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Christiansen, Jesper Riis; Barrera Romero, Alejandro Jose; Jørgensen, Niels O. G.; Glaring, Mikkel Andreas; Jørgensen, Christian Juncher; Berg, Louise Kristine; Elberling, Bo

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Methane fluxes and the functional groups of methanotrophs and methanogens in a young Arctic landscape on Disko Island, West Greenland

Jesper Riis Christiansen · Alejandro Jose Barrera Romero · Niels O. G. Jørgensen · Mikkel Andreas Glaring · Christian Juncher Jørgensen · Louise Kristine Berg · Bo Elberling

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Abstract Arctic soils are known to be important methane (CH_4) consumers and sources. This study integrates in situ fluxes of CH_4 between upland and wetland soils with potential rates of CH_4 oxidation and production as well as abundance and diversity of the methanotrophs and methanogens measured with pyrosequencing of 16S DNA and rRNA fragments in soil and permafrost layers. Here, the spatial patterns of in situ CH_4 fluxes for a 2,000 years old Arctic landscape in West Greenland reveal similar CH_4 uptake rates (-4 ± 0.3 μmol m^{-2} h^{-1}) as in other Arctic sites, but lower CH_4 emissions (14 ± 1.5 μmol m^{-2} h^{-1}) at wetland sites compared to other Arctic wetlands. Potential CH_4 oxidation was similar for upland and wetland soils, but the wetter soils produced more CH_4 in active and permafrost layers. Accordingly, the abundance of methanogenic archaea was highest in wetland soils. The methanotrophic community also differed between upland and wetland soils, with predominant activity of Type II methanotrophs in the active layer for upland soils, but only Type I methanotrophs for the wetland. In the permafrost of upland and wetland soils, activity of the methanotrophs belonging to Type I and Type II as well as methanogens were detected. This study indicates that the magnitude of CH_4 oxidation and the direction of the flux, i.e. uptake or emission, are linked to different methanotrophic communities in upland and wetland soils. Also, the observed link between production/consumption rates and the microbial abundance and activity indicates that the age of an Arctic landscape is not important for the CH_4 consumption but can be very important for CH_4 production. Considering the prevalence of dry landscapes and contrasting ages of high Arctic soils, our results highlight that well-drained soils should not be overlooked as an important component of Arctic net CH_4 budget.

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J. R. Christiansen · A. J. B. Romero · C. J. Jørgensen · L. K. Berg · B. Elberling (✉)
Center for Permafrost (CENPERM), Department of Geosciences and Natural Resource Management, University of Copenhagen, Øster Voldgade 10, 1350 Copenhagen, Denmark
e-mail: be@ign.ku.dk

J. R. Christiansen
Department of Forest and Conservation Science, University of British Columbia, Main Mall 2424, Vancouver, BC V6T 1Z4, Canada

A. J. B. Romero
Department of Biology and Geology, Universidad de Almería, Carretera Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain

N. O. G. Jørgensen · M. A. Glaring
Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

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Introduction

Microbial CH_4 oxidation in soils is a large terrestrial sink of atmospheric CH_4 and changes in soil CH_4 oxidation rates in natural ecosystems could significantly impact the global methane budget (Torn and Harte 1996). Arctic upland soils and polar deserts cover more than 1,358,000 km^2 or 26 % of the ice free area in the arctic (Walker et al. 2002) and have been shown to be sinks of atmospheric CH_4 (Whalen and Reeburgh 1990; Curry 2009; Bárcena et al. 2011; Brummell et al. 2014) and Olefeldt et al. (2013) reported average rates of atmospheric CH_4 uptake in upland tundra soils to be -1.8 μmol CH_4 m^{-2} h^{-1}. In Greenland and Canada, ice-free land areas with favorable conditions for CH_4 uptake are typically between 1,000 and 10,000 years old and are dominated by moist or dry upland tundra, or scarce vegetation (Whalen and Reeburgh 1990). In contrast, the proportion of true fens and wetland is limited, and organic rich layers seldom reach to the depth of the permafrost (Funder and Hansen 1996). Arctic upland soils are generally underrepresented in published studies and it was recently shown that CH_4 uptake in the drier Arctic upland soils offset CH_4 production in regions with a high upland to wetland ratio (Emmerton et al. 2014). Hence, a better understanding of the magnitude and drivers of CH_4 uptake in Arctic upland soils compared to wetlands in these young Arctic landscapes is needed in order to elucidate their importance for the Arctic CH_4 budget in a broader context.

