High peatland methane emissions following permafrost thaw: enhanced acetoclastic methanogenesis during early successional stages

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Abstract

Permafrost thaw in northern peatlands often leads to increased methane (CH_4) emissions, but ~~gaps remain in our understanding of the~~the underlying controls responsible for increased emissions and the duration for which they persist have yet to be fully elucidated. We assessed how shifting ~~ecological conditions~~environmental conditions affect microbial communities, and the magnitude and stable isotopic signature ($\delta^{13}C$) of CH_4 emissions along a thermokarst bog transect in boreal western Canada. Thermokarst bogs develop following permafrost thaw when dry, elevated peat plateaus collapse and become saturated and dominated by *Sphagnum* mosses. We differentiated between a young and a mature thermokarst bog stage (~30 and ~~years~~ ~200 years since thaw, respectively). The young bog located along the thermokarst edge, was wetter, warmer and dominated by hydrophilic vegetation compared to the mature bog. Using high throughput 16S rRNA gene sequencing~~16S rRNA gene high throughput sequencing~~, we show that microbial communities were distinct near the surface and converged with depth, but lesser differences remained down to the lowest depth (160 cm). Microbial community analysis and $\delta^{13}C$ data from CH_4 surface emissions and dissolved gas depth profiles show that hydrogenotrophic methanogenesis was the dominant pathway at both sites. However, mean $\delta^{13}C$-CH_4 signatures of both dissolved gases profiles and surface CH_4 emissions ~~the young bog was~~ were found to ~~have~~ be isotopically heavier in the young bog (-63 ‰ and -65 ‰, respectively) compared to the mature bog (-69 ‰ and -75 ‰, respectively) ~~$\delta^{13}C$-CH_4 in both dissolved gases profiles and surface CH_4 emissions~~, suggesting that acetoclastic methanogenesis was relatively more enhanced throughout the young bog peat profile. Furthermore, mean young bog CH_4 emissions of 82 mg CH_4 m^{-2} day^{-1}, were ~~almost~~ three times greater than the 32 mg CH_4 m^{-2} day^{-1}, observed in the mature bog. Our study suggests that interactions between the methanogenic community, ~~and~~ hydrophilic vegetation, warmer temperatures, and saturated surface conditions enhance CH_4 emissions in young

thermokarst bogsecological conditions and methanogenic communities enhance CH_4 emissions in young thermokarst bogs, but that these favorable conditions only persist for the initial decades after permafrost thaw.

Keywords

Permafrost, peatland, thermokarst, 16S RNA, isotope, methanogenesis, microbial community, methane emissions

1. Introduction

Methane (CH_4) emissions in northern peatlands are typically thought to be of as being driven by environmental and ecological conditions such as temperature, water table position, and vegetation community (Bellisario et al., 1999). However, CH_4 emissions are ultimately the result of microbial activity and understanding the interactions between environmental conditions and microbial processes is key to understanding the impact of disturbances on peatland CH_4 emissions. Increased disturbances such as permafrost thaw are transforming northern latitude peatlands (Helbig, Pappas , & Sonnentag, 2016), through the disruption of the frozen landscape and ecological conditionsenvironmental conditions responsible for the regional accumulation of large peatland carbon (C) stores. Rapidly rising northern air temperatures (Mudryk et al., 2018) are predicted to lead to widespread gradual thawing of permafrost (Schaefer et al., 2011) and subsequent thermokarst development in high C density permafrost peatlands (Olefeldt et al., 2016). Thermokarst formation in ice-rich permafrost peatlands is characterized by ground subsidence and surface inundation (Camill, 1999). and This exposes previously frozen C to anaerobic microbial decomposition and potential mineralization into greenhouse gases (Schuur et al., 2015). Redox conditions following thermokarst formation are an important control of decomposition, with 3 – 4 times greater C

mineralization occurring as aerobic respiration compared to anaerobic respiration (Schädel et al., 2016). Increased emissions of methane (CH_4) due to thermokarst formation are projected to result in a positive feedback with climate warming (Turetsky et al., 2020). However, the magnitude of peatland CH_4 emissions and the metabolic pathways responsible for these emissions in response to permafrost thaw remain uncertain, as does the period for which these conditions and emissions persist.

Methanogenesis, conducted by methanogenic archaea belonging to phylum Euryarchaeota, is one of the most prominent microbial processes contributing to the anaerobic decomposition of organic matter in water-logged permafrost soils (Cai et al., 2016; Knoblauch et al., 2018). Methanogenesis occurs primarily via two pathways: acetoclastic methanogenesis and hydrogenotrophic methanogenesis (Whiticar et al., 1986; Whiticar, 1999). Acetoclastic methanogenesis involves the cleavage of acetate into CH_4 and CO_2 and when considering these two species, causes less apparent fractionation than the hydrogenotrophic methanogenesis pathway. This results in acetoclastic methanogenesis yielding comparatively isotopically heavy $\delta^{13}C$-CH_4 ($\delta^{13}C$ = -65 to -50‰). The reduction of CO_2 and H_2 in hydrogenotrophic methanogenesis typically produces CH_4 lighter in ^{13}C ($\delta^{13}C$ = -110 to -60‰) (Hornibrook et al., 1997, 2000). While the two pathways are stoichiometrically equal (Conrad, 1999; Corbett et al., 2013), the activity of acetoclastic and hydrogenotrophic methanogens are governed by different extrinsic controls (Bridgham et al., 2013).

Hydrogenotrophic methanogenesis is thought to be the main pathway of CH_4 formation in northern peatlands (Hornibrook et al., 1997; Galand et al., 2005). However, the acetoclastic pathway can dominate in the upper layers of more minerotrophic, nutrient rich peatlands (Popp et al., 1999; Chasar et al., 2000) where there are sufficient levels of acetate (Ye et al., 2012). ~~Acetoclastic methanogenesis accounts for two-thirds of peatland CH_4~~

~~production in northern peatlands (Conrad, 1999; Kotsyurbenko et al., 2007) and is favoured in more minerotrophic, nutrient-rich conditions, where there are sufficient levels of acetate required to fuel this pathway (Ye et al., 2012).~~During the initial decades following thaw, surface runoff of nutrients from surrounding intact peat plateaus (Keuper et al., 2012; 2017) and increased connectivity to regional hydrology (Connon et al., 2014), can result in more minerotrophic conditions. ~~These~~ Such shifts in hydrology, temperature, nutrients, redox conditions, and vegetation communities following permafrost thaw have been shown to increase the prevalence of acetoclastic methanogenesis and CH_4 emissions (Hodgkins et al., 2014; McCalley et al., 2014). However, this potential post-thaw enhancement of acetoclastic methanogenesis needs to be considered in context of the existing methanogenic community that developed in the peat profile before thaw. For example, historical ~~ecological conditions~~environmental conditions have been shown to have a legacy effect on the methanogenic community following thaw and can therefore be a key constraint on methanogenic community structure and activity post-thaw (Holm et al., 2020; Lee et al., 2012). Overall, an understanding of the methanogenic community's response following thaw to shifts in both surface conditions and exposure to previously frozen organic matter is key to estimating CH_4 emissions from thermokarst peatlands.

Environmental conditions following permafrost thaw in peatlands are characterized by a drastic shift in water table position and increased wetness, increased soil temperatures, and a change in vegetation community associated with increased labile inputs (Beilman, 2001; Burd et al., 2020; Camill, 1999). These shifts may provide optimal conditions for CH_4 production and emissions, particularly in the initial decades following thaw. Peatland CH_4 emissions are constrained by ~~the~~ water table position (Huang et al., 2021; Strack et al., 2004), and surface inundation leads to increased CH_4 emissions (Tuittila et al., 2000). Methane production and emissions are positively influenced by soil temperatures (Hopple et al., 2020;

Olefeldt et al., 2017), and peatland CH_4 emissions have been shown to increase when both ~~the~~ water table position and temperatures are high (Grant, 2015). The colonization of vegetation associated with fresh, labile inputs has also been shown to increase both the magnitude and temperature sensitivity of CH_4 emissions in peatlands (Leroy et al., 2017; McNicol et al., ~~2020~~2019). As such, many studies have focussed on the relationship between water table position, soil temperature and vegetation communities in determining CH_4 fluxes following thaw (Johnston et al., 2014; Turetsky et al., 2007; Wickland et al., 2006). However, while these environmental conditions are key drivers of CH_4 emissions, they are unable to fully account for the variability in permafrost peatland CH_4 emissions (Juottonen et al., 2021; Kuhn et al., 2021). Some of this unaccounted variance may be in part explained by microbial activity, as changes in the composition and abundance of methanogenic community members can contribute significantly towards peatland CH_4 emissions (Fritze et al., 2021). Relatively few studies have assessed how shifts in ~~ecological conditions~~environmental conditions and ensuing changes in methanogenic community structure influences CH_4 emissions following thaw (McCalley et al., 2014), an interaction that may be significant both at the local and circumpolar scale.

In this study we assess the impact of permafrost thaw on peatland methanogenic community composition and CH_4 emissions along a space-for-time thaw gradient that includes an intact peat plateau and an adjacent thermokarst bog with areas that have thawed ~30 and ~200 years ago (herein referred to as young bog and mature bog, respectively). Thermokarst formation has resulted in distinct environmental conditions at each stage along this thaw gradient. We herein define these distinct environmental conditions as water table position and surface wetness, soil temperatures, and vegetation community. Along this gradient we assessed methanogenic community structure down to 160 cm. We hypothesize that: (1) shifting ~~ecological conditions~~environmental conditions along the permafrost thaw

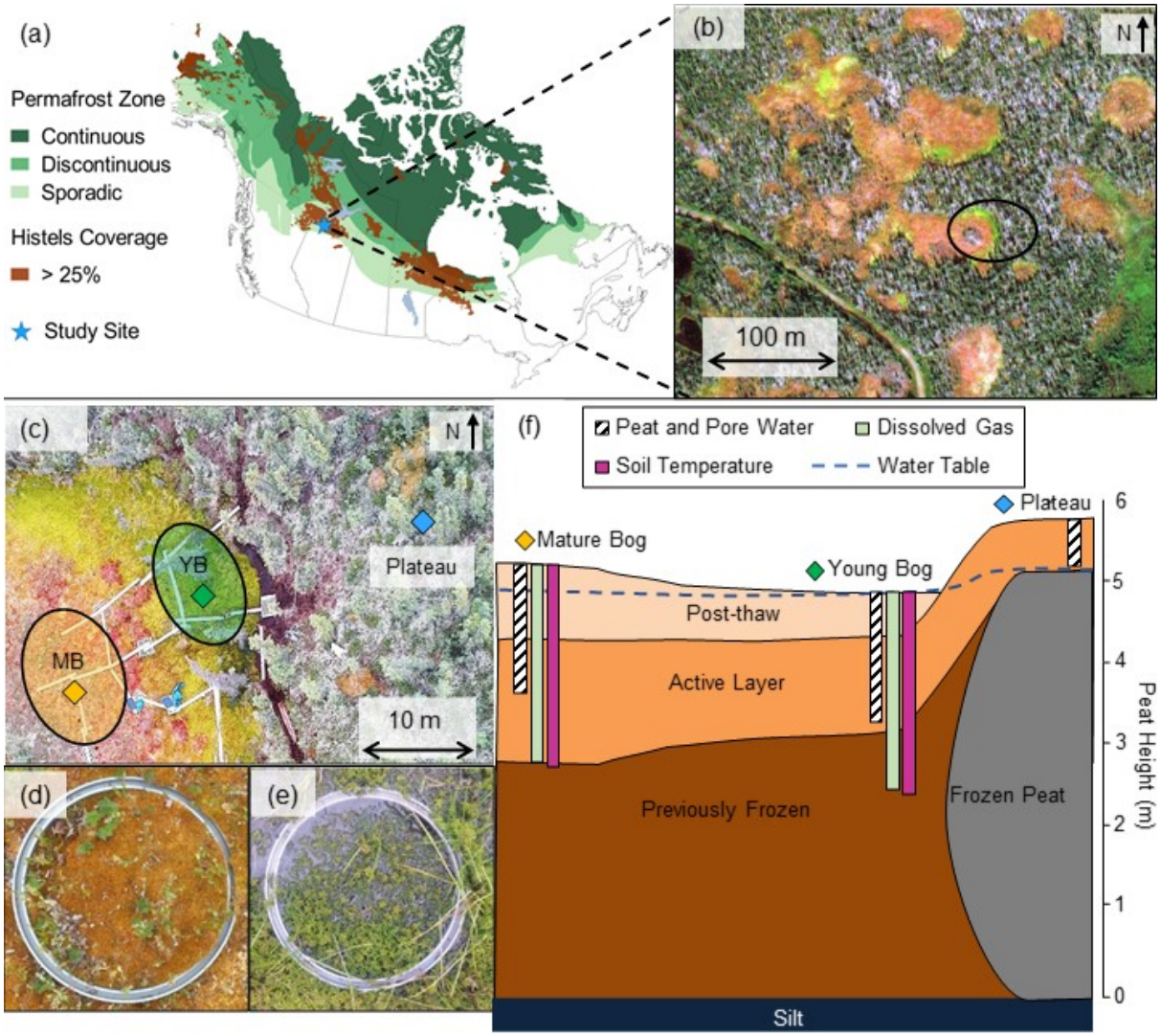
gradient results in a successional microbial community and a restructuring of the methanogenic community, and (2) the warmer conditions and hydrophilic vegetation community in the young bog, along with the exposure of previously frozen peat, will result in a greater relative abundance of acetoclastic methanogens throughout the depth profile, and subsequently greater overall CH_4 emissions. In the young bog and mature bog, we measured the concentration and $\delta^{13}C$-signature of dissolved CH_4 and CO_2 down to 245 cm, and the rates and $\delta^{13}C$-signature of both CH_4 and CO_2 land-atmosphere fluxes. The combined approach of measuring dissolved gas depth profiles and surface emissions, in tandem with assessing the structure of the methanogenic community along a depth profile, allows us to determine how changing ~~ecological conditions~~environmental conditions following thaw impacts methanogenic pathways and community composition. Utilizing this approach, we can subsequently gain further insight into how long elevated surface CH_4 emissions may persist post-thaw. Furthermore, this approach highlights that while environmental and ~~ecological conditions~~environmental conditions are important in determining CH_4 emissions, microbial community composition, and changes in the methanogenic community structure are likely to significantly influence CH_4 emissions following thaw.

