https://doi.org/10.5194/egusphere-2025-2570
Preprint. Discussion started: 26 June 2025


# Addition of brackish water to tundra soils does not inhibit methane production: implications for Arctic coastal methane production

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**Abstract.** In Arctic regions where coastal sediments contain permafrost, global climate change drives processes such as erosion and subsidence. The contribution of these processes to carbon emissions are still uncertain. Relative sea level rise can lead to more waterlogged environments, promoting anoxic degradation of organic matter but it can also lead to a greater exposure of coastal sediments to seawater. This could alter methane ($CH_4$) production dynamics, although the controls remain poorly understood. For instance, sulfates contained in seawater may have a tampering effect on methanogenesis through competitive inhibition but the increase in microbial abundance could enhance methanogenesis. In this study, we present $CH_4$ production rates alongside geochemical analyses in a rapidly evolving coastal landscape near the community of Tuktoyaktuk, NWT, Canada, which is located in the continuous permafrost zone. To better constrain $CH_4$ production dynamics along the land to ocean continuum, sediment profiles were collected from nearshore marine sediments, as well as from the active layer of the coastal (intertidal) zone and inland soils. Anoxic incubations were performed, amended with brackish water to simulate the effect of seawater on the breakdown of organic matter and the production of $CH_4$. We found marine sediments expectedly led to negligible $CH_4$ production rates, while the inland sites showed variable rates between null and 35 nmol $cm^{-3}$ $d^{-1}$. The coastal (intertidal) zone had the highest rates reaching 415 nmol $cm^{-3}$ $d^{-1}$. Interestingly, sulfate present in brackish water and sediments did not suppress methanogenesis in the incubations of the coastal and inland zones. Analyses of stable carbon isotopes from $CH_4$ produced in the incubation experiment indicated greater acetotrophy and higher organic matter lability in the coastal zone, possibly contributing to higher $CH_4$ production rates. This study highlights the potential for significant $CH_4$ emissions even with high sulfate concentrations which are classically thought to inhibit methanogenesis. This suggests that Arctic coastal microbial $CH_4$ production might be an understudied source to the atmosphere.

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## 1 Introduction

Arctic coastal ecosystems are impacted by sea level rise, coastal erosion, land submersion, higher frequency in storm events and permafrost degradation (AMAP 2019; Guimond et al., 2021; Irrgang et al., 2022; Lantuit et al., 2013; Lim et al., 2020). The amplification of coastal environmental changes has impacts on biogeochemical cycles (AMAP, 2017) and on organic matter (OM) degradation processes and fluxes at the land-ocean continuum (Tanski et al., 2021). Furthermore, the progressive thawing of permafrost exposes long frozen organic matter to microbial decomposition (Lapham et al., 2020; Pellerin et al., 2022; Schuur et al., 2015), leading to the release of greenhouse gases like carbon dioxide ($CO_2$) and methane ($CH_4$). Inputs and outputs of the Arctic carbon biogeochemical cycle are known to be reshaped by rapid environmental changes (Couture et al., 2018), but processes in coastal settings are still poorly understood.

Rates of coastal change vary according to the morphology of coastal landscapes (Manson et al., 2019). The average rate of land retreat measured in the Tuktoyaktuk Coastlands (North-West Territories, Canada), our study site, between 1985 and 2020 was -1.06 m/yr, while processes of ground subsidence and submersion induced retreat rates higher than -4 m/yr (Costa, 2022) which can inundate large swaths of land. Inundated tundra flats and polygons are widespread landforms in the landscape (Costa, 2022). Polygon tundra flats are characterized by ice-wedge polygons, which are formed by the repeated thermal contraction and expansion of the upper layers of the permafrost (Steedman et al., 2016). At the surface, the polygons are expressed as minor topographic features separated by lower-lying, often wet or inundated channels called troughs (Fig 1). Polygons can be classified as low-centered (with a low, wet center and raised rims) or as high-centered (with well-drained centers and lower well-drained rims) (Fig 1), exhibiting strong thermal, hydrological and geochemical gradients (Vaughn et al., 2016).

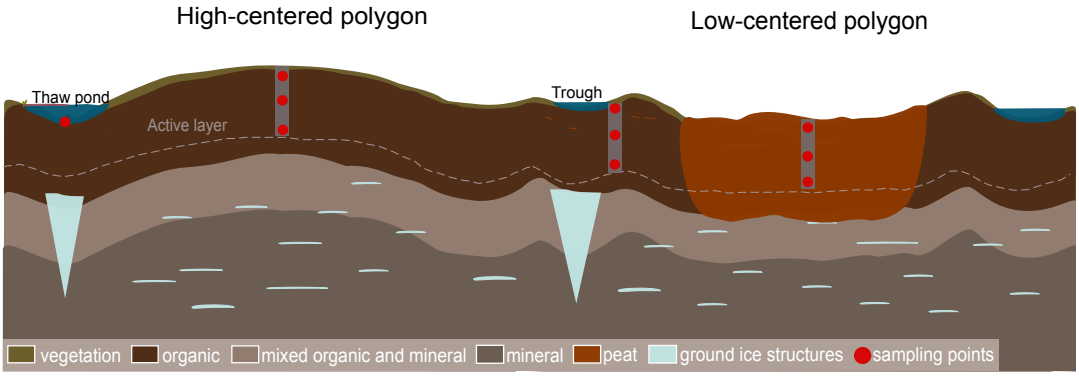


**Figure 1**. Schematic representation of polygonal tundra with peat accumulation as seen in continuous permafrost environments. High-centered polygons are associated with drier conditions, while low-centered polygons, troughs and pondlets are associated with humid or water-saturated conditions. Vegetation cover and OM reflect the hydrology of sites. Not to scale.

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Hydrological conditions in polygons play a pivotal role in shaping the pathways of OM decomposition and consequently influence the resulting $CO_2$ and $CH_4$ production. Well drained oxic conditions allow microbes to decompose OM rapidly, leading to the production of $CO_2$ (Jones et al., 2020). Conversely, water saturation restricts oxygen availability, promoting anaerobic respiration and fermentation, thus inducing both $CO_2$ and $CH_4$ production (Lipson et al., 2012; Turetsky et al., 2008). Thus, coastal changes can swiftly alter water saturation conditions in polygons, in many cases significantly enhancing fermentation and $CH_4$ production (Elberling et al., 2013; Holm et al., 2020; Treat et al., 2015).

Furthermore, coastal changes can also influence the chemistry of the water within soils, which can affect OM degradation. In anaerobic conditions, OM degradation processes follow a sequence of electron acceptors of decreasing energetic yields with nitrate, manganese oxides, iron oxides and sulfate as the most abundant electron acceptors (Froelich et al., 1979). It is when all alternative electron acceptors are depleted that fermentation takes place, leading to the production of $CH_4$; methanogenesis. For example, in beach, estuarine mudflats and marsh mudflats on the Brittany coast (France), OM degradation is dominated by sulfate reduction; the abundance in sulfate from seawater inhibits methanogenesis through competitive inhibition (Winfrey and Ward, 1983). By contrast, OM degradation in sediments below thermokarst lakes, which are anoxic and devoid of alternative electron acceptors, is completely dominated by methanogenesis (Sepulveda-Jauregui et al., 2015). The aqueous chemistry therefore plays a critical role in determining OM degradation. $CH_4$ produced in soils or sediments can also be oxidized by anaerobic methanotrophic archaea and sulfate-reducing bacteria (Boetius et al., 2000; La et al., 2022) present in the soils or sediment, contributing to lower $CH_4$ emissions in coastal environments. Thus, on or near the coast, the interaction with seawater, which contains electron acceptors such as sulfate, can shift the OM mineralization pathway and the resulting $CO_2$ and $CH_4$ productions. Consequently, a nuanced understanding of biogeochemical processes and their drivers is paramount in determining the magnitude of permafrost carbon emissions, especially from coastal environments.