Because CH_4 oxidation and production in soils is mainly driven by microbial activity it is further necessary to compare CH_4 dynamics to the responsible functional microbial groups in these soils. The importance of microbial CH_4 oxidation in both well-drained and poorly drained Arctic soils for net surface exchange of CH_4 with the atmosphere is two-fold: (1) In the aerated top part of the soil, the process removes atmospheric CH_4 while (2) in the deeper parts of the soil profile at the interface with the water table, the process consumes CH_4 produced at depth (Whalen and Reeburgh 1990; Moosavi and Crill 1998). Thus, the activity and diversity of methane-oxidizing bacteria (MOB) within the soil profile to a large extent determines the net fluxes of CH_4 from Arctic soils (Tveit et al. 2013). Soil hydrology is likely a major factor regulating the functional diversity of the MOBs community in the Arctic landscape as well as it has been shown for temperate grassland soils (Shrestha et al. 2012). However, little is known about how the functional diversity and activity of methanotrophic communities in upland Arctic soils differ from the more well-known communities studied in Arctic wetlands.

Studies of aerobic methanotrophs in the Arctic have focused on wet soil environments such as moist tundra, fens and bogs. Predominantly low-affinity MOBs belonging to the lineages known as Type I (Trotsenko and Khmelenina 2005), e.g. active at high soil CH_4 concentrations, have been found and to a lesser extent of Type II methanotrophs (Liebner et al. 2009; Martineau et al. 2010; Graef et al. 2011; Barbier et al. 2012; Tveit et al. 2013). However, other studies have documented the presence and activity of Type II MOBs in Arctic soils (Liebner and Wagner 2007; Yergeau et al. 2010; Mackelprang et al. 2011).

Type I and Type II MOBs are not closely related, but belong to the γ- and α-subclasses of proteobacteria, respectively. Also, their metabolic pathways differ, implying that Type I and Type II MOBs are adapted to different environmental conditions (Hanson and Hanson 1996). Some Type II MOB species, e.g. *Methylocapsa acidiphila* (Dedysh et al. 2002; Ricke et al. 2005), are able to oxidize CH_4 at or below atmospheric concentrations, i.e., below app. 2 ppm, and are related to uncultured high-affinity MOBs of the *Beijerinckiceae* family predominantly found in upland temperate forest soils that consume atmospheric CH_4 (Kolb 2009; Bengtson et al. 2009; Shrestha et al. 2012). The presence of high-affinity MOBs was also indicated in cold glacier forefield soils in Eastern Greenland (Barcena et al. 2010) and found in high Arctic upland soils in Canada (Martineau et al. 2014).

These studies across contrasting soil types in the Arctic and cold soils suggest that well-drained upland and wetland soils harbor different MOB communities and that result in zones of uptake and emission of CH_4, respectively. Although there is compelling evidence that MOB in Arctic upland soils drive uptake of atmospheric CH_4 it is still unclear how the functional diversity of high- and low-affinity MOBs compare to CH_4 dynamics measured in Arctic upland and wetland

soils. Furthermore, only few studies (Wagner et al. 2003; Barcena et al. 2010; Nauer et al. 2012) have compared MOB community diversity, abundance and activity to in situ CH_4 fluxes in Arctic environments and laboratory incubation studies specifically targeting high-affinity MOBs (Martineau et al. 2014).This study is based on the hypothesis that young High Arctic ecosystems typically found in Greenland may release less methane in wetlands than atmospheric methane being oxidized when net fluxes are calculated on a landscape scale. Thus, we aim to quantify spatial patterns of in situ CH_4 fluxes, including potential rates of high- and low-affinity CH_4 oxidation rates and CH_4 production in permafrost upland and wetland soils during the peak of the growing season in 2011 and secondly, to compare the observed CH_4 dynamics to depth-specific diversity, abundance and activity of microbial populations including, the methanotrophic and methanogenic communities. This can provide new evidence of the importance of CH_4 uptake and oxidation in young Arctic soils as well as give new insight to how the eco-physiological adaptation of functionally important groups of methanotrophs and methanogens in upland and wetland soils is linked to CH_4 dynamics.

