Modeling CH₄ and CO₂ cycling using porewater stable isotopes in a thermokarst bog in Interior Alaska: Results from three conceptual reaction networks

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ABSTRACT

Quantifying rates of microbial carbon transformation in peatlands is essential for gaining mechanistic understanding of the factors that influence methane emissions from these systems, and for predicting how emissions will respond to climate change and other disturbances. In this study, we used porewater stable isotopes collected from both the edge and center of a thermokarst bog in Interior Alaska to estimate in situ microbial reaction rates. We expected that near the edge of the thaw feature, actively thawing permafrost and greater abundance of sedges would increase carbon, oxygen and nutrient availability, enabling faster microbial rates relative to the center of the thaw feature. We developed three different conceptual reaction networks that explained the temporal change in porewater CO₂, CH₄, δ¹³C-CO₂ and δ¹³C-CH₄. All three reaction-network models included methane production, methane oxidation and CO₂ production,
and two of the models included homoacetogenesis — a reaction not previously included in isotope-based porewater models. All three models fit the data equally well, but rates resulting from the models differed. Most notably, inclusion of homoacetogenesis altered the modeled pathways of methane production when the reaction was directly coupled to methanogenesis, and it decreased gross methane production rates by up to a factor of five when it remained decoupled from methanogenesis. The ability of all three conceptual reaction networks to successfully match the measured data indicate that this technique for estimating in-situ reaction rates requires other data and information from the site to confirm the considered set of microbial reactions. Despite these differences, all models indicated that, as expected, rates were greater at the edge than in the center of the thaw bog, that rates at the edge increased more during the growing season than did rates in the center, and that the ratio of acetoclastic to hydrogenotrophic methanogenesis was greater at the edge than in the center. In both locations, modeled rates (excluding methane oxidation) increased with depth. A puzzling outcome from the effort was that none of the models could fit the porewater dataset without generating “fugitive” carbon (i.e., methane or acetate generated by the models but not detected at the field site), indicating that either our conceptualization of the reactions occurring at the site remains incomplete or our site measurements are missing important carbon transformations and/or carbon fluxes. This model–data discrepancy will motivate and inform future research efforts focused on improving our understanding of carbon cycling in permafrost wetlands.

Keywords: carbon fluxes; homoacetogenesis; methanogenesis; methanotrophy; microbial rates; peat; model; $^{13}$CO$_2$; $^{13}$CH$_4$; carbon isotopes
Wetlands represent the largest natural source of methane to the atmosphere, responsible for roughly one third of global methane emissions (Ciais et al. 2013), and emissions from northern high latitudes have recently increased with atmospheric warming (Bloom et al. 2010). Because methane is a potent greenhouse gas with strong radiative forcing, it is important to understand the factors that modulate the magnitude of wetland methane emissions and to predict how emissions may change in the future. Currently, the response of wetland methane emissions to future climate change is unknown, with uncertainty surrounding both the magnitude and direction of the climate—methane feedback (Wuebbles and Hayhoe 2002; Bridgham et al. 2013).

Emitted methane represents the end product of various microbial processes operating within the largely anaerobic subsurface of wetlands. In anaerobic environments, methanogens produce methane primarily from acetate (acetoclastic methanogenesis) or from hydrogen and carbon dioxide (hydrogenotrophic methanogenesis). The substrates for methanogenesis (acetate, hydrogen and CO$_2$) are produced from subsurface organic matter (e.g., peat and plant root exudates) by communities of fermenting and syntrophic microbes (Conrad 1999). Acetate can also be generated from H$_2$ and CO$_2$ by homoacetogenic microbes (Ye et al. 2014). Once generated, methane can be oxidized to CO$_2$ by methanotrophs who use oxygen or other compounds (e.g., nitrate, sulfate, Fe(III)) as their electron acceptor (Popp et al. 2000; Blazewicz et al. 2012). Determining the rates at which these various microbial reactions occur within the subsurface of wetlands is challenging, making it difficult to gain a mechanistic understanding of the factors and conditions that influence these rates and ultimately methane emissions. Most studies predict wetland methane emissions based on empirical relationships with factors such as water table position, soil temperature, peat humification and vegetation (structure and
productivity) (Dise et al. 1993; Turetsky et al. 2014). However, these relationships often have low explanatory power due to, for example, temporal lags between microbial rates and environmental factors (Van Hulzen et al. 1999), and different sensitivities of microbial communities to these predictors (e.g., different $Q_{10}$ temperature responses of methanogens and methanotrophs (van Winden et al. 2012)).

Potential microbial reaction rates are often assessed with laboratory incubations that take soil or peat from the wetland of interest and track the generation of CO$_2$ and CH$_4$ (methanogenesis and respiration), consumption of methane (methanotrophy), and/or generation of acetate (homoacetogenesis) (e.g., Segers 1998; Hodgkins et al. 2014; Ye et al. 2014). While this method allows for an intercomparison of potential reaction rates between samples, it relies on disturbed samples and does not provide information about in-situ reaction rates. Actual reaction rates are influenced by site conditions (e.g., plant structure and productivity, redox conditions, temperature) that cannot be mimicked in incubation experiments. A powerful approach for estimating in-situ reaction rates involves tracking the temporal change in porewater concentrations and stable carbon isotopes of methane and CO$_2$ (Shoemaker and Schrag 2010; Shoemaker et al. 2012; Corbett et al. 2012, 2015; Holmes et al. 2015). Microbes preferentially use isotopically depleted carbon substrates, which causes the carbon product pool to become depleted in $^{13}$C and the carbon substrate pool to become enriched in $^{13}$C. Different microbial biochemical pathways fractionate the carbon pools to different extents (Whiticar 1999), which allows for differentiation between the microbial reactions of interest. Thus, by simultaneously tracking both the rate of change of stable carbon isotopes and porewater concentrations of the carbon pools (i.e., CH$_4$ and CO$_2$), it is possible to calculate rates of multiple microbial reactions. This technique is extremely useful for estimating in-situ rates, but, as we show in this paper, it is
possible for different combinations of reaction rates to provide equally good fits to the temporal change in porewater data. The solution is non-unique and depends on the set of considered reactions (i.e., the reaction network).

Previous applications of the *in-situ* rate-estimation technique (Shoemaker and Schrag 2010; Shoemaker et al. 2012; Corbett et al. 2012, 2015; Holmes et al. 2015) considered the first four reactions listed in Table 1: acetoclastic methanogenesis, hydrogenotrophic methanogenesis, methane oxidation, and non-methanogenic CO$_2$ production (e.g., respiration and/or CO$_2$ shedding by organic matter (Tfaily et al. 2013)). However, recent work has demonstrated that homoacetogenesis can occur in peatlands at rates that rival or even exceed that of methanogenesis (Ye et al. 2014), and this reaction fractionates dissolved CO$_2$ (Blaser et al. 2013). Given these findings, and the fact that the genes and transcripts involved in homoacetogenesis (tetrahydrofolate metabolism) were observed in the metagenome and metatranscriptome of microorganisms present at our site (Hultman et al. 2015), we developed alternative conceptual reaction networks that included homoacetogenesis. Homoacetogenesis has not been a reaction previously considered in isotope-based porewater models.

We used the *in-situ* rate-estimation technique with three different conceptual reaction networks (two with and one without homoacetogenesis) to estimate microbial reaction rates during the summer growing season in a thermokarst bog at the Alaska Peatland Experiment (APEX) outside of Fairbanks, AK. We applied the three models to porewater data collected from multiple depths in two locations — edge and center of the thaw bog feature. The edge location was closer to the actively thawing permafrost margin and, as is expected for wet, recently thawed locations (Camill 1999), hosted a greater abundance of sedges relative to the center of the thaw bog (Klapstein et al. 2014). We hypothesized that all considered microbial rates would be faster
at the edge of the bog because we expected that 1) the recently degraded permafrost would increase carbon and nutrient availability (Turetsky et al. 2007; Keuper et al. 2012; Abbott et al. 2014), enabling faster rates of fermentation and methanogenesis, and 2) the greater proportion of sedges would increase oxygen delivery to the subsurface (Armstrong 1964), enabling faster rates of methane oxidation. With regards to methane production pathways, we anticipated that the greater sedge abundance at the edge would favor acetoclastic methanogenesis over hydrogenotrophic methanogenesis due to plant root exudation of acetate and other carbon substrates (Popp et al. 1999; Galand et al. 2005).

METHODS

Field Site

Our study site is located in Interior Alaska, 30 km to the southwest of Fairbanks (64.70 °N, -148.3 °W). The site is within the Bonanza Creek LTER system and is a component of the Alaska Peatland Experiment (APEX) research program. The APEX sites include a number of collapse scar bogs formed as a result of permafrost thaw, subsidence, and thermokarst within a larger complex of forested bog underlain by permafrost (Klapstein et al. 2014; Euskirchen et al. 2014; Hultman et al. 2015). These bogs vary in timing of thaw and range in approximate age from 800 years post-thaw to 50-100 years post-thaw (Jones et al. 2012). Across our chronosequence of time following thaw, the thermokarst bogs are primarily composed of Sphagnum mosses, as well as vascular species Carex aquatilis, Eriphorum chamissonis, Chamaedaphne calyculata, and Carex chordorrhiza, and contain recently deceased black spruce (Picea mariana) trees from the former forested permafrost peatland. In the area where this study
was conducted, the onset of thaw and collapse scar bog formation based on radiocarbon and peat stratigraphy occurred 50 to 200 years ago (USGS unpublished data), though there is ongoing permafrost thaw on several active margins of the collapse scar feature. In this study, we contrasted two sampling locations: the edge of the thaw feature where permafrost thaw is active and recent versus the center of the thaw bog where permafrost thawed in the last several decades to hundreds of years. Methane emissions from the bog, measured with eddy flux towers, range between 0.5 and 5 mmol/m$^2$/day (Euskirchen et al. 2014).

**Porewater Samples**

*Collection*

We collected porewater at a monthly interval (June, July and August 2013) from multiple depths in the center and near the edge of the studied bog. To collect porewater, we used passive diffusion samplers (peepers) built following a previous design (Thomas and Arthur 2010). Briefly, the peepers consisted of an outer perforated PVC sheath permanently installed in the bog and anchored to the mineral soil, and a removable solid PVC inner rod that contained inset holes for holding sample collection cells. Collection cells were made from 5 cm$^3$ plastic Nalgene cups with snap cap lids. The lids, with the tops cut out, held a 0.2 µm, 45 mm polypropylene filter membrane (Pall Life Sciences) over each cup. In each location (i.e., edge and center of the bog), we installed three peeper rods and sheaths with sample collection cells placed at the same depths, generating triplicate samples for each depth at each location.

We filled the peeper cells with 200 µM potassium bromide (KBr) solution made from distilled, deionized water and visually inspected the cells to ensure they were free of gas bubbles. We used bromide as a reverse tracer to allow for correction of measured data within cells that
may not have reached chemical equilibrium during deployment in the bog (see *Post-processing*).

Once filled, we submerged the cells in KBr solution and sealed them in an air-tight container fitted with two bulkhead Swagelok valves. One valve was connected to an N\textsubscript{2} tank and the other acted as an exhaust vent. We purged the solution for 3 days with N\textsubscript{2} to remove any dissolved oxygen. After 3 days, we slightly over pressurized the airtight container with N\textsubscript{2}, sealed it, and transported it to the field. We placed the peeper cells into the peeper rods within five minutes of opening the container, and then quickly inserted the rods into the previously installed perforated PVC sheaths.

After at least one week of deployment, we removed the peeper rods and sampled the water from the peeper cells. In the field, we transferred peeper solution for dissolved gas analysis (concentration and isotopic composition) to preweighed 30mL serum bottles flushed with N\textsubscript{2}, sealed with blue butyl rubber stoppers (Bellco Glass, Vineland, NJ), and pre-acidified with 100 µL of 85% phosphoric acid to ensure cessation of microbial activity and to degas dissolved inorganic carbon as CO\textsubscript{2} (final pH of peeper solution was approximately 1). Peeper solution was also transferred to sealed Vacutainers for dissolved anion quantification. Samples for gas analysis were stored at room temperature in the dark; samples for anion analysis were kept cool in the field and were stored at -20 °C in the laboratory until they were analyzed.

*Analysis*

We measured CO\textsubscript{2} and CH\textsubscript{4} concentrations in the headspace of the serum vials on a SRI 8610C GC with FID-Methanizer and ECD (Column 3.2 mm I.D. x 6 m HayeSep® D, 1 mL sample loop; SRI Instruments, Torrance, CA), which was calibrated using a 3 point line for both CO\textsubscript{2} (10, 100, and 1000 ppmv CO\textsubscript{2}) and CH\textsubscript{4} (10, 100, and 1000 ppmv CH\textsubscript{4}; Air Liquide
America Specialty Gases, Plumsteadville, PA). All samples were removed from the serum vials using an air-tight syringe sealed with high-performance vacuum grease and fitted with a Luer-lok™ stopcock and directly injected into the GC. We used the concentration of the headspace analyte along with Henry’s law constants to calculate the concentration of the analyte originally present in porewater.

We measured carbon isotopic composition ($\delta^{13}$C relative to the VPDB standard) of $\text{CH}_4$ and $\text{CO}_2$ using cavity ring-down spectrometry (CRDS). We used a Picarro model G2132-i CRDS to measure $\delta^{13}$C-$\text{CH}_4$. To make discrete measurements, we connected a tube with a three-way stopcock on one end to the inlet port of the instrument; the three-way stopcock allowed for controlled entry of room air, a reference gas (Isometric Instruments, T-iso3, 250ppmv $\text{CH}_4$, $\delta^{13}$C-$\text{CH}_4$ = -38.3 ± 0.2‰), or a sample from a syringe. We introduced standards and samples to the CRDS over a three-minute period, at the end of which we recorded concentration and isotope composition. The CRDS continuously records, updates, and improves the output data, and we determined that three minutes were needed for the instrument to reach steady concentration and isotope composition for the standard gas. We ran room air between each sample and ran standards between every 10 samples. We used 60 mL syringes fitted with a three-way stopcock and needle to pull headspace samples from the stoppered serum vials described above. We diluted these samples with $\text{N}_2$ gas to a concentration between 100 and 180 ppmv $\text{CH}_4$ based on previously measured methane concentrations. The syringes typically contained 30mL of gas after dilution, and never contained less than 20 mL. We connected the syringe to the three-way stopcock on the CRDS, and, after the instrument returned to baseline conditions, opened the valve to the instrument. Over a three-minute sampling interval, the CRDS pulled in gas from the
syringe. Standard readings were \(-37.8 \pm 0.1\%e\) \((\pm 1 \text{ S.D})\) and room air was 2.2 ppmv CH\(_4\) and -
\(50.8 \pm 1.3\%e\). Based on the standard reading, we adjusted \(\delta^{13}\text{C-CH}_4\) values by -0.5\%e.