2. Methods

2.1 Study Site and Design

The Lutose peatland study site (59.5°N, 117.2°W; Figure 1) is located on the Interior Plains of western Canada, within the zone of discontinuous permafrost (Brown et al., 1997; Heginbottom et al., 1995). The climate is continental with a monthly average summer high temperature of 16.1 °C (July), winter low of -22.8 °C (January), and annual average air temperature of -1.8 °C (Climate-Data.org, 2019 – data from site located ~50 km south of Lutose). Annual average precipitation is 391 mm, of which three quarters fall as rain between

May and September. In the discontinuous permafrost zone of the Interior Plains in boreal western Canada, ~40% of the landscape is covered by permafrost peatlands that have between 2 and 6 m deep peat deposits (Gibson et al., 2018; Vitt et al., 2000). The peatland complexes in this area are a fine-scale mosaic of permafrost peat plateaus, and permafrost-free ponds, fens, and bogs (Zoltai, 1993; Bauer et al., 2003; Vitt et al., 2000; Pelletier et al., 2017),~~.~~ and they are similar to those found in the Hudson Bay Lowlands (Kuhry, 2008) and Alaska (Jones et al., 2017). The Lutose peatland complex is representative of the peatlands found in the discontinuous permafrost zone of the Interior Plains in western Canada (~~Zoltai, 1993; Bailman, 2001; Bauer et al., 2003; Vitt et al., 2000~~Heffernan et al., 2020). The site has 5 – 6 m deep peat and has transitioned through multiple developmental stages since it began accumulating organic matter ~8,800 years ago. It transitioned from a marsh, through a fen and a bog stage prior to permafrost aggradation ~1,800 years ago (Heffernan et al., 2020). Peatlands in the Interior Plains in western Canada are one of the three largest stores of organic carbon found in peatlands within the permafrost zone, the other two being the Hudson Bay Lowlands and the West Siberian Lowlands (Hugelius et al., 2020; Olefeldt et al., 2021). Within the sporadic and discontinuous permafrost zone of our study region >15% of the total peat plateau area has thawed and formed thermokarst bogs in the last 30 years (Baltzer et al., 2014; Gibson et al., 2018). Projections for this area suggests total permafrost lost from plateaus by 2050 (Chasmer and Hopkins, 2017).


(a)
Permafrost Zone
Continuous
Discontinuous
Sporadic
Histels Coverage
> 25%
Study Site
(b)
N
100 m
(c)
N
YB
MB
Plateau
10 m
(d)
(e)
(f)
Peat and Pore Water
Dissolved Gas
Soil Temperature
Water Table
Plateau
Mature Bog
Young Bog
Post-thaw
Active Layer
Previously Frozen
Frozen Peat
Silt
Peat Height (m)
0
1
2
3
4
5
6

191

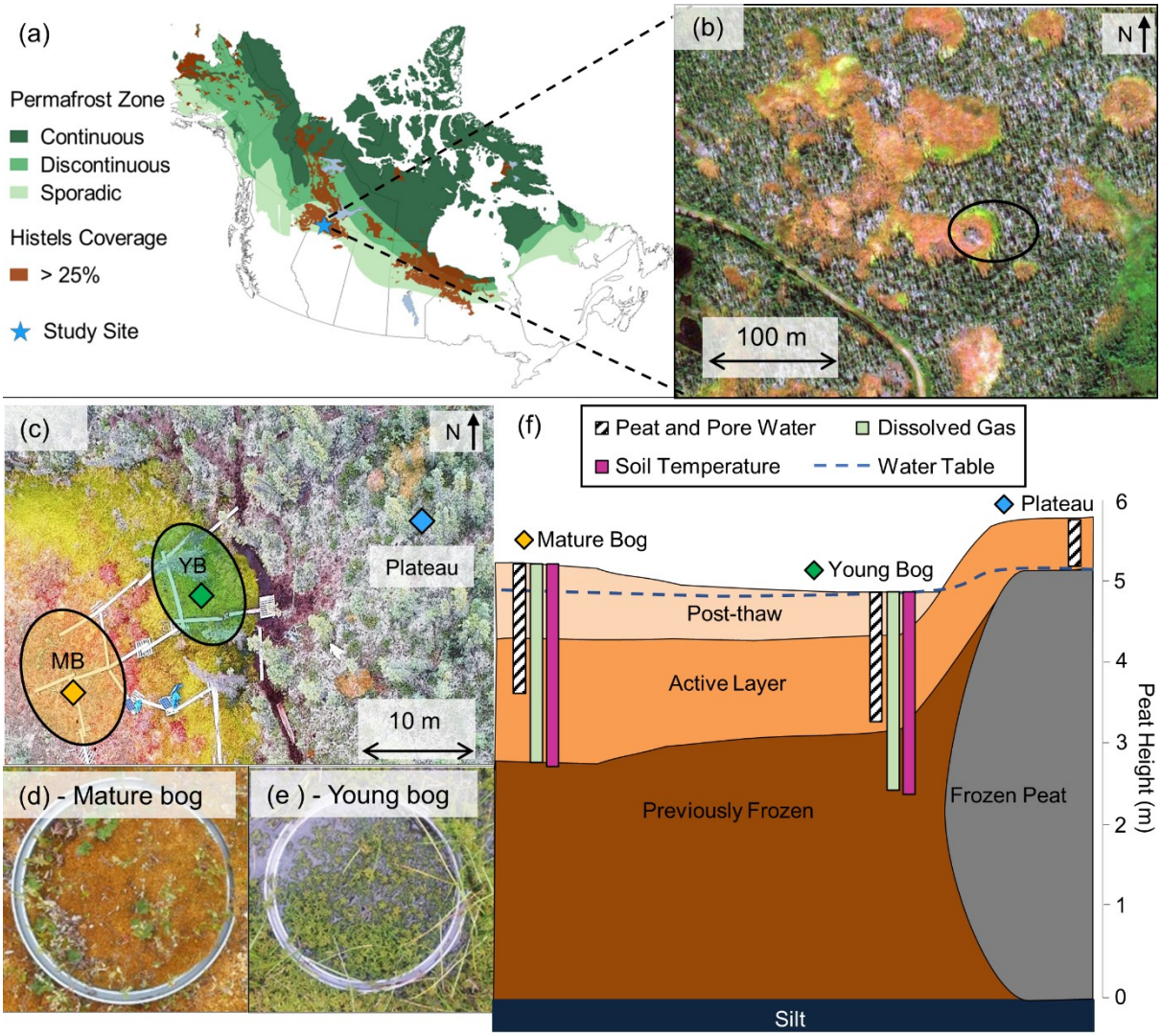


Figure 1. Lutose peatland site location and study design. (a) Site location (Lutose, Alberta, Canada 59.5°N, 117.2°W) in boreal western Canada. Green shading represents permafrost zonation (Brown et al., 1997) and brown shading represents areas with >25% permafrost peatland (histels) extent (Hugelius et al., 2014). (b) Geoeye satellite image of study site (image from https://zoom.earth/), 0.46 m resolution. Circle represents the area where sampling took place. (c) Aerial image of study transect, locations of peat and dissolved gas sampling in the plateau (blue diamond), young bog (green diamond), and mature bog (orange diamond), and area where collars for gas flux measurements were located in the young bog (YB, green) and mature bog (MB, orange) (Aerial photo credit: Olefeldt, David). (d, e) Surface vegetation in the mature bog and young bog (f) Soil profile of thaw transect based on (Heffernan et al., 2020). The transition to Post-thaw peat occurs at 29 cm and 71 cm in the young bog and mature bog respectively. Peat (core) and pore water (pore water peepers), including microbial community, sampling depth profile 0 – 160 cm shown as white column with diagonal black lines. Dissolved gas (diffusive samplers) sampling depth profile 0 – 245 cm shown as light green column. Soil temperature depth profile 0 – 250 cm shown as purple column. Average water table depth shown as dashed blue line.

The studied transect represents a space-for-time gradient of permafrost thaw that includes three thaw stages: a permafrost peat plateau, and a young (~30 years since thaw) and mature (~200 years since thaw) part of an adjacent thermokarst bog. The timing of permafrost thaw was previously determined by ^{14}C dating the shift in macrofossil vegetation indicative of thaw, at 29 cm in the young bog and at 71 cm in the mature bog (Figure 1f) (Heffernan et al., 2020). The peat plateau has an active layer thickness of ~70 cm and its surface is raised 1 – 2 m above the adjacent thermokarst bog due to the presence of excess ground ice, resulting in relatively dry surface conditions where the water table generally follows the deepening of the seasonally thawed peat layer (Zoltai, 1972). This thaw stage is characterized by a stunted, open black spruce (*Picea mariana*) canopy and ground cover of lichens (*Cladonia* spp.), *Sphagnum fuscum* hummocks, and low-lying ericaceous shrubs as is characteristic of the peat plateaus in the area (Vitt et al., 1994). The young bog stage is narrow (<5 – 10 m wide) and is located next to the actively thawing area of the peat plateau. The young bog has an average growing season water table position of 1.3 ± 4.9 cm below the peat surface. These inundated conditions result in the dominance of a hydrophilic vegetation community (Figure 1e) consisting of *Sphagnum riparium*, bog-sedge (*Carex limosa*), and rannoch rush (*Scheuchzeria palustris*). The mature bog is ~10 – 15 m from the young bog and is ~~relatively~~ drier, compared to the young bog, with an average growing season water table position of 22.9 ± 9.3 cm below the surface. The dominant vegetation reflects these drier conditions and consists of *Sphagnum fuscum, Sphagnum magellanicum,* leather leaf (*Chamaedaphne calyculata),* cloudberry *(Rubus chamaemorus), Eriophorum vaginatum* tussocks, and some black spruce (*Picea mariana)* regrowth (Figure 1d). The mature bog is located >10 – 20 m from the thawing plateau edge.

2.2 Site Preparation and Monitoring of Environmental Conditions

The Lutose peatland study site was established in 2015 and a boardwalk was constructed to minimize disturbances along the peat plateau - thermokarst bog transect. Three collars for ~~measurements of~~surface greenhouse gas flux~~es~~ (39 cm diameter) measurements were permanently installed to a depth of 20 cm in both the young and mature thermokarst bog stages. The top of each collar was aligned with the peat surface. PVC wells (2 cm diameter) were installed directly next to each collar and were used to manually monitor the water table position during each gas flux measurement. We monitored soil temperature (°C) at 10, 30, 50, 75, 100, 150, 200, and 250 cm every 30 min from May – September 2018 using permanently installed loggers (Hobo 8k Pendant Onset Computer, Bourne, MA, USA) in ~~the young and mature bog~~both thermokarst bog stages. Temperature depth profiles were established centrally among collars in each thermokarst bog stage, in areas that had similar vegetation, water table position, and distance from the thawing edge as the collars.

Custom made plexiglass pore water suction (Heffernan et al., 2021) and diffusive equilibration gas sampling devices (Knorr et al., 2009) were installed in July 2016 in the young ~~bog~~ and mature bog. These devices were installed in ~~the~~ both ~~young and mature~~thermokarst bog stage~~ss,~~ ~1 m from the nearest flux measurement collar. Pore water suction devices were installed to a depth of 160 cm ~~deep~~ and consisted of 15 sampling depths, with each sampling depth connected to the surface via silicone tubing. This allowed for repeated non-destructive pore water sampling. Three diffusive gas sampling devices ~~each~~ were installed in ~~the young and mature~~each thermokarst bog stage, where ~~two~~ two collected dissolved soil gas samples ~~collected dissolved soil gas samples~~ from 5 – 95 cm deep and ~~the~~ a third from 115 – 245 cm. Each diffusive gas sampler consisted of a PVC pipe with a 10 cm long sampling section centred at each sampling depth. Sampling sections consisted of ~2 m of silicon tubing (3 mm i.d., 5 mm o.d.) wrapped around the PVC pipe and kept in place by PVC-spacers at the top and bottom of each interval. Silicone tubes were sealed at one end

259 whereas the other end was connected to polyurethane tubing (1.8 mm i.d.) that ran back up
260 inside the PVC tube to reach the peat surface where it was sealed with a three-way stopcock.
261 Silicone tubing has been shown to be permeable to gases such as CO_2 and CH_4 within a
262 number of hours, while remaining impermeable to water, making it suitable for sampling of
263 dissolved soil gases (Kammann et al., 2001).