Numerous $CH_4$ emissions monitoring programs are in operation, but remote-sensing methods lack the ability to comprehensively capture the microbial, biogeochemical and environmental processes involved. In specific regions, estimates of methane production from the breakdown of OM is possible by carefully studying degradation pathways and production rates (Pellerin et al., 2022; Heslop et al., 2015; Knoblauch et al., 2018; Treat et al., 2014). To reduce the knowledge gap of $CH_4$ biogeochemistry in coastal permafrost settings, we collected material from the active layer for incubation experiments, which were coupled to physical and chemical characterizations. The main objective of this study was to assess microbial $CH_4$ production dynamics in a coastal permafrost setting and apply it at the landscape level, since methane production is well documented in inland thermokarst but is not well understood in a land-ocean interaction context. We hypothesized that methanogenesis in coastal active layer incubations would be suppressed by the addition of sulfate. Consequently, we discuss the influence of environmental conditions on microbial $CH_4$ production with an emphasis on brackish water addition in coastal soils and sediments along with the microbial pathways involved. We then apply these results at the landscape level to provide an estimate of $CH_4$ production in the event that a natural process like a storm inputs brackish water over a large area of polygonal patterned ground. We use the region around Tuktoyaktuk as an example.

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## 2 Methods

### 2.1 Site description and sampling

Tuktoyaktuk (69°26'24'' N, 133°01'52''W) is located in the Inuvik region of the North-West Territories, adjacent to the Arctic Ocean in the Kugmallit Bay, east of the Mackenzie Delta. The region experiences prolonged cold winters, short cool summers, and year-round low precipitation, fostering low-arctic tundra vegetation. Lying in the continuous permafrost zone, its coastal areas feature thick Quaternary and glaciogenic unconsolidated deposits (Rampton, 1988), where permafrost thickness averages 400 m (Hu et al., 2013) and is characterized by prevalent ground ice structures (Mackay and Dallimore, 1992; Martin et al., 2018; Murton, 1996; Rampton, 1988). The area has been ice-free for the past 13 000 years, with evidence indicating that early Holocene summer temperatures were up to 6°C warmer than today, fostering vegetation and peat accumulation (Dallimore et al., 1997; Vardy et al., 1997). During that same period, sea level was considerably lower than it is today and the Tuktoyaktuk area was located approximately 100 km inland (Vardy et al., 1997). Currently, ground subsidence and coastal erosion are major causes of rapid land retreat (Hynes et al., 2014; Lapham et al., 2020; Lim et al., 2020). Combined with sea level rise (Hill et al, 1993), it is projected that a substantial amount of terrestrial soil will become part of the ocean seafloor either by erosion and deposition or by subsidence of land and submersion. Over the past 15 years, extensive studies on Tuktoyaktuk's coastal environment, driven by the region's vulnerability to climate change, highlighted challenges for the Inuvialuit population relying on hunting, fishing, trapping and harvesting (Andrachuk and Smit, 2012).

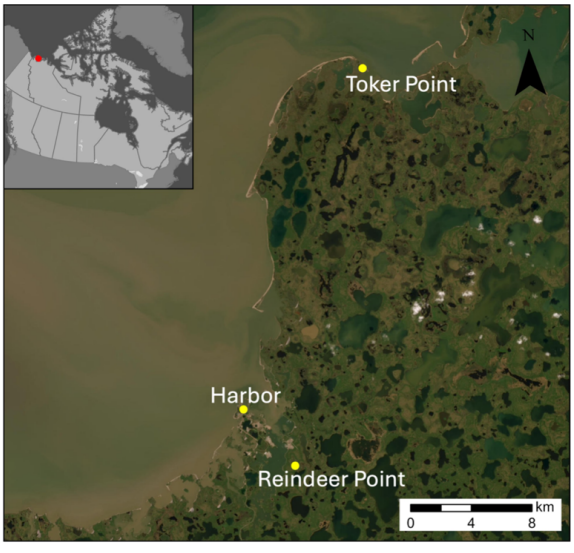


**Figure 2**. Map of study area indicating the sampled sites with yellow dots (ESRI, 2022). Harbor site is located in the marine waters, Toker Point site is located in the coastal (intertidal) zone and Reindeer Point site is located inland.

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Active layer samples were collected from three sites: an inland site, Reindeer Point (RP), a coastal site, Toker Point (TP) and a marine site, Harbor. RP was selected as the inland site because it features a polygonal patterned ground typical of the region, and is located in a stable region not directly affected by coastal processes such as storm surges, tides, seawater intrusion, erosion etc. The thermokarst lake margin, about 300m south of RP has remain unchanged since aerial photos began recording the evolution of the landscape in 1947 (Fig S1). TP was selected as the coastal site because of the strong coastal processes such as tides and storm surge that regularly lead to seawater intrusion in this polygonal patterned ground, strongly influenced by ground subsidence. The Harbor site was selected about 400 m offshore in the Harbor of Tuktoyaktuk where cold marine bottom waters are overlain at about 10m depth by a surface brackish water layer. The water depth was 20 m. Sediments were collected using a UWITEC gravity corer. The sediments consisted of recently deposited silty sands originating from the strong erosional processes occurring in the region (Whalen et al., 2022). The site was accessible by small watercraft. At RP and TP sites, the soil profiles were extracted from the active layer by digging a soil pit with a shovel. To retain an intact stratigraphic relationship, samples were taken from the wall of the soil pit. Biogenic ebullition gases were collected from pondlets at RP and TP. Pondlets were located within sampled polygonal patterned ground and are defined as small (1 to 3 $m^2$) and shallow standing bodies of water, potentially draining seasonally. Samples were trapped using a plastic funnel attached to a 20 mL glass vial. Surface soil lying at the bottom of the pondlets (Fig. 1) were poked until the vial was filled with gas. Once full, vials were crimped with 20 mm butyl rubber stoppers and aluminum caps. Samples were kept frozen until the time of analyses.

The inland site, (RP), was located 750 m from the coast and 2 km East of Tuktoyaktuk in a polygonal patterned ground. This patterned ground is located in a depression, surrounded by elevated plateaus with observable ground water flowing into the valley. In this area, low-centered polygons exhibited higher moisture levels compared to high-centered polygons. High-centered polygons were colonized by shrubs and small flowering plants like *Ericaceae*, while low-centered polygons were dominated by hydrophilic plants such as grasses and sedges. Wet troughs delimited the polygons, with vegetation reflecting waterlogged conditions. The mean active layer thickness across RP was about 35 cm. Profile 10A was collected from a trough and presented water-saturated conditions with brown OM. Profiles 10B and 10D were collected from high-centered polygons and characterized by unsaturated conditions with dark brown OM and presence of roots until 20 cm depth. Profile 10C was collected from a low-centered polygon and consisted of reddish-brown peat throughout. Profiles 10A, 10B and 10D did not consist of peat.

The coastal site (TP) is located 20 km NW of Tuktoyaktuk, featuring a polygonal patterned ground, largely colonized by *Carex sp,* a type of graminoid plant common near Arctic coastlines. The mean active layer depth was 35 cm. The site's dynamics are influenced by the twice-daily ebb and flow of tides. Profile 07 was collected from a water-saturated low-centered polygon, located in the intertidal zone. The soil color was very dark greyish black. Profile 08 was collected from a water-saturated polygonal trough not immediately located in the intertidal zone, but which floods during storms The soil was characterized by dark greyish-brown OM mixed with sand. Finally, profile 09, was collected from the center of a higher-centered polygon situated in the middle intertidal zone. The active layer appeared

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water unsaturated. The soil from this site consisted of a mixture of black organic-rich material and sand. The sand found in samples from TP appeared to be wind-deposited from nearby dunes.