To determine \(\delta^{13}\text{C-CO}_2\), we used a Picarro model G2101-i CRDS in a similar manner to
that described above, with the following modifications: 1) headspace gas from the acidified
sample was diluted with ultra-high purity N\(_2\) to a concentration of 1000 ppmv in a final volume
of 60 mL, and 2) the \(\delta^{13}\text{C}\) value was taken as the average CRDS reading over a 2 minute period.
We corrected isotopic compositions based on in-house standards of variable \(\delta^{13}\text{C}\) values at a
range of concentrations calibrated against known reference materials. At the low pH values
found at our site (average of pH 4.9, measured early August 2013 from 10–80 cm depths with a
sipper sampler and flow-through electrode (Microelectrodes Inc.)), CO\(_2\) is the predominant form
of dissolved inorganic carbon (DIC) (Stumm and Morgan 1996) and thus, throughout the
manuscript, we use CO\(_2\) and DIC interchangeably.

We simultaneously measured the concentrations of bromide tracer and environmental
acetate in the peeper cells using high performance ion chromatography on a Metrohm 881
Compact IC pro (Anion) with a Metrosep A Supp 7-250/4.0 column. The eluent was 3.6 mM
Na\(_2\)CO\(_3\) with a flow of 0.8 mL/min. Detection was by suppressed conductivity, the column
temperature was 45 °C, and sample injection volume was 20 \(\mu\text{L}\).

Post-processing

The ~1 week deployment period for the peepers was not long enough to achieve complete
equilibrium between the peat porewater and peeper water. Therefore, following the approach of
Thomas and Arthur (2010), we used porewater bromide concentrations to correct the
concentrations of CH\(_4\), CO\(_2\) and acetate. The approach assumes exponential decrease of bromide
concentrations in the peeper cells over time and assumes that the movement of bromide and the solute of interest (e.g., CH$_4$, CO$_2$, acetate) through the peeper membrane and peat are related by the ratio of their free diffusion coefficients in water. We did not correct $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$ using this method because in water or water-filled soil, diffusion causes little fractionation ($\varepsilon_C = 1\%$ for CH$_4$ (Preuss et al. 2013) and $\varepsilon_C = 0.87\%$ for CO$_2$ (Jahne et al. 1987)).

After the bromide correction, we calculated average concentration and isotopic values from the three replicate samples at each peeper depth. During the sampling season, the peat surface moved up and down, but the outer sheath for the peepers did not move because they were anchored to the mineral soil. We translated our peeper measurements to depth below the peat surface and, in our modeling, tracked how concentrations and isotopic composition changed over time (month to month) for a given depth below the peat surface. In most cases, we could align, within a 5-cm window, the translated peeper depth (i.e., translated to depth below peat surface) from one month with translated depths for the following month. In six cases, we could not align the peeper depths. In these instances, we linearly interpolated between measurements above and below the target depth to calculate a concentration or isotopic value for the target depth. Our initial data set included 95 samples, which resulted in a total of 32 concentration and isotopic values for our studied bog (six of the 32 sets of values were interpolated).

**Rate Estimation**

We determined microbial rates of interest using three different conceptual reaction networks that described temporal changes in porewater data for concentrations and stable carbon isotopes of CH$_4$ and CO$_2$. 
Conceptual Reaction Network 1: Original Fugitive Methane Model

The first conceptual reaction network was built from previous applications of the in-situ rate-estimation technique (Shoemaker and Schrag 2010; Shoemaker et al. 2012; Corbett et al. 2012, 2015; Holmes et al. 2015), which considered the following sets of reactions that are summarized in Table 1:

- Acetoclastic methanogenesis:

\[ CH_3COOH \rightarrow CH_4 + CO_2 \]  

(1)

Acetoclastic methanogenesis produces CH$_4$ and CO$_2$ in equal proportions and has a carbon isotopic enrichment factor ($\varepsilon_C$) of 17‰ to 35‰ between the substrate (acetate) and products (Gelwicks et al. 1994; Whiticar 1999; Valentine et al. 2004; Londry et al. 2008). This reaction produces CH$_4$ that is depleted and CO$_2$ that is enriched in $^{13}$C by 20–30‰ relative to the acetate. Fractionation between the produced $^{13}$CH$_4$ and $^{13}$CO$_2$ is 34‰ to 70‰.

- Hydrogenotrophic methanogenesis:

\[ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \]  

(2)

When the generation of H$_2$ is considered, this reaction also produces methane and carbon dioxide in equal proportions. Hydrogen is generated from fermentation and syntrophic reactions in the subsurface, and when a glucose-like molecule is used in these reactions, the outcome is (Conrad 1999):

\[ C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \]  

(3)

This fermentation/syntrophic reaction in combination with hydrogenotrophic methanogenesis produces CO$_2$ and CH$_4$ in equimolar concentrations:

\[ C_6H_{12}O_6 \rightarrow 2CH_3COOH + CO_2 + CH_4 \]  

(4)
Fermentation/synthetic reactions result in minimal carbon fractionation (Blair et al. 1985; Balesdent et al. 1987; Penning and Conrad 2006; Botsch and Conrad 2011; Conrad et al. 2014), while hydrogenotrophic methanogenesis has a carbon isotopic enrichment factor of 46‰ to 80‰ between δ^{13}CO\textsubscript{2} and δ^{13}CH\textsubscript{4} (Games et al. 1978; Botz et al. 1996; Whiticar 1999; Valentine et al. 2004; Londry et al. 2008). Thus for each molecule of CH\textsubscript{4} produced via this reaction, two CO\textsubscript{2} molecules are added to the dissolved inorganic carbon (DIC) pool that have an isotopic composition similar to that of the fermented substrate (e.g., peat). One CO\textsubscript{2} molecule is then removed from the DIC pool to produce methane that is 46‰ to 80‰ depleted relative to the DIC pool, leaving the DIC pool 46‰ to 80‰ enriched relative to its initial composition. In this sequence, the methanogenesis reaction fractionates the DIC pool with an isotopic signature already altered by the addition of CO\textsubscript{2} from the fermentation/synthetic reactions.

- Aerobic methane oxidation:

\[ \text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} \] (5)

Aerobic methane oxidation converts methane into carbon dioxide with a carbon enrichment factor of 5‰ to 31‰ between δ^{13}CO\textsubscript{2} and δ^{13}CH\textsubscript{4} (Whiticar 1999; Feisthauer et al. 2011). In contrast to the methane generation reactions, methane oxidation enriches the methane pool and depletes the DIC pool. Anaerobic methane oxidation can also occur, but was not explicitly considered in previous isotope-based porewater models (Shoemaker and Schrag 2010; Shoemaker et al. 2012; Corbett et al. 2012, 2015; Holmes et al. 2015). At our study site, anaerobic methane oxidation is 0.3% of methane production (Blazewicz et al. 2012), and thus we assumed it minimally affected carbon fractionation.

- Non-methanogenic CO\textsubscript{2} production (e.g., respiration and/or CO\textsubscript{2} shedding by organic matter):
respiration: substrate (e.g., peat) + oxidant $\rightarrow$ CO$_2$ + reduced oxidant

shredding: substrate (e.g., peat) $\rightarrow$ CO$_2$ + reduced substrate

This reaction accounts for the production of dissolved inorganic carbon from all pathways that do not end up producing methane. Potential pathways include respiration with inorganic electron acceptors (e.g., oxygen, sulfate, ferric iron) and/or humic acid electron acceptors (Keller et al. 2009). In addition, CO$_2$ can directly be shed from organic matter during decomposition (Tfaily et al. 2013). These numerous reaction pathways minimally fractionate carbon (i.e., less than 10‰ enrichment between substrate and product) (Blair et al. 1985; Balesdent et al. 1987; Penning and Conrad 2006; Botsch and Conrad 2011; Conrad et al. 2014), and thus they contribute CO$_2$ to the DIC pool that has a similar $\delta^{13}$C as the substrate.

With the above reaction network, the DIC pool can only get $^{13}$C enriched through the production of methane (Table 1). Within this framework, the development of $^{13}$C enriched DIC and the growth of dissolved CO$_2$ concentrations over time in porewater can indicate greater rates of methanogenesis than can be accounted for by the increase in dissolved methane concentrations over the same time period (given the 1:1 production ratio of methane and CO$_2$ via both methanogenic pathways). Corbett et al. (2012) used this difference between isotopically modeled (i.e., $^{13}$C-CO$_2$ constrained) and measured methane concentrations to determine the quantity of methane lost from porewater, terming the lost portion “fugitive methane.” Possible mechanisms for loss of this dissolved methane include ebullition and plant mediated transport.

With the above reaction network, our porewater data similarly indicated greater rates of methanogenesis than could be accounted for by dissolved methane concentrations, and thus we refer to this first conceptual reaction network as the "Original Fugitive Methane Model" within the remainder of the manuscript.
For this model, we used the following four finite difference equations to describe temporal changes in concentration and isotopic composition of methane and CO\textsubscript{2} dissolved in porewater. The equations included four unknown rates for the four reactions described above, as well as diffusive transport. Equations 7 and 8 track the temporal change of dissolved methane (\textsuperscript{12}CH\textsubscript{4}) and carbon dioxide (\textsuperscript{12}CO\textsubscript{2}) concentrations, respectively. Equations 9 and 10 track the temporal change of \textsuperscript{13}CH\textsubscript{4} and \textsuperscript{13}CO\textsubscript{2} concentrations, respectively.

\[
\frac{^{12}\text{CH}_4^{\text{final}} - ^{12}\text{CH}_4^{\text{initial}}}{t^{\text{final}} - t^{\text{initial}}} = \text{DiffCH}_4 + CH4Acet\_i + CH4Hydro\_i - CH4Oxid\_i
\]  

\[
\frac{^{12}\text{CO}_2^{\text{final}} - ^{12}\text{CO}_2^{\text{initial}}}{t^{\text{final}} - t^{\text{initial}}} = \text{DiffCO}_2 + CH4Acet\_i + CH4Hydro\_i + CH4Oxid\_i + \text{Resp\_i}
\]  

\[
\frac{^{13}\text{CH}_4^{\text{final}} - ^{13}\text{CH}_4^{\text{initial}}}{t^{\text{final}} - t^{\text{initial}}} = r\text{CH}_4 \cdot \text{DiffCH}_4 + r\text{ORG} \left(1 - \frac{\varepsilon_{\text{CH4Acet}}}{1000}\right)CH4Acet\_i +
\]

\[
r\text{CO}_2^{\text{frac}} \left(1 - \frac{\varepsilon_{\text{CH4Hydro}}}{1000}\right)CH4Hydro\_i - r\text{CH}_4 \left(1 - \frac{\varepsilon_{\text{CH4Oxid}}}{1000}\right)CH4Oxid\_i
\]  

\[
\frac{^{13}\text{CO}_2^{\text{final}} - ^{13}\text{CO}_2^{\text{initial}}}{t^{\text{final}} - t^{\text{initial}}} = r\text{CO}_2^{\text{data}} \cdot \text{DiffCO}_2 + r\text{ORG} \left(1 + \frac{\varepsilon_{\text{CH4Acet}}}{1000}\right)CH4Acet\_i +
\]

\[
2 \cdot r\text{ORG} - r\text{CO}_2^{\text{frac}} \left(1 - \frac{\varepsilon_{\text{CH4Hydro}}}{1000}\right)CH4Hydro\_i +
\]

\[
r\text{CH}_4 \left(1 - \frac{\varepsilon_{\text{CH4Oxid}}}{1000}\right)CH4Oxid\_i + r\text{ORG} \cdot \text{Resp\_i}
\]  

Where,

\text{\textsuperscript{12}CH}_4^{\text{initial}} and \text{\textsuperscript{12}CO}_2^{\text{initial}} are the initial concentrations of \textsuperscript{12}CH\textsubscript{4} and \textsuperscript{12}CO\textsubscript{2}, respectively, in the \textsuperscript{i}th depth of a given peeper profile for time increment \textit{t}^{\text{initial}} to \textit{t}^{\text{final}};

\text{\textsuperscript{12}CH}_4^{\text{final}} and \text{\textsuperscript{12}CO}_2^{\text{final}} are the final concentrations of \textsuperscript{12}CH\textsubscript{4} and \textsuperscript{12}CO\textsubscript{2}, respectively;
\[^{13}CH_4_{initial}\] and \[^{13}CO_2_{initial}\] are the initial concentrations of \(^{13}\text{CH}_4\) and \(^{13}\text{CO}_2\), respectively; and

\[^{13}CH_4_{final}\] and \[^{13}CO_2_{final}\] are the final concentrations of \(^{13}\text{CH}_4\) and \(^{13}\text{CO}_2\), respectively.

All concentrations have dimensions of mass per volume. We collected porewater data in June, July and August. When we considered the rates responsible for the temporal change in data from that measured in June to that measured in July, the June data represented initial concentrations and the July data represented final concentrations. When considering the rates responsible for the temporal changes in data from that measured in July to that measured in August, the July data represented initial concentrations and the August data represented final concentrations.

In this model, the four rates (dimensions of mass per volume per time) responsible for the temporal change in these concentrations and concentration differences were:

\(^{CH4}\text{Acet}_i\) — acetoclastic methanogenesis (Eqn. 1),

\(^{CH4}\text{Hydro}_i\) — hydrogenotrophic methanogenesis (Eqn. 4),

\(^{CH4}\text{Oxid}_i\) — aerobic methane oxidation (Eqn. 5),

\(^Resp_i\) — respiration and/or \(\text{CO}_2\) shedding, i.e., any non-methanogenic production of \(\text{CO}_2\) (Eqn. 6).

We modeled diffusive transport of methane (\(^{DiffCH4}_i\)) and DIC (\(^{DiffCO2}_i\)) with finite differences using the average of the measured initial and final concentration profiles to drive transport (see Supplemental Information, Section 1). We did not include diffusive isotopic
fractionation in the model (i.e., different diffusion rates for $^{12}\text{C}$ versus $^{13}\text{C}$) because in water or water-filled soil, diffusion causes little fractionation (Jahne et al. 1987; Preuss et al. 2013).

The model captured the impact of the microbial reactions on the concentrations of $^{13}\text{CH}_4$ and $^{13}\text{CO}_2$ using isotope ratios ($r$ terms in Eqns. 9 and 10) and enrichment factors ($\epsilon$ terms in Eqns. 9 and 10). The $r$ terms included:

$r_{\text{ORG}}$ — $^{13}\text{C}:{^{12}\text{C}}$ ratio of substrate (i.e., peat) used in fermentation, acetoclastic methanogenesis and non-methanogenic $\text{CO}_2$ production. This ratio was set to a constant value between 0.0109113 to 0.0109450 (i.e., -29‰ and -26‰) (O’Leary 1988), and for a given simulation, this value did not vary with depth or location.

$r_{\text{CH}_4_i}$ — $^{13}\text{CH}_4:{^{12}\text{CH}_4}$ ratio measured in a given peeper cell. We used the average ratio for the time increment of interest: $\frac{1}{2} \left( \frac{^{13}\text{CH}_4_i^{\text{initial}}}{^{12}\text{CH}_4_i^{\text{initial}}} + \frac{^{13}\text{CH}_4_i^{\text{final}}}{^{12}\text{CH}_4_i^{\text{final}}} \right)$.