264 *2.3 Pore water chemistry and peat enzyme activity*

265 Pore water dissolved organic matter (DOM) chemistry and peat enzyme activity
266 presented in this study have previously been published (Heffernan et al., 2021), and are
267 briefly described here. Pore water samples for DOM chemistry were taken monthly from
268 May – September 2018 using the previously described pore water suction devices in the
269 young bog and mature bog. Three 60 mL samples were taken from all 15 measurement
270 depths by applying a vacuum at the surface and collecting water with syringes via a three-
271 way stopcock. Each water sample was immediately filtered through 0.7 µm pore size glass
272 fiber filters (GF/F Whatman) into two acid-washed amber glass bottles, with one sample
273 acidified with 0.6 mL 2N HCl to prevent further microbial activity. Pore water samples were
274 transported in a cooled container and stored at 4 °C prior to analysis. Pore water DOM was
275 analyzed for pH, phosphate (PO_4^{3-}; µg L^{-1}), dissolved organic carbon (DOC; mg L^{-1}), total
276 dissolved nitrogen (TDN; mg L^{-1}) concentrations, phenolic contents, specific UV absorbance
277 at 254 nm (SUVA, L mg C^{-1} m^{-1}; Weishaar et al., 2003) and spectral slope between 250 – 465
278 nm ($S_{250-465}$, nm^{-1}; Helms et al., 2008). SUVA and $S_{250-465}$ values are used to indicate
279 aromaticity, with high SUVA indicating a high aromatic content and lower $S_{250-465}$
280 indicating low molecular weight and decreasing aromaticity (Hansen et al., 2016).
281 Peat cores extracted to a depth of 160 cm were stored at 4 °C for less than one week in the
282 laboratory before homogenization to determine potential soil enzyme activities. We

283 performed hydrolytic enzyme assays for four enzymes; phosphatase, β-N-glucosaminidase, β-
284 glucosidase, and β-cellobiosidase using fluorogenic 4-methylumbelliferone labelled
285 substrates (Dunn et al., 2014). We assayed oxidative enzyme activity by measuring laccase
286 activity using syringaldazine (Criquet et al., 2000; Jassey et al., 2012). We summarized the
287 activity of all enzymes using a multi-functionality index based on *z*-scores (Allan et al., 2015;
288 Heffernan et al., 2021).

289 *2.4 Surface Land-Atmosphere Gas Fluxes*

290 We measured surface land-atmosphere greenhouse gas fluxes (CH_4 and carbon dioxide;
291 CO_2) monthly from May – September 2018 at the 3 collars in each peatland stage using the
292 static chamber method (Carroll & Crill, 1997). The chamber used to capture land-atmosphere
293 fluxes was a transparent cylindrical Plexiglass chamber with a basal area of 0.12 m^2, height
294 of 0.40 m, and volume of 47.8 L. The chamber was equipped with three fans (Micronel
295 Ventilator D341T012GK-2, BEDEK GmbH, Dinkelsbühl, Germany) to mix air during
296 measurements and a temperature sensor (Hobo RH Smart Sensor, S-THB-M002, Onset
297 computers, Bourne, USA) that was shaded from direct sunlight (Burger et al., 2016). An
298 airtight seal was formed between the chamber and collar by pouring water in a ~1.5 cm deep
299 well around the upper circumference of each collar. Land-atmosphere fluxes of CO_2
300 (ecosystem respiration) and CH_4 were captured simultaneously in darkened conditions by
301 covering the chamber with a reflective shroud. Gas concentrations were determined at a
302 temporal resolution of 1 s using an Ultraportable Greenhouse Gas Analyser (Los Gatos
303 Research, CA, USA) and real-time fluxes were monitored using the VNV® Viewer
304 (RealVNC® Limited, UK) application with an iPad mini 2 (Apple Inc.).

305 The rates of CH_4 and CO_2 land-atmosphere fluxes (*Flux*) were calculated using ~~the~~
306 ~~change in gas concentration over time inside the chamber (linear regression),~~ the ideal gas

law following~~, average air temperature inside the chamber during the measurement, and a constant atmospheric pressure value of 0.96 atm in Eq. (1):~~:

$$Flux = slope \frac{P.V}{R.T.A} \quad (1)$$

where slope is the linear rate of change of gas concentration (μmol mol^{-1} $second^{-1}$) over the measurement period inside the chamber; P is an atmospheric pressure (atm) constant of 0.96 atm; V is chamber volume (L); R is the universal gas constant (L atm K^{-1} mol^{-1}); T is the average temperature (K) inside the chamber during the measurement; and A is the chamber basal area (m^2). Chamber closure for each flux measurement was 5 minutes with the first 2 minutes discarded to ensure fluxes (i.e., change in concentration over time) with $R^2 > 0.75$. We report CO_2 fluxes in g CO_2 m^{-2} day^{-1} and CH_4 fluxes in mg CH_4 m^{-2} day^{-1}, with positive values indicating fluxes to the atmosphere. To quantify the proportion of C being emitted as CH_4, we standardized our CO_2 and CH_4 fluxes per g C emitted. The proportion of C emitted as CH_4 (CH_4:C emissions) was calculated as

~~Slope is the linear rate of change of gas concentration (μmol mol^{-1} $second^{-1}$) over the measurement period inside the chamber; P is atmospheric pressure (atm); V is chamber volume (L); R is the universal gas constant (L atm K^{-1} mol^{-1}); T is the temperature (K); and A is the chamber basal area (m^2). Chamber closure for each flux measurement was 5 minutes with the first 2 minutes discarded to ensure fluxes (i.e., change in concentration over time) with $R^2 > 0.75$. We report CO_2 fluxes in g CO_2 m^{-2} day^{-1} and CH_4 fluxes in mg CH_4 m^{-2} day^{-1}, with positive values indicating fluxes to the atmosphere. To quantify the proportion of C being emitted as CH_4 we standardized our CO_2 and CH_4 fluxes per g C emitted. The proportion of C emitted as CH_4 was calculated as$44 - 2 - 1 g\ C - C2\ - 2\ da - 1\ +\ g\ C - C4\ - 2\ da - 1$ CH_4:C emissions = g C CH_4 m^{-2} day^{-1}/(g C CO_2 m^{-2} day^{-1} + g C CH_4 m^{-2} day^{-1}~~

[1]).~~CH_4: C emissions~~ $= \frac{CH_4\ m^{-2}\ day^{-1}}{CH_4\ m^{-2}\ day^{-1} + CO_2\ m^{-2}\ day^{-1}}$ (2)

2.5 δ-^{13}C-signature of CH_4 emissions

We assessed the δ^{13}C-CO_2 and δ^{13}C-CH_4 signatures of ecosystem respiration (CO_2) and CH_4 emissions. This was done similarly to regular measurements of CO_2 and CH_4 fluxes, but using a smaller, opaque chamber of 31.1 L and discrete syringe-samples for δ^{13}C analysis in combination with the continuous monitoring of gas concentrations described above. Gas syringe samples were taken using a 20 mL syringe via a three-way stopcock placed between the sealed chamber and gas inlet port on the Ultraportable Greenhouse Gas Analyser. Gas samples were then injected into a 37.5 mL sealed glass-vial that had been flushed with nitrogen gas prior to sealing. Chamber enclosure time ranged from 30 – 50 minutes with 4 – 5 samples being taken during this time. Samples were taken either every 10-minutes or once a minimum change in CO_2 (30 µmol mol^{-1}) and CH_4 (1 µmol mol^{-1}) concentrations was observed. An atmospheric gas sample was used as a time-zero measurement when assessing the change in concentration over time. Glass-vials containing samples were stored at 4 °C until analysis. These measurements were taken in September and October 2016 from 1 collar in both the young and mature bog, with each collar measured twice.

We measured the δ^{13}C values of gas samples from both the chamber fluxes and atmospheric background~~Gas vials containing both the chamber and atmospheric gas samples were analysed in the laboratory for ^{13}C isotopic signatures~~. To assess whether the gas concentration of each sample fit within the measurement range required for δ^{13}C analysis we measured CO_2 and CH_4 concentrations using 1 – 3 mL from each vial. ~~Concentrations of CO_2 and CH_4, using between 1 – 3 mL from each vial, were checked in order to validate the tightness of the containers and to ensured that concentrations fit within the measurement~~

~~range required for ^{13}C analysis. Subsequently, after measurement of the concentration of CO_2 and CH_4 in each sample~~Following these concentration measurements~~, the ^{13}C-CO_2 and ^{13}C-CH_4 signature was quantified in-line with a cavity ring-down spectrometer (G2201-L, Picarro, California, USA) that had been calibrated using certified standards. To this end~~, the remaining sample (17 – 19 ml) was diluted with nitrogen gas to a final volume of 20 mL and injected ~~for analysis~~ into a Small Sample Introduction Module (SSIM, Picarro, California, USA) system to measure ~~$\delta^{13}C$~~ signatures. The $\delta^{13}C$-CO_2 and $\delta^{13}C$-CH_4 signature was measured in-line with a cavity ring-down spectrometer (G2201-L, Picarro, California, USA) that had been calibrated using certified standards.

We then used the time-series of $\delta^{13}C$-CH_4 and CH_4 concentrations to estimate the $\delta^{13}C$-CH_4 signature of the CH_4 released to the atmosphere using Keeling plots (Keeling, 1958). Using this approach, the $\delta^{13}C$-CH_4 signature of gas in each sample is plotted on the *y*-axis against the inverse of CH_4 gas concentrations ($1/[CH_4]$). The *y*-axis intercept of the linear regression represents the mean isotopic signature of the CH_4 source (Fisher et al., 2017). While fractionation during diffusive transport may influence these estimates, it has been shown in similar systems to be of minor importance compared to other contributing processes (Preuss et al., 2013; Nielsen et al., 2019).

2.6 Dissolved gas depth profiles

Dissolved gas samples were ~~taken~~ collected using ~~the~~ diffusive equilibration gas sampling devices. Samples were taken from the following 15 ~~depths~~depths: ~~that include 5 – 15 cm and then~~ every 10 cm down to 95 cm starting at 5 – 15 cm ~~down to 95 cm~~, and then at 115 cm, 140 cm, 165 cm, 195 cm, and 245 cm. Once a month from May – September 2018 a ~~Samples of~~ ~7 mL gas sample ~~were~~ was drawn from each depth ~~monthly from May – September in 2018~~ using a 10 mL plastic syringe~~s~~. These gas ~~Samples~~ samples were immediately injected into a 10 mL sealed glass-vial that had been flushed with nitrogen gas

prior to sealing~~.~~ , and then were ~~Glass vials containing~~These gas samples were stored at 4 °C until analysis. A total of ~~Concentrations of~~ 214 ~~dissolved~~ CO_2 and 211 CH_4 dissolved ~~for each~~gas concentration measurements were made ~~s were analysed~~ measured by injecting 1 – 3 mL of gas into ~~using~~ a gas chromatograph with an FID and CO_2 methanizer (8610C Gas Chromatograph, SRI Instruments, California, USA). ~~for a total of 214 and 211 concentrations of CO_2 and CH_4, respectively. Between 1 – 3 mL of gas was injected into the analyser.~~ We measured ~~Signatures of~~ δ^{13}C-CO_2 and δ^{13}C-CH_4 signatures using ~~were measured with~~ the previously mentioned ~~method using the~~ cavity ringdown spectrometer and SSIM system. As with surface chamber gas samples, dissolved gas samples were diluted with N_2 to 20 ml. However, dissolved gas concentrations were considerably higher than gas concentrations found in the surface chambers, and some were well above the optimal ~~range~~ concentration range required for accurate δ^{13}C analysis for the SSIM system even after dilution. ~~Due~~ To fit within~~to the optimal operational CH_4 concentration range of the SSIM~~measurement range of the system~~,m used~~, further dilution ~~of samples to CH_4 concentrations within the systems measurable range~~ resulted in CO_2 concentrations below detectable limits. As such, we were able to obtain 90 and 75 measurements of δ^{13}C-CH_4 in the young and mature bog, respectively, and 93 measurements of δ^{13}C-CO_2 in both.

We used the δ^{13}C-CO_2 and δ^{13}C-CH_4 signature of each gas sample to calculate the apparent fraction factor α_c, where $\alpha_c = [^{13}\text{C-CO}_2 + 1000]/[^{13}\text{C-CH}_4 + 1000]$. The α_c can serve as an isotopic indicator of the pathway of methanogenesis, with typical values of 1.060 – 1.090 observed for hydrogenotrophic methanogenesis and 1.040 – 1.060 for acetoclastic methanogenesis (Chanton et al., 2005).

2.7 Peat and pore water sample collection for microbial community composition analyses

Microbial community composition was characterized in both peat and peat pore water samples from depths between 0 – 160 cm in the young bog and mature bog. Focussing on peat samples, microbial community composition in the active layer of the peat plateau was assessed from depths between 0 – 30 cm. Peat cores were extracted in June and September 2018. Near-surface cores were extracted using a cutting tool to 30 cm deep in the peat plateau and young bog, and 50 cm deep in the mature bog. Surface cores were limited to 30 cm in the plateau due to the presence of ground ice during sampling in June. Surface core depths differed between the young bog and mature bog due to differences in the water table position. Deeper core sections (down to 160 cm) in the young bog and mature bog were extracted using a Russian peat corer (4.5 cm inner-diameter, Eijkelkamp, Giesbeek, The Netherlands). Cores were extracted from two boreholes located ~20 cm apart, alternating between boreholes to avoid disturbance contamination from the 10 cm corer tip during the coring process. To do so, 50 cm long core sections were taken alternatively from each borehole, with each core having a 10 cm overlap with the previous core taken from the adjacent borehole. In the field, immediately after the entire core was extracted, cores were divided into 15 subsections. The first two subsections contained peat from 0 – 5 cm and 5 – 10 cm, followed by 10 cm increments down to 120 cm, and two further subsections from 130 – 140 cm and 150 – 160 cm. Peat from each interval was sub-sampled using sterilized forceps and placed directly into Whirl-Pak® bags, and frozen within 3 hours of sampling for transportation back to the laboratory. Once samples reached the laboratory, they were frozen at -80 °C until analysis.