### 2.2 Sulfates and chlorine concentrations in sediments

The extraction of sulfate and chloride from sediments and soils pore-water was conducted through a leaching experiment following Lacelle (2019). Frozen aliquots of sediments and soils were thawed at 4°C overnight, then weighed, dried in the oven at 60°C for 24 hours and re-weighed to determine the densities. Aliquots of dried material were put in 50 mL falcon tubes with nanopure water following a 1:10 ratio. Tubes were then shaken for one hour to promote leaching of anions towards the aqueous phase of the solution. Once the leaching process was done, 2 mL of the aqueous solution was filtered using 0.2 µm pore size Whatman 25 mm GD/X syringe filters and transferred in disposable microtubes. Concentrations of sulfate and chloride were measured by ion chromatography using a Thermo Dionex Integrion at UQAR's Chemistry department facilities with a limit of detection of 0,01 µg/mL. The measured concentrations are expressed in mmol $g^{-1}$ wet-$weight^{-1}$ of material (mmol $g^{-1}$ $wweight^{-1}$). Only one measurement per sample was performed as stability tests revealed variability of less than 3% between measured samples. The error on each value was calculated by the least squares method (Skoog et al., 2014).

### 2.3 Methane production rates in incubations

Long-term sediment and soil incubations under anoxic conditions were used to assess $CH_4$ production rates over several months by measuring $CH_4$ accumulation in the vials' headspace. The objective was to simulate the increased connectivity between the land and the ocean in the coastal environment of the Canadian Arctic, which represents an important aspect of the ongoing regional environmental transition. Collected sediments and soil profiles were immediately sub-sampled based on depth, at 5 or 10 cm intervals, according to shifts in sedimentary units. To prepare incubations, about 4 mL of sediment and exactly 2 mL of brackish water (collected from the coast) were immediately transferred into 20 mL glass vials. Incubation vials were crimped with 20 mm blue chlorobutyl rubber stoppers and aluminum caps. The bottles were flushed with nitrogen gas (Alpha Gaz 1) at a rate of 300mL/min for 2 minutes in the field to replace the air with a nitrogen atmosphere. Four incubations were prepared for each sampled depth; 3 were kept for measurements of $CH_4$ production rates (triplicates) and one served for isotopic analyses. Incubations were kept at 4°C and incubated for 339 days in total. The brackish water added to all incubations contained $5.7 \pm 0.0$ mmol $g^{-1}$ $wweight^{-1}$ of sulfate and $28.7 \pm 0.5$ mmol $g^{-1}$ $wweight^{-1}$ of chloride.

Analyses of the $CH_4$ concentrations in the headspace of the vials were performed on a gas chromatograph (Agilent 8900) equipped with a flame ionization detector (GC-FID) at UQAR facilities. The GC-FID is equipped with a 100 µL injection loop to ensure a consistent volume of sample is analyzed. To saturate the injection loop, 300 µL are taken from the headspace of the vials and transferred to the injection loop with a gas-tight syringe. Prior to injection, samples were shaken for 30 seconds to equilibrate headspace and sediment gases. This procedure was done every two weeks for 16 weeks to measure $CH_4$ accumulation in the headspace. The resulting production rates were back calculated, and values are expressed in nmol of $CH_4$ per cubic centimeters of wet material per day (nmol $cm^{-3}$ $d^{-1}$). The density of the collected samples varied widely, with some being organic deposits and peat, while others

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contained higher mineral content. Consequently, the $CH_4$ production rates were expressed volumetrically to account for these discrepancies which are more representative of the volume they occupy in the soils profiles and landscape. The limit of detection of the GC-FID is 0.3 ppm and all samples had higher concentrations. Each value represents the mean of triplicate measurements and the reported uncertainty on the measurement is the standard deviation on triplicates.

To estimate the potential total active layer $CH_4$ production (T), the active layer production rates were vertically integrated to obtain the total production of each profile. Values are reported in mmol $m^{-2}$ $d^{-1}$ and were calculated using eq. 1:

$$T = \frac{1}{100} \sum_{i=1}^{n} P_i \cdot e_i \qquad [mmol\ m^{-2}\ d^{-1}]$$

Where $P_i$ represents $CH_4$ production rate in layer i (nmol $cm^{-3}$ $d^{-1}$), $e_i$ represents the thickness of layer i (cm) and $n$ represents the numbers of layers in the profile.

Using aerial imagery from 2022, the polygonal tundra at RP was mapped in QGIS, allowing for the discrimination between high-centered polygons, low-centered polygons and throughs (Fig S2). The total area of each geomorphological form was calculated based on the map data (Table S1). Landforms total areas were multiplied by the corresponding potential total active layer methane production (T) to estimate the total $CH_4$ produced in the polygonal tundra of RP over a day (mol $d^{-1}$).

### 2.4 Elemental and isotope composition of the sediment

The total organic carbon (TOC) content of the sediments was measured by combustion using an elemental analyzer (ECS 8020, NC Technologies) combined with a gas chromatograph equipped with a thermal conductivity detector at ULaval facilities (The International Research Laboratory Takuvik). A 100 mg aliquot of sediment was thawed and weighed for each sample. They were then dried in an oven at 60°C for 48 hours and re-weighed to determine their water content. Sediments were then ground using a granite mortar pestle and homogenized using a 1.18 mm pore size sieve to remove roots and rootlets. Instruments were cleaned with ethanol between manipulations. Inorganic carbon was removed from sediments by adding 2.2 mL of 12M HCl in every sample. After reacting for 24 hours, around 8 mg was encapsulated in tin foil capsules. Samples were kept in a desiccator until analyses. Values are expressed as % of carbon contained in the weighed sample (wt. %).

The organic carbon ($\delta^{13}$C-TOC) isotopic compositions were measured at UOttawa facilities (Jàn Veizer Stable Isotope Laboratory) using EA-IRMS (Delta Advantage, Thermo Germany). The sample preparation method was the same used for elemental analyses. $\delta^{13}$C-TOC values are denoted as $\delta‰ = 10^3$ (($R_{sample}/R_{standard}$) - 1), where R is $^{13}C/^{12}C$ and standards refer to the Vienna Pee Dee Belmnite (VPDP).

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### 2.5 Stable carbon isotopic composition of methane

One incubation vial was analyzed for stable carbon isotopic composition of headspace methane ($\delta^{13}$C-$CH_4$). Stable carbon from methane ebullition samples collected from pondlets were also analyzed. Both types of samples were analyzed with a cavity ring-down spectrometer (PICARRO G2201-i isotopic $CO_2$/$CH_4$) equipped with a 16-port distribution manifold and small sample introduction module (SSIM) at McGill (McGill Isotope Biogeochemistry Laboratory). Incubations were kept at 4°C in the dark for 8 months to let the microbial community stabilize and produce sufficient $CH_4$ for analysis. To stay in the detection range of the analyzer (1.8-1000 ppm $CH_4$), a small volume of the headspace, proportional to $CH_4$ concentration in sample, was drawn from the incubation vial (0.2-6 mL). The sample was introduced to the 16-port manifold with a 21G needle connected to a disposable luer lock plastic syringe. Samples were diluted with zero air by the SSIM to reach a volume of 20 mL. Two or three measurements per sample were conducted depending on headspace concentration. Ebullition gases samples were analyzed following the same method. Measured values were corrected with internal certified methane standards (-59 ‰ and -42‰) from AirLiquide and stability of the analyzer was tested with injections of ambient air. Measured values were more precise than ± 1.2‰. All $\delta^{13}$C-$CH_4$ values are expressed relatively to VPDB.