$r_{\text{CO}_2_i}^{\text{data}}$ — $^{13}\text{CO}_2:{^{12}\text{CO}_2}$ ratio measured in a given peeper cell. We used the average ratio for the time increment of interest: $\frac{1}{2} \left( \frac{^{13}\text{CO}_2_i^{\text{initial}}}{^{12}\text{CO}_2_i^{\text{initial}}} + \frac{^{13}\text{CO}_2_i^{\text{final}}}{^{12}\text{CO}_2_i^{\text{final}}} \right)$. This ratio was used with diffusive transport, but not with fractionating microbial reactions that use $\text{DIC}$ as a substrate.

$r_{\text{CO}_2_i}^{\text{frac}}$ — $^{13}\text{CO}_2:{^{12}\text{CO}_2}$ ratio used with fractionating microbial reactions that use $\text{DIC}$ as a substrate (i.e., hydrogenotrophic methanogenesis). The isotopic impact of hydrogenotrophic methanogenesis is convoluted. When the production of hydrogen is considered, the reaction contributes two equivalents of $\text{CO}_2$ to the $\text{DIC}$ pool that are generated via fermentation of peat substrate. We assumed that fermentation minimally fractionates carbon, and thus the isotopic
signature of these contributed CO$_2$ equivalents match that of the peat. The reaction then utilizes one isotopically depleted CO$_2$ equivalent from the DIC pool to generate methane, fractionating the DIC pool. We assumed that the methanogenic microbes have access to and fractionate the entire DIC pool rather than just the two equivalents of fermented CO$_2$ generated with the hydrogen that is used in the reaction. Difficulty arises in determining the isotopic composition of the DIC pool used by the methanogenic microbes. Conceptually, we have an initial $\delta^{13}$C-CO$_2$ measured in porewater that is incrementally altered by the addition of CO$_2$ from fermentation and the removal of isotopically depleted CO$_2$ for methanogenesis. However, the resolution of our data set and our modeling approach does not capture small incremental changes, and thus, we chose to handle this progression of events by assuming that the fermentation reactions add CO$_2$ to the DIC pool first before the methanogenic microbes remove CO$_2$ and fractionate the DIC pool. The isotopic composition of the DIC pool after the addition CO$_2$ from fermentation was represented by $r_{CO_2,fract}^{i} = \frac{^{13}CO_2^{initial}}{^{12}CO_2^{initial}} + 2 \cdot r_{ORG \cdot CH_4,Hydro}^{i}$, which was the isotopic ratio of the DIC pool made available to hydrogenotrophic methanogenesis in the model.

The enrichment factors included:

$\varepsilon_{CH_4,acet} = [\delta^{13}C_{acetate} - \delta^{13}C_{CH_4}] = [\delta^{13}C_{acetate} - \delta^{13}C_{CO_2}]$ — for acetoclastic methanogenesis

$\varepsilon_{CH_4,Hydro} = [\delta^{13}C_{CO_2} - \delta^{13}C_{CH_4}]$ — for hydrogenotrophic methanogenesis, and

$\varepsilon_{CH_4,Oxid} = [\delta^{13}C_{CH_4} - \delta^{13}C_{CO_2}]$ — for methane oxidation.
Values for enrichment factors were obtained from published literature and are listed in Table 1.

Table 2 summarizes the various components of this reaction network model, as well as the other two models described below, and includes a description of how the components were used in our rate estimation approach.

**Homoacetogenesis Models**

A potential weakness of the modeling framework presented above is the assumption that methanogenesis is the only microbial process responsible for the generation of $^{13}$C enriched DIC (Table 1), which is not always the case. Homoacetogenesis,

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$  \hspace{1cm} (11)

causes carbon isotopic fractionation of 38\% to 68\% between $\delta^{13}$CO$_2$ and $\delta^{13}$C-acetate (Blaser et al. 2013), depleting acetate and enriching the DIC pool. When the production of hydrogen from fermentation/syntrophy is considered (Eqn. 3), homoacetogenesis has no net impact on DIC concentrations because CO$_2$ is produced:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$  \hspace{1cm} (3)

and then consumed:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$  \hspace{1cm} (11)

resulting in a net reaction of:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \rightarrow 3CH_3COOH + 2H_2O$$  \hspace{1cm} (12)

However, it is possible that $^{13}$C enriched DIC is generated over time due to non-methanogenic CO$_2$ production (Eqn. 6) and homoacetogenesis operating together, with the former increasing DIC concentrations and the later fractionating the DIC pool (Table 1). In the net reaction (Eqn. 12), two of the produced acetates have a similar isotopic composition to the peat (i.e., the two
produced via fermentation/syntrophy) and one of the produced acetates is $^{13}$C depleted (i.e., the one produced via homoacetogenesis).

We developed two alternative reaction network models that included homoacetogenesis as a possible microbial reaction. One of these models (Model 2) coupled homoacetogenesis with acetoclastic methanogenesis; the acetate formed via homoacetogenesis directly fueled methane production. The other model (Model 3) allowed these two reactions to remain uncoupled. Model 2 simulated fast cycling of acetate between acetogens and methanogens (Jones and Simon 1985), while Model 3 simulated acetate as a terminal metabolism product and implicitly allowed acetate to accumulate over time (Hines et al. 2008). As we discuss in the Results section, with our porewater data set, Model 2 indicated greater rates of methanogenesis than could be accounted for by dissolved methane concentrations, and thus we refer to this model as the “Acetogenesis Fugitive Methane Model.” Model 3 implied concentrations of acetate that exceed those measured at our site, and thus we refer to this model as the “Acetogenesis Fugitive Acetate Model.”

**Conceptual Reaction Network 2: Acetogenesis Fugitive Methane Model**

In the Acetogenesis Fugitive Methane Model, the direct coupling between homoacetogenesis and methanogenesis means that homoacetogenesis ultimately produces both methane and CO$_2$ in equal proportions:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \rightarrow CH_4 + CO_2 + 2H_2O$$

(13)

Isotopically, the generated acetate is $^{13}$C depleted by 38‰ to 68‰ relative to the initial DIC pool, the produced methane is depleted by 17‰ to 35‰ relative to the $^{13}$C-depleted acetate, and the produced CO$_2$ is enriched by 17‰ to 35‰ relative to the $^{13}$C-depleted acetate. The finite difference equations below describe this direct coupling between homoacetogenesis and
methanogenesis. Equations 14 and 15 track the temporal change of dissolved methane ($^{12}\text{CH}_4$) and carbon dioxide ($^{12}\text{CO}_2$) concentrations, respectively. Equations 16 and 17 track the temporal change of $^{13}\text{CH}_4$ and $^{13}\text{CO}_2$ concentrations, respectively. Equation 18 defines the difference between $^{12}\text{CO}_2$ and $^{12}\text{CH}_4$ concentrations. This additional equation is required to solve for the additional unknown rate added to the model (i.e., five unknown rates require five equations). Modeling the temporal change in this concentration difference accounts for the fact that methanogenesis produces CH$_4$ and CO$_2$ in equal proportions (Corbett et al. 2013).

\[
\frac{^{12}\text{CH}_4^{\text{final}} - ^{12}\text{CH}_4^{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = \text{Diff} CH_4 + CH_4\text{Acet}_i + CH_4\text{Hydro}_i + \text{AcetGen}_i - CH_4\text{Oxid}_i \quad (14)
\]

\[
\frac{^{12}\text{CO}_2^{\text{final}} - ^{12}\text{CO}_2^{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = \text{Diff} CO_2 + CH_4\text{Acet}_i + CH_4\text{Hydro}_i + \text{AcetGen}_i + CH_4\text{Oxid}_i + \text{Resp}_i \quad (15)
\]

\[
\frac{^{13}\text{CH}_4^{\text{final}} - ^{13}\text{CH}_4^{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = rCH_4_i \cdot \text{Diff} CH_4 + r\text{ORG} \left(1 - \frac{\epsilon_{\text{CH}_4\text{Acet}}}{1000}\right) CH_4\text{Acet}_i + rCO_2^{\text{frac2}} \left(1 - \frac{\epsilon_{\text{CH}_4\text{Hydro}}}{1000}\right) CH_4\text{Hydro}_i + rCO_2^{\text{frac2}} \left(1 - \frac{\epsilon_{\text{AcetGen}}}{1000}\right) \left(1 - \frac{\epsilon_{\text{CH}_4\text{Acet}}}{1000}\right) \text{AcetGen}_i - rCH_4_i \left(1 - \frac{\epsilon_{\text{CH}_4\text{Oxid}}}{1000}\right) CH_4\text{Oxid}_i \quad (16)
\]

\[
\frac{^{13}\text{CO}_2^{\text{final}} - ^{13}\text{CO}_2^{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = rCO_2^{\text{frac2}} \cdot \text{Diff} CO_2 + r\text{ORG} \left(1 + \frac{\epsilon_{\text{CH}_4\text{Acet}}}{1000}\right) CH_4\text{Acet}_i + \left(2 \cdot r\text{ORG} - rCO_2^{\text{frac2}} \left(1 - \frac{\epsilon_{\text{CH}_4\text{Hydro}}}{1000}\right) \right) CH_4\text{Hydro}_i + \left(2 \cdot r\text{ORG} - rCO_2^{\text{frac2}} \left(1 - \frac{\epsilon_{\text{AcetGen}}}{1000}\right) \left(1 - \frac{\epsilon_{\text{CH}_4\text{Acet}}}{1000}\right) \right) \text{AcetGen}_i + rCH_4_i \left(1 - \frac{\epsilon_{\text{CH}_4\text{Oxid}}}{1000}\right) CH_4\text{Oxid}_i + r\text{ORG} \cdot \text{Resp}_i \quad (17)
\]
Variable definitions from Eqns. 7–10 apply to Eqns. 14–18. Undefined variables in the Acetogenesis Fugitive Methane Model include:

- \( \text{AcetGen}_i \) — rate of homoacetogenesis (dimensions of mass per volume per time).

- \( \varepsilon_{\text{AcetGen}} = [\delta^{13}C_{\text{CO}_2} - \delta^{13}C_{\text{acetate}}] \) — isotopic enrichment factor for homoacetogenesis. See Table 1 for values of this parameter.

- \( r\text{CO}_2^{\text{frac2}} \) — \(^{13}\text{CO}_2:^{12}\text{CO}_2\) ratio used with fractionating microbial reactions that use DIC as a substrate (i.e., hydrogenotrophic methanogenesis and homoacetogenesis). Similar to that discussed above for the Original Fugitive Methane model, we allowed all the fermentation reactions to add \( \text{CO}_2 \) to the DIC pool before the reactions that use DIC as the substrate accessed and fractionated the pool. For the Acetogenesis Fugitive Methane Model, the isotopic ratio of the DIC pool made available for hydrogenotrophic methanogenesis and homoacetogenesis was:

\[
r\text{CO}_2^{\text{frac2}} = \frac{^{13}\text{CO}_2^{\text{initial}} + 2 \cdot r\text{ORG} \left( \text{CH}_4\text{Hydro}_i + \text{AcetGen}_i \right)}{^{12}\text{CO}_2^{\text{initial}} + 2 \left( \text{CH}_4\text{Hydro}_i + \text{AcetGen}_i \right)}
\]

\[(19)\]

Conceptual Reaction Network 3: Acetogenesis Fugitive Acetate Model

In our final model, acetate produced via homoacetogenesis does not necessarily fuel methanogenesis. Instead, we allow a variable portion of the acetogenic acetate to generate methane. While we do not explicitly track acetate production, the model framework results in a
build up of acetate over time. The finite difference equations below describe this model. As was the case for the Original Fugitive Methane Model, Equations 7 and 8 track the temporal change of dissolved methane ($^{12}$CH$_4$) and carbon dioxide ($^{13}$CO$_2$) concentrations, respectively. Equations 20 and 21 track the temporal change of $^{13}$CH$_4$ and $^{13}$CO$_2$, respectively. Equation 18 defines the difference between $^{12}$CO$_2$ and $^{12}$CH$_4$ concentrations, as it did in the Acetogenesis Fugitive Methane Model.

\[
\frac{^{12}CH_4_{\text{final}} - ^{12}CH_4_{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = \text{DiffCH}_4 + CH_4Acet_i + CH_4Hydro_i - CH_4Oxid_i \tag{7}
\]

\[
\frac{^{12}CO_2_{\text{final}} - ^{12}CO_2_{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = \text{DiffCO}_2 + CH_4Acet_i + CH_4Hydro_i + CH_4Oxid_i + Resp_i \tag{8}
\]

\[
\frac{^{13}CH_4_{\text{final}} - ^{13}CH_4_{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = rCH_4 \cdot \text{DiffCH}_4 + rACET \left(1 - \frac{E_{CH_4Acet}}{1000}\right) CH_4Acet_i \\
+ rCO_2^{frac2} \left(1 - \frac{E_{CH_4Hydro}}{1000}\right) CH_4Hydro_i \\
- rCH_4 \left(1 - \frac{E_{CH_4Oxid}}{1000}\right) CH_4Oxid_i \tag{20}
\]

\[
\frac{^{13}CO_2_{\text{final}} - ^{13}CO_2_{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = rCO_2^{data} \cdot \text{DiffCO}_2 + rACET \left(1 + \frac{E_{CH_4Acet}}{1000}\right) CH_4Acet_i \\
+ \left(2 \cdot rORG - rCO_2^{frac2} \left(1 - \frac{E_{CH_4Hydro}}{1000}\right)\right) CH_4Hydro_i \\
+ \left(2 \cdot rORG - 2 \cdot rCO_2^{frac2} \left(1 - \frac{E_{AcetGen}}{1000}\right)\right) AcetGen_i \\
+ rCH_4 \left(1 - \frac{E_{CH_4Oxid}}{1000}\right) CH_4Oxid_i + rORG \cdot Resp_i \tag{21}
\]

\[
\frac{^{12}CO_2_{\text{final}} - ^{12}CH_4_{\text{final}}}{t_{\text{final}} - t_{\text{initial}}} - \frac{^{12}CO_2_{\text{initial}} - ^{12}CH_4_{\text{initial}}}{t_{\text{final}} - t_{\text{initial}}} = \left(\text{DiffCO}_2 - \text{DiffCH}_4\right) + 2 \cdot CH_4Oxid_i + Resp_i \tag{18}
\]
Variable definitions from Eqns. 7–10 (Original Fugitive Methane Model) and Eqns. 14–18 (Acetogenesis Fugitive Methane Model) apply to the Eqns. 20 and 21. Variable unique to the Acetogenesis Fugitive Acetate Model include:

\[ r_{ACET} = \frac{\text{acetate used by acetoclastic methanogens}}{\text{acetate generated during fermentation/syntrophy and acetate generated by homoacetogenesis}} \]

The isotopic composition of the former is similar to that of the peat while the isotopic composition of the later is \(^{13}\text{C}\) depleted relative to the initial DIC pool. The proportional usage of the two types of acetate is not linked to modeled rates. Instead, we used a factor \(f_{AcetGen}\) to represent the fractional usage of acetogenic acetate used by methanogens. Thus, the isotopic ratio of acetate used by acetoclastic methanogens in this model is:

\[ r_{ACET} = r_{ORG} (1 - f_{AcetGen}) + r_{CO_{2}}^{frac2} \left(1 - \frac{\epsilon_{AcetGen}}{1000}\right) f_{AcetGen} \]

Incubation experiments conducted by Ye et al. (2014) demonstrated that acetate generated by homoacetogenesis can make up 0 to 60% of the acetate used by acetoclastic methanogens. We allowed \(f_{AcetGen}\) to vary across this percentage range (Table 2).