We also sampled peat pore water at all 15 peat sampling depths in September 2018 from the pre-installed pore water suction sampling devices mentioned above. We extracted 60 mL pore water samples by applying a vacuum at the surface and collecting water with new plastic 60 mL syringes. Pore water was immediately filtered through sterile 0.2 μM pore size

Polyvinylidene difluoride (PVDF) ~~PVDF~~ membrane sterivex filters (MilliporeSigm~~n~~a). Microbial cells were retained on the filter, and remaining porewater in the sterivex was removed via extrusion using a 60 mL sterile syringe. Sterivex filters were then immediately flash-frozen at -80 °C in a liquid nitrogen dry-shipper to preserve microbial community members until analysis could take place.

2.8 DNA extraction

~~Microbial~~ Genomic DNA was extracted from all peat and pore water samples using the DNeasy PowerSoil kit (Qiagen) and the PowerWater DNeasy kit (Qiagen), respectively, to assess the differences in microbial community structure. Extraction of DNA from both sample types was followed as described by the manufacturer (Qiagen), with two modifications: (i) for peat samples, prior to mechanical lysis using bead beating, the prepared samples were chemically lysed by incubation at 70 °C for 10 minutes in the provided lysis solution, and (ii) sterivex (pore water) samples were incubated with rotation at 37 °C following addition of lysis buffer. These modifications were made to increase total DNA yield. The amount of isolated DNA from each sample was then determined using a Qubit fluorometer (model 2.0, using the 1×HS dsDNA kit), with concentrations ranging between ~0.1 and 22.4 ng μL^{-1}. This extracted DNA served as the template for polymerase chain reaction (PCR) analyses described below.

2.9 *Sequencing and computational analyses*

We amplified 16S rRNA genes using universal prokaryotic primers 515F (Parada, Needham & Fuhrman, 2016) and 926R (Quince et al., 2011). Each primer also contained a six-base index sequence for sample multiplexing (Bartram et al., 2011). The PCR mix (25µL total volume) contained 1 × Q5 reaction buffer, 0.5 µM forward primer, 0.5 µM reverse primer, 200 µM dNTPs, 0.500 U Q5 polymerase (New England Biolabs, Ipswich,M.A, U.S.A) and 2.5 µL of genomic template. Genomic extracts with DNA concentrations of greater than 2 ng

453 μL^{-1} were diluted 1:100 in nuclease-free water. The PCR was performed as follows: 95 °C for
454 3 minutes, 35 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, 70 °C for 1 minute and a
455 final extension of 70 °C for 10 minutes. Pooled 16S rRNA gene amplicons were purified
456 using Nucleomag beads and a 4.5 pM library containing 50% PhiX Control v3 (Illumina,
457 Canada Inc., NB, Canada) was sequenced on a MiSeq instrument (Illumina Inc., CA, USA)
458 using a 2 × 250 cycle MiSeq Reagent Kit v3 (Illumina Canada Inc) at the Molecular Biology
459 Service Unit (MBSU, University of Alberta). The MiSeq reads were demultiplexed using
460 MiSeq Reporter software version 2.5.0.5. Each read pair was assembled using the paired-end
461 assembler for Illumina sequences (PANDAseq; Masella, Bartram & Truszkowski, 2012) with
462 a quality threshold of 0.9, dictating that 90% of overlapping reverse and forward reads must
463 match in order to assemble reads into read pairs. Assembled reads were analyzed using the
464 Quantitative Insights Into Microbial Ecology II pipeline (QIIME2; Boylen et al., 2020).
465 Sequences were clustered into amplicon sequence variants (ASVs) with chimeric sequences,
466 singletons and low abundance ASVs removed using DADA2 (Callahan et al., 2019). All
467 representative sequences were classified with the Greengenes reference database, using the
468 most recent release (version 13.8; McDonald et al., 2012). Although Greengenes is not
469 updated as frequently as the SILVA database, we chose to use it to classify our ASVs as a
470 comparison of both databases revealed that they captured a similar number of archaea (total
471 of 51187 methanogenic read counts attributed to SILVA *versus* 51141 methanogenic read
472 counts attributed to Greengenes). The taxonomic resolution between both databases was also
473 similar, identifying the same kinds of phyla, families and genus, and methanogens (e.g.,
474 methanoregula, methanosarcinales, etc~~.~~…~~,~~). ~~, etc,..)~~ Given these similarities, and the fact that
475 methanogen nomenclature has not changed significantly over time, we ultimately chose to
476 use Greengenes because it was able to resolve more methanogenic families belonging to
477 ~~M~~Methanocelalles and Methanomassiliicoccaceae particularly, compared to SILVA. The

Greengenes database is also still commonly used to explore methanogenic archaeal communities in current literature (Vanwonterghem et al., 2016, Lin et al., 2017, Carson et al., 2019). Furthermore, ~~Because~~ since 1021 methanogenic reads were captured per sample, on average, using Greengenes and are comparable to other studies (Vishnivetskaya et al., 2018; Holm, et al., 2020) ~~,~~ we believe that our approach is sufficient for covering methanogen diversity.

2.10 Statistical analyses~~s~~

All statistical analyses were carried out in R (Version 3.4.4, R Core Team, 2015) using the *nlme*, *vegan*, *factoextra*, *ggplot2*, *VariancePartition* and *ggpubr* packages (Pinheiro et al., 2017; Oksanen et al., 2013; Kassambara & Mundt, 2017; Wickham, 2016; Hoffman & Schadt, 2016; Kassambara, 2018). For Analysis of Variance (ANOVAs), distribution of the data was inspected visually for normality along with the Shapiro-Wilk test. We tested homogeneity of variances using the *car* package and Levene's test (Fox and Weisberg, 2011). We report uncertainty as ± 1 standard deviation, except for land-atmosphere greenhouse gas fluxes which we report as ± 95% confidence intervals. We here define the statistical significance level at 5%.

We used ANOVAs and Bonferroni post-hoc tests on linear mixed effects models to address our second hypothesis and to evaluate significant differences and seasonal trends in greenhouse gas fluxes and dissolved gas depth profiles. ~~These were~~We performed ~~with a focus on assessing~~these tests to assess whether thaw stage (young bog or mature bog) influenced greenhouse gas fluxes and dissolved gas depth profiles. This approach was used to test for significant differences in CH_4 fluxes, ratio of CH_4:C emissions, and source ^{13}C-CH_4 signature intercepts of Keeling plots between young bog and mature bog stages. In each linear mixed effect model, sampling month and peatland stage were defined as fixed effects whereas sampling collar was defined as a random effect. Similarly, we ~~assessed~~ tested for

significant differences between the ~~in depth profiles~~young and mature bog depth profiles with respect to ~~of~~ dissolved CH_4 and CO_2 concentrations, $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 ~~signatures~~values, ~~and~~ α_c values, and pore water chemistry. ~~between the young bog and mature bog~~ . ~~In~~ these models, sampling month and peatland stage were defined as fixed effects while sample depth was defined as a random effect.

Following microbial 16S rRNA gene sequencing ~~on an Illumina Miseq~~, sample reads were rarefied to the lowest read count of 28,129 for all subsequent analyses. These sequences represent whole microbial community data that was used to determine whether there was evidence of changes in microbial community structure representing the successional peatland stages following permafrost thaw throughout the 160 cm depth peat profile. In addition, to address our first hypothesis, ~~s~~ we assessed differences in community composition across both peat and pore water and to determine whether seasonality impacted microbial community structure in both sample matrices. Here, Bray Curtis dissimilarity matrices for overall microbial community data were used, at 999 permutations, to identify distinct groupings assessed at the 95% confidence interval in NMDS ordinations. These distinct groupings were further evaluated for significance using the non-parametric ~~Analysis of Similarities~~permutational analysis of variance (PERMANOVA) ~~(ANOSIM)~~ test.

To further test our first hypothesis, ~~Methanogens~~ methanogens were selected at the order level from our whole community data using Greengenes-assigned taxonomy. Utilizing their assigned taxonomy, the pathways through which identified methanogens conduct methanogenesis was determined by comparing our findings with the literature (Berghuis et al., 2019; Stams et al, 2019; Kendall & Boone, 2006; Zhang et al., 2020). Focusing on the methanogenic community allowed us to specifically assess how permafrost thaw affects the microbial community responsible for CH_4 production and net CH_4 emissions following thaw. We utilized our methanogenic community data to construct redundancy analyses (RDA) and

relative abundance bar plots. RDAs were conducted using a Hellinger-transformed methanogenic community. Explanatory variables (i.e., dissolved concentrations of CO_2, CH_4, DOC, temperature, enzymatic activity estimate, thaw stage, depth, and distance to water table) were scaled about the mean. These explanatory variables had variance standardized, were checked for collinearity (parameters with variance inflation value > 10 were removed) and selected for significance using backward selection, set at 1,000 permutations. The significance of the RDA model, and of each axis was tested using ANOVAs, set at 999 permutations. Variance partitioning analyses were conducted to assess the contribution of significant environmental parameters (i.e., thaw stage and distance to water table) on the structuring of the Hellinger-transformed methanogenic community. Distance from water table reflects the distance (in cm) a certain sample is from the water table in different stages of thaw (young bog and mature bog). Due to the smaller size of our methanogenic community relative to the total community, and the lack of some data at certain depths, we combined pore water and peat samples together for these analyses. Relative abundance, which measures how common or rare a particular microorganism is relative to ~~others in~~ the entire microbial community, of methanogenic orders related to acetoclastic or hydrogenotrophic methanogenesis processes were plotted according to depth. Significant differences in methanogenic community composition between depths were assessed using the non-parametric Kruskall-Wallis test with a Benjamini-Hochberg correction for multiple comparisons, after running a Wilcox rank sum test.

3. Results

3.1 Site environmental conditions

The young bog was wetter and warmer than the mature bog throughout the May – September 2018 study period. In June, following snowmelt, the water table was at its highest

at 2.2 ± 0.6 cm above the surface in the young bog. The highest water table position in the mature bog was 17.5 ± 1.9 cm below the peat surface and observed in July. The water table dropped during the season and in September was 5.7 ± 2.2 cm and 27.3 ± 1.2 cm below the peat surface, in the young bog and mature bog respectively. In the plateau, the seasonally thawed layer gradually deepened during the growing season, with an active layer depth of 79.5 ± 13.7 cm measured in September. The water table in the peat plateau followed the deepening of the seasonally thawed layer.

Soil temperatures followed the seasonal climate but were dampened and had temporal lags in deeper peat layers (Figure S1a). The highest young bog and mature bog soil temperatures at 10 cm depth occurred in July, at 14.3 and 14.1 °C, respectively. At 100 cm depth the maximum temperatures occurred in August and September, at 8.6 and 6.9 °C, respectively for the young and mature bog. Soil temperatures at 250 cm were still rising at the end of September, peaking at 4.1 and 3.2 °C in the young bog and mature, respectively. The young bog was consistently warmer than the mature bog throughout the study by on average 0.9 ± 0.9 °C, 1.8 ± 1.0 °C, and 0.5 ± 0.4 °C at 10 cm, 100 cm, and 250 cm depths, respectively.

Across all depths and sampling occasions, average pH -was higher (ANOVA: $F_{(1, 77)} = 35.2$, $P < 0.001$) in the young bog than in the mature bog at 4.1 ± 0.2 and 3.9 ± 0.2 respectively. In contrast, DOC at 69.2 ± 18.4 and 53.8 ± 5.4 mg C L^{-1} (ANOVA: $F_{(1, 82)} = 38.7$, $P < 0.001$) and total dissolved nitrogen at 1.5 ± 1.4 and 0.9 ± 0.1 mg L^{-1} (ANOVA: $F_{(1, 82)} = 12.8$, $P < 0.01$) were higher in the mature bog than in the young bog, respectively. ~~Average SUVA was also higher in the young bog (3.2 ± 0.4 L mg C^{-1} m^{-1}) compared to the mature bog (2.6 ± 0.4 L mg C^{-1} m^{-1}), indicating DOM with a greater aromatic content in the young bog. In contrast, DOC (69.2 ± 18.4 and 53.8 ± 5.4 mg C L^{-1}) and total dissolved nitrogen (1.5 ± 1.4 and 0.9 ± 0.1 mg L^{-1}) were higher in the mature bog than in the young~~

~~bog, respectively.~~ Average SUVA values were higher (ANOVA: $F_{(1, 82)} = 103.5$, $P < 0.001$) in the young bog (3.2 ± 0.4 L mg C^{-1} m^{-1}) compared to the mature bog (2.6 ± 0.4 L mg C^{-1} m^{-1}), indicating DOM with a greater aromatic content in the young bog. However, average spectral slope ($S_{250-465}$) values were also greater (ANOVA: $F_{(1, 81)} = 6.9$, $P < 0.05$) in the young bog (-0.016 ± 0.002 nm^{-1}) compared to the mature bog (-0.017 ± 0.003 nm^{-1}), indicating lower molecular weight and decreasing aromaticity. Average phenolics (0.6 ± 0.2 and 0.6 ± 0.2 mg L^{-1}) ~~, spectral slope ($S_{250-465}$: -0.016 ± 0.002 and -0.017 ± 0.003 nm^{-1}),~~ and phosphate (PO_4^{3-}: 9.0 ± 14.3 and 6.7± 3.0 µg L^{-1}) were similar between the young bog and mature bog, respectively, across all depths and sampling occasions. Full details of DOM chemistry results can be found in Heffernan et al., (2021). Of note is the fact that the pore water chemistry was compared across all depths in this study, in contrast to Heffernan et al., (2021) in which pore water found above and below the transition indicating permafrost thaw was compared.