Methods and materials

Study site

The study site is located at Flakkerhuk, Disko Island in West Greenland (69°40′N, 52°00′W; Fig. 1) within the transition zone from continuous to discontinuous permafrost. The mean annual air temperature (1991-2000) is −4.4 °C and the annual precipitation as rain is 150–200 mm (Hansen et al. 2006). As a result of relative sea level changes during the Holocene epoch, several marine terraces, ranging up to 60 m above present sea level, were identified in the study area (Nielsen 1969). This study focuses on the youngest terrace (Fig. 1), dated to 2000 BP (Jensen et al. 2006). Four major land cover types were recognized on this terrace: (a) wind exposed abrasion plateau with sparse

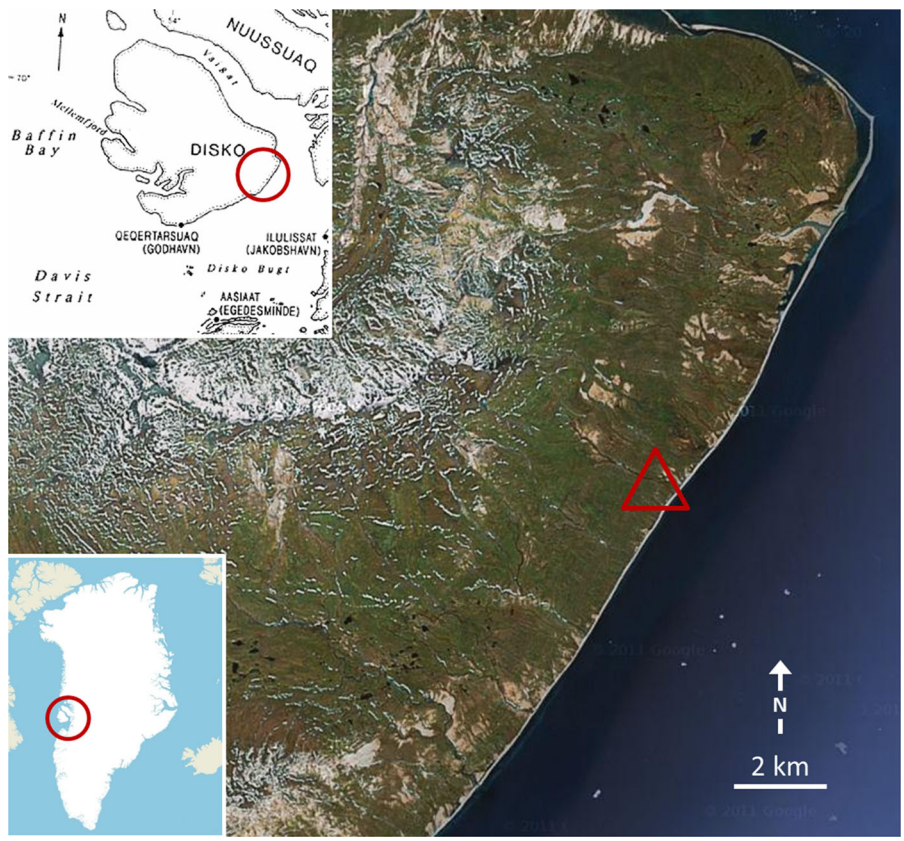

Fig. 1 Satellite image of the study area Flakkerhuk at Disko Island, West Central Greenland (Google Maps, Google Inc., 18 February 2014). The triangle shows the location of base camp at Flakkerhuk. The bottom insert map indicates the location in Greenland (modified from mapsopensource.com, 2013). Top insert map shows Disko Island with a circle indicating Flakkerhuk (modified from Humlum et al. (1995))

vegetation, (b) heath areas dominated by *Betula nana* and *Cassiope tetragona*, (c) tundra dominated by *Salix arctica* and *Salix glauca*, (d) permanently wet fens dominated by *Sphagnum* mosses and grasses, predominantly *Carex L., Deschampsia alpine*, *Eriophorum vaginatum* and *Arctophila fulva*. Within the fens, two types were identified: fens with standing water and a vegetation cover below 25 % (Fen 1) and fens with tussock grasses with a 100 % vegetation cover (Fen 2). The heath and tundra areas were defined in the text as upland soils and the fens as wetlands.