Data Fitting Approach

We applied the three conceptual models described above to determine the microbial rates responsible for transforming the concentration and isotope profiles measured in June to the ones measured in July, and transforming the profiles measured in July to the ones measured in August. We used the measured profiles from the start of the time period of interest as our initial condition and applied the reaction networks, with unknown rates, to generate modeled
concentration and isotope profiles for the end of the time period. The modeled profiles were fit to the measured profiles from the end of the time period using a non-linear least squares routine (lsqnonlin in MATLAB) that solved for the unknown rates. Each depth was treated independently (i.e., we applied the fitting routine to individual depths below the peat surface). The objective functions that least squares routine simultaneously minimized relied on differences between measured and modeled values:

\[
\left(\frac{^{12}\text{CH}_4^{\text{final}} - ^{12}\text{CH}_4^{\text{final, meas}}}{N}\right) \quad (22)
\]

\[
\left(\frac{^{12}\text{CO}_2^{\text{final}} - ^{12}\text{CO}_2^{\text{final, meas}}}{10}\right) \quad (23)
\]

\[
\left(\frac{\delta^{13}\text{CH}_4^{\text{final}} - \delta^{13}\text{CH}_4^{\text{final, meas}}}{0.1}\right) \quad (24)
\]

\[
\left(\frac{\delta^{13}\text{CO}_2^{\text{final}} - \delta^{13}\text{CO}_2^{\text{final, meas}}}{0.1}\right) \quad (25)
\]

\[
\left[\left(\frac{^{12}\text{CO}_2^{\text{final}} - ^{12}\text{CH}_4^{\text{final}}}{N}\right) - \left(\frac{^{12}\text{CO}_2^{\text{final, meas}} - ^{12}\text{CH}_4^{\text{final, meas}}}{N}\right)\right] \quad (26)
\]

Model 1 (Original Fugitive Methane Model) minimized Eqns. 22–25, while Models 2 and 3 (the two acetogenesis models) minimized Eqns. 22–26. These equations include weighting factors (i.e., the denominator) that, in most instances, ensured that the isotopic differences (Eqns. 24 and 25) carried a similar weight in the objective function as the concentration differences (Eqns. 22, 23 and 26). In our modeled data set, concentrations values ranged between ~100 to ~10,000 µmol/L while isotopic values ranged between 0 and -70‰. However, Eqns. (23) and (26) are presented with a weighting factor of N because in some simulations this factor was set to 10 while in others it was set to 100 (Table 3). As discussed in the Results section, for Model 1 and Model 2, we could not achieve a good fit unless we decreased the importance of the methane
concentration data in the objective function (i.e., set $N = 100$), which allowed the model to generate excess methane beyond that measured in porewater (i.e., fugitive methane).

The *lsqnonlin* routine in MATLAB requires lower and upper bounds and an initial guess for the unknown values. We set the lower bound for all the rates at 0 $\mu$mol L$^{-1}$ month$^{-1}$ and the upper bound at 20,000 $\mu$mol L$^{-1}$ month$^{-1}$, and initialized all of the unknown reaction rates at 0 $\mu$mol L$^{-1}$ month$^{-1}$. After a solution was reached, the *lsqnonlin* routine reported residuals ($res$) of the objective function. We used these residuals to calculate root mean square error for the entire peeper profile as:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{i} \sum_{j=1}^{j} res_{i,j}}{i \cdot j}},$$

where $i$ is number of peeper depths in given profile and $j$ is number of objective functions (Eqns. 22–26) solved for each peeper depth.

**Parameter Uncertainty**

All three applied reaction networks include parameters with values obtained from published literature: enrichment factors ($\varepsilon_C$, Table 1), isotopic ratio of peat ($r_{ORG}$, Table 2), and, in Model 3, the fraction of acetate used by methanogens that was generated by homoacetogenesis ($f_{AcetGen}$, Table 2). We employed a Monte Carlo approach to deal with uncertainty regarding values for these parameters. For each model, we ran 500 simulations. In each simulation, we used a random number generator to randomly choose a value for each of the needed parameters. The considered parameter ranges were constrained to that reported in the literature (see Table 1 and 2 for considered ranges). The result was 500 simulations, each with a different combination
of parameter values. We used the root-mean square error (RMSE) of the modeled fits to the data to identify simulations that performed well. For each simulation, we calculated a joint RMSE value that was the sum of the individual RMSE values (Eqn. 27) for each of our four datasets (i.e., both locations, center and edge, during both time periods, June to July and July to August). We kept the simulations with joint root-mean square errors that were within 5% of the joint root-mean square error of the best performing simulation. Tables 1 and 2 list the range of parameter values used in these top simulations for each of our models.

**Methane Oxidation**

In addition to parameter variation, we found that our modeled rates were sensitive to the depth to which we allowed methane oxidation to occur. Because isotopic fractionation from methane oxidation is smaller than that from the other modeled reactions (Table 1), it is possible for the least-squares fitting routine to use the reaction as a way to reduce methane concentrations and generate CO$_2$, while minimally impacting the isotopic composition of these carbon pools. Thus, the fitting routine sometimes predicted high rates of methane oxidation at deep depths (>60 cm, below the rooting zone of plants) where we know oxygen was not present (Figure S1). Given this outcome, we conducted a series of simulations where we sequentially increased the depth to which we allowed methane oxidation to occur. We found that in general, methane oxidation only improved the RMSE for fits at depths where methane grew $^{13}$C enriched and DIC grew $^{13}$C depleted over time (See Results: Depth of Methane Oxidation). We restricted methane oxidation to these identified depths for all following model runs.

**RESULTS**
In order to fit our porewater data, Model 1 (Original Fugitive Methane Model) and Model 2 (Acetogenesis Fugitive Methane Model) had to generate methane at rates that resulted in dissolved concentrations exceeding those measured in porewater at our site. Figure 1 shows measured and modeled porewater profiles for data from the July to August time period in the center of the bog. The grey profiles indicate the top modeled fits to the entire dataset (i.e., $^{12}\text{C-CH}_4$, $^{13}\text{C-CH}_4$, $^{12}\text{C-CO}_2$ and $^{13}\text{C-CO}_2$) without any constraint on the depth of methane oxidation (see Depth of Methane Oxidation). The grey profiles nicely match the $^{12}\text{C-CO}_2$ data and adequately match the $^{12}\text{C-CH}_4$ and $^{13}\text{C-CH}_4$ data. However, they do a poor job of matching the $^{13}\text{C-CO}_2$ data, particularly at depths where $^{13}\text{C-CO}_2$ grew enriched over the modeled period. This result reflects the fact that in both models, the generation of $^{13}\text{C}$-enriched CO$_2$ required methanogenesis. In Model 1, methanogenesis was the only reaction that could generate $^{13}\text{C}$-enriched CO$_2$ (Table 1). In Model 2, homoacetogenesis could $^{13}\text{C}$-enrich CO$_2$ (Table 1), but in this model, homoacetogenesis ultimately produced methane because the two reactions were coupled. With the data equally weighted (i.e., $N=10$ in Eqn. 22), both models limited methanogenesis to balance the $^{13}\text{C-CO}_2$ isotopic constraint and the $^{12}\text{C-CH}_4$ concentration constraint.

Our models explicitly considered vertical diffusive transport of methane, but did not include other non-reactive loss mechanisms (e.g., ebullition and plant mediated transport). Because methane is lost from peatlands via non-diffusive pathways, we reduced the importance of the $^{12}\text{C-CH}_4$ data in the fitting routine (i.e., set $N=100$ in Eqn. 22), which enabled the model to over generate methane in order to fit the other data components. The black profiles in Figure 1 indicate top fits for Model 1 and Model 2 with this modified weighting scheme. The profiles
nicely match the $\delta^{13}$C-$\text{CH}_4$, $^{12}$C-\text{CO}_2 and $\delta^{13}$C-\text{CO}_2 data and, as expected, over predict $^{12}$C-$\text{CH}_4$ concentrations. The difference between modeled and measured $^{12}$C-$\text{CH}_4$ concentrations represents fugitive methane (i.e., methane generated by the models but not measured in porewater). For Model 1 and Model 2, the generation of fugitive methane improved modeled fits for all four data sets (i.e., both locations during both time periods), not just the data set presented in Figure 1. Table 3 indicates a reduction in the RMSE of fits to the $\delta^{13}$C-$\text{CH}_4$, $^{12}$C-\text{CO}_2 and $\delta^{13}$C-\text{CO}_2 profiles for all four datasets when the weight of $^{12}$C-$\text{CH}_4$ in the fitting routine was reduced (i.e., $N$ in Eqn. 22 was increased from 10 to 100). Constraining the depth of methane oxidation (see Depth of Methane Oxidation) did not alter the fit improvement that accompanied the generation of fugitive methane (Figure 1 and Table 3), emphasizing that fugitive methane represents methane that has physically escaped porewater rather than methane that was oxidized within porewater. The former (physically escaped methane) does not alter the $^{13}$C signature of methane or $\text{CO}_2$ in porewater while the later (oxidized methane) does. Note that our models provide no information about processes occurring outside of sampled porewater, and therefore, fugitive methane could get oxidized after it has escaped porewater (e.g., within the roots of plants or within unsaturated peat).

Model 3 (Acetogenesis Fugitive Acetate Model) did not generate fugitive methane. As indicated in Figure 1, it was able to fit the entire data set with all components equally weighted (i.e., $N=10$ in Eqns. 22 and 26). This result reflects that fact that Model 3 can generate $^{13}$C-enriched $\text{CO}_2$ without simultaneously generating methane. In this model, homoacetogenesis is decoupled from methanogenesis. Homoacetogenesis can $^{13}$C enrich $\text{CO}_2$ and non-methanogenic $\text{CO}_2$ production (Eqn. 6) can increase $\text{CO}_2$ concentrations without impacting methane concentrations. However, the de-coupling between homoacetogenesis and methanogenesis in this
model along with the modeled rates of homoacetogenesis (see Modeled Rates and Ratios — Comparison Between Conceptual Models for a more in depth discussion of modeled rates) imply an accumulation of acetate to concentrations that exceed those measured in porewater at our site (compare Table 6 and Figure S2). Thus, Model 3 required the generation of fugitive acetate rather than fugitive methane to fit our datasets.

Peeper Data and Modeled Fits to Data

With the generation of fugitive methane in Models 1 and Model 2 and fugitive acetate in Model 3, all three reaction networks could describe the temporal change in concentration and isotope data for both locations (center and edge) and time periods (June to July and July to August). The modeled profiles presented in Figure 2 are the top fits to measured data given constraints on the depth of methane oxidation (see Depth of Methane Oxidation). We defined the top fits as having a joint RMSE value within 5% of the joint RMSE value of the best performing simulation. In many instances, top fits from all three models were visually indistinguishable from each other (compare grey, black and white filled circles, Figure 2), and indistinguishable from the final measured profiles (compare grey, black and white filled circles to red circles, Figure 2).

Statistically speaking, all three models performed equally well; RMSE values for top fits to the δ^{13}C-CH_{4}, ¹²C-CO_{2} and δ^{13}C-CO_{2} profiles varied more across the four data sets than it did between the three conceptual reaction network models (Table 3). We focus on fits to these three data profiles and not the ¹²C-CH_{4} profile because generation of fugitive methane necessitated a change to the least-squares objective function for equations involving ¹²C-CH_{4} (i.e., increasing N from 10 to 100 in Eqns. 22 and 26). We cannot directly compare RMSE values that include residuals resulting from different fitting equations.
While fits to the $\delta^{13}$C-CH$_4$, $^{12}$C-CO$_2$ and $\delta^{13}$C-CO$_2$ profiles from the top simulations were equally good among the three models, the number of top simulations varied. Out of the 500 simulations conducted for each model, Model 1 had 70 simulations, Model 2 had 97 simulations and Model 3 had only 6 simulations that qualified as a top simulation (Table 3). This result indicates Model 3 was more sensitive to parameter values than Model 1 and 2; each of the 500 simulations run for each model had a different, randomly chosen combination of parameter values. Figure S3A visually depicts this difference in parameter sensitivity. It plots joint RMSE values, from smallest to largest, for all 500 simulations run for each model. The joint RMSE values for Model 1 and Model 2 gradually increased across 300 to 400 simulations, and then rapidly increased for remaining simulations. In contrast, the values for Model 1 immediately began increasing at a rapid rate.

Interestingly, Model 3 was not sensitive to an individual parameter value. It was instead sensitive to the combination of parameter values. Tables 1 and 2 show both the considered ranges of all parameter values (i.e., $\varepsilon_{CH4Acet}$, $\varepsilon_{CH4Hydro}$, $\varepsilon_{CH4Oxid}$, $\varepsilon_{AcetGen}$, $r_{ORG}$, $f_{AcetGen}$) and ranges that resulted in top fits to the data. All three models produced good fits to the data across much of the considered parameter ranges; though the ranges that resulted in top simulations were wider for Model 1 and 2 than for Model 3. Similarly, Figure S3B indicates that no individual parameter value dominated the performance of Model 3. This figure plots joint RMSE values against each parameter value for all three models, and no clear optimum exists for Model 3 in any of the parameter plots.

While Model 1 and 2 were relatively insensitive to parameter combinations, Figure S3B does demonstrate a clear improvement in the performance of both models as $\delta^{13}$C of the peat increased from -29‰ to -26‰, and a clear diminishment in the performance of both models once
the enrichment factor for acetoclastic methanogenesis increased beyond ~30‰. Performance of Model 1 also diminished when the enrichment factor for hydrogenotrophic methanogenesis decreased below ~60‰.

Overall, the consistency of RMSE values across top simulations from all three models, and the fact that these top fits did not depend on a single parameter combination, demonstrate that all three models plausibly explained our datasets.