3.2 Concentrations and isotopic signatures of dissolved gases

Dissolved CH_4 increased with depth ~~under~~ below the water table in both the young and mature bog (Figure 2a). ~~Concentrations~~ Dissolved CH_4 concentrations ~~of CH_4~~ in the young bog increased with depth, from 19 µmol L^{-1} at 5 cm depth, to a peak of 5,400 µmol L^{-1} at 195 cm. Dissolved CH_4 concentrations ~~Concentrations of CH_4~~ in the mature bog remained low above the water table (<6 µmol L^{-1} below 25 cm), but then increased to 4,100 ± 1,700 µmol L^{-1} between 115 and 250 cm depth and peaked at 6,800 µmol L^{-1}. Dissolved CO_2 concentrations followed a very similar pattern to CH_4, increasing with depth in both the young and mature bog (Figure 2b). Again, the mature bog had overall higher concentrations, with mean average values ranging from 340 – 1,295 µmol L^{-1} and peaking at 1,500 µmol L^{-1}

at 85 cm. ~~while~~ Whereas in the young bog average values ranged from 113 – 960 µmol L^{-1} and peaked at 1,200 µmol L^{-1} at 95 cm (Figure 2b).

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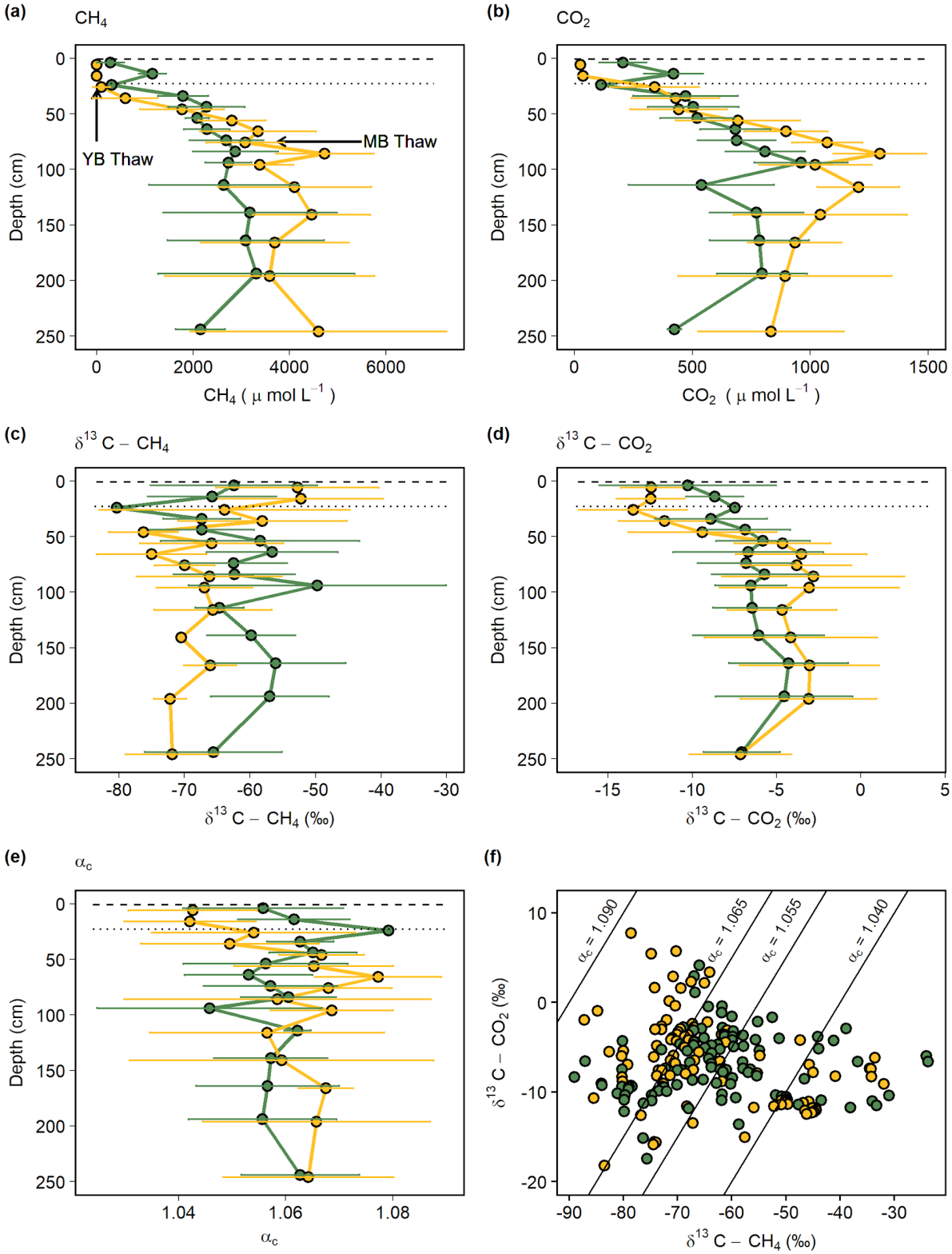

(a)
CH4
YB Thaw
MB Thaw
Depth (cm)
CH4 (μ mol L−1)
(b)
CO2
CO2 (μ mol L−1)
(c)
δ13 C − CH4
δ13 C − CH4 (‰)
(d)
δ13 C − CO2
δ13 C − CO2 (‰)
(e)
αc
(f)
αc = 1.090
αc = 1.065
αc = 1.055
αc = 1.040
δ13 C − CO2 (‰)
δ13 C − CH4 (‰)

604

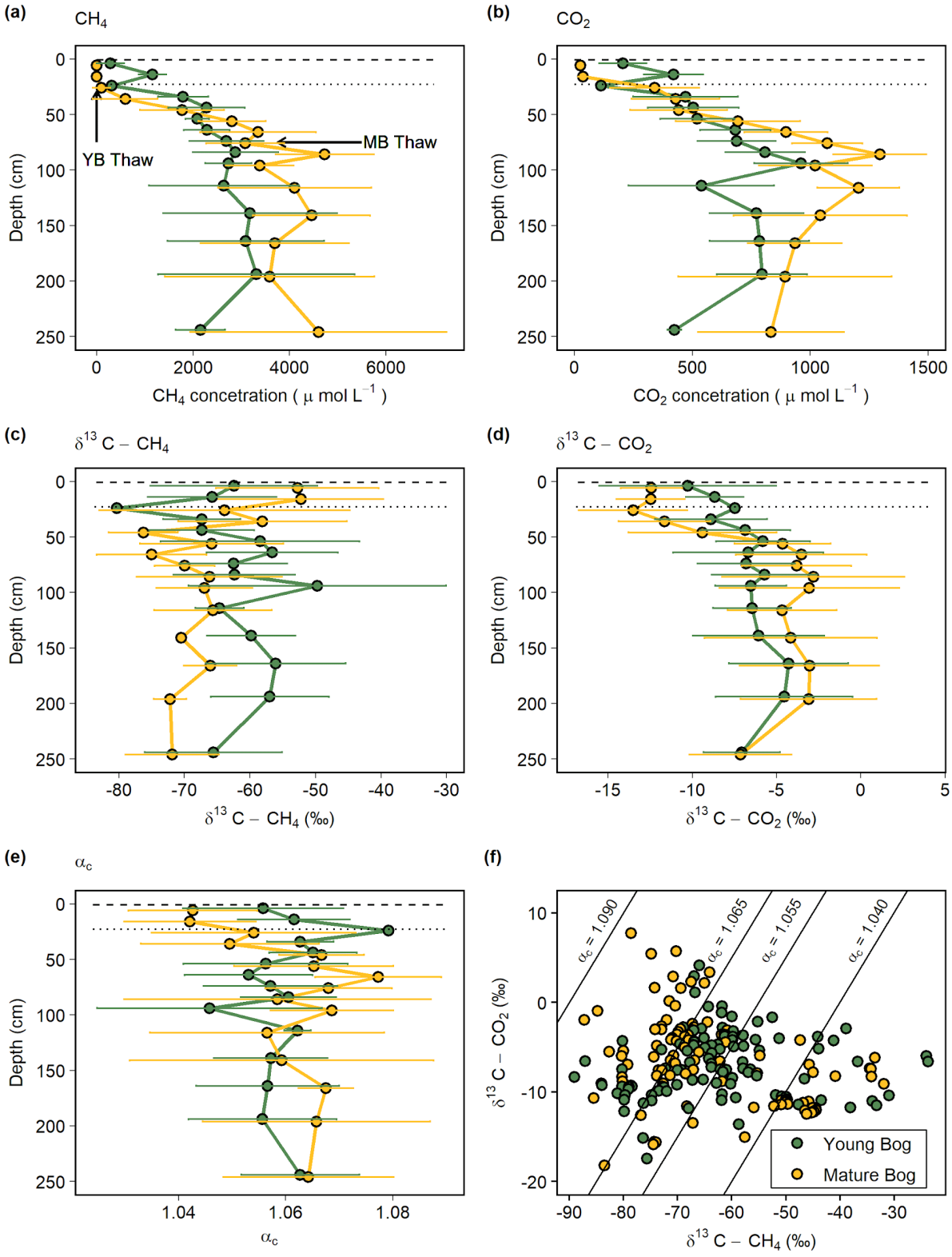


Figure 2. Average seasonal (May – September) depth profiles in the young (green, black circles) and mature (yellow, black circles) bog of (a) dissolved CH_4 concentration (μmol L^{-1}), (b) dissolved CO_2 concentration (μmol L^{-1}), (c) δ^{13}C-CH_4 (‰), (d) δ^{13}C-CO_2 (‰), and (e) apparent fractionation factor (α_c) between dissolved CH_4 and CO_2. (f) Cross-plot of corresponding δ^{13}C-CH_4 and δ^{13}C-CO_2 values (‰) in the young bog and mature bog, from

raw data used in panels (c) and (d). Diagonal lines represent different α_c where α_c 1.040 – 1.065 represents acetoclastic methanogenesis, and α_c 1.055 – 1.09 represents hydrogenotrophic methanogenesis (Whiticar, 1999). (a) – (e) Dashed and dotted horizontal lines represent water table depth in the young (YB) and mature bog (MB) respectively. Arrows in panel (a) represent depth of thaw transition in both the young (29 cm) and mature bog (71 cm), i.e., the transition from deep peat (accumulated prior to thawing) and shallow peat (accumulated post thawing).

The young bog and mature bog had distinct profiles of $\delta^{13}C$ ~~isotopic signatures~~ values for both CH_4 and CO_2 (Figure 2c, d). The young bog had no apparent trend ~~in~~with depth for both $\delta^{13}C$-CH_4 ~~by depth~~(ANOVA; $F_{(14, 45)} = 1.75$, $P = 0.08$) and $\delta^{13}C$-CO_2 (ANOVA; $F_{(14, 46)} = 1.79$, $P = 0.07$), averaging -62.4 ± 7.0 ‰ and -6.8 ± 1.6 ‰, respectively ~~ranging between -49.7 ‰ and -80.3 ‰~~ (Figure 2c, d). In the mature bog we observed significant depth trends for both ~~isotopically heavy~~ $\delta^{13}C$-CH_4 (ANOVA: $F_{(14, 43)} = 3.19$, $P < 0.01$) and $\delta^{13}C$-CO_2 (ANOVA: $F_{(14, 49)} = 6.22$, $P < 0.001$). These significant depth trends are due to isotopically heavy $\delta^{13}C$-CH_4 and light $\delta^{13}C$-CO_2 above the water table, which suggest~~ed~~ an influence from CH_4 oxidation. When comparing $\delta^{13}C$ depth profiles between the thermokarst bogs we focused on those values taken from under the water table to avoid the effect of CH_4 oxidation observed above the water table in the mature bog. Under the water table, $\delta^{13}C$-CH_4 values in the mature bog ~~had~~ were significantly lighter (ANOVA: $F_{(1, 64)} = 18.72$, $P < 0.001$) ~~^{13}C-CH_4~~ compared to the young bog at an average of -68.7 ± 5.0 ‰ and -62.4 ± 7.0 ‰, respectively ~~($F_{(1, 92)} = 17.25$, $P < 0.001$). In the young bog, ^{13}C-CO_2 had no apparent trend with depth (average -6.8 ± 1.6 ‰). The~~ Conversely, the mature bog had isotopically ~~lighter ^{13}C-CO_2 above the water table and was isotopically~~ heavier $\delta^{13}C$-CO_2 than the young bog below the water table (ANOVA: $F_{(1, 9971)} = 5.33$13.86, $P < 0.05$001).