## 3 Results

### 3.1 Soil description and composition

TOC content in the sampled soils ranged from 2 to 47 wt. %, with no clear trend in relation to depth (Fig 3, a, c). The RP polygonal patterned ground featured organic soils with TOC content ranging from 14 to 47 wt. % (Fig 3, c). The TP coastal polygonal patterned ground also featured organic soils with TOC content ranging from 2 to 37 wt. % (Fig 3, a).

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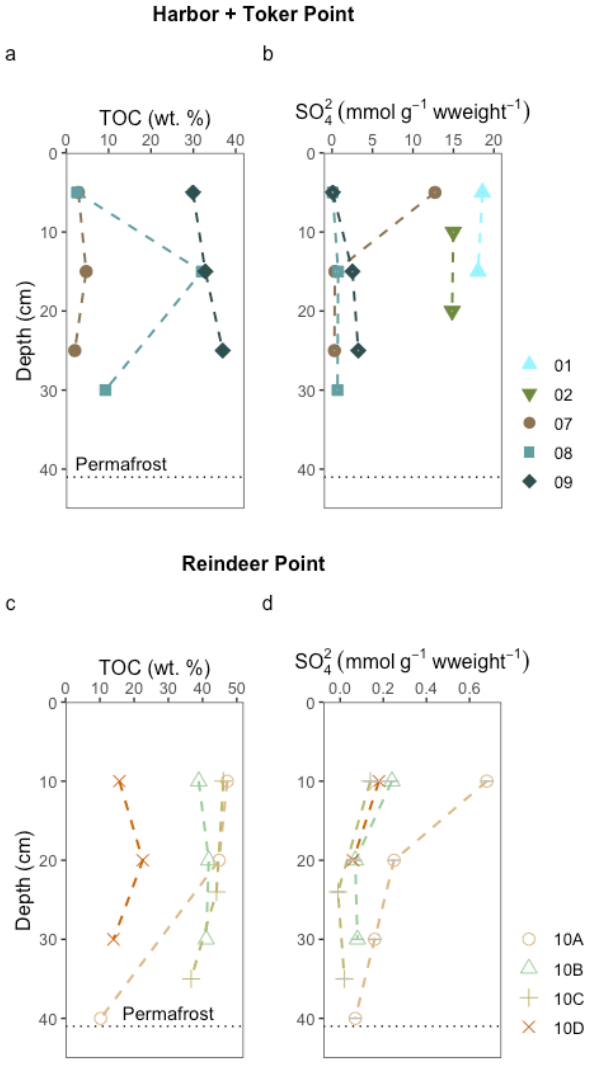


**Figure 3**. Total organic carbon and sulfate ($SO_4^{2-}$) concentrations in sediment or soil from the active layer of different sites in this study. The datasets are separated into two for clarity. The upper part of the figure (panels A and B) displays the data of the marine site Harbor (profile 01 and 02), and the coastal site, Toker Point (profile 07, 08 and 09). The lower part of the figure (panel C and D) displays the data from the inland site Reindeer Point (profile 10A, 10B, 10C and 10D). The black horizontal dotted line in each graph represents the permafrost-active layer interface except for the Harbor site, where the active layer is much deeper but not measured. TOC data from Harbor site is not available. A uniform color pattern is used throughout this article.

RP, the inland site, had low sulfate and chloride concentrations relative to TP, the coastal site (Fig 3 (b), (d) and S2). Sulfates at RP ranged from null concentrations to 0,68 ± 0.03 mmol $g^{-1}$ wweight$^{-1}$, while at TP, profiles

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exhibited varying concentrations and patterns in relation to depth. Sulfate concentrations, ranged from 0.07 ± 0,03 to 12.72 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$. Profile 07, the low-centered polygon, exhibited the highest sulfates concentrations of all TP site at its surface (12.72 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$), with concentrations decreasing drastically with depth, reaching 0.29 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$ at 25 cm (Fig 3, a). In profile 09, the high-centered polygon, sulfate concentrations increased with depth ranging from 0.09 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$ at 5 cm to 3.2 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$ at 25 cm. Finally, profile 08, characterized as a polygonal trough, had sulfate concentrations ranging from 0.07 ± 0.03 to 0.75 ± 0.03 mmol $g^{-1}$ $wweight^{-1}$. The highest sulfate concentrations measured in this study were found in the sediments of the Harbor site, with a mean value of 16.6 mmol $g^{-1}$ $wweight^{-1}$ (Fig 3, a).

### 3.2 Methane production

Rates of $CH_4$ production in incubations of sediment and soil with brackish water were undertaken at the three studied sites: RP, TP and Harbor. Production rates ranged from null to 415.4 ± 69.2 nmol $cm^{-3}$ $d^{-1}$ (Fig 4) throughout all samples in this study. At RP, the maximum $CH_4$ production rate of 35.2 ± 15.7 nmol $cm^{-3}$ $d^{-1}$ was measured in the trough profile (10B) at a depth of 20 cm. Lower values were obtained for the surface and at the active layer-permafrost interface. The low-centered polygon (10C) had its maximum $CH_4$ production rate in the surface, decreasing with depth. High-centered polygons (10A and 10D) had very low production rates along their depth profiles ranging between null to 1.2 ± 0.2 nmol $cm^{-3}$ $d^{-1}$. Both water-saturated trough and low-centered polygon (10B,10C) had relatively high $CH_4$ production rate compared with the high-centered polygon profiles (10A, 10D), which were water-unsaturated.

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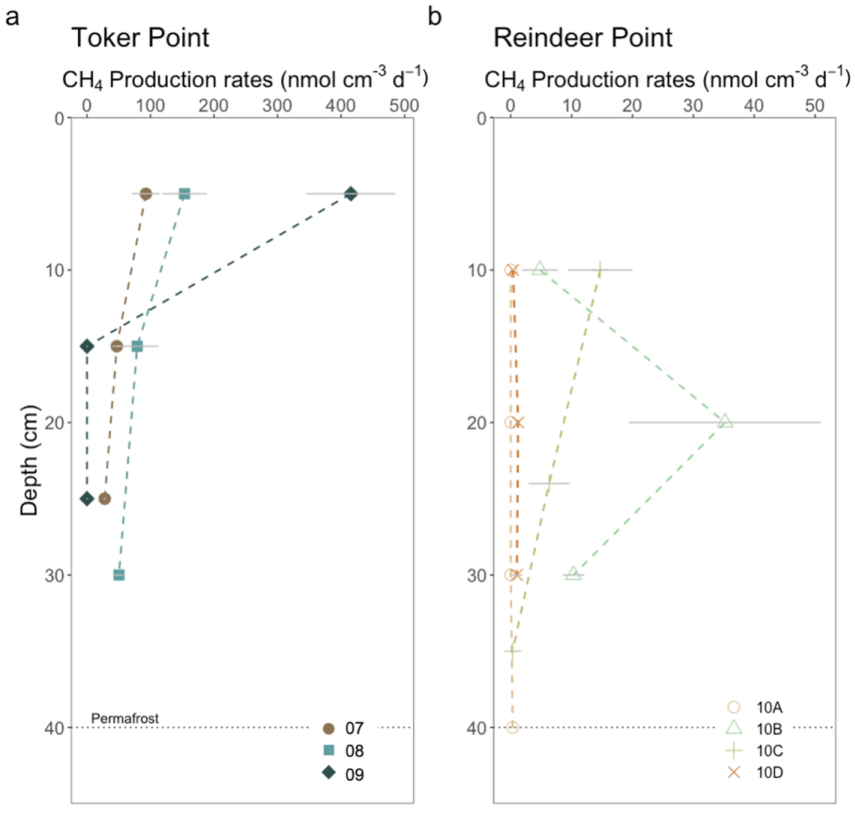


**Figure 4**. $CH_4$ production in incubations of soil and sediment with brackish water from (a) TP and (b) RP. Each datapoint represent the mean value of three incubations. The error bars equal to the standard deviation of the three separate incubations. Each profile corresponds to a specific landform. At Toker Point (panel A), profile 07 is from a low-centered polygon, profile 08 is from a trough and profile 09 is from a high-centered polygon. At Reindeer point (panel B), profile 10A is from a high-centered polygon, profile 10B is from a trough, profile 10C is from a low-centered polygon and profile 10D is from a high-centered polygon.