**Depth of Methane Oxidation**

When we did not constrain the depth to which methane oxidation could occur, all three conceptual models converged on solutions that involved methane oxidation throughout much of the depth profile, and for some data sets, even estimated that rates of methane oxidation increased with depth. The yellow plotted profiles in Figure 3 demonstrate this result for Model 3 (Acetogenesis Fugitive Acetate Model) fit to data from the edge of the bog during the June to July time period. A similar result is presented in the Supplemental Information (Figure S4) for Model 1 and Model 2 fit to the same data set. This modeled outcome is unrealistic if oxygen is the electron acceptor for methane oxidation. Oxygen is transported into wetland soils either via diffusion at the land surface, resulting in shallow penetration of oxygen, or via hollow aerenchyma tissue in wetland plants, resulting in penetration of oxygen down through the rooting zone. At our site, oxic methane oxidation is likely constrained to the top 50–60 cm where a majority of the root biomass is located and where we have measurable concentrations of dissolved oxygen (Figure S1). Anaerobic methane oxidation that relies on other electron acceptors (e.g., nitrate, sulfate, ferric iron) can occur throughout the depth profile, but incubations of peat material collected from our site found that rates of anaerobic methane
oxidation were ~0.3% of methane production rates (Blazewicz et al. 2012). Our modeled oxidation rates at depth were approaching 80% of modeled methane production rates (yellow profiles, Figure 3), leading us to conclude that, in the deeper depths, our modeled rates of methane oxidation were incorrect. Given these lines of evidence (rooting depth, dissolved oxygen concentrations and slow rates of anaerobic methane oxidation), we chose to constrain the depth to which methane oxidation was allowed to occur in our models.

To determine the appropriate depth-constraint for methane oxidation, we initially excluded methane oxidation from the entire peeper profile and then sequentially increased the depth to which we allowed methane oxidation to occur, one peeper cell depth at a time for all three models. The RMSE of all of the modeled fits either initially decreased and then plateaued as methane oxidation was allowed to occur deeper in the profile or showed no response to the inclusion of methane oxidation (Figure S5). The exercise demonstrated that we could exclude methane oxidation at deeper depths without noticeably impairing the quality of the fit.

Comparison of results from this effort (Figure S5) to measured profiles of $^{12}$C-$\text{CH}_4$, $\delta^{13}$C-$\text{CH}_4$ and $\delta^{13}$C-$\text{CO}_2$ over time in the peepers (Figure 2) showed that for Model 1 and Model 2, the inclusion of methane oxidation at a given depth only improved modeled fits if $^{12}$C-$\text{CH}_4$ concentration decreased, $\delta^{13}$C-$\text{CH}_4$ became $^{13}$C enriched and $\delta^{13}$C-$\text{CO}_2$ became $^{13}$C depleted. This combination of concentration and isotopic changes occurred only once in our data set, in the shallowest peeper cell in the center of the bog for the June to July time period (Figure 2A). The fit improvement reflects the fact that methane oxidation is the only considered reaction that can simultaneously decrease methane concentrations, $^{13}$C-enrich methane and $^{13}$C-deplete CO$_2$ (Table 1). Model 3 was more sensitive to methane oxidation than Model 1 and 2. For Model 3, the inclusion of methane oxidation at a given depth improved modeled fits for depths where
\[ ^{13}\text{C-CH}_4 \text{ became } ^{13}\text{C enriched and } ^{13}\text{C-CO}_2 \text{ became } ^{13}\text{C depleted over time, regardless of the change in methane concentration (Figure S5 and Figure 2). During the June to July time period, these isotopic changes occurred in the shallowest peeper cell in the center of the bog and in the two shallowest peeper cells at the edge of the bog. During the July to August time period, these isotopic changes occurred only in the shallowest peeper cell at the edge of the bog. We used results from the more sensitive model (Model 3) to set our depth-constraint for methane oxidation. Thus, for all three models, we restricted methane oxidation to depths where \(^{13}\text{C-CH}_4\) became \(^{13}\text{C enriched with time and } ^{13}\text{C-CO}_2 \text{ became } ^{13}\text{C depleted with time, regardless of the change in } ^{12}\text{CH}_4 \text{ concentration. These depths are marked with red circles in Figure S5.}

The restriction of methane oxidation to shallow depths impacted all of our modeled rates, not just the rate of methane oxidation. Results from Model 3 (Acetogenesis Fugitive Acetate Model) were particularly sensitive to the allowed depth of methane oxidation; the model estimated large rates of methane oxidation at deeper depths when not restricted (e.g., \(~1.5 \text{ mmol/L/month in the deepest peeper, see yellow profile in Figure 3)\). Figure 3 demonstrates that for this conceptual framework, rates of methane production and rates of non-methanogenic CO\(_2\) production all noticeably changed as the allowed depth of methane oxidation increased (compare progression of profiles, blue to yellow, Figure 3). Rates from Model 1 and 2 (the fugitive methane models) only slightly changed as the depth of methane oxidation increased due to smaller rates of methane oxidation estimated by the model (e.g., \(~0.4–0.6 \text{ mmol/L/month in the deepest peeper, see yellow profile in Figure S4).\)
Although top fits from all three conceptual models generated equally good fits to the measured data (i.e., similar RMSE values, Table 3), rates resulting from the models were different from each other. Modeled rates are plotted in Figure 4 for the peeper profiles, and depth-integrated rates are presented in Tables 4 – 6. The most noticeable difference existed in the gross rate of methane production between Model 3 and the two fugitive methane models (Model 1 and 2). The depth integrated gross methane production rates were 3 to 5 times greater in the two fugitive methane models (Model 1 and 2) than in the fugitive acetate model (Model 3) (Tables 4 – 6). This outcome directly reflects differences in the conceptual reaction networks. As discussed previously, in the fugitive methane models (Model 1 and 2), generation of $^{13}$C-enriched CO$_2$, only occurred in tandem with methane production, and in much of our measured dataset, CO$_2$ became $^{13}$C enriched with time (Figure 2). Thus, with these two modeling frameworks, methane generation was needed to account for CO$_2$ enrichment rather than growth in methane concentration. Methane produced in excess of that explained by measured changes in methane concentration was designated as fugitive methane. For our data set, ~80% of the generated methane in both Model 1 and 2 was fugitive (Tables 4 and 5). In contrast, in Model 3, the generation of $^{13}$C enriched CO$_2$ was uncoupled from methanogenesis. As a consequence, with this third modeling framework, methane generation responded primarily to changes in dissolved methane concentrations while homoacetogenesis and non-methanogenic CO$_2$ production (Eqn. 6) accounted for changes in concentrations of $^{12}$C-CO$_2$ and $^{13}$C-CO$_2$. The depth-integrated rates of non-methanogenic CO$_2$ production were 1.6 to 6.5 times greater in Model 3 than in the fugitive methane models (Model 1 and 2) (Tables 4 – 6). Given these tradeoffs, it is not surprising that the gross CO$_2$:CH$_4$ ratio was greater for Model 3 than for the two fugitive methane models (Model 1 and 2). The former model predicted ratios that ranged
between 5.2 and 8, while the latter two models predicted ratios that ranged between 1.2 and 2.2 (Tables 4 – 6).

Interestingly, while the gross methane production rates were similar between the two fugitive methane models (Model 1 and 2), the pathway by which methane was produced varied. In Model 1 (Original Fugitive Methane Model), rates of acetoclastic methanogenesis and hydrogenotrophic methanogenesis were roughly equal (the ratio of acetoclastic to hydrogenotrophic methanogenesis varied between 0.7 and 1.3, Table 4). In Model 2 (Acetogenesis Fugitive Methane Model), rates of acetoclastic methanogenesis were almost three times faster than rates of hydrogenotrophic methanogenesis (the ratio of acetoclastic to hydrogenotrophic methanogenesis varied between 2.5 and 2.9, Table 5). Conceptually, a shift toward the acetoclastic pathway and away from the hydrogenotrophic pathway is expected when homoacetogenesis occurs because homoacetogenesis and hydrogenotrophic methanogenesis reactions compete with each other for substrate (H₂ and CO₂). In agreement with this conceptualization, rates of acetoclastic methanogenesis were generally faster than rates of hydrogenotrophic methanogenesis in the other model that included homoacetogenesis, Model 3 (the ratio of acetoclastic to hydrogenotrophic methanogenesis varied between 0.8 and 2.7, Table 6). However, dominance of the acetoclastic pathway was greatest in Model 2 due to the direct coupling of homoacetogenesis with methanogenesis; acetate generated by homoacetogenesis directly fueled acetoclastic methanogens.

Depth-integrated rates of methane oxidation predicted by the two fugitive methane models (Model 1 and 2) were the same for all four data sets (within error estimates). The rates predicted by Model 3 matched those from the two fugitive methane models for data collected from the center of the bog, but differed by a factor of 1.4 to 3.1 for data collected from the edge.
of the bog. At the edge, Model 3 predicted greater rates of methane oxidation than the two
fugitive methane models. Agreement by all the models in the center of the bog was due, in part,
to the fact that at this location, for the July to August time period, methane oxidation was not
allowed to occur (see Depth of Methane Oxidation and Figure S5). Methane oxidation accounted
for 0 to 2% of methane produced in the two fugitive methane models (Model 1 and 2) and 0 to
28% of methane produced in the fugitive acetate model (Model 3). The smaller percentage of
oxidation with the two fugitive methane models reflects the models’ greater rates of methane
production (Tables 4 – 6).

Acetate production was not explicitly simulated by any of the models. However, we
calculated an implied minimum rate of acetate production based on modeled rates of
methanogenesis and homoacetogenesis. Both methanogenic pathways require fermentation,
which produces acetate (Eqn. 3). Surprisingly, all three models, regardless of the inclusion of
homoacetogenesis, implied the same gross rates of acetate production (within error estimates)
(Tables 4 – 6). However, the implied rate of net acetate production was different between the
fugitive acetate model (Model 3) and the two fugitive methane models (Model 1 and 2). The
fugitive methane models implied no net production of acetate, while the fugitive acetate model
implied net acetate production rates that ranged between 16 to 28 mmol/L/month. These rates,
when spatially averaged across the modeled depth ranges, implied a concentration build up of 0.4
to 0.9 mmol/L/month of acetate, which exceeded the nano to micro-molar concentrations of
acetate measured at our site (Figure S2). It was this implied build up of acetate that gave Model 3
the designation of a fugitive acetate model.

Patterns in Modeled Rates
Despite differences in the magnitude of modeled rates, results from the two conceptual models told a similar story with respect to relative changes in rates with time (June to July versus July to August), depth below bog surface, and location in the bog (center versus edge). Figure 4 indicates that for both time periods (June to July and July to August) and both locations (center and edge), modeled rates of methane production, non-methanogenic CO$_2$ production, and acetogenesis increased with depth below the peat surface. (Methane oxidation decreased with depth below the peat surface). For a given time period, all rates (including methane oxidation) were greater at the edge of the bog than in the center (compare squares to circles in Figure 4). In addition, rates (excluding methane oxidation) tended to increase more at the edge of the bog than in the center of the bog as the summer progressed (compare empty symbols to filled symbols in Figure 4). All three models indicated that for both time periods, the ratio of acetoclastic to hydrogenotrophic methanogenesis was greater at the edge of the bog than in the center of the bog, even though the actual ratio of these utilization pathways varied between the models (Table 4–6).

**DISCUSSION**

**Reaction Network Models**

The conceptual reaction network used to explain the temporal changes in dissolved gas and stable isotope data had a large impact on the modeled rates. We found that the inclusion of a single reaction, homoacetogenesis, altered the modeled production pathway for methane when the reaction was directly coupled with methanogenesis (Model 1 versus Model 2), and reduced modeled rates of methane production and increased modeled rates of non-methanogenic CO$_2$.
production when the reaction was uncoupled from methanogenesis (Model 1 versus Model 3). All three conceptual networks provided equally good fits to the measured data, and thus, the achievement of a good fit by any single reaction network does not necessarily mean that the represented processes and modeled rates correctly reflect in-situ conditions. The ability of all three conceptual reaction networks to successfully match the measured data indicate that this technique for estimating in-situ reaction rates requires other data and information from the site to confirm the considered set of microbial reactions. In particular, the concentration and composition of acetate in porewater could help confirm the occurrence of homoacetogenesis.

While we are lacking direct evidence that homoacetogenesis occurred at our site, genes and transcripts involved in homoacetogenesis (tetrahydrofolate metabolism) have been observed in the metagenome and metatranscriptome of microorganisms present within this bog (Hultman et al. 2015), and this process can play an important role in carbon cycling within Sphagnum dominated peatlands (Ye et al. 2014). Thus, we believe the inclusion of homoacetogenesis in Model 2 and Model 3 likely improved the representation of reactions occurring at the site. However, it is unclear which of these two model versions did a better job of simulating site conditions. In Model 2, homoacetogenesis was directly coupled with methanogenesis, while in Model 3 the reaction was uncoupled from methanogenesis. Results from both the coupled and uncoupled versions were consistent with some and inconsistent with other pieces of data from our field site.

For the coupled version (Model 2), Figure 4 shows that maximum modeled methane production rates reached 2 to 5 mmol L\(^{-1}\) month\(^{-1}\), which aligned with the potential production rate of \(-1.9\) mmol L\(^{-1}\) month\(^{-1}\) measured in an incubation experiment conducted with peat collected at a 45- to 60-cm depth within the center of the bog (Hultman et al. 2015; conversion
assumed bulk density of 0.07 g cm$^{-3}$ (Chambers et al. 2011) and porosity of 1). While alignment with rates from the incubation experiment is promising for this model, it is puzzling that the modeled methane production rates far exceeded measured emission rates at the site. The depth-integrated net methane production rates for Model 2 were between 48 and 81 mmol m$^{-2}$ day$^{-1}$ (Table 5), while measured emission rates at the site were between 0.5 and 5 mmol m$^{-2}$ day$^{-1}$ (Euskirchen et al. 2014). Further, ~80% of the model-produced methane (39 to 66 mmol m$^{-2}$ day$^{-1}$) was designated as fugitive, which means that it non-diffusively escaped the porewater either into gas bubbles or through the roots of plants. Bubble traps installed belowground at the site captured an equivalent of ~0.5 mmol m$^{-2}$ day$^{-1}$ of methane (Klapstein et al. 2014) and flux chambers installed on individual vascular plant tillers indicated that plants at the site emit an equivalent of ~3 to 5 mmol m$^{-2}$ day$^{-1}$ of methane (Shea 2010). Because we do not measure 39 to 66 mmol m$^{-2}$ day$^{-1}$ of non-diffusively emitted methane, the results imply that if the model is correct, 85% to 95% of the fugitive methane (33 to 63 mmol m$^{-2}$ day$^{-1}$) was oxidized into CO$_2$ after it left the porewater and before it reached the atmosphere. However, preliminary data from the site that compares CH$_4$ fluxes under oxic and anoxic (nitrogen flushed) chamber conditions (i.e., method of van der Nat and Middelburg 1998) indicate that methane oxidation reduced emissions, at most, by ~3 mmol m$^{-2}$ day$^{-1}$, which is an order of magnitude lower than that implied by Model 2.