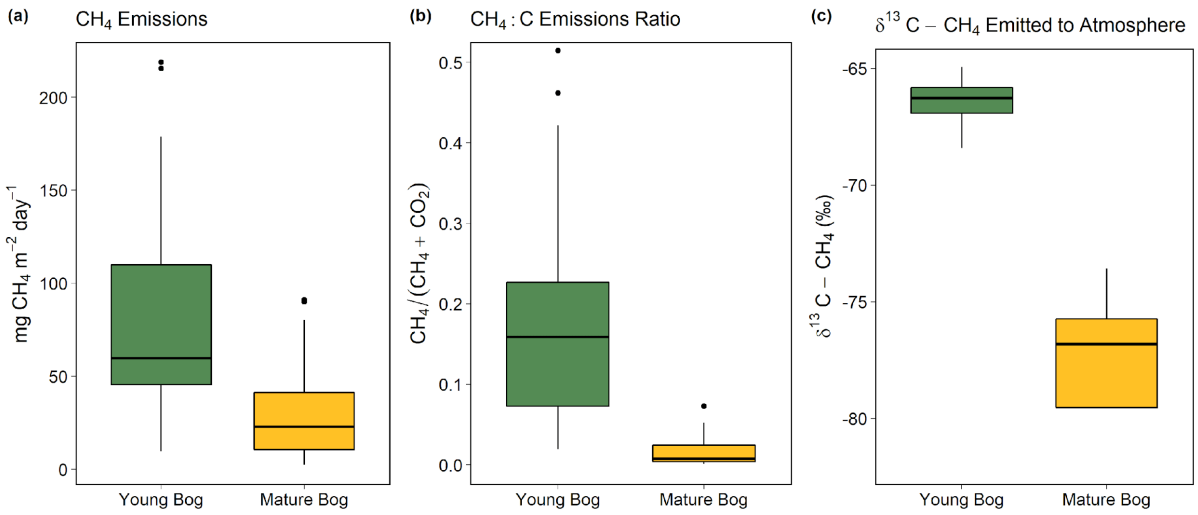
The apparent fractionation factor (α_C) is a robust parameter to characterize the relative contribution of CH_4 production pathways, with values of 1.040 – 1.060 indicating acetoclastic methanogenesis and 1.060 – 1.090 for hydrogenotrophic methanogenesis

(Chanton et al., 2005). Similar to the gas $\delta^{13}C$ depth-profiles, we found no clear trend with depth ~~in~~ for α_C values ~~with depth~~ in the young bog (ANOVA; $F_{(14, 44)} = 0.87$, $P = 0.59$) with an average of 1.058 ± 0.012 and range of 1.018 – 1.079 (Figure 2e). In the mature bog, we found a clear depth trend in α_C values (ANOVA: $F_{(14, 43)} = 5.71$, $P < 0.001$). Similar to the $\delta^{13}C$ depth profiles in the mature bog, this significant depth trend ~~the average~~in α_C ~~was lowest in samples collected above the water table at 5, 15, and 25 cm, likely~~ is due to the influence of CH_4 oxidation above the water table, with the lowest α_C values being those from samples collected above the water table at 5, 15, and 25 cm. The average α_C beneath the water table in the mature bog was 1.064 ± 0.017 and ranged from 1.015 – 1.094. When comparing α_C values from beneath the water table between the young and mature bog, we found that α_C values were significantly lower in ~~similar to the average values found in~~ the young bog (ANOVA: $F_{(1, 99}$~~$_{63)}$~~ = ~~0.7~~30.8, P = < 0.~~4~~001).

In the isotopic ratio cross-plot of $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 (Figure 2f), most of the young bog had α_C values of between 1.055 – 1.065 (29 in total), with a greater number of samples (21) between α_C =1.040 – 1.055, compared to the mature bog (15). In contrast, a greater proportion of the mature bog samples had α_C > 1.065 (42 in the young bog and 52 in the mature bog). There was no clear depth trend in the α_C values and no samples in this study had α_C ~~></~~ > 1.090. Several samples (13) from the young bog and mature bog had α_C values of < 1.040, likely due CH_4 oxidation (Knorr et al., 2009). ~~The $\delta^{13}C$-CH_4 signature of CH_4 emissions (intercept values from Keeling plots), in the young bog were significantly greater than those observed in the mature bog (Figure 3c; $F_{(1, 4)} = 20.67$, $P < 0.05$), suggesting a greater influence of acetoclastic CH_4 production. Overall, the isotopic data indicates a general dominance of hydrogenotrophic methanogenesis in both sites, but a greater contribution of acetoclastic methanogenesis in the young bog relative to the mature bog.~~

3.3 Magnitude and isotopic signature of land-atmosphere gas fluxes

The young bog had almost three times greater average CH_4 fluxes than the mature bog during the May – September study period, at 82.3 ± 21.9 mg CH_4 m^{-2} day^{-1} and 30.8 ± 10.6 mg CH_4 m^{-2} day^{-1}, respectively (Figure 3a). Fluxes of CH_4 in the young bog were greatest between June and August, ranging from 80.6 ± 40.3 mg CH_4 m^{-2} day^{-1} to 100.9 ± 63.1 mg CH_4 m^{-2} day^{-1}. The lowest young bog CH_4 fluxes were observed in September at 55.0 ± 17.7 mg CH_4 m^{-2} day^{-1} (Figure S3a). Mature bog CH_4 fluxes were greatest in September (55.8 ± 21.1 mg CH_4 m^{-2} day-1) and lowest in May (5.6 ± 2.7mg CH_4 m^{-2} day^{-1}). Ecosystem respiration (CO_2 emissions measured with dark chambers) was significantly lower in the young bog than mature bog, with study period averages of 0.6 ± 0.3 and 1.9 ± 0.3 g CO_2 m^{-2} day^{-1}, respectively (Figure S32b). Maximum ecosystem respiration in the young bog occurred in August (1.6 g CO_2 m^{-2} day^{-1}) and was much lower during the other four months (monthly averages of 0.2 to 0.4 g CO_2 m^{-2} day^{-1}). Maximum eEcosystem respiration rates in the mature bog were elevated was found for the periodfrom June to August (monthly averages between 2.1 and 2.6 g CO_2 m^{-2} day^{-1}), with lower emissionsand decreased in September (0.8 g CO_2 m^{-2} day^{-1}). The proportion of total C emissions (sum of CH_4 and CO_2 emissions) released as CH_4 wereas an order or of magnitude greater in the young bog than mature bog stage, at 18 and 2% respectively. This, resulting fromwas a result of both the young bog higher CH_4 emissions and lower ecosystem respiration (Figure S3) in the young bog. The $\delta^{13}C$-CH_4 signature of CH_4 emissions (intercept values from Keeling plots), in the young bog were significantly greater than those observed in the mature bog (Figure 3c; ANOVA: $F_{(1, 4)} = 20.67$, $P < 0.05$)., suggesting a greater influence of acetoclastic CH_4 production. The average $\delta^{13}C$-CH_4isotopic signature of CH_4 emissions in the young bog CH_4 emissions (n = 4) was -66.5 ± 1.4‰ (Figure 3c95% CI) and 78.5 ± 5.6‰ (95% CI; Figure 3c) in the , whereas the average from mature bog emissions (n = 4).) was -78.5 ± 5.6‰ (95% CI).



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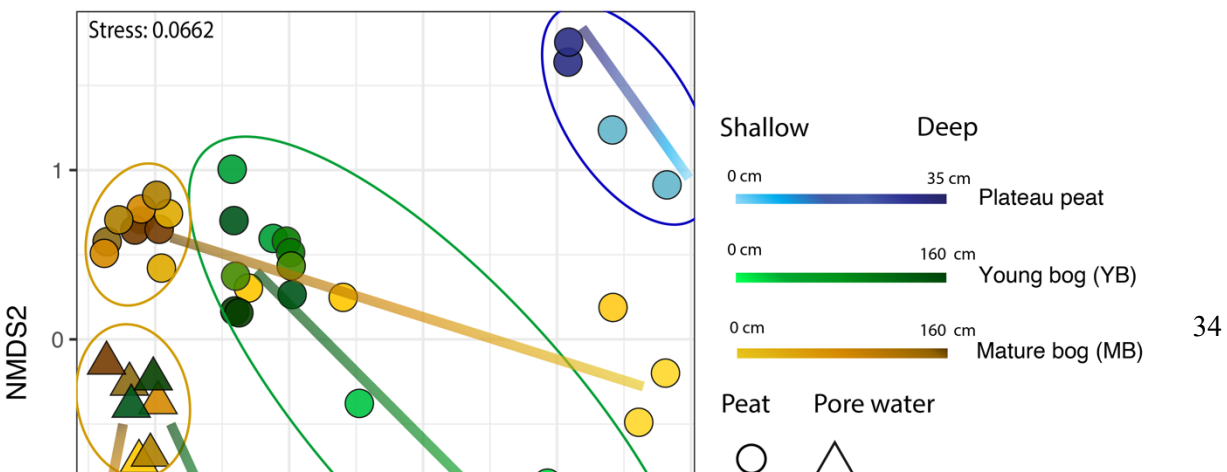
689 **Figure** 3. Magnitude and isotopic signature of greenhouse gas fluxes from the young bog
690 (green) and mature bog (yellow) shown as boxplots. Boxes represents the interquartile range
691 (25 – 75%), with median shown as black horizontal line. Whiskers extend to 1.5 times the
692 interquartile range (distance between first and third quartile) in each direction, with outlier
693 data plotted individually as black dots (a) The magnitude of net land-atmosphere CH_4
694 emissions as measured by soil chambers. (b) The ratio between CH_4 emissions and the sum of
695 CO_2 emissions (ecosystem respiration) and CH_4, both standardized to per g C. (c) Intercept
696 values of Keeling plots indicating the δ^{13}C-CH_4 signature of CH_4 emissions. Isotopically
697 heavier (i.e., less negative) δ^{13}C-CH_4 is produced via acetoclastic methanogenesis, whereas
698 isotopically lighter (i.e., more negative) δ^{13}C-CH_4 is produced via hydrogenotrophic
699 methanogenesis, The CH_4 and CO_2 land-atmosphere fluxes shown in (a) and (b) were
700 measured once a month from May – September 2018. The δ^{13}C-CH_4 of CH_4 emitted to the
701 atmosphere was measured in September and October 2016 (see methods for details and
702 Figure S4 for Keeling plots).

703

704 *3.4 Microbial community structure along the permafrost peatland thaw gradient*

705 We used NMDS ordinations to assess differences in microbial community structure
706 between solid peat and pore water samples, between sampling depths, and between the
707 plateau, young bog, and mature bog. The only exception was the plateau, where only peat
708 samples were collected (~~i.e~~i.e.,~~.~~ no pore water samples). Microbial community structure in
709 peat was determined to be significantly different from porewater microbial communities
710 (PERMANOVA, R^2= 0.13, $P < 0.05$, Figure 4). The differences observed in the microbial
711 community structure between peat and pore water samples could be a function of the

different extraction methods used to extract DNA (Carrigg et al., 2007). Among the pore water samples, distinct microbial communities were found to be associated with the young bog and mature bog. Similarly, microbial community structure in peat was found to be significantly distinct between the three successional stages (plateau peat, young bog and mature bog; Figure 4; PERMANOVA, $R^2 = 0.18$, ~~,~~ $P < 0.05$). There is also a common trend in vertical community structuring for all sample matrices according to depth. Changes in overall microbial community composition in both peat and pore water, across a vertical profile (to a maximum depth of 160 cm), illustrate a confluence in microbial community structure with depth in both the young and mature bog (Figure 4). In other words, community structure was most dissimilar at depths closer to the surface (Figure 4, Figure S2~~b~~, c; PERMANOVA; $R^2 = 0.16$, $P < 0.05$). This trend was particularly evident in the porewater samples (Figure 4). In the peat samples, though microbial communities did not fully converge, deeper young bog peat (i.e., 90 – 160 cm) communities did become more similar to communities found in the mature bog at intermediate depths (i.e., 30 – 70 cm), based on the nearness of sample points on the NMDS (Figure 4). We also observed that the mature bog near-surface peat samples were located closer to the plateau peat on the NMDS (Figure 4, PERMANOVA,~~;~~ $R^2 = 0.4$, $P = 0.$~~266~~1). It was not possible to assess the presence of this cyclic succession (from young bog to mature bog to plateau) in the pore water samples since we did not characterize the microbial community in the plateau pore water. Finally, we also assessed the effect of seasonality on microbial community structure ~~diversity~~ and found no effect with regards to sampling month (PERMANOVA~~ANOSIM~~; $R^2 = 0.02$, ~~P~~ $= 0.$~~559~~090).



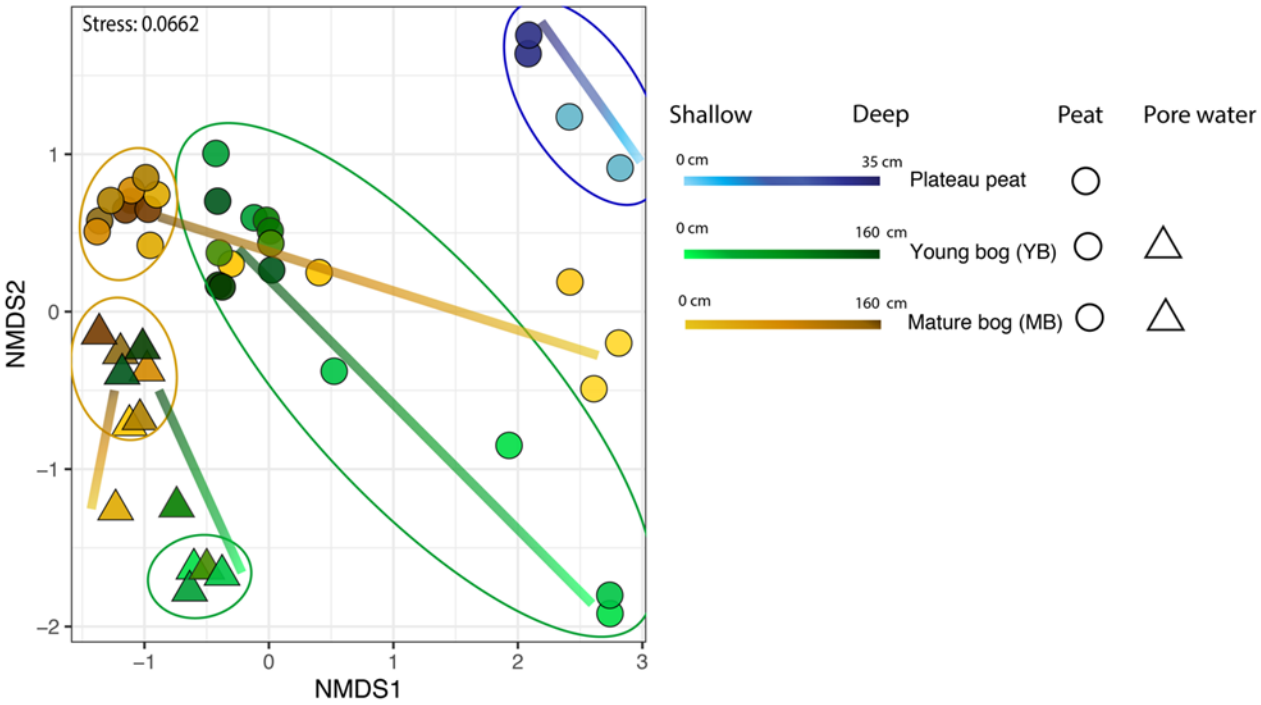


Figure 4. Microbial community distribution according to stage of peat/pore water. NMDS ordinations of amplicon sequencing variant (ASV) data demonstrate significant community dissimilarities (PERMANOVA, $R^2 = 0.13$, $P < 0.05$~~PERMANOVA; $P < 0.05$~~) according to thaw stage for both pore water (shown by the triangles) and peat (shown by the circles) samples, encircled by 95% confidence intervals. Colour gradient and lines demonstrate the shift in microbial community structure along vertical depth profiles where lighter shades indicate samples closer to the surface.