CH_4 flux measurements

Fluxes of CH_4 were determined using closed static chamber technique (Pihlatie et al. 2013) in two permanently wet fens (Fen1 and Fen2) and two drier sites dominated by *Salix arctica* (Salix) and *Betula nana* (Betula). The sites are representative of the wetland and upland soil types found in the Flakkerhuk area (Fig. 1). Black cylindrical chambers (3 mm polycarbonate, inner diameter 31 cm) were installed to a depth of approximately 10 cm below the surface for more than five days prior to the first measurements. CH_4 fluxes were measured over a 10 min period with 10 s sampling frequency (60 samples per enclosure) using a DLT-100 Fast Methane Analyzer spectrometer (Los Gatos Research Inc., California, USA). During the measurements, air volume in the chamber headspace was circulated in a closed loop at a flow rate of approximately 0.3 L min^{-1}. Air temperatures inside and outside the chambers were measured using temperature sensors mounted in the lid (107 temperature probe; Campbell Scientific, UK). A total of 40 chambers were installed in the four vegetation types (Fen1, n = 20; Fen2, n = 10; Salix, n = 5; Betula, n = 5). Wooden boardwalks were installed at the study sites to access the chambers, and flux measurements were conducted between July 9th and July 17th in 2011. Chambers in Fen1, Fen2 and Salix were measured four times, and Betula three times. Soil moisture and soil temperature in the upper 5 cm were measured in 5 replicates for each chamber measurements using a ThetaProbe soil moisture sensor (ML2x Delta-T Devices ltd., Cambridge, UK) and portable thermometer.

In situ CH_4 flux estimates were calculated on basis of 60 sample points over the 10 min chamber enclosure using a quadratic regression model to account for potential non-linearity in the diffusion gradient (Wagner et al. 1997). Flux estimates were corrected using the chamber headspace, temperature and atmospheric pressure (Askaer et al. 2011), and significance of the regression was tested at the 95 % significance level. In situ CH_4 fluxes were expressed as μmol CH_4 m^{-2} h^{-1}.

The flux detection limit (FDL) for the chamber measurements was estimated by first defining the FDL, the minimal concentration change (minimum quantification limit (MQL)), that could be detected over one chamber enclosures of 10 min or 0.17 h. The MQL is defined as (Corley 2003):

$$MQL[\text{ppm}] = 3 * t_{99\%} * S_{\text{dev}} \quad (1)$$

where $t_{99\ \%}$ is the t value at the 99 % confidence interval at df = 0 (2.66) and S_{dev} is the standard deviation of 61 atmospheric concentration measurements by the Los Gatos.

The FDL was calculated for each of the chamber volumes (from 6 to 21 L) and a uniform chamber area of 0.048 m^2.

$$FDL\left[\mu\text{molCH}_4 m^{-2} h^{-1}\right] = \frac{MQL}{t} * \frac{V}{A * V_m} * \frac{273.16}{273.16 + T_a} \quad (2)$$

where t is the enclosure time in hours, V is the chamber volume in m^3, A is the chamber area m^2, V_m is ideal gas molar volume [0.0224 m^3 mol^{-1}], T_a was the temperature in °C inside the chamber headspace. Exchange rates falling within the interval [−FDL:FDL] could not be detected above the instrument noise and were discarded. All 318 flux measurements were made manually to verify that ebullition, a sudden release of methane bubbles, was not occurring during measurements. Only four out of the 318 flux measurements were excluded in the analysis due to unknown errors during measurements.

Soil sampling and analyses

Soil from CH_4 flux chambers was sampled within each chamber at two or three depths, based on appearance of soil profile at the end of the study period. The first samples were retrieved from the top 0–5 cm soil characterized by a high content of organic matter and a noticeable amount of roots. The second sample was taken from the mineral soil 10–20 cm below the

surface. A third sample was retrieved deeper from the layer assumed to represent the parent material. These soil samples were used to quantify soil characteristics for upland and wetland soils.

Adjacent to the sites for CH_4 flux measurements, active layer (0–5 cm) and permafrost soil were sampled in three separate upland (*Salix* vegetation) and wetland sites. These samples were used to quantify potential rates of CH_4 oxidation and production. The wetlands represented both dry and wet fens, with the water table at the surface and below (Supplementary Table S1). In the wetland, intact cores (Ø 6.8 cm) were collected from the upper active layer from pits. In the upland sites, active layer samples were taken using a sterile spatula. Intact cores of permafrost soil (Ø 5.5 cm) were collected using a handheld motorized diamond drill (STIHL Model BT 121; Stihl, Germany). A wooden box was used to cover the drilling hole to prevent contamination of the core material. Permafrost cores represent sediment about 10 cm below the frost table (top permafrost, TP) and deeper samples typically 1 m below TP and considered deeper permafrost (DP) at each of the wetland and upland sites. Characteristics of the sampled sites are given in Supplementary Table S1. In the field the permafrost cores were kept below 0 °C and stored at −70 °C in the laboratory until analysis. In total six sites were sampled, providing three replicate samples of the active layer, the upper and deeper permafrost soils for the upland and wetland sites, respectively. All active layer soil samples were sealed in plastic bags to minimize contact with the surrounding air and kept at 4 °C.