In contrast, when homoacetogenesis was decoupled from methanogenesis (Model 3), methane production rates were slower (10 to 28 mmol m$^{-2}$ day$^{-1}$, Table 6), aligning better with measured emission rates at the site (0.5 to 5 mmol m$^{-2}$ day$^{-1}$). However, we note that the only emission pathway included in the model was diffusive transfer from the porewater surface to the atmosphere, and because other emission pathways exist at the site, rates from this model should
be interpreted as lower-bound estimates. While the better alignment with measured emission rates at the site is promising for this model, it is puzzling that the model implied a buildup of acetate to concentrations exceeding those measured in porewater at our site (Table 6 and Figure S2).

**Homoacetogenesis**

If the inclusion of homoacetogenesis in our reaction network is correctly representing conditions at our site, results indicate that homoacetogenesis is an important microbial process in our studied bog. On a depth-integrated basis, modeled rates of homoacetogenesis were, when coupled to methanogenesis (Model 2), roughly equal to modeled rates of hydrogenotrophic methanogenesis, and when decoupled from methanogenesis (Model 3), 4 to 9 times faster than modeled rates of hydrogenotrophic methanogenesis (Tables 4 and 5). In both cases, the acetogenic microbes were successfully competing with, and, in some cases, outcompeting methanogens for H₂ and CO₂. This result aligns with those from published incubation studies. For example, Ye et al. (2014) found that in incubated bog peat, homoacetogenesis was 1 to 6 times faster than hydrogenotrophic methanogenesis. The competitive advantage of homoacetogenesis over hydrogenotrophic methanogenesis in our system could be related to the fact that much of our soil profile remained below 10 °C during porewater sampling events (Figure S6); homoacetogens outcompete methanogens for H₂ in low temperature conditions (Conrad et al. 1989; Kotsyurbenko et al. 1993; Kotsyurbenko et al. 2001). Both models also indicated that homoacetogenesis could have contributed acetate to acetoclastic methanogens. In the coupled model (Model 2), 30 to 40% of acetate used by acetoclastic methanogens came from homoacetogenesis, while in the decoupled model (Model 3), 0 to 30% of the acetate by
acetoclastic methanogens came from homoacetogenesis (Table 5 and 6). Ye et al. (2014) found homoacetogenesis was responsible for between 5 and 20% of all the generated acetate in their incubated bog peat.

Our model results further indicate that homoacetogenesis can alter methane production pathways without altering the $^{13}$C composition of porewater. The apparent fractionation factor for $\delta^{13}$C-CO$_2$ and $\delta^{13}$C-CH$_4$ in porewater,

$$\alpha = \left(\delta^{13}C_{CO_2} + 1000\right) / \left(\delta^{13}C_{CH_4} + 1000\right),$$

(28)
is often used to determine the dominant pathways of methanogenesis (Whiticar 1999; Conrad 2005; Prater et al. 2007). Alpha values smaller than 1.055 ($\alpha = 1.04$ to 1.055) are typically associated with acetoclastic methanogenesis, while $\alpha$ values larger than 1.055 ($\alpha = 1.055$ to 1.09) are typically associated with hydrogenotrophic methanogenesis. At our site, the peeper profile in the center of the bog had depth-averaged monthly $\alpha$ values that ranged from 1.054 to 1.058 and the peeper profile at the edge of the bog had depth-averaged monthly $\alpha$ values that ranged from 1.051 to 1.052. According to previous interpretation, these values indicate roughly equal utilization of the two production pathways in the center of the bog (i.e., the $\alpha$ values spanned the 1.055 threshold) and a slight dominance of acetoclastic methanogenesis at the edge of the bog (i.e., values were slightly smaller than the 1.055 threshold). This interpretation aligns with rates resulting from Model 1 (Original Fugitive Methane Model, Table 4), but disagrees with rates resulting from Models 2 and 3, the two models that included homoacetogenesis. Models 2 and 3 predicted notably greater rates of acetoclastic methanogenesis than hydrogenotrophic methanogenesis in both locations (Tables 5 and 6). These results demonstrate that larger $\alpha$ values do not necessarily indicate the occurrence of hydrogenotrophic
methanogenesis. Homoacetogenesis in combination with acetoclastic methanogenesis can similarly generate a larger $\alpha$ value.

*Methane Oxidation*

Methane oxidation was difficult to detect isotopically given the small fractionation factor for the reaction (Table 1). However, methane oxidation was an important reaction in our modeling effort. In the shallow depths, the reaction was required to get a good fit to our data set. In the deeper depths, it was not a required reaction, but inclusion of it impacted other modeled rates, particularly in Model 3 (Figure 3). Future modeling efforts would benefit from site data that constrain the depth to which methane oxidation actually occurs.

*Fugitive Carbon*

All three models required the generation of carbon that we could not account for with our site measurements. Model 1 and 2 generated more methane than we could account for with porewater and surface flux measurements; Model 3 implicitly generated more acetate than we could account for with porewater measurements. These fugitive carbon pools indicate that our models misrepresented the set of microbial reactions occurring at the site, and/or the fugitive carbon was transformed before we could measure it, and/or our field measurements failed to capture an important carbon flux. As with any modeling study, it is possible that our models were incorrect. However, with the addition of homoacetogenesis, our models included all the reactions we are aware of that can impact concentration and isotopic composition of methane and CO$_2$ in porewater. It is also possible that one of the models was correct, and that we did not detect the generated carbon because it was oxidized by oxygen residing around the root of plants.
and/or near the bog surface. However, in all three models, much of the fugitive methane and acetate was generated at deeper depths. In particular, in the center of the bog, most of the fugitive carbon was generated below a depth of 80 cm (Figure 4; note that implied acetate production scales with rates of methanogenesis and homoacetogenesis), and at our site, plant roots do not reach below a depth of 50 – 60 cm and oxygen was only detected down to a depth of 40 cm (Figure S1). Finally, it is possible that our field measurements failed to capture an important flux of carbon leaving the site. We have measured ebullition (Klapstein et al. 2014), plant mediate methane transport (Shea 2010), and total methane emission off the surface of the bog (Euskirchen et al. 2014), but we have not fully constrained site hydrology, and thus, it is possible we missed an advective flux of water and dissolved carbon leaving the site. However, these collapse scar bogs are internal features with no prominent outflows and little opportunity for overland flow during springmelt.

**Patterns in Modeled Rates**

Even though process rates estimated by the three models differed from each other, the patterns associated with depth, location and time were similar. In all three models, rates of methane production (as well as non-methanogenic CO₂ production and homoacetogenesis) increased with depth regardless of location or time. Studies from other wetland systems (not just thermokarst bogs) have similarly reported increased rates of methane production with depth or increased abundance of methanogenic Achaea with depth (Dinel et al. 1988; Kotsyurbenko et al. 2004; Tveit et al. 2012; Tveit et al. 2014; Johnston et al. 2014). However, this trend is not universal; many wetland studies have reported faster rates of methane production or greater abundance of methanogens in shallow depths (Moore and Knowles 1990; Valentine et al. 1994;
Segers 1998; van den Pol-van Dasselaar and Oenema 1999; Cadillo-Quiroz et al. 2006; Shoemaker et al. 2012), which often is interpreted as evidence that peat quality declines with depth, or that surface peat receives inputs of labile carbon from root exudates. At our site, the faster modeled rates of methane production at depth indicate either more favorable conditions for methanogenesis at deeper depths and/or a greater loss of produced methane and carbon dioxide from shallower depths. Conditions become more reducing with depth in our studied bog (Figure S1), which could explain the modeled depth trend in methanogenesis, and other studies have demonstrated that downward transport of surface-derived biologically available organic carbon can help fuel methanogenesis and fermentation reactions at depth (Chanton et al. 1995; Chanton et al. 2008; Levy et al. 2013). However, our models used changes in dissolved gas concentrations (CH$_4$ and CO$_2$) to track microbial reaction rates. Non-diffusive escape of these gases (i.e., ebullition and plant mediated transport) from porewater between sampling events would result in an underestimation of actual rates because the models do not explicitly consider these non-diffusive loss pathways. Model 1 and 2 do account for non-diffusive methane loss (i.e., fugitive methane), but they do so by assuming that carbon dioxide produced during methanogenesis was not lost from porewater. Thus, it is possible that the modeled increase in microbial methane production with depth reflects an inability of the models to account for non-diffusive emission of CH$_4$ and CO$_2$ near the surface. Plant mediated transport of gases is necessarily greater in near-surface zone where plants are rooted, and Klapstein et al. (2014) showed that at our site, ebullition is greater in shallow peat (20–60 cm depth) than in deeper peat (greater than 60 cm depth). However, CO$_2$ is not often transported to the atmosphere through these non-diffusive pathways. While the bubbles captured by Klapstein et al. (2014) contained both CH$_4$ and CO$_2$,
which indicates a potential loss mechanism for CO$_2$ in shallow depths, the concentrations of CO$_2$
in the bubbles were less than 4% while concentrations of methane were 20.3 – 26.4%.

As hypothesized, our models indicated that all microbial rates were greater at the edge of
the bog than in the center of the bog. The peeper profile at the edge of the bog was closer to the
actively thawing permafrost margin at our site and had a denser sedge community. The greater
abundance of sedges explains the faster modeled rates of methane oxidation at this location;
aerenchyma tissue inside sedges facilitates oxygen transport belowground (Armstrong 1964). We
infer that for the other reactions, the faster modeled rates at the edge reflect an input of
biologically available organic carbon and nutrients from thawing permafrost. Previous work has
indicated that dissolved organic carbon (DOC) in actively thawing permafrost is more
biologically available than that in stabilized thermokarst (Abbott et al. 2014), and permafrost
peat has more nitrogen and phosphorous than peat from locations that have thawed or were never
frozen (Turetsky et al. 2007; Keuper et al. 2012). This interpretation aligns with findings of
Klapstein et al. (2014) that rates of ebullition were greater in a younger collapse site than in an
older collapse site, and that in the younger site, a great proportion of carbon contained in the
captured bubbles was derived from thawed permafrost. However, our model results are in
contrast to those of Corbett et al. (2015) who applied the equivalent of Model 1 (Original
Fugitive Methane Model) to porewater data collected from bog moats (i.e., edge) and midbog
locations (i.e., center) throughout the Glacial Lake Agassiz Peatlands. Their isotopic data
indicated that moat locations produced half as much methane than midbog locations, and they
hypothesized that the recently thawed permafrost in moat locations could not fuel
methanogenesis because it was instead undergoing initial phases of anaerobic decomposition (i.e.,
fermentation). Our model results indicate faster rates of both non-methanogenic CO$_2$ production
and methanogenesis at the edge location. The discrepancy among these results indicates that the chemical composition of organic matter entering thermokarst bogs and the development of methanogenic microbial communities therein influence the resulting pathways of carbon fluxes. The availability of nutrients and organic carbon at the edge of the bog also explains other patterns in our modeled rates. All three models indicated that microbial rates (excluding methane oxidation) increased more during the growing season at the edge of the bog than in the center of the bog. Temperatures increased in both locations during the season (Figure S6), enabling faster microbial reactions at both locations. However, we suspect that microbial rates in the center of the bog were substrate limited, which reduced the ability of temperature to enhance rates at this location. In contrast, we suspect that such a substrate limitation was not present at the edge of the bog. All three models also indicated a shift in methanogenic pathways across the bog; acetoclastic methanogenesis was more important at the edge of the bog than in the center of the bog. This shift is similarly explained by an availability of labile organic carbon (i.e., acetate) at the edge of the bog released either by sedge roots or thawing permafrost. Prater et al. (2007) saw a similar shift toward acetoclastic methanogenesis in the moats of many collapse scar bogs, but only those colonized by sedges. They concluded the sedges stimulated acetate fermentation while active permafrost thaw facilitated the wet conditions favorable to sedge colonization.

CONCLUSIONS

Modeling the temporal change in dissolved gas concentrations and stable carbon isotopes is a valuable tool for estimating in-situ microbial rates; but, as should be expected, the resulting rates depend on the initial conceptualization of the reactions occurring at the site. For our data set, the inclusion of a single reaction, homoacetogenesis — a reaction not included in previous rate-
estimation efforts — notably impacted our modeled rates. Beyond conceptualization of the reaction network, we found that our modeled rates were also sensitive to the depths across which methane oxidation was allowed to occur. Despite differences in the magnitude of the modeled rates by the three reaction network models, all models indicated that microbial rates at the site increased with depth below the bog surface and were greater at the edge of the bog than at the center of the bog. Rates at the edge of the bog increased more over the summer season than those in the center of the bog. The ratio of acetoclastic to hydrogenotrophic methanogenesis was greater at the edge of the bog than in the center of the bog. A majority of these trends were explained by the proximity of the edge location to the actively thawing permafrost margin where the proportion of sedges was greater and recently thawed permafrost provided bioavailable carbon and nutrients. However, the modeled trends with depth might reflect the inability of porewater-based modeling approaches to estimate microbial rates for depths where the non-diffusive loss of methane and CO$_2$ from porewater is prevalent (i.e., shallower depths at our site).

Collectively, the results provide information about the various microbial processes operating within a collapsed permafrost wetland, and provide insight into how these processes vary over a season and with proximity to the actively thawing permafrost margin. A puzzling outcome from the effort was the modeled generation of carbon (methane or acetate) that we could not account for with porewater and flux measurements from the site. None of the models could fit the porewater dataset without generation of ‘fugitive’ carbon, indicating that our conceptualization of the reactions occurring at the site remains incomplete, despite developing and testing three different reaction networks, and/or our site measurements are missing important carbon transformations and/or fluxes. This model–data discrepancy will motivate and inform future
research efforts focused on improving our understanding of carbon cycling in permafrost wetlands.

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**FIGURE CAPTIONS**

**Figure 1: Fit Performance.** Concentrations and $^{13}$C isotopic compositions of CH$_4$ and CO$_2$ both measured in and modeled for porewater collected from the center of the bog during the July to August time period. Measured profiles are presented with red symbols (empty squares for initial profiles, i.e., measured in July, and filled circles for final profiles, i.e., measured in August).

**Panels A & B:** Grey circles indicate top fits from Model 1 (panel A) and Model 2 (panel B) to measured data when the entire dataset was equally weighted (i.e., $N = 10$ in Eqns. 22 and 26) and the depth of methane oxidation was not constrained. Black circles indicate top fits when the importance of methane was decreased in the objective function (i.e., $N = 100$ in Eqns. 22 and 26, which allowed for the generation of fugitive methane) and the depth of methane oxidation was not constrained. White circles indicate top fits when the importance of methane was decreased in the objective function (i.e., $N = 100$ in Eqns. 22 and 26) and methane oxidation was constrained to shallow depths. **Panel C:** Black circles indicate top fits when the entire dataset was equally weighted and the depth of methane oxidation was not constrained. White circles (often co-located with black circles) indicate top fits when the entire dataset was equally weighted and the depth of methane oxidation was constrained to shallow depths. RMSE from these plotted fits, as well as fits to the other datasets, are presented in Table 3.