The total archaeal community comprised 6% of the entire microbial dataset. Methanogen-related orders comprised 54% of this archaeal dataset and demonstrated marked differences in the relative abundance of acetoclastic-related methanogens according to thaw stage and depth in both peat and pore water samples (Figure 5; Figure S2). In the young and mature bog peat samples, hydrogenotrophic-related methanogens were ubiquitously present throughout both depth profiles (Figure 5a). In comparison, acetoclastic-related methanogens exhibited a relatively restricted presence, only present at specific depths (Figure 5a). These communities were most abundant (>25% of the total methanogenic community) near the surface in the young bog, just above and below the thaw transition zone (Figure 5a). In the pore water, hydrogenotrophic methanogens were also dominant throughout depths in both stages of thaw (Figure 5b). However, in contrast to peat samples, acetoclastic methanogens were virtually absent in the pore water, although minimally present (i.e., $\leq$ 10% relative abundance) at depths between 35 and 155 cm, all found below the thaw transition zone (Figure 5b).

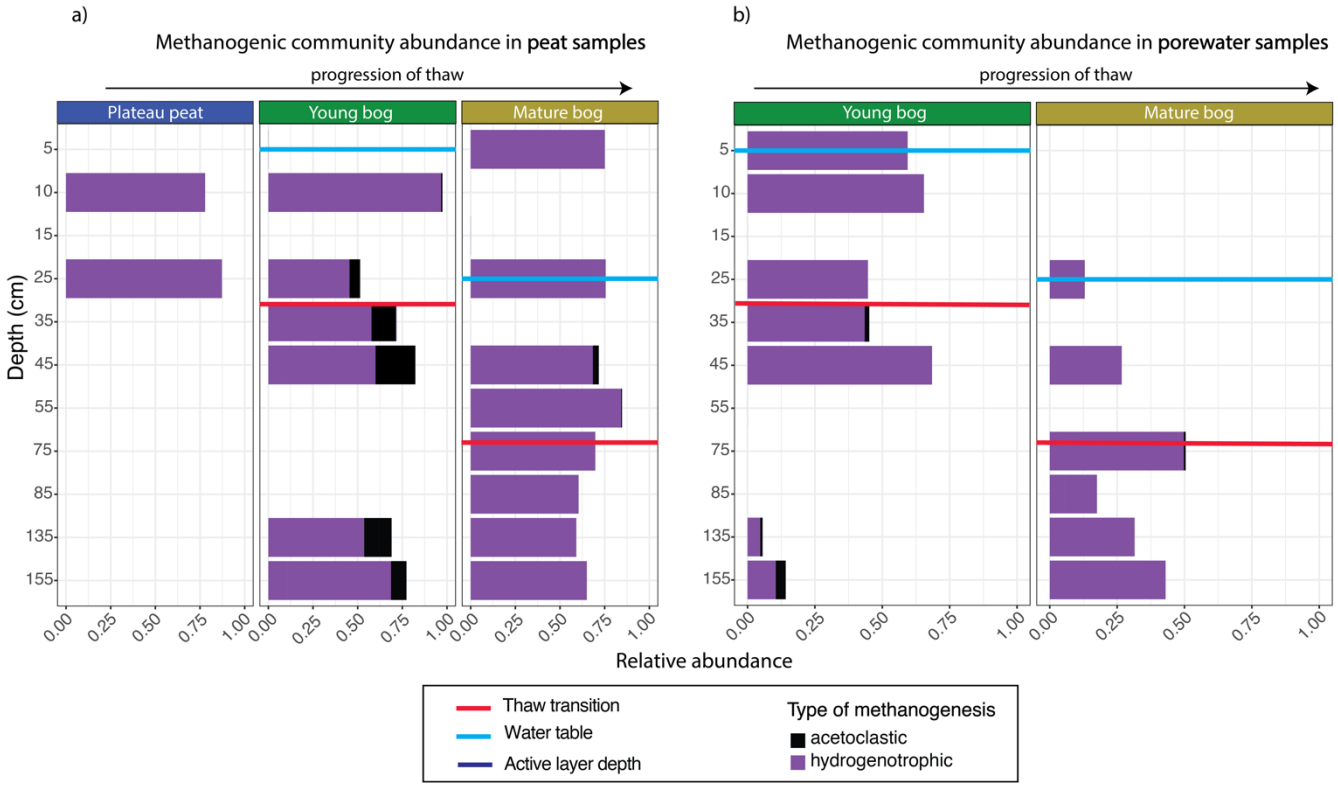


Figure 5. Relative abundance of archaeal orders according to putative methanogenic capability, along a depth profile for peat and pore water samples. Samples are arranged according to depth (y axis), with the relative abundance of methanogenic archaea resolved shown on the x axis. Note that the y axis does not uniformly progress in 10 cm increments. Progression of thaw is shown from plateau peat to young bog to mature bog at the top of the figures, with position of water table shown in blue for each panel. Red lines demonstrate thaw transition zone for the young bog and mature bog. (a) Stacked bar plot of methanogenic Archaea for all peat samples. Samples demonstrate significant differences in putative methanogen composition between all stages (Kruskall-Wallis test & Wilcox rank sum test, with Benjamini-Hochberg corrected p-values, $P < 0.05$). (b) Stacked bar plot of methanogenic Archaea for all pore water samples. Samples do not demonstrate significant differences in putative methanogen composition between stages (Kruskall-Wallis test, with Benjamini-Hochberg corrected p-values, $P = 0.965$).

Using a redundancy analysis (RDA, Figure 6) we found that 27.6% of variation in the methanogenic community was explained by two variables: thaw stage (ANOVA, $P < 0.05$) and depth from the water table (ANOVA, $P < 0.05$). Although these were the only two parameters that were identified as significant variables impacting microbial community structure when using a backward stepping model, it should be noted that there may be more variation in the community that our experimental design does not take into account as a result of unconstrained variation represented by plant-microbe and/or microbe-microbe interactions (Boon et al., 2014). Nonetheless, ~~T~~the 27.6% variation explained is in accordance with other studies conducted in permafrost impacted regions using similar methods, where the ~~percentage of~~percentage of explained variation falls between ~~-~~6% (low) to 43% (high) (Comte et al., 2015; Hough et al., 2020). Next, we used variance partitioning to assess the extent to which thaw stage and depth from the water table (~~i.e.~~i.e., the significant environmental variables identified by the RDA) explained the variation in only the methanogenic community structure (Figure 6). Based on this analysis, thaw stage explained 18.4% and distance to the water table explained 4.3% of methanogenic community variation, respectively.

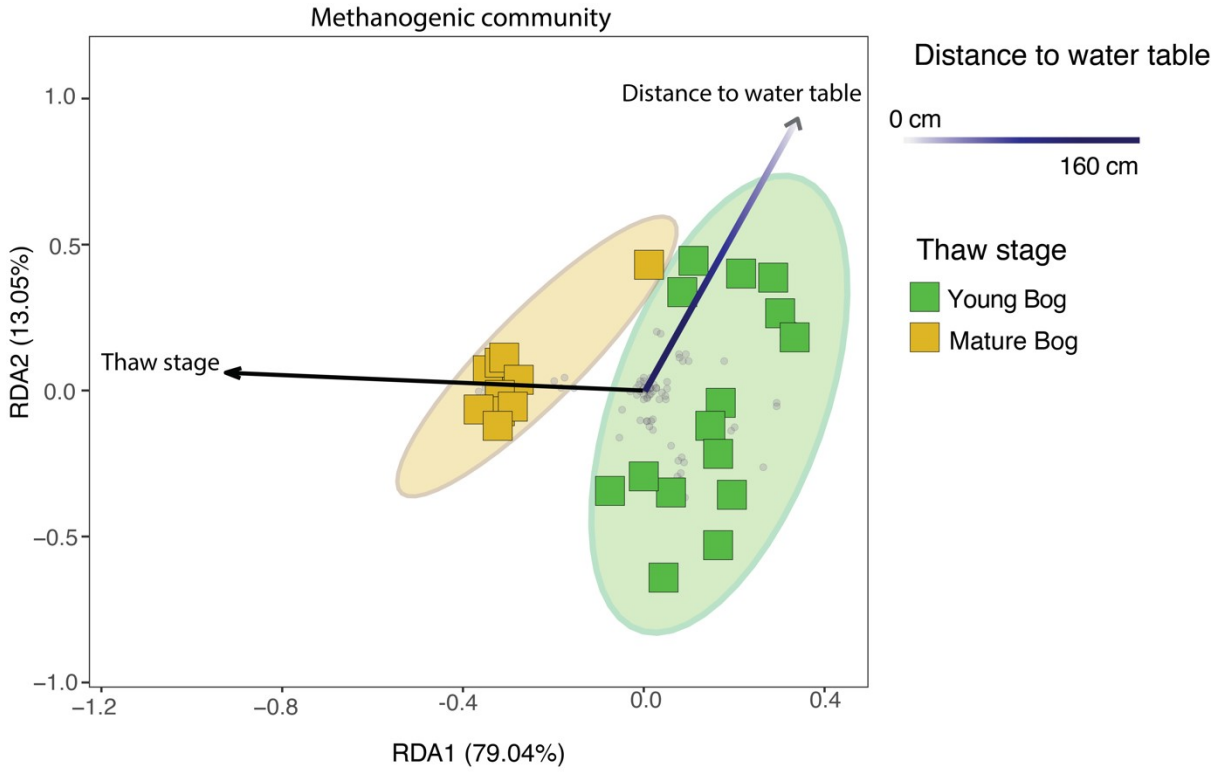


Figure 6. Redundancy analysis (RDA) exploring ~~biotic~~significant biotic and abiotic variables influencing the total methanogenic community (adjusted R^2 = 27.6%), as determined by a backward stepping RDA model in the peat and pore water samples**.** All parameters that were used in model are described in section 2.10 of the Methods. Grey dots in the panel demonstrate the distribution of all ASVs in the methanogenic dataset. Shaded ellipses represent the 95% confidence intervals for microbial community structure ~~diversity~~ according to peatland thaw stage (young bog vs mature bog). Only significant (ANOVA, $P < 0.05$) variables are shown. Using variation partitioning, we found that peatland thaw stage significantly explains about 18.4% of methanogenic community variation whereas distance to water table explained 4.3%. Both axes are significant (ANOVA, $P < 0.05$).

4. Discussion

Our study shows that high CH_4 emissions from thermokarst bogs in the initial decades following permafrost thaw (young bog) are not only linked to environmental conditions (wetness, soil temperature, vegetation), but also driven by relatively increased microbial CH_4 production through the energetically more favourable acetoclastic methanogenesis pathway.

Evidence of acetoclastic methanogens and CH_4 produced via the acetoclastic metabolic pathway was found in the young bog both near the surface and at depths below the thaw transition (i.e., in peat that accumulated prior to permafrost thaw). We are unable to determine whether these greater CH_4 emissions in the initial decades following thaw are due to the mineralization of labile organic matter released from previously frozen peat, or are driven solely by fresh, labile DOM derived from surface vegetation leached throughout the peat profile. However, previous work in the discontinuous permafrost region in the Interior Plains of western Canada has found a limited contribution of previously frozen organic matter contributing to surface CH_4 emissions in thermokarst bogs (Cooper et al., 2017). Elevated CH_4 emissions then slow over the following centuries with succession into a mature thermokarst bog stage where CH_4 production is almost exclusively through the hydrogenotrophic pathway.