For each soil samples from the CH_4 chamber, subsamples of 20 g were mixed with 20 g of deionised water and shaken for 15 min before measurement of pH (Metrohm model: 691, Metrohm AG, Switzerland). The soil suspensions were subsequently centrifuged at 8,000×g for 10 min. The supernatants were stored at −20 °C until analysis of dissolved organic carbon (DOC), ammonium (NH_4^+), nitrate (NO_3^-) and total organic nitrogen (TON) on a FIA Star 5000 system according to methods provided by the manufacturer (Foss, Denmark). Parallel subsamples of the soil were dried, crushed and analysed for total C and N content using an ELTRA CS500 carbon analyzer (ELTRA Gmbh, Germany) and a LECO TruSpec Carbon Nitrogen Determinator (LECO Corporation, MI, USA).

Potential CH_4 oxidation

For determination of high-affinity CH_4 oxidation rates from the permafrost sites, 10 g of fresh, frozen permafrost soil was placed into 120 mL glass bottles, allowed to thaw and flushed with atmospheric air for 2 min. The bottles were sealed with butyl rubber stoppers and incubated at atmospheric CH_4 concentration ($\sim$1.8 ppm). The soil samples were either kept at 0–1 °C by immersing the samples into crushed ice, or at room temperature (22–25 °C). For both temperatures, 600 µL headspace was sampled at 0, 24, 48, 96 and 168 h (removing about 2.7 % of the headspace), but only 500 µL was injected into the GC.

For analysis of the low-affinity oxidation, bottles from the high-affinity experiment were opened and flushed with laboratory air and sealed with rubber stoppers. One mL of pure CH_4 was injected into the headspace to obtain a concentration of app. 10,000 ppm. Headspace volumes of 300 µL were sampled at 0, 24, 72, 144, 168, 192, 240 h, and 200 µL were transferred to 2.7 mL crimped vials and analysed as above. On average 1.9 % of the headspace was removed during the incubation. Low-affinity oxidation rates for permafrost samples were normalised to 7 °C using a Q_{10} of 1.7 for CH_4 oxidation rates determined at 1,000 ppm CH_4 (Dunfield et al. 1993). Similarly, to the vials three empty bottles were used to check for gas leakages in the incubation setup.

The headspace concentration of 5,000 and 10,000 ppm does not necessarily reflect in situ conditions at Flakkerhuk but is used in order to target and stimulate low-affinity methanotrophs that generally are not active at atmospheric levels (Bender and Conrad 1992). In extension to this, we assumed that incubating the soil samples at ambient CH_4 we would only target high-affinity methanotrophs and not affect the potential activity of low-affinity methanotrophs at elevated headspace concentrations.

Potential CH_4 production

Production of CH_4 was measured using a published protocol by Wagner et al. (2005). 10 g of soil was transferred to 120 mL incubation bottles and 3 mL (permafrost) 10 mM sodium acetate were added to the soil to obtain an initial acetate amount of 0.18 g bottle^{-1} for permafrost site samples. Dissolved oxygen in the acetate solution was removed by sonification for

10 min under vacuum before addition to the bottles. After closure, the bottles were evacuated to a constant partial vacuum of −700 mbar for 3 min and subsequently flushed with pure N_2 for additional 3 min. The bottles were then pressurised with 10 mL a H_2/CO_2 gas mixture (80/20 % v/v) to obtain an overpressure of ca. 15 mbar. Three empty bottles with acetate solution were used to check for potential leakages during incubation. Gas volumes of 600 µL headspace were extracted, followed by rejection of 100 µL before a final injection of 500 µL into a crimp sealed vial with atmospheric air. The gas samples were analysed using the auto sampler on GC.

For the permafrost soils triplicate samples from active layer, TP and DP, were incubated over 160 days and sampled at 0, 1, 2, 3, 6, 8, 9, 15, 34, 38, 49, 64, 90 and 160 days after start. On average 7.6 % of the headspace was removed during the incubation. As for the oxidation experiment, the samples were incubated at 1 °C. The CH_4 production rates for the permafrost soils were normalized to 7 °C using an average Q_{10} of 3 for wetlands on permafrost (Lupascu et al., 2012).