**Figure 2: Top Fits.** Concentrations and $^{13}$C isotopic composition of CH$_4$ and CO$_2$ measured in and modeled for porewater collected in the center and at the edge of the bog during the two considered time periods (June to July and July to August). Initial measured profiles (empty red squares) are data from the start of the designated time period and final measured profiles (filled red circles) are data from the end of the time period. Modeled profiles are grey for Model 1,
black for Model 2, and white for Model 3. They represent top fits to measured data given constraints on the depth of methane oxidation.

**Figure 3: Modeled Rates and Methane Oxidation.** Average modeled rates for top fits from Model 3 to data collected from the edge of the bog during the June to July time period as methane oxidation was allowed to occur in sequentially increasing depths. Error bars represent plus and minus one standard deviation around the mean. Blue points at the front of the 3D plots present modeled rates when methane oxidation was not allowed to occur anywhere along the peeper profile. Yellow points at the back of the 3D plots present modeled rates when methane oxidation was allowed to occur in any of the four peeper depths. Points in between present modeled rates as the depth where methane oxidation was allowed to occur sequentially increased, one peeper depth at a time. The rate of fugitive methane production was calculated as the difference between modeled and measured concentrations of methane over the monthly time step.

**Figure 4: Modeled Rates.** Average modeled rates for top fits to all four porewater datasets (two time periods at two locations within the bog) for Model 1 (panel A), Model 2 (panel B), and Model 3 (panel C). Error bars represent plus and minus one standard deviation around the mean. The rate of fugitive methane production was calculated as the difference between modeled and measured concentrations of methane over the monthly time step. Note the scale-change for the x-axis in panel C relative to panels A and B.


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Res: Biogeo 120:280–293. doi: 10.1002/(ISSN)2169-8961


<table>
<thead>
<tr>
<th>Eqn.</th>
<th>Process</th>
<th>([\text{CH}_4]) relative to substrate</th>
<th>(^{13}\text{C})</th>
<th>([\text{CO}_2]) relative to substrate</th>
<th>(^{13}\text{C})</th>
<th>Considered fractionation factor range (‰)</th>
<th>Range fitting data (‰)</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetoclastic Methanogenesis</td>
<td>↑ ↑</td>
<td>Depleted</td>
<td>Enriched</td>
<td>17 – 35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17–32</td>
<td>17–32</td>
<td>17–27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hydrogenotrophic Methanogenesis (considering (\text{H}_2) generation)</td>
<td>↑ ↑</td>
<td>Depleted</td>
<td>Enriched*</td>
<td>46 – 80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56–80</td>
<td>46–79</td>
<td>67–79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Methane Oxidation</td>
<td>↓ ↑</td>
<td>Enriched</td>
<td>Depleted</td>
<td>5 – 31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5–31</td>
<td>5–31</td>
<td>11–23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Non-methanogenic (\text{CO}_2) Production</td>
<td>– ↑</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Homoacetogenesis (considering (\text{H}_2) generation)</td>
<td>– –</td>
<td>Depleted (indirectly via depleted acetate)</td>
<td>Enriched*</td>
<td>38 – 68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>–</td>
<td>38–68</td>
<td>41–62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* microbial reactions enrich the DIC pool by listed fractionation factor, however, the net reaction, which includes generation of \(\text{H}_2\), involves the addition of \(\text{CO}_2\) into the DIC pool from fermentation of peat. Thus, the net impact on the isotopic composition of the DIC pool depends on the initial isotopic composition of the DIC pool and the isotopic composition of the peat.

a. \(\epsilon_r = (\delta^{13}\text{C}_{\text{substrate}} - \delta^{13}\text{C}_{\text{product}})\)

b. (Gelwicks et al. 1994; Whiticar 1999; Valentine et al. 2004; Londry et al. 2008)

c. (Games et al. 1978; Botz et al. 1996; Whiticar 1999; Valentine et al. 2004; Londry et al. 2008)

d. (Whiticar 1999; Feisthauer et al. 2011)

e. (Blaser et al. 2013)

f. Model 1 = Original Fugitive Methane Model

g. Model 2 = Acetogenesis Fugitive Methane Model

h. Model 3 = Acetogenesis Fugitive Acetate Model
Table 2: Summary of Components in Conceptual Reaction Networks

<table>
<thead>
<tr>
<th>Components</th>
<th>Description</th>
<th>Role in model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{12}$CH$<em>4</em>{\text{initial}}$, $^{13}$CH$<em>4</em>{\text{initial}}$, $^{12}$CO$<em>2</em>{\text{initial}}$, $^{13}$CO$<em>2</em>{\text{initial}}$</td>
<td>Concentrations at start of time period of interest [µmol L$^{-1}$]</td>
<td>Known values measured in peepers. Used as the starting concentration in the models.</td>
</tr>
<tr>
<td>$^{12}$CH$<em>4</em>{\text{final}}$, $^{13}$CH$<em>4</em>{\text{final}}$, $^{12}$CO$<em>2</em>{\text{final}}$, $^{13}$CO$<em>2</em>{\text{final}}$</td>
<td>Concentrations at end of time period of interest [µmol L$^{-1}$]</td>
<td>Modeled values that represent the result of the evolution of initial concentrations over the time period of interest given rates of considered microbial reactions. Modeled concentrations were fit (see Data Fitting Approach) to measured peeper concentrations to determine the microbial reaction rates.</td>
</tr>
<tr>
<td>CH4Acet; CH4Hydro; CH4Oxid; Resp; AcetGen</td>
<td>Microbial reaction rates [µmol L$^{-1}$ month$^{-1}$]</td>
<td>Unknowns solved for in the models by fitting modeled final concentrations to measured final concentrations (see Data Fitting Approach).</td>
</tr>
<tr>
<td>DiffCH$_4$; DiffCO$_2$</td>
<td>Diffusion Rates [µmol L$^{-1}$ month$^{-1}$]</td>
<td>Known rates calculated using initial and final concentrations measured in peepers (see SI, section 1).</td>
</tr>
<tr>
<td>rORG</td>
<td>$^{13}$C:$^{12}$C ratio of peat</td>
<td>Constant defined value taken from O’Leary (1988). Considered range for all models spanned -29‰ to -26‰ (rORG = 0.0109113 to 0.0109450). Range that fit data for: Model 1 = -27.2‰ to -26.0‰ Model 2 = -27.0‰ to -26.0‰ Model 3 = -29.0‰ to -27.0‰.</td>
</tr>
<tr>
<td>rCH$_4$; rCO$_2^{\text{data}}$</td>
<td>$^{13}$C:$^{12}$C ratios of CH$_4$ and DIC</td>
<td>Known ratios calculated from initial and final concentrations measured in peepers.</td>
</tr>
<tr>
<td>rCO$_2^{\text{fract}}$; rCO$_2^{\text{fract2}}$</td>
<td>$^{13}$C:$^{12}$C ratio of DIC after CO$_2$ input from fermentation reactions</td>
<td>Unknown ratios solved for in the models because values depend on modeled microbial reaction rates.</td>
</tr>
<tr>
<td>rACET</td>
<td>$^{13}$C:$^{12}$C ratio of acetate considering input from homoacetogenesis. Used in Model 3.</td>
<td>Unknown ratio solved for in Model 3 because the value depended on rCO$_2^{\text{fract2}}$.</td>
</tr>
<tr>
<td>$f_{\text{AcetGen}}$</td>
<td>Fraction of acetate used by methanogens that is generated by homoacetogenesis. Used in Model 3.</td>
<td>Constant defined value taken from Ye et al. (2014). Considered range spanned from 0 to 60%. Range that fit data was 0 to 30%.</td>
</tr>
<tr>
<td>$\varepsilon_{\text{CHAcet}}$; $\varepsilon_{\text{CHHydro}}$; $\varepsilon_{\text{CHOxid}}$; $\varepsilon_{\text{AcetGen}}$</td>
<td>Carbon isotopic fractionation factors for microbial reactions</td>
<td>Constant defined values taken from literature. See Table 1 for values and literature sources.</td>
</tr>
</tbody>
</table>
Table 3: RMSE (Mean ± Standard Deviation) for Fits from Reaction Network Models

<table>
<thead>
<tr>
<th>Run</th>
<th>Num. Top Fits&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RMSE Jun-Jul Center</th>
<th>RMSE Jul-Aug Center</th>
<th>RMSE Jun-Jul Edge</th>
<th>RMSE Jul-Aug Edge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1: Original Fugitive Methane Model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data equally weighted (&lt;em&gt;N&lt;/em&gt;=10)&lt;sup&gt;b&lt;/sup&gt;; No oxid. constraint.</td>
<td>91</td>
<td>22.5 ± 0.4</td>
<td>22.9 ± 0.5</td>
<td>37.1 ± 0.7</td>
<td>41.1 ± 0.6</td>
</tr>
<tr>
<td>&lt;sup&gt;12&lt;/sup&gt;CH&lt;sub&gt;4&lt;/sub&gt; data de-weighted (&lt;em&gt;N&lt;/em&gt;=100)&lt;sup&gt;b&lt;/sup&gt;; No oxid. constraint.</td>
<td>104</td>
<td>10.9 ± 0.5</td>
<td>1.3 ± 0.4</td>
<td>2.6 ± 0.4</td>
<td>20.5 ± 0.1</td>
</tr>
<tr>
<td>&lt;sup&gt;12&lt;/sup&gt;CH&lt;sub&gt;4&lt;/sub&gt; data de-weighted (&lt;em&gt;N&lt;/em&gt;=100)&lt;sup&gt;b&lt;/sup&gt;; Yes oxid. constraint.</td>
<td>70</td>
<td>11.0 ± 0.5</td>
<td>1.3 ± 0.4</td>
<td>2.4 ± 0.1</td>
<td>20.6 ± 0.1</td>
</tr>
<tr>
<td><strong>Model 2: Acetogenesis Fugitive Methane Model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data equally weighted (&lt;em&gt;N&lt;/em&gt;=10)&lt;sup&gt;b&lt;/sup&gt;; No oxid. constraint.</td>
<td>132</td>
<td>26.6 ± 0.4</td>
<td>26.5 ± 0.5</td>
<td>42.3 ± 0.6</td>
<td>42.4 ± 0.6</td>
</tr>
<tr>
<td>&lt;sup&gt;12&lt;/sup&gt;CH&lt;sub&gt;4&lt;/sub&gt; data de-weighted (&lt;em&gt;N&lt;/em&gt;=100)&lt;sup&gt;b&lt;/sup&gt;; No oxid. constraint.</td>
<td>125</td>
<td>11.1 ± 0.5</td>
<td>2.2 ± 0.1</td>
<td>4.6 ± 0.5</td>
<td>21.7 ± 0.1</td>
</tr>
<tr>
<td>&lt;sup&gt;12&lt;/sup&gt;CH&lt;sub&gt;4&lt;/sub&gt; data de-weighted (&lt;em&gt;N&lt;/em&gt;=100)&lt;sup&gt;b&lt;/sup&gt;; Yes oxid. constraint.</td>
<td>97</td>
<td>11.3 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>21.7 ± 0.2</td>
</tr>
<tr>
<td><strong>Model 3: Acetogenesis Fugitive Acetate Model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data equally weighted (&lt;em&gt;N&lt;/em&gt;=10)&lt;sup&gt;b&lt;/sup&gt;; No oxid. constraint.</td>
<td>100</td>
<td>11.0 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.0 ± 0.04</td>
<td>19.9 ± 0.0</td>
</tr>
<tr>
<td>Data equally weighted (&lt;em&gt;N&lt;/em&gt;=10)&lt;sup&gt;b&lt;/sup&gt;; Yes oxid. constraint.</td>
<td>6</td>
<td>11.3 ± 0.5</td>
<td>2.4 ± 0.1</td>
<td>7.0e-7 ± 3e-8</td>
<td>20.1 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>. Number of simulations with root-mean square errors that were within 5% of the best performing simulation for the model run.

<sup>b</sup>. Value of <em>N</em> in Eqns. (22) and (26).
Table 4: Total Rates and Ratios for Model 1, Original Fugitive Methane Model

<table>
<thead>
<tr>
<th>Rates (mmol m^{-2} day^{-1})^a</th>
<th>Jun-Jul Center^b</th>
<th>Jul-Aug Center^b</th>
<th>Jun-Jul Edge^c</th>
<th>Jul-Aug Edge^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₄ prod. acetate</td>
<td>26 ±5</td>
<td>20 ±6</td>
<td>44 ±7</td>
<td>44 ±9</td>
</tr>
<tr>
<td>CH₄ prod. H₂/CO₂</td>
<td>24 ±5</td>
<td>31 ±6</td>
<td>34 ±7</td>
<td>43 ±9</td>
</tr>
<tr>
<td>CH₄ oxid.</td>
<td>0.9 ±0.3</td>
<td>0.0 ±0.0</td>
<td>1.4 ±0.6</td>
<td>1.0 ±0.1</td>
</tr>
<tr>
<td>Gross CH₄ prod.</td>
<td>50 ±1</td>
<td>51 ±1</td>
<td>78 ±1</td>
<td>88 ±2</td>
</tr>
<tr>
<td>Net CH₄ prod. due to rxns.</td>
<td>49 ±1</td>
<td>51 ±1</td>
<td>77 ±1</td>
<td>87 ±2</td>
</tr>
<tr>
<td>Fugitive Methane^d</td>
<td>39 ±1</td>
<td>40 ±1</td>
<td>66 ±1</td>
<td>62 ±2</td>
</tr>
<tr>
<td>Net CH₄ prod. with CH₄ loss</td>
<td>10 ±0</td>
<td>11 ±0</td>
<td>11 ±0</td>
<td>25 ±0</td>
</tr>
<tr>
<td>CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-methanogenic CO₂ Prod.</td>
<td>7 ±1</td>
<td>37 ±1</td>
<td>30 ±2</td>
<td>92 ±2</td>
</tr>
<tr>
<td>Gross &amp; Net CO₂ Prod.^e</td>
<td>58 ±7</td>
<td>88 ±8</td>
<td>110 ±10</td>
<td>181 ±13</td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. Gross Acetate Prod.^f</td>
<td>33 ±6</td>
<td>34 ±7</td>
<td>52 ±9</td>
<td>59 ±11</td>
</tr>
<tr>
<td>Min. Net Acetate Prod.^g</td>
<td>0.0 ±0.0</td>
<td>0.0 ±0.0</td>
<td>0.0 ±0.0</td>
<td>0.0 ±0.0</td>
</tr>
<tr>
<td>Ratios^h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross CO₂ prod. : CH₄ prod.</td>
<td>1.2 ±0.2</td>
<td>1.7 ±0.3</td>
<td>1.4 ±0.2</td>
<td>2.1 ±0.3</td>
</tr>
<tr>
<td>Net CO₂ prod. : CH₄ prod.^i</td>
<td>6 ±4</td>
<td>8 ±6</td>
<td>10 ±9</td>
<td>7 ±4</td>
</tr>
<tr>
<td>CH₄ prod. acetate : H₂/CO₂</td>
<td>1.1 ±0.3</td>
<td>0.7 ±0.2</td>
<td>1.3 ±0.3</td>
<td>1.0 ±0.4</td>
</tr>
<tr>
<td>CH₄ oxid. : CH₄ prod.</td>
<td>0.02 ±0.01</td>
<td>0.02 ±0.01</td>
<td>0.02 ±0.01</td>
<td>0.01 ±0.001</td>
</tr>
<tr>
<td>Fugitive CH₄ : CH₄ prod.</td>
<td>0.77 ±0.02</td>
<td>0.78 ±0.03</td>
<td>0.84 ±0.02</td>
<td>0.71 ±0.03</td>
</tr>
</tbody>
</table>

a. Mean and standard deviation of rates from 70 simulations
b. Depth of integration = 132.5 cm
c. Depth of integration = 89.5 cm
d. Calculated for each simulation as difference between modeled dissolved methane concentration and measured dissolve methane concentration.
e. Includes CO₂ generated from methanogenesis and methane oxidation. See Equations (1), (4) and (5).
f. Implicit rate that accounts for acetate generated by fermentation/syntrophy. See Eqn. (3).
g. The model structure implies that all generated acetate is immediately consumed.
h. Calculated from total rates listed in table with error propagation.
i. After accounting for loss of fugitive methane.
Table 5: Total Rates and Ratios for Model 2, Acetogenesis Fugitive Methane Model