4.1 Shift in microbial community assemblages along a permafrost thaw gradient

Microbial communities varied along the permafrost thaw gradient; among different thaw stages (permafrost peat plateau, young bog, and mature bog), with peat depth (surface down to 160 cm), and between different sample types (solid peat and pore water). We found clear differences in microbial communities between the young bog and mature bog, despite similar peat stratigraphy up to the surficial vegetation (Heffernan et al., 2020), where dominant *Sphagnum* species varied. The greater height of the peat surface above the water table and ~~relatively~~ drier conditions in the mature bog, due to the slow accumulation of new peat over centuries, leads to a shift in vegetation composition from hydrophilic *Sphagnum* and graminoids towards more drought resistant *Sphagnum* spp. and ericaceous shrubs. This shift in water table position and vegetation community, along with a decrease in temperatures (Figure S1a) due to the thermal insulating properties of *Sphagnum* peat (Kujala, Seppälä, &

Holappa, 2008) appears to have caused the observed differences in microbial communities between the young and mature bog, even at depths >1 m. Microbial communities were most dissimilar between the peat plateau and young bog. This was unsurprising given the abrupt shift from the elevated, frozen, and relatively dry peat plateau forest to the young bog where the surface was saturated, dominated by hydrophilic vegetation and had warmer temperatures. We further noted that the microbial community of the mature bog was more similar with the peat plateau than with the young bog. Paleo-records in the region (Heffernan et al., 2020; Pelletier et al., 2017; Zoltai, 1993) show that many peatlands have undergone cyclical permafrost developments, as thermal insulating properties of *Sphagnum* peat in mature bogs leads to the re-aggradation of permafrost peat plateaus. Our study suggests that the peat plateau microbial community is influenced by the preceding mature bog microbial community as permafrost aggrades.

The most dissimilar microbial community composition was observed between samples near the surface and those at depth (i.e., down to 160 cm), as has also been ~~also~~ observed in other permafrost ecosystems (Frey et al., 2016; Monteux et al., 2018). Shifts in microbial community composition along the thaw gradient were most evident nearer the surface, whereas communities found at depth were similar between the young bog and mature bog (Figure 4). At the surface, microbial community structure is influenced by the successional vegetation community (Hodgkins et al., 2014) and the role that vegetation, particularly graminoids which are found in the young bog, has on microbial community structure has been well documented in northern peatlands (Robroek et al., 2015, 2021; Bragazza et al., 2015). Moderately acidic, saturated peatlands with hydrophilic vegetation, similar to the young bog, have been shown to harbour acid tolerant fermenting bacteria that produce substrates for methanogenesis and are trophically linked with methanogens (Wüst et al., 2009). Thus, the interaction between water table position, pH, and vegetation community

influences the substrates available to the microbial community, which in turn impacts the surface community's structure (Kotiaho et al., 2013). In contrast, communities at depth are known to be influenced by peat properties, such as peat chemistry and degree of decomposition, and the paleoenvironment under which they originally colonized (Lee et al., 2012; Holm et al., 2020). In the young and mature bog both peat properties (humification indices including FTIR 1630/1090 cm^{-3} and C:N ratios) and the paleoenvironment at depth are similar (Heffernan et al., 2020), which may explain the observed convergence of microbial community structure. Nonetheless, although there are some similarities at depth between both young and mature bog, microbial communities inhabiting either are still distinct (Figure 4). This is emphasized by the differing abundance of Archaea that participate in hydrogenotrophic or acetoclastic methanogenesis (Figure 5) in both stages down the peat profile.

As has been shown previously in other thermokarst peatlands (McCalley et al., 2014), the young and mature bog stages were dominated by hydrogenotrophic methanogens. However, acetoclastic methanogens were relatively more abundant in the young bog (Figure 5), particularly at or below the transition in peat that accumulated prior to permafrost thaw. Thaw stage and distance from the water table were found to influence the methanogenic community composition (Figure 6), with distance from the water table dictating where anoxic conditions persist (Blodau et al., 2004) and thus where methanogenic colonization can occur. The influence of vegetation communities associated with different thermokarst peatland stages on methanogenic community composition has previously been attributed to the role of plant derived DOM serving as the substrate for CH_4 production (Liebner et al., 2015; McCalley et al., 2014). The presence of hydrophilic vegetation, particularly graminoids, in the saturated young bog provides the precursors for fermentation, yielding acetate (Liebner et al., 2015; Ström et al., 2003, 2012, 2015) and serving as the substrate for acetoclastic CH_4

production. The downward transport from the surface of plant derived DOM in the young bog (Chanton et al., 20088) likely provides sufficient acetate for the establishment of acetoclastic methanogens at depth in this environment.

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4.2 Production and emissions of CH_4 along a peatland thaw gradient

Isotopic signatures ($\delta^{13}C$) of dissolved CO_2 and CH_4 and α_C values in porewater and the of $\delta^{13}C$ signature of CH_4 emitted to the atmosphere provided further evidence of relatively elevated acetoclastic methanogenesis in the young bog stage. The general increase in $\delta^{13}C$-CO_2 with depth observed at both sites (Figure 2d) indicates accumulation of

isotopically heavier $\delta^{13}C\text{-}CO_2$ which is likely explained by the preferential use of isotopically lighter $\delta^{13}C\text{-}CO_2$ during hydrogenotrophic methanogenesis (Hornibrook et al., 2000). As a result, CH_4 tends to become lighter with depth and this was particularly apparent in the mature bog (Figure 2c). This leads to the average α_C values of 1.064 ($\delta^{13}C\text{-}CH_4$; -68.7‰) in the mature bog, which were significantly higher than the 1.058 ($\delta^{13}C\text{-}CH_4$; -62.4‰) observed in the young. Together, the $\delta^{13}C\text{-}CH_4$ and $\delta^{13}C\text{-}CO_2$ data and the resulting α_C depth profiles suggest that the majority of CH_4 is produced via the hydrogenotrophic methanogenic pathway, which supports the findings of the microbial community analysis (Figure 5). Our isotope data also suggests that a greater proportion of CH_4 is produced via acetoclastic methanogenesis throughout the profile in the young bog compared to the mature bog (Figure 2c – f). This is evident from lower average α_C values found in the young bog compared to the mature bog, and greater number of these young bog α_C values falling between 1.040 – 1.065 which represents acetoclastic methanogenesis (Whiticar, 1999).~~,~~ ~~which~~ These findings again agree~~s~~ with the relatively greater abundance of acetoclastic methanogens observed at that site (Figure 5).

In this study we found that average CH_4 emissions in the initial decades following thaw, in the young bog stage, were 2.5 – 3 times greater than emissions measured in the mature bog stage which had thawed ~200 years ago (Figure 3a). Furthermore, the proportion of CH_4 to overall C emissions (Figure 3b) was considerably greater in the young bog than in the mature bog. In the mature bog the lower water table position leads to both increased CO_2 emissions and decreased CH_4 emissions, resulting in a reduced fraction of C emissions as CH_4. Previous studies have shown similarly increased CH_4 emissions in the initial decades following thaw (Johnston et al., 2014; Wickland et al., 2006). While our pore water chemistry data is inconclusive with regards to organic carbon characteristics, other work in thermokarst bogs in the Interior Plains of western Canada has shown that the organic matter derived from the

young bog vegetation community is highly labile (Burd et al., 2020). Previous work at our study site has shown that the vegetation community in the young bog is associated with greater potential enzymatic degradation of organic matter (Heffernan et al., 2021). Hydrolysis of plant derived organic matter by extracellular enzymes leads to the formation of monomers (Kotsyurbenko, 2005). These monomers can be further degraded to form acetate and other percussors for methanogenesis when present with anaerobic fermenting bacteria (Hamberger et al., 2008) and near the surface and vegetation inputs (Hädrich et al., 2012). Our study shows that these higher CH_4 emissions are likely linked to increased wetness, temperatures, and a vegetation community associated with more labile organic matter which favour a greater proportion of CH_4 produced via acetoclastic methanogenesis, as shown by our $\delta^{13}C$-CH_4, α_c depth profiles and microbial community composition analyses.

Many factors, including environmental conditions and microbial community structure likely contribute to the differences in net CH_4 emissions from the young and mature bog (Figure 3a). Methane oxidation has been shown to be an important regulator of post-thaw CH_4 emissions (Perryman et al., 2020) and to result in isotopically heavier (i.e., less negative) $\delta^{13}C$-CH_4 and lighter (i.e., more negative) $\delta^{13}C$-CO_2 (Whiticar, 1999). Our data suggests the role of CH_4 oxidation was different between sites. Methane oxidation was apparent in the $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 signatures above the water table in the mature bog but no CH_4 oxidation is evident in the young bog (Figure 2c, d). The difference in gas flux $\delta^{13}C$ signatures (Figure 3c) also suggests a greater prevalence of CH_4 oxidation in the mature bog. However, increased oxidation above the water table in the mature bog is likely not fully responsible for the observed differences in CH_4 surface emissions and depth profiles between the young and mature bog. Lower soil temperatures, a vegetation community associated with reduced substrate availability, the dominance of hydrogenotrophic methanogenesis throughout the peat profile, and a deeper water table position all contribute to the lower CH_4

production and higher CH_4 oxidation observed in the mature bog. However, the observed differences between the young and mature bog CH_4 emissions and depth profiles are likely not due solely to increased CH_4 oxidation above the water table in the mature bog. The lower CH_4 emissions and greater dominance of hydrogenotrophic methanogenesis in the mature bog relative to the young bog presumably arise from lower soil temperatures, a vegetation shift and associated reduction in labile C substrates as peat aggrades in the mature bog in addition to a deeper water table that contributes both to lower CH_4 production and higher potential for CH_4 oxidation. HoweverNonetheless, using this interdisciplinary approach, we are unable to determine the relative contribution of acetoclastic methanogenesis at each depth to the overall emissions at the surface.

Our results, and those of others (Euskirchen et al., 2014; Johnston et al., 2014), have shown that CH_4 emissions exhibit seasonal variation (Figure S32a, c) & Figure S3cb). However, in contrast to some previous findings (Ebrahimi & Or, 2017), we did not observe a corresponding seasonal response in the microbial community composition (Figure S23a). This may be a sampling design effect since our study spanned only two months (June and September), compounded by the fact that we did not have replicate samples to test the robustness of this finding. However, other studies have also shown that soil microbial community growth is not impacted by seasonal variations in temperature (Simon et al., 2020) and that microbial communities require a longer time scale (years-decades-centuries) to respond to temperature following thaw (Feng et al., 2020). Our results corroborate these observations, suggesting a long-term response in the microbial community composition to the ecological shifts associated with autogenic peatland succession following permafrost thaw. Autogenic peatland succession following thaw occurs on the decade to century timescale, shifting from recently thawed to mature thermokarst bogs (Camill, 1999). Both recently thawed (young) and mature thermokarst bogs have distinct hydrological regimes, vegetation

communities, and peat chemistry. Following thaw, associated changes in vegetation and litter input alters microbial community composition and activity (Adamczyk et al., 2020; Kirkwood et al., 2021). Such changes in microbial community structure thus impact CH_4 emissions from thermokarst peatlands. Under predicted climatic warming scenarios differences in microbial community composition have been shown to be increasingly driven by seasonally independent variables such as substrate quality and the legacy effects of soil temperatures (Luláková et al., 2019). This study suggests that the ~~ecological conditions~~environmental conditions required for increased methanogenic activity at depth is limited to the initial decades following thaw, after which the microbial community structure changes in response to lowering of the water table, lower soil temperatures and shifts in the vegetation community.

5. Conclusion

This study demonstrates that higher CH_4 emissions in thermokarst bogs in the initial decades following thaw are driven by shifts in vegetation communities that produce organic matter inputs of varying lability (Burd et al., 2020) and prevalence of anoxic conditions, which was associated with an increase of acetoclastic methanogenesis in our site. The influence of this pathway was apparent at depth throughout the peat profile. With succession following thaw towards a mature thermokarst bog, a shift in water table position and vegetation composition seems to reduce the role of acetoclastic methanogenesis pathway. Previous work at this site (Heffernan et al., 2021) and other thermokarst peatlands in the discontinuous permafrost zone of boreal western Canada (Burd et al., 2020) have indicated that the vegetation community found in the initial decades following permafrost thaw is associated with increased potential enzymatic degradation and biodegradability of organic matter compared to that found in the mature bog. Average growing season CH_4 emissions

were 2.5 – 3 times greater in the recently thawed young bog. Overall, C emissions in the young bog contained proportionally more CH_4 than those from the mature bog, due to greater CH_4 production and also reduced CO_2 emissions. These greater CH_4 emissions in the young bog are driven by a higher contribution to surface emissions from CH_4 produced throughout the peat profile by acetoclastic methanogens. The response of the microbial community to permafrost thaw is tied to the shifting ~~ecological conditions~~environmental conditions associated with peatland autogenic succession. Warmer and wetter conditions in the initial decades following thaw, in conjunction with a vegetation community associated with greater availability of labile plant leachates (Bragazza et al., 2015), provides favourable conditions for acetoclastic methanogens throughout the peat profile. Given the projected increases in thermokarst peatland formation (Olefeldt et al., 2016), our study suggests that we can expect a pulse of CH_4 emissions from current regions of the discontinuous permafrost zone. This pulse will be driven, in part, by increased acetoclastic methanogenesis from labile substrates in recently thawed thermokarst peatlands. However, this rapid increase in CH_4 emissions will only remain at the decadal to century scale as autogenic peatland succession results in relatively drier mature thermokarst bogs, where lower temperatures and less labile substrate availability leads to a dominance of hydrogenotrophic methanogenesis.

Data availability

All biogeochemical and enzyme datasets generated and analyzed during this study are available in the UAL Dataverse repository, [https://doi.org/10.5683/SP3/5TSH9V]. Microbial sequences used in this study can be accessed from the NCBI database, using accession number PRJNA660023.

Author contributions

All authors contributed to the conception of the work. LH and CEA performed the field work component. LH performed the biogeochemistry measurements. MAC performed the microbial measurements. LH and MAC analyzed the data and wrote the manuscript draft. All authors reviewed and edited the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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