CH_4 concentrations were measured on a Shimadzu GC-2014 gas chromatograph (GC) (Shimadzu, Kyoto, Japan) equipped with flame ionization detector. Detector temperature was 200 °C and carrier gas was 100 % pure N_2 at a flow of 25 mL min^{-1}, and a 60/80 Carboxen 1000 column (15ft, 1/8 in.) at a constant temperature of 40 °C. A gastight syringe (SGE, Australia) was used for all headspace samplings from incubation vessels. In order to correct the measured rates for soil moisture contents, parallel samples of 10–20 g of fresh soil were oven-dried at 55 °C for 48 h.

Calculation of CH_4 oxidation and production rates

Potential CH_4 oxidation rates were calculated using linear regression and CH_4 production rates determined from a logistic model (see Supplementary materials). Initially, all rates (ppm h^{-1}) were compared to the observed rates for the empty bottles. Any rates larger than the average rate of the empty bottles were assumed to be above detection limit and expressed in nmol CH_4 g dw^{-1} day^{-1} using headspace of the bottles and incubation temperature. The maximum CH_4 production rate was assumed to be equal to the maximum rate of concentration change at the theoretical time (t_{max}) during the logistic growth. In cases where t_{max} occurred after termination of the experiments the maximum CH_4 production rate was set to t = 150 or 160 days.

Bacterial production (incorporation of ^{3}H-leucine)

Bacterial cell production in the soils at different temperatures was measured by the ^{3}H-leucine incorporation method, using the micro-centrifugation approach (Bååth et al. 2001; Brandt et al. 2004). All handling of the soils was done at 5 °C (close to the 4 °C storage temperature for the active soils). The permafrost soils were thawed from −70 to 5 °C overnight. In brief, samples of 5 g soil were transferred to 50 mL sterile tubes after which 45 mL 7.5 mM sodium pyrophosphate (pH 7.5) was added. After shaking for 15 min (to extract microorganisms) and centrifugation at 10,000×*g* for 10 min, triplicate 1.5 mL soil suspensions received a mixture of 50 µL ^{3}H-leucine and unlabeled leucine to a final concentration of 100 nM. Killed controls (soil suspension with 160 µL 50 % trichloroacetic acid (TCA)) were treated similarly. Five sets of triplicate samples (and controls) were incubated at 1, 5, 10, 15 °C and room temperature (RT, 20 °C). The samples were incubated for 1 h (incubation at room temperature) to 20 h (incubation at 1 °C), after which the samples were killed by addition of 200 µL 50 % TCA. The samples were centrifuged at 20,000×*g* and re-extracted in 5 % TCA and 80 % ethanol. The pellets was digested in 1 M NaOH for 1 h at 90 °C before redissolution in 1 mL of liquid scintillation cocktail and counting of radioactivity. The incorporated leucine was converted to bacterial cell production using a conversion factor of 1×10^{16} cells mol $leucine^{-1}$ (Michel and Bloem 1993).

Coextraction of DNA and RNA from soil samples

The DNA assemblages from active layer, upper and permafrost soil samples at the permafrost sampling site were extracted from the soil samples following incubation in the laboratory with the protocol modified from Griffiths et al. (2000). DNA and RNA nucleic acids were coextracted from the natural soil samples following the incubation experiments. Environmental samples were snapshot frozen in liquid nitrogen and stored at −80 °C prior to extraction. Twenty vials containing 0.5 g of fresh soil were submerged in liquid

nitrogen for 5 min and stored at −80 °C. Soil samples were freeze-dried (Hetosicc, Heto Lab equipment) for 72 h. We acknowledge that extracting DNA and RNA at this stage does not reflect in situ conditions as samples have been subjected to various environmental conditions different than they came from. However, we assume that the relative differences in abundance and activity still represent the basic differences in the function of the methanotrophic and methanogenic communities in these contrasting soil types.