<table>
<thead>
<tr>
<th>Rates (mmol m(^{-2}) day(^{-1}))(^{a})</th>
<th>Jun-Jul Center(^{b})</th>
<th>Jul-Aug Center(^{b})</th>
<th>Jun-Jul Edge(^{c})</th>
<th>Jul-Aug Edge(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH(_4) prod. acetate</td>
<td>36 ± 3</td>
<td>35 ± 3</td>
<td>56 ± 5</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>CH(_4) prod. H(_2)/CO(_2)</td>
<td>13 ± 3</td>
<td>14 ± 3</td>
<td>19 ± 4</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>CH(_4) oxid.</td>
<td>0.9 ± 0.3</td>
<td>0</td>
<td>1.6 ± 0.7</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Gross CH(_4) prod.</td>
<td>49 ± 1</td>
<td>49 ± 1</td>
<td>75 ± 1</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>Net CH(_4) prod. due to rxns.</td>
<td>48 ± 1</td>
<td>49 ± 1</td>
<td>73 ± 1</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Fugitive Methaned(^{d})</td>
<td>37 ± 1</td>
<td>38 ± 1</td>
<td>63 ± 1</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>Net CH(_4) prod. with CH(_4) loss</td>
<td>10 ± 0</td>
<td>11 ± 0</td>
<td>11 ± 0</td>
<td>25 ± 0</td>
</tr>
<tr>
<td>CO(_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-methanogenic CO(_2) Prod.</td>
<td>8.1 ± 0.7</td>
<td>39 ± 1</td>
<td>33 ± 2</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Gross &amp; Net CO(_2) Prod.(^{e})</td>
<td>58 ± 4</td>
<td>88 ± 5</td>
<td>110 ± 7</td>
<td>181 ± 6</td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homoacetogenesis</td>
<td>11 ± 4</td>
<td>15 ± 5</td>
<td>15 ± 5</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>Min. Gross Acetate Prod.(^{f})</td>
<td>36 ± 3</td>
<td>35 ± 3</td>
<td>56 ± 5</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>Min. Net Acetate Prod.(^{g})</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ratios(^{h})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross CO(_2) prod. : CH(_4) prod.</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Net CO(_2) prod. : CH(_4) prod.(^{i})</td>
<td>6 ± 2</td>
<td>8 ± 3</td>
<td>10 ± 6</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>CH(_4) prod. acetate : H(_2)/CO(_2)</td>
<td>2.7 ± 0.7</td>
<td>2.5 ± 0.6</td>
<td>2.9 ± 0.7</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>CH(_4) oxid. : CH(_4) prod.</td>
<td>0.02 ± 0.01</td>
<td>0</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>Fugitive CH(_4) : CH(_4) prod.</td>
<td>0.77 ± 0.02</td>
<td>0.78 ± 0.02</td>
<td>0.83 ± 0.02</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>(f_{AcetGen}), resulting from model</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Acetogenesis : CH(_4) prod. H(_2)/CO(_2)</td>
<td>0.8 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean and standard deviation of rates from 97 simulations  
\(^{b}\) Depth of integration = 132.5 cm  
\(^{c}\) Depth of integration = 89.5 cm  
\(^{d}\) Calculated for each simulation as difference between modeled dissolved methane concentration and measured dissolve methane concentration.  
\(^{e}\) Includes CO\(_2\) generated from methanogenesis and methane oxidation. See Equations (1), (4) and (5).  
\(^{f}\) Implicit rate that accounts for acetate generated by fermentation/syntrophy (see Eqn. (3)) and homoacetogenesis.  
\(^{g}\) The model structure implies that all generated acetate is immediately consumed.  
\(^{h}\) Calculated from total rates listed in table with error propagation.  
\(^{i}\) After accounting for loss of fugitive methane.
<table>
<thead>
<tr>
<th>Table 6: Total Rates and Ratios for Model 3, Acetogenesis Fugitive Acetate Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rates (mmol m$^{-2}$ day$^{-1}$)</strong>$^a$</td>
</tr>
<tr>
<td><strong>Methane</strong></td>
</tr>
<tr>
<td>CH$_4$ prod. acetate</td>
</tr>
<tr>
<td>CH$_4$ prod. H$_2$/CO$_2$</td>
</tr>
<tr>
<td>CH$_4$ oxid.</td>
</tr>
<tr>
<td>Gross CH$_4$ prod.</td>
</tr>
<tr>
<td>Net CH$_4$ prod. due to rxns.</td>
</tr>
<tr>
<td>Fugitive Methane$^d$</td>
</tr>
<tr>
<td>Net CH$_4$ prod. with CH$_4$ loss</td>
</tr>
<tr>
<td><strong>CO$_2$</strong></td>
</tr>
<tr>
<td>Non-methanogenic CO$_2$ Prod.</td>
</tr>
<tr>
<td>Gross &amp; Net CO$_2$ Prod.$^e$</td>
</tr>
<tr>
<td><strong>Acetate</strong></td>
</tr>
<tr>
<td>Homoacetogenesis</td>
</tr>
<tr>
<td>Min. Gross Acetate Prod.$^f$</td>
</tr>
<tr>
<td>Min. Net Acetate Prod.$^g$</td>
</tr>
<tr>
<td>Min. Avg. Acetate Conc. Change (mmol/L/month)$^h$</td>
</tr>
<tr>
<td><strong>Ratios</strong>$^i$</td>
</tr>
<tr>
<td>Gross CO$_2$ prod. : CH$_4$ prod.</td>
</tr>
<tr>
<td>Net CO$_2$ prod. : CH$_4$ prod.$^j$</td>
</tr>
<tr>
<td>CH$_4$ prod. acetate : H$_2$/CO$_2$</td>
</tr>
<tr>
<td>CH$_4$ oxid. : CH$_4$ prod.</td>
</tr>
<tr>
<td>Fugitive CH$_4$ : CH$_4$ prod.</td>
</tr>
<tr>
<td>$f_{AcetGen}$ parameter value</td>
</tr>
<tr>
<td>Acetogenesis : CH$_4$ prod. H$_2$/CO$_2$</td>
</tr>
</tbody>
</table>

a. Mean and standard deviation of rates from 6 simulations
b. Depth of integration = 132.5 cm
c. Depth of integration = 89.5 cm
d. Calculated for each simulation as difference between modeled dissolved methane concentration and measured dissolved methane concentration.
e. Includes CO$_2$ generated from methanogenesis and methane oxidation. See Equations (1), (4) and (5)
f. Implicit rate that accounts for acetate generated by fermentation/syntrophy (see Eqn. (3)) and homoacetogenesis.
g. Assumes $f_{AcetGen} = 30\%$, the maximum value resulting in a top fit to the data.
h. Spatially averaged concentration change given depth of integration and a porosity of 1.
i. Calculated from total rates listed in table with error propagation.
j. After accounting for loss of fugitive methane.
Figure 1

Model 1: Original Fugitive Methane Model

Model 2: Acetogenesis Fugitive Methane Model

Model 3: Acetogenesis Fugitive Acetate Model

Initial data    Final data
Fit to all data
Fit without [CH₄]
Fit without [CH₄] + oxidation constrained

depth [cm]

CH₄ [mmol/L]

δ¹³C-CH₄ [%]
Figure 2
Figure 3
Figure 4

Model 1: Original Fugitive Methane Model (mmol/L/month)

Model 2: Acetogenesis Fugitive Methane Model (mmol/L/month)

Model 3: Acetogenesis Fugitive Acetate Model (mmol/L/month)
SUPPLEMENTAL INFORMATION

Modeling CH₄ and CO₂ cycling using porewater stable isotopes in a thermokarst bog in interior Alaska: Results from three conceptual reaction networks

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Section 1: Diffusive Transport Formulation

Diffusive transport of methane (\(\text{DiffCH}_4\)) and DIC (\(\text{DiffCO}_2\)) was also included in both models [dimensions of M L^{-3} T^{-1}], and was represented as finite differences using the average of the measured initial and final concentration profiles to drive vertical transport.

\[
\text{DiffCH}_4 = D_{CH4} \left[ \frac{1}{2} \left( \frac{12\text{CH}_4^{\text{final}} - 12\text{CH}_4^{\text{initial}}}{(z_{i+1/2} - z_{i-1/2})(z_{i+1} - z_i)} \right) - \frac{12\text{CH}_4^{\text{initial}} - 12\text{CH}_4^{\text{final}}}{(z_{i+1/2} - z_{i-1/2})(z_{i} - z_{i-1})} \right]
\]

\[
\text{DiffCO}_2 = D_{CO2} \left[ \frac{1}{2} \left( \frac{12\text{CO}_2^{\text{initial}} - 12\text{CO}_2^{\text{initial}}}{(z_{i+1/2} - z_{i-1/2})(z_{i+1} - z_i)} \right) - \frac{12\text{CO}_2^{\text{initial}} - 12\text{CO}_2^{\text{final}}}{(z_{i+1/2} - z_{i-1/2})(z_{i} - z_{i-1})} \right]
\]

Where \(D_{\text{CH}_4}\) and \(D_{\text{CO}_2}\) are the diffusion coefficients for methane and carbon dioxide in water. We chose to set both \(D_{\text{CH}_4}\) and \(D_{\text{CO}_2}\) equal to 1.5 \times 10^{-5} \text{ cm}^2/\text{sec}, which is the average diffusion coefficient for solutes in water. The presence of porous material (e.g., peat) can decrease diffusion relative to free water (Hemond and Fechner 2014), but other papers indicate that pockets of dissolved gas in peat can enhance diffusion relative to free water (Stephen et al. 1998; Elberling et al. 2011). Given these conflicting behaviors, we chose to simply use the value for diffusion in free water. In the equations above, \(z_i\) represents the depth of the \(i^{th}\) peeper in the profile (\(z\) is positive with increasing depth below the peat surface [dimension L]). The subscript \(i-1\) and \(i+1\) represent the peeper above and below the peeper of interest, while \(i-1/2\) and \(i+1/2\) represent the mid point between the peeper of interest and the peeper above \((i-1/2)\) and the peeper below \((i+1/2)\). In the diffusive flux calculation, we assumed a zero concentration gradient (i.e., no change in concentration with depth) for the bottom boundary (i.e., below the deepest peeper in the profile). At the top boundary (i.e., at the peat surface above the shallowest peeper in the profile) we assumed a constant concentration of methane and DIC. We set these concentrations to match those expected for water in equilibration with the atmosphere (Morel and Hering 1993), which was 0.002 \(\mu\text{mol/L}\) for methane and 13.5 \(\mu\text{mol/L}\) for DIC.
Figure S1: Dissolved oxygen concentrations

To test for the presence of oxygen at deep depths (i.e., below the rooting zone of plants) dissolved oxygen was measured at one location in the bog in September 2014. A 4.5-cm wide hole was drilled into the peat. Three days later, a dissolved oxygen probe was lowered down the hole and oxygen readings were taken at specified depths. Below are the measured oxygen values plotted in both mg/L and in µmol/L.
Figure S2: Acetate concentrations measured in porewater

A) Center of bog:

B) Edge of bog:
Figure S3: Normalized RMSE for fits for all three models

A) Joint RMSE from 500 simulations run for each of the three models normalized by the joint RMSE value from the best performing simulation. Simulations were ordered and plotted in ascending order. Solid thick lines indicate the leading simulations for each model that had a joint RMSE value within 5% of the best performing simulation. The rapid increase in the joint RMSE value for Model 3 indicates that it was more sensitive to parameter combinations than Models 2 and 3.

B) Normalized joint RMSE values from each model plotted against model parameter values (i.e., \(\varepsilon_{CH4Acet}\), \(\varepsilon_{CH4Hydro}\), \(\varepsilon_{CH4Oxid}\), \(f_{AcetGen}\), \(r_{ORG}\), \(f_{AcetGen}\)). No individual parameter value dominated the performance of Model 3; it appears sensitive to the combination of parameters rather than a single parameter values. The performance of Models 1 and 2 improved as \(\delta^{13}C\) of the peat increased from -29% to -26% and diminished as the fractionation factor for acetoclastic methanogenesis increased beyond ~0.03. Performance of Model 1 also diminished when the fractionation factor for hydrogenotrophic methanogenesis decreased below ~0.06.
**Figure S4: Modeled rates with increasing depths of methane oxidation**

Average modeled rates for data collected from the edge of the bog during the June to July time period given increasing depths of allowed methane oxidation. The average values incorporate only the well performing simulations (i.e., those with RMSE within 5% of best performing simulation). Error bars represent plus and minus one standard deviation around the mean. Blue points at the front of the 3D plots present modeled rates when methane oxidation was not allowed to occur anywhere along the peeper profile. Yellow points at the back of the 3D plots present modeled rates when methane oxidation was allowed to occur anywhere in the four peeper depths. Points in between present modeled rates as the depth of allowed methane oxidation was sequentially increased, one peeper cell depth at a time.
Figure S5: RMSE given increasing depths of methane oxidation

Average RMSE for modeled fits from all three models to data from the designated peeper locations and time periods given increasing depths of allowed methane oxidation. The average values incorporate the best performing simulations (i.e., those with RMSE within 5% of best performing simulation); error bars represent plus and minus one standard deviation around the mean. The red circles on the bottom figure mark the chosen depths of allowed methane oxidation for all three models.
Beginning August 2013, peat temperatures were continuously monitored using thermistors. Duplicate thermistors were permanently installed near the peepers at each location (edge and center) at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 135, and 150 cm below the surface and data were collected using a CR800 datalogger (Campbell Scientific). The data indicate the temperatures at both peeper locations increase over the season.

A) August 2013

B) June 2014
C) July 2014

D) August 2014
REFERENCES