At TP, a maximum $CH_4$ production rate was recorded in profile 09, the high-centered polygon at 415.4 ± 69.2 nmol $cm^{-3}$ $d^{-1}$ at the uppermost depth but it quickly decreased in the subsurface. Profile 08, the trough, and profile 07, the low-centered polygon, had lower sub-surface $CH_4$ production rates, but rates decreased less drastically with depth with values being relatively high at the permafrost-active layer interface. Profile 07 had values ranging from 27.9 ± 1.5 nmol $cm^{-3}$ $d^{-1}$ to 92.8 ± 21.2 nmol $cm^{-3}$ $d^{-1}$ and profile 08 had values ranging from 50.4 ± 7.2 nmol $cm^{-3}$ $d^{-1}$ and 153.7 ± 33.9 nmol $cm^{-3}$ $d^{-1}$ (Fig 4). In general, at TP, the coastal site, much higher $CH_4$ production rates were measured than at RP, the inland site (Fig 4). The mean $CH_4$ production rate measured in the active layer of RP was 5.7 nmol $cm^{-3}$ $d^{-1}$, while at TP it was 96.2 nmol $cm^{-3}$ $d^{-1}$. The incubations with silty-clay Harbor sediments did not have measurable $CH_4$ production rates (Fig S5).

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Estimated total active layer $CH_4$ production rates were calculated for each geomorphological landforms of RP and TP sites. At RP, the total $CH_4$ production estimated for the high-centered polygons (profile 10A and 10D), low-centered polygon (profile 10C) and trough (profile 10B) were 0.3 ± 0.1 mmol $m^{-2}$ $d^{-1}$, 2.4 ± 1.0 mmol $m^{-2}$ $d^{-1}$ and 5 ± 2 mmol $m^{-2}$ $d^{-}$, respectively (Fig 5). At TP, the total $CH_4$ production estimated for the high-centered polygon (profile 09), the low-centered polygon (profile 07) and the through (profile 08) were 41.5 ± 6.9 mmol $m^{-2}$ $d^{-1}$, 16.8 ± 3.0 mmol $m^{-2}$ $d^{-1}$ and 28.3 ± 7.4 mmol $m^{-2}$ $d^{-1}$, respectively (Fig 5). In all landforms, the active layer $CH_4$ production rates were higher in the coastal site, TP, than the inland site, RP.

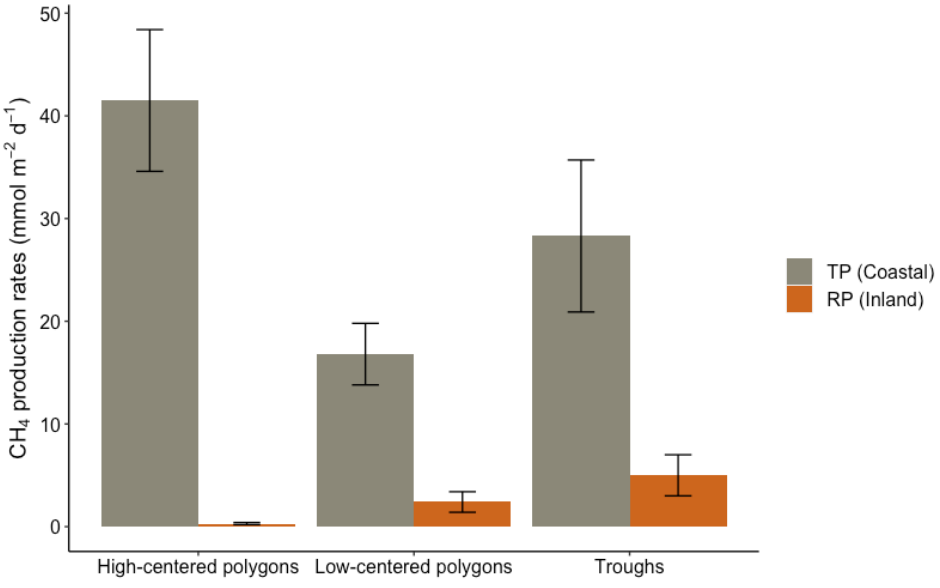


**Figure 5**. Total active layer production rates (T) at Toker Point (Coastal) and Reindeer Point (Inland) organized by geomorphological forms of high-centered polygons, low-centered polygons and throughs. High-centered polygons at RP is the mean of two profiles. All other landforms at RP and TP are one profile. The uncertainty on total active layer production rate is propagated from the uncertainty of individual $CH_4$ production rates, not averages from replicate sites.

### 3.3 Isotopic composition of $^{13}C$-$CH_4$

In parallel with $CH_4$ production rates, one incubation vial per depth was used to measure the stable carbon isotopic composition of the $CH_4$ produced. At RP, the $\delta^{13}C$-$CH_4$ of the first sampled depth (10 cm) ranged from -81.3‰ to -89.4‰. At TP, the coastal site, the $\delta^{13}C$-$CH_4$ signature of the first sampled depth (5 cm) ranged from -47.1‰ and -51.6‰. The values cluster together based on site, suggesting surface OM degradation processes are most similar within sites than between sites (Fig. 6). Profiles at RP became progressively enriched in $^{13}C$ with depth, except for profile 10C where a more depleted value was observed at 35 cm. Conversely, at TP, profiles became depleted in $^{13}C$ with depth, except for profile 08 where an enrichment was measured between 15 and 30 cm.

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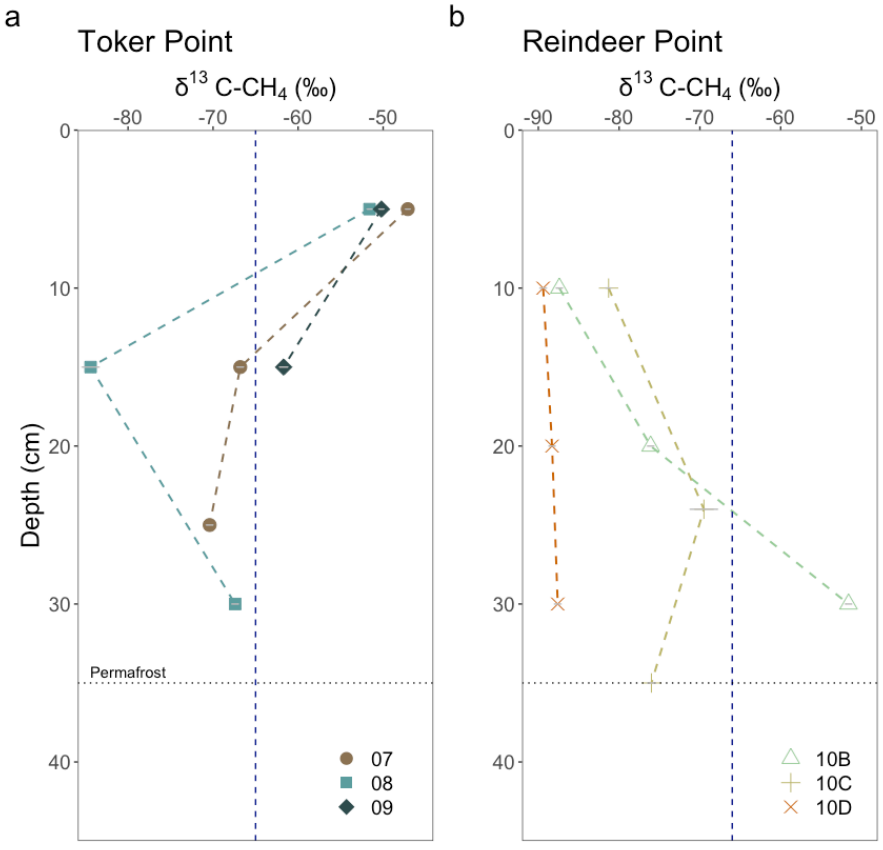