About 0.5 g of soil (wet weight equivalent) was transferred into 1.4 mm ceramic bead tubes (MO BIO Laboratories, CA, USA). Extractions were performed by the addition of 0.5 mL Phenol–chloroform-isoamyl alcohol (25:24:1; Sigma, pH 8) to the soil followed by 0.5 mL of CTAB extraction buffer (Sigma-Aldrich, Denmark). Soil samples were lysed by shaking 2 times during 20 s (ice in between) at speed 4 in BIO 101 Fast prep FP 120. The aqueous phase with nucleic acids was separated by centrifugation (16,000×*g*) for 5 min at 4 °C (Thermo Scientific, Heraens Fresco 21 Centrifuge). Approximately 425 µL of the supernatant was transferred into 2.0 mL tubes and kept on ice. Phenol was removed by inverting 10 times with an equal volume of Chloroform-isoamyl alcohol (24:1) followed by centrifugation (16,000×*g*) for 5 min at 4 °C. The aqueous phase was transferred into a second set of tubes. Total nucleic acids were precipitated with 800 µL of 30 % PEG and 1 µL of glycogen (Roche, Denmark) mixed by inverting and placed on ice for 2 h. Centrifuge tubes (16,000 *g*) for 30 min at 4 °C. Pellets were resuspended in 150 µL DEPC treated dH_20 by shaking (1,400 rpm) in an Eppendorf shaker (Thermomixer comfort) at 4 °C until dissolved. A series of washing steps were performed by adding 150 µL of Clean up solution (NucleoSpin RNA clean-up XS kit; Macherey–Nagel) and vortex 2 times for 5 s. The entire volume was transferred to filter tubes and spin (10,000 rpm) for 1 min. Filters were washed by adding 400 µL of Wash solution and spin (10,000 rpm) 1 min. 200 µL of Wash solution was added followed by centrifugation. Filters were transferred into new collection tubes and RNA was eluted in 20 µL DEPC treated water. Extracted nucleic acids (DNA/RNA) were divided into two aliquots for the preparation of DNA or RNA templates. Transfer 8 µL to an RNase free tube for DNase treatment (RNA template) and 10 µL to another tube for DNA template. Templates were not diluted and DNA templates were stored directly at −80 °C. The same day DNase treatment and reverse transcriptase reactions were performed.

For the synthesis of cDNA, DNA was first degraded in the RNA template samples by using the RST DNase kit (MO BIO Laboratories, Inc., CA, USA) according to the manufacturer's instructions. In brief, volumes of 12 µL master mix (2 µL reaction buffer, 9 µL RNase-free water and 1 µL RTS DNase) were added to each tube containing 8 µL RNA/DNA extracts. Tubes were placed on the PCR cycler (Biorad, iCycler, Denmark) at 37 °C for 20 min. 5 µL RTS DNase removal resin was added to each tube, inverted manually and centrifugation (13,000×*g*) for 2 min. The resultant supernatant was divided into two aliquots: 6 µL of the volume was transferred into a new tube for the synthesis of cDNA and the remaining 10–12 µL volume to another tube (RNA) and directly frozen at −80 °C. The later was used for determination of RNA quality (Agilent Bioanalyzer, Agilent, CA, USA).

The RNA was converted into cDNA with the RevertAid Premium RT Kit (Fermentas, Denmark) according to the manufacturer's instructions. In brief, 6 µL of the DNase treated extracts were added to 4 µL master mix in RNase-free tubes and placed in the PCR cycler. The RT-PCR reaction was run in a PCR cycler (Biorad, iCycler, Denmark) with a 3 cycle's program of 25 °C during 10 min, 50 °C during 30 min, and lastly 85 °C during 5 min. Tubes containing cDNA assemblages for downstream analysis were stored at −80 °C.

Pyrosequencing of 16S rDNA amplicons

Pyrosequencing of the 16S rRNA gene was conducted for 24 samples; six DNA samples extracted from the active layer soil of both wetlands and uplands and 18 samples of cDNA from both sites and the three sample depths (active layer, top and deep permafrost). A 466 bp fragment covering the V3 and V4 hypervariable regions of 16S rDNA from bacteria and archaea was amplified using the primers 341F (5′-CCTAYGGGRBGCAS-CAG-3′) and 806R (5′-GGACTACNNGGGTATC-TAAT-3′) (Yu et al. 2005). For cDNA samples, the concentration was adjusted to 5 ng μL^{-1} and the PCR reaction (25 µL) was performed using 10 ng of cDNA template, 1 U of Phusion HotStart DNA polymerase (Finnzymes, Vantaa, Finland), 1 × Phusion HF Buffer, 200 µM of each dNTP and 0.5 µM of each primer

with the following cycle conditions: 98 °C for 30 s, followed by 30 cycles of 98 °C for 5 s, 56 °C for 20 s and 72 °C for 20 s and a final extension of 72 °C for 5 min. For DNA samples, amplification was performed using a commercial PCR beads kit (Illustra PureTaq Ready-To-Go PCR Beads, GE Healthcare, UK) in a total volume of 25 µL containing 0.5 µM of each primer and 5 ng DNA template. PCR conditions were: 94 °C for 2 min, followed by 32 cycles of 94 °C for 20 s, 55 °C for 30 s and 72 °C for 1 min. All PCR products were incubated at 70 °C for 3 min, immediately separated on an agarose gel and gel purified using a Montage DNA Gel Extraction Kit (Millipore, Hellerup, Denmark).

