^{-1}\) g C\(^{-1}\) [Isidorova et al. 2019; Praetzel et al. 2020 and ca. 20 – 40 μmol d\(^{-1}\) g OM\(^{-1}\) [Berberich et al. 2019]. In earlier sediment incubation experiments, POM was typically added to the sediment only one time (e.g., Kankaala et al. 2003; West et al. 2012; Grasset et al. 2018). Our results

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**Fig 6.** Prediction of CH4 production rates (mg C d\(^{-1}\)) derived from the decomposition of phytoplankton and terrestrial POM, pre-aged during 3 weeks and added at different frequencies to an anoxic sediment (experiment II). The models are represented by lines while the measurements are represented by dots. CH4 production rates are predicted according to the logistic model in Eq. 3 before (gray line) and after (black line) optimization of the model parameters \(x_{mid}\) and \(scal\) obtained with experiment I. The pink dashed line represents the CH4 production rates as calculated from the average of \(x_{mid}\) and \(scal\) after optimization of all 12 treatments (i.e., \(x_{mid} = 17\) and \(scal = 4.3\)).

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indicate that such experiments can accurately predict the extent of POM transformation into CH_{4} (Table 2), but they are likely to underestimate peak CH_{4} production rates in anoxic sediments (Figs. 5, 6). This is especially important for modeling CH_{4} ebullition, because CH_{4} can accumulate in the sediment only if CH_{4} production rates exceed the rate of CH_{4} loss through diffusion, ebullition and oxidation (Schmid et al. 2017; Langenegger et al. 2019). Consequently, underestimating the peak in CH_{4} production rate following POM deposition onto a sediment is likely to result in an underestimation of the CH_{4} ebullition flux. Interestingly, the average kinetic parameters of the different POM sources in this study returned CH_{4} production rates relatively similar to the measured CH_{4} production rates (Figs. 5, 6). This suggests that when POM is frequently supplied to the sediment (more often than monthly), the average of the kinetic parameters given in Figs. 5, 6 (i.e., 17 for xmid and 4.3 for scal) can thus be used to give an approximate prediction of CH_{4} production rates for phytoplankton-derived or terrestrially derived POM. Studies are nevertheless needed to further investigate how the kinetic parameters of CH_{4} production can vary when POM is added regularly to a sediment, in order to improve this prediction.

Applicability of the model

The equations and parameters derived from our empirical experiments (Asym given in Table 1, xmid = 17 and scal = 4.3) can be used to predict the short-term (< ca. 100 d) CH_{4} production rates in anoxic inland water surface sediments, based on POM supply (i.e., POM sedimentation rate) and reactivity (C/N ratio). The CH_{4} production rates derived from our model can be directly used to model CH_{4} ebullition in combination with physical models that predicts bubble growth and migration through the sediment column (e.g., Ramirez et al. 2015; Scandella et al. 2011). Our results can also be used to model diffusive CH_{4} flux from the sediment to the water column, but the limited knowledge on the drivers of CH_{4} oxidation is currently limiting the prediction of CH_{4} loss during CH_{4} diffusion over the sediment and water column.

Several aspects need to be taken into account when using our model to predict CH_{4} production. Firstly, in-situ CH_{4} production extends beyond the time scale of the experiment, and a substantial part of CH_{4} production can occur after 100 d. For example, a study on reservoir sediments has shown that POM can fuel significant CH_{4} production for up to 6–12 years for tropical sediments rich in aquatic and terrestrial POM, respectively (Isidorova et al. 2019). For an exhaustive model of CH_{4} production in inland water sediments, our short-term model that predicts CH_{4} production according to POM supply and reactivity should consequently be completed by a long-term model of CH_{4} production during sediment diagenesis (e.g., Clayer et al. 2018; Isidorova et al. 2019). Furthermore, our model is directly applicable to inland waters with anoxic bottom waters where POM is decomposed at anoxic condition as soon as it reaches surface sediments. However, in systems with oxygenated bottom water, the aerobic decomposition of POM in the surface sediment needs to be accounted for, and CH_{4} production will be lower than reported in our study (Table 2) because the POM has undergone aerobic decomposition in oxygenated surface sediment before reaching anoxic sediment layers. Lastly, our study focused on POM reactivity in terms of intrinsic composition, but POM reactivity is also likely to differ between different sediment matrices with different biological, geochemical and physical characteristics (LaRowe et al. 2020). Further studies should consequently investigate in how far this model applies to different inland water sediments.

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Conflict of interest

None declared.

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