**Figure 6**. Isotopic composition of $CH_4$ produced in brackish water incubations from (a) TP and (b) RP. Each datapoint corresponds to the mean value of two or three measurements done on one incubation, depending on the headspace concentration. The dashed vertical lines correspond to in situ ebullition $CH_4$ collected in pondlets at each sampling site (n=1). These values give information on the pathways used by the soil microbes to produce $CH_4$. $\delta^{13}C$ between -65‰ and -50‰ is typically associated with acetoclastic methanogenesis, while $\delta^{13}C$ between -110‰ and -60‰ is associated with hydrogenotrophic methanogenesis (Hornibrook et al., 1997, 2000). The error bars on each point represents the analytical uncertainty on the measured value, otherwise the uncertainty is smaller than the point.

Ebullition samples from pondlets were also measured for stable isotopes. The ebullition samples represent the net $\delta^{13}C$ signature of methane produced in the sediments of pondlets at RP and TP. At RP, $CH_4$ ebullition from a sampled thaw pond had a $\delta^{13}C$ of -66.1‰ (fig 6). At TP, $CH_4$ ebullition from a sampled pondlet had a $\delta^{13}C$-$CH_4$ of -65.0‰ (fig 6).

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## 4 Discussion

### 4.1 Addition of brackish water to anoxic incubations did not strongly suppress methanogenesis

Despite the addition of brackish water containing sulfate to incubations, the range of $CH_4$ production rates measured in this study is consistent with reports for anaerobic incubations of recently thawed permafrost soils, suggesting that the input of brackish water to some costal systems may not inhibit $CH_4$ production. For example, in the talik of Big Trail Lake, a young thermokarst lake in the interior of Alaska, $CH_4$ production rates based on incubations ranged between 4.7 and 16.1 nmol $cm^{-3}$ $d^{-1}$ (Pellerin et al., 2022), while in incubations from Vault Lake, another thermokarst lake in the interior of Alaska, $CH_4$ production rates varied between 11.1 and 275 nmol $cm^{-3}$ $d^{-1}$ (Heslop et al., 2015). In active layer incubations from the Yamal Peninsula in NW Siberia (Russia), $CH_4$ production rates of incubations varied between 0.1 and 33.8 nmol $cm^{-3}$ $d^{-1}$ (Heyer et al., 2002). This indicates that overall, the $CH_4$ production rates measured at both TP and RP are within the range observed in typical ice-rich permafrost settings and reasonable for the environment studied (Fig. 4). Control incubations without the addition of brackish water were not included in this study. However, Lapham et al. (2021) conducted sediment incubation experiments using a core collected from the coast of the Tuktoyaktuk Peninsula. In their study, $CH_4$ production was measured under anaerobic conditions at 15 °C, without the addition of water, over a 35-day period. The reported $CH_4$ production rate was 0.07 nmol $cm^{-3}$ $d^{-1}$. Although their incubations were performed over a shorter duration and at a significantly higher temperature than those in the present study, the measured rate reflects $CH_4$ production under relatively natural, unamended conditions. This value is comparable to the lowest $CH_4$ production rates measured in our incubations at both the coastal (TP) and inland (RP) sites and provides a useful reference for $CH_4$ production under unamended conditions.

The novel aspect of this study is that it attempts to understand marine influences on OM degradation by addition of brackish water in anoxic active layer sediment and soil incubations between a marine site (Harbor), one that is periodically submerged (TP) and never submerged (RP). This simulates the input of seawater to the active layer of tundra soils (RP) as well as providing reference sites with a high marine influence (Habor and TP). We hypothesized that the addition of locally obtained brackish water, which contained sulfate (5.7 mmol $L^{-1}$), to the incubations, would suppress $CH_4$ production in RP, the inland site and potentially also at TP, the coastal site. This reasoning is because supplying sulfate to low sulfate organic-rich sediment would promote sulfate reduction, which is thermodynamically more favorable than methanogenesis, thereby competitively inhibiting it (Lovely and Klug, 1983; Oremland and Polcin, 1982). This hypothesis is also consistent with field observations; organic matter mineralization in brackish wetlands is consistently dominated by bacterial sulfate reduction (Bridgham et al., 2013; Torres-Alvarado et al., 2005) where little to no $CH_4$ emissions are observed (Pönisch et al., 2022; Petersen et al., 2023; Kroeger et al., 2017).

RP had low sulfate concentrations before addition of the brackish water but so did many of the profiles from TP (Fig 3). In the Harbor sediments, no methane production was observed (Fig S5). This is consistent with the competitive inhibition of methanogenesis by energetically favorable redox reactions with electron acceptors like oxygen, nitrate, iron oxides or sulfate that is typical of marine systems e.g. (Martens and Berner, 1974) as well as the potential for anaerobic oxidation of methane (AOM). Given that the Harbor sediments already had high sulfate concentrations, the

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lack of methane production with addition of brackish water was expected. However, strong $CH_4$ production was observed in the incubations of both the coastal site TP and the inland site RP, indicating that $CH_4$ production was not halted by the addition of sulfate via the brackish water addition at those sites. While sulfate reduction rates were not measured, a strong sulfide smell was recorded when opening most of the incubations at the end of the experiment, likely indicating the coexistence of sulfate reduction and methanogenesis during the incubations.

Coexistence of sulfate and methanogenesis within complex sediment systems such as estuarine, coastal and salt marsh sediments has been widely reported (Lovely et al., 1982; Oremland and Taylor, 1978; Sela-Adler et al., 2017). Two main mechanisms are invoked to explain this co-existence in our incubation experiment: (1) noncompetitive methanogenesis (i.e. methylotrophic methanogenesis) and (2) syntrophic methanogenesis. (1) Noncompetitive substrates are substrates like methanol and methylamines that are used by methanogens alone and cannot be used with electron acceptors like sulfate (Lovley and Klug, 1983; Oremland et al., 1982). Noncompetitive substrates are thus microbially converted to $CH_4$, even in sediments with high sulfate concentrations (Maltby et al., 2018; Yuan et al., 2019). For example, in salt marshes, where high sulfate concentrations are often found, elevated $CH_4$ emissions are suggested to mainly stem from noncompetitive methanogenesis (Comer-Warner et al., 2022; Poffenbarger et al., 2011; Yuan et al., 2019). In the sulfate reducing zone of sediments from the Baltic Sea, where ample sulfate is found in the porewaters, seasonal methanogenesis rates were measured up to 1.3 nmol $cm^{-3}$ $d^{-1}$ due to noncompetitive substrates (Maltby et al., 2018). In permafrost soils, methanol, methylamines and the microorganisms capable of degrading them have been observed but their concentrations are typically low (Coolen and Orsi, 2015; Kramshoj et al., 2018). However, our study sites are on a coast undergoing a rapid transgression which may be driving imbalances between substrate supply and microbial abundances. The rates of methane production observed at RP and TP of up to 154 nmol $cm^{-3}$ $d^{-1}$ contrast with reported values for methylotrophic methanogenesis (Maltby et al., 2018). Based on these numbers, noncompetitive substrates likely play a small role in the total methane production at our study sites but further investigation into methylotrophic methane production in coastal environments will allow to document the overall role of methylotrophic methane production in coastal permafrost settings.

(2) Syntrophic methanogenesis occurs when molecular hydrogen produced by acetoclastic sulfate-reducing bacteria is used by hydrogenotrophic methanogens. In this syntrophy, the chemical energy is shared via interspecies hydrogen transfer (Ozuolmez et al., 2015). For instance, in permafrost soils of Sweden, it was demonstrated that syntrophic methanogenesis was favored in anoxic and water-saturated soils by an elevated abundance in methanogens and their syntrophic partners (Keuschnig et al., 2022). As the incubation experiment in our study at RP and TP featured water-saturated and anoxic environments, syntrophic methanogenesis could participate in the co-occurrence of sulfate-reduction and methanogenesis. This mechanism is consistent with most incubations producing methane with a $\delta^{13}C$ value in the range of hydrogenotrophic methanogenesis (see below).

Measuring methane production through incubations inherently has limitations as they prevent continuous inputs of microorganisms, fresh OM and nutrients that would occur in the natural environment. This can create a “bottle effect”, which leads to restrictions in microbial community composition, limits the input of nutrients and leads to the accumulation of metabolites which would normally be degraded (Ionescu et al., 2015). Typically, overestimation of

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microbial processes rates is observed compared to *in situ* data (Sherr et al., 1999). The overestimation of $CH_4$ production rates by incubations relative to the *in situ* rates are difficult to assess because of a lack of data in permafrost environments (Heslop et al., 2020). Furthermore, a lag time between the start of anaerobic incubations and maximum $CH_4$ production rate is widely documented, which appears to be the case for both active layer and thawed permafrost incubations (Holm et al., 2020; Knoblauch et al., 2018; Knoblauch et al., 2013; Roy Chowdry et al., 2015). Drier or water-unsaturated conditions lead to a longer lag time before the onset of maximum $CH_4$ production (Treat et al., 2015). Microbial community composition in the soil or sediment also exerts a strong control on the organic carbon degradation and has been shown to change throughout the incubations (Holm et al., 2020). Low initial methanogen population in soils can contribute to this lag time, but other factors such as disturbance of sediment during sampling, substrate availability and redox state can also contribute to the observed lag time in some incubations (Treat et al., 2015; Roy Chowdry et al., 2015).

Furthermore, it is also possible that a "priming effect" from the addition of brackish water in incubations could have supercharged OM degradation with marine organic carbon, nutrients and microorganisms (Bianchi, 2011), which may have enhanced $CH_4$ production. However, this priming effect was not observed in the Harbor sediments which were amended with the same brackish water. Furthermore, $CH_4$ ebullition samples collected from pondlets adjacent to RP and TP exhibited broadly similar $\delta^{13}C$ values to methane produced in incubations (Fig 6), suggesting a similitude in microbial degradation pathways to methane *in situ* and in the incubations. Despite these uncertainties, our dataset shows clear depth trends and landscape-level variations, indicating that even under brackish water addition, local conditions will strongly influence $CH_4$ production.

### 4.2 $CH_4$ production pathways depend on hydrology and organic matter lability

The addition of brackish water resulted in incubation conditions being water-saturated in all cases, but it appears that biological and hydrological conditions of the polygonal patterned grounds influenced the magnitude of $CH_4$ production, nonetheless.

In all landforms, the active layer $CH_4$ production rates were lower at the inland site, RP than at TP, the coastal site (Fig 4). Inland, low-centered polygons and troughs have typically higher $CH_4$ fluxes than unsaturated landforms like high-centered polygons (Roy Chowdry et al., 2015; Martin et al., 2018; Zheng et al., 2018) which indicates they likely also have higher $CH_4$ production rates. Within sites in our study, brackish water amended incubations of high-centered polygon soils had lower $CH_4$ production rates, while brackish water amended incubations of troughs and low-centered polygons had higher $CH_4$ production rates (Fig 4). This indicates that for the degradation of organic matter into $CH_4$ in tundra soils, increasing seawater interactions through coastal processes, such as submersion due to subsidence or increased storm severity, resulting in the input of seawater in terrestrial soils, does not halt $CH_4$ production. It also shows that landforms and local hydrology remain important in controlling the microbial communities which affects the resulting $CH_4$ production.

Marine OM and nutrient inputs from tides and storm surges may contribute to the higher lability of OM and could fuel greater fermentation (Valdemarsen and Kristensen, 2010). It was reported that 8.7% of the organic carbon

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in nearshore sediments of Herschel Island, Beaufort Sea, came from marine sources (Couture et al., 2018). This is relevant for the TP site because while $\delta^{13}C$ signature of soils showed that terrestrial OM is dominant (Fig S4), marine OM may get transported and deposited in coastal soils during high tides and storm surges. Although our analyses could not detect the presence of marine OM in TP soils, the higher $CH_4$ production rates recorded in the incubations of TP, relatively to those of RP could in part be explained by marine OM and nutrient inputs. Interestingly, the high-centered polygon at TP, profile 09 (Fig 4), did not behave in a predictable manner, since it had very high $CH_4$ production rates on the surface. This may be due to the presence of high levels of geese fecal matter on the surface at TP, profile 09, which may provide a significant source of labile organic carbon or nutrients like N and P and a different microbial community composition on surface sediments, capable of high $CH_4$ production rates immediately following the start of the incubations. Lower in the profile, $CH_4$ production rates were very low, characteristic of the $CH_4$ production rates observed in water-unsaturated high-centered polygons (Fig 4). Therefore, in this instance, proximity with the coast may have influenced $CH_4$ production through the presence of fauna.

Stable carbon isotopic signature of $CH_4$ provides insights on the microbial processes involved in methanogenesis and on substrates used. $\delta^{13}C$-$CH_4$ between -65‰ and -50‰ is typically associated with acetoclastic methanogenesis, while $\delta^{13}C$-$CH_4$ between -110‰ and -60‰ is associated with hydrogenotrophic methanogenesis (Hornibrook et al., 1997, 2000). The stable isotopic signature of methylotrophic methanogenesis is between -83‰ and -72‰ (Penger et al., 2012), which overlaps with the hydrogenotrophic interval, precluding us from separating these two metabolic pathways. At RP, except for profile 10B, $\delta^{13}C$-$CH_4$ had more negative values, consistent with the processing of recalcitrant organic matter through the hydrogenotrophic production pathway (Heffernan et al., 2022; Hodgkins et al., 2014). Profile 10B, a polygonal trough, had less negative $^{13}C$-$CH_4$ values more consistent with acetoclastic methanogenesis (Hornibrook et al., 1997). At TP, the coastal polygonal tundra, $\delta^{13}C$-$CH_4$ at 5 cm depth is less negative, consistent with methanogenesis with more labile organic carbon and the acetoclastic production pathway (Hodgkins et al., 2014), transitioning to more negative values, associated to hydrogenotrophic production with depth. This shift suggests an input of labile OM in TP surface and sub-surface soils. This may be due to the labile OM from abundant geese fecal matter that was observed in the surface. It is also possible that *Carex sp.*, the dominant plant species of the site, may be a source of labile fermentation precursors (Galand et al., 2010; Liebner et al., 2015).

By comparing the $CH_4$ production rates from brackish water incubations from RP and TP we infer that the simple addition of brackish water is not the only parameter controlling $CH_4$ production between inland and coastal sites. Other interactions from coastal processes must enhance $CH_4$ production in the coastal site. Investigating the potential role of additional marine organic matter inputs will be crucial to better understanding the carbon cycling in Arctic coastal environments.

### 4.3 Active layer $CH_4$ production rates are comparable to the net $CH_4$ fluxes measured in similar environments

In a polygonal terrain of the Tuktoyaktuk Coastlands, net $CH_4$ fluxes from the center of high-centered polygons and troughs derived from flux chambers were measured to be $1.9 \pm 20.4$ mmol $m^{-2}$ $d^{-1}$ and $13.0 \pm 20.4$ mmol $m^{-2}$ $d^{-1}$ respectively (Martin et al., 2018). These overlap with values of estimated total $CH_4$ production derived from the

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brackish water amended incubation experiments (Fig. 5). It is clear from the large variations in measured $CH_4$ emissions from the study of Martin et al., (2018) that incubations to estimate total active layer $CH_4$ production rates can discern small differences due to local variations that stem mostly from the polygonal features. For example, at RP a comparable polygonal terrain located in the same study area of Martin et al., (2018), the active layer of high-centered polygons and trough, produced $0.3 \pm 0.1$ mmol $m^{-2}$ $d^{-1}$ and $5.0 \pm 2.0$ mmol $m^{-2}$ $d^{-1}$ (Fig. 5), respectively which are significantly different. This indicates the role of polygonal forms in controlling the activity of microbial communities which controls $CH_4$ production and the potential to scale more accurately $CH_4$ production at the landscape level based on landform distributions.

Interestingly, TP, the coastal site, had an estimated total active layer $CH_4$ production rate comparable to emissions of a St. Lawrence estuary salt marsh which had a $CH_4$ flux of $24 \pm 14.4$ mmol $m^{-2}$ $d^{-1}$ (Comer-Warner et al., 2022). The St. Lawrence estuary salt marshes are affected by freeze-thaw cycles associated with seasons comparable to the freeze-thaw cycles observed in the active layer of Tuktoyaktuk coastlands despite lacking some characteristics features of our site like the presence of permafrost and rapid coastal erosion rates. $CH_4$ emissions and production within areas of coastal influence thus appear of similar magnitude. By comparison, mangrove forests, which are a major global source of $CH_4$ but a very different environment from coastal Arctic polygon terrain, had average $CH_4$ fluxes to the atmosphere of 0.3 +/- 0.1 mmol $m^{-2}$ $d^{-1}$ (Rosentreter et al., 2018). In another study, the average measured $CH_4$ flux from a Yangtze Estuary (China) tidal salt marsh, with a subtropical monsoon climate, was 2.4 mmol $m^{-2}$ $d^{-1}$ (Li et al., 2021). These reported values are similar to our study as well as other studies in the region and adds to the notion that Arctic coasts are an important source of $CH_4$ globally that warrant further investigation.

The calculated total active layer methane production rates (T) from TP and RP do not take into account aerobic and anaerobic oxidation of $CH_4$, which will most likely reduce fluxes of $CH_4$ from these sites. Studies and models of Arctic soils emissions have highlighted that aerobic methanotrophy could consume more than half of the $CH_4$ produced in soils, greatly limiting surface emissions (Oh et al., 2020; Zheng et al., 2018). Furthermore, AOM has been shown to play an important role in attenuating $CH_4$ production in soils and sediments (Segarra et al., 2013; Winkel et al., 2019). However, AOM did not appear to influence significantly $CH_4$ production in incubations with thermokarst lake sediments (Lotem et al., 2023). For the discussion of this study, we compared results of brackish water incubations to $CH_4$ emissions measured in other landscapes. Such comparisons provide valuable context by comparing long-term microbial production processes with net atmospheric fluxes. However, we emphasize that $CH_4$ production rates cannot be directly equated to $CH_4$ emissions.

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**Table 1**. Total methane active layer production in a context of brackish water addition in high-centered polygons, low-centered polygons and troughs during growing season applied to the spatial scale of the polygonal landscape of RP. Two samples were taken for the high-centered polygon. The mean active layer depth of the region is 35 cm. The error represents the propagation of the analytical uncertainty from the incubations results.

| Geomorphological form | Relative area of each landform ($km^2$) | Estimated Total $CH_4$ production in active layer (mol $d^{-1}$) |
|---|---|---|
| High-centered polygons | 0.0803 | 20.7 ± 10.3 |
| Low-centered polygons | 0.119 | 284 ± 123 |
| Troughs | 0.0362 | 182 ± 73.4 |

In order to better frame the potential impact of brackish water addition at scale, we extrapolated its consequence to a 25 hectare area of polygonal tundra surrounding RP (Fig S2). This estimate simulates $CH_4$ production following the infiltration of brackish water into the terrestrial polygonal landscape around Tuktoyaktuk. This event could result from coastal flooding during storm surges, which are frequent in the Mackenzie River delta (Kokelj et al., 2012; Solomon et al., 2005). Taking into consideration the distribution of the polygonal features within RP and the relative areas of each landform, the $CH_4$ production rates in the active layer, excluding pondlets, for an area of 25 ha was calculated to be 487 mol $d^{-1}$ (Table 1) or 22 nmol $m^{-2}$ $s^{-1}$ and is consistent with the $CH_4$ emissions measured from various wetland types (Cui et al., 2024). The increasing sensitivity of wetlands to climate change and the preponderant role of carbon substrate availability in controlling global methane emissions (Hu et al., 2024) warrants further investigating $CH_4$ dynamics in thawing continuous permafrost landscapes and the role of coastal processes influencing these emissions. More polygonal tundra in various settings should be investigated as a comparison to the studied region. Further research on aerobic and anaerobic $CH_4$ oxidation is necessary to provide a more precise estimate of the $CH_4$ cycle inputs and outputs in a scope of the evaluation of its impacts on the greenhouse gas feedback loop.

## 5 Conclusions

The primary hypothesis for this study was that an increase in waterlogged environments due to coastal flooding and inundation processes would not enhance $CH_4$ production because of sulfate present in coastal waters. However, our incubation experiments revealed high $CH_4$ production rates in the presence of sulfates. Additionally, waterlogged conditions attributed to the ebb and flow of tides, seems to favor anoxic OM degradation and may potentially provide inputs of fresh OM and nutrients from marine sources, contributing to the elevated $CH_4$ production rates measured in the coastal setting of TP. Moreover, no conclusive explanation for the co-occurrence of sulfate-reduction and methanogenesis in our brackish water incubations was identified, but based on evidence, we suggest syntrophic methanogenesis could support this co-occurrence. More investigation on methylotrophic methanogenesis in coastal soils are needed as it can be an important process in saline environments (Conrad, 2020). Future studies

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should investigate $CH_4$ oxidation processes in greater detail, as they could provide crucial insights into Arctic coastal carbon cycling in sediments and soils affected by changing sea level.

## Data availability

All raw data of incubation experiment and other analyses performed and generated by study are available as supplementary information.

## Competing interests

The authors declare that they have no conflict of interest.

## Author contributions

AP designed the experiment; ARL executed the experiments and analyses. AP, ARL, DW, RL participated in the fieldwork. PMJD provided lab space, equipment and insights for the stable carbon isotopes analyses on incubation $CH_4$. RL performed all GIS analyses and maps. ARL performed the data interpretation and generated all figures. AP provided expertise on the writing and interpretation of figures. All authors reviewed and edited the manuscript.

## Acknowledgements

We thank Santiago Mareque for assistance during field sampling. Mathieu Babin and Thi Hao Bui are acknowledged for assisting with the laboratory work performed at Université du Québec à Rimouski and at McGill University, respectively. Takuvik Laboratory is acknowledged for providing analyses and results on $\delta^{13}C$ and TOC content of sediments. We also thank the community of Tuktoyaktuk for providing wildlife monitors with insightful information on the territory during field sampling. This research was funded by NSERC Discovery Grant and Northern Supplement to AP. ARL acknowledges financial support from the NSERC Northern Scientific Training Program. PMJD acknowledges support from the NSERC Discovery Grant and the Canadian Foundation for Innovation.

## Financial support

Support funds and grant agreement numbers are listed as specified upon manuscript registration.

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