Adapters and tags for pyrosequencing were added in a second 15-cycle PCR on 5 ng of purified PCR product, using the conditions described above for the cDNA samples with primers 341F and 806R carrying sequencing adapters and tags for multiplexing. The amplified fragments were gel-purified as above, quantified using the Quant-iT dsDNA HS Assay Kit (Invitrogen, Life Technologies Europe, Naerum, Denmark) and mixed in equal amounts before sequencing on a Genome Sequencer FLX pyrosequencing system (454 Life Sciences, Roche, Branford, CT, USA).

Sequence handling and phylogenetic analysis

Trimming and quality-filtering of pyrosequencing results were performed using Biopieces (www.biopieces.org). Initially, tags and primer sequences were removed, discarding any sequences that did not show a match to both a tag and the forward primer. Low quality bases were trimmed from both ends and sequences shorter than 250 bases, containing more than one ambiguous nucleotide, or with an average Phred quality score lower than 25 were discarded. OTU clustering was performed in USEARCH v.6.1 (Edgar 2010), which included: 1) Dereplication and subsequent error-correction by outputting the consensus sequences of an initial clustering-step at 97 % identity, 2) Reference-based chimera detection by comparison to a Greengenes dataset of OTUs clustered at 97 % (http://greengenes.lbl.gov), and 3) A final OTU clustering step at 97 % (roughly corresponding to species level). Phylogenetic analysis was performed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (www.qiime.org) (Caporaso et al. 2010). The USEARCH generated OTUs were used as a reference set for OTU picking at 97 % identity using the original trimmed and filtered sequences. Taxonomy was assigned using the RDP classifier with a confidence threshold of 50 % and a training set from the Greengenes database (version 13_05) (DeSantis et al. 2006) and all OTUs containing only one sequence (singletons) were discarded before further analysis.

Data and statistical analyses

To test for significant differences of in situ CH_4 fluxes, volumetric soil moisture content and soil temperature (at 5 cm depth), respectively, between soil types (wetland and upland) a 1-way repeated measures ANOVA using the Mixed procedure in SAS 9.2 (SAS Institute Inc., USA) with soil type as class variable and investigation site (Betula, Salix, Fen1 and Fen2) as a random factor was applied.

Using a 1-way ANOVA with the GLM procedure in SAS 9.2 it was tested whether mean values of soil pH_{H2O}, log_{10}-transformed concentrations of TON, NH_4^+, %C and soil C:N ratio for the entire active layer (A, B and C horizons) were significantly different between upland and wetland soils. A non-parametric analysis using the Wilcoxon test was applied using the NPAR1WAY procedure in SAS 9.2 for mean values of NO_3^-, DOC and %N concentrations due to non-normal distribution. In the manuscript, average values are presented with standard error of the mean as uncertainty measure.

Q_{10} values of microbial growth, e.g. the rate of $^3H^-$leucine incorporation, for the permafrost upland and wetland sites were calculated using the rates at 5 and 15 °C with the following formula:

$$Q_{10} = \left(\frac{R_{15}}{R_5}\right)^{\frac{10}{15-5}} \quad (3)$$

Differences in Q_{10} values between active layer, TP and DP for upland and wetland sites were tested using *t* test assuming equal variances.

Results and discussion

Soil characteristics

Total carbon (% C), total nitrogen (% N) and C:N ratio decreased with depth as observed for both soil types

(Fig. 2; Table S1). Overall, soil carbon and nitrogen contents were significantly higher in wetland (21.3 ± 1.9, 0.78 ± 0.05 %) compared to upland soils (8.4 ± 1.7, 0.29 ± 0.05 %) (Fig. 2). Accordingly, C:N ratios were significantly higher for wetland soils (26.3 ± 2.3), as compared to upland soils (17.1 ± 2.0) (Fig. 2). Total dissolved organic carbon (DOC) in the two soil types was rather similar and ranged from 10 to 12 mg L^{-1}, except for the upland A horizon in which about 50 mg DOC L^{-1}. Soil bulk densities in the upland soils ranged from approximately 0.4 g cm^{-3} in the A horizon and main root zone to approximately 1.6 g cm^{-3} in the underlying mineral B horizon. In the wetland soils, bulk densities ranged from approximately 0.2 to 0.6 g cm^{-3} in the A and B horizons, respectively (Fig. 2). However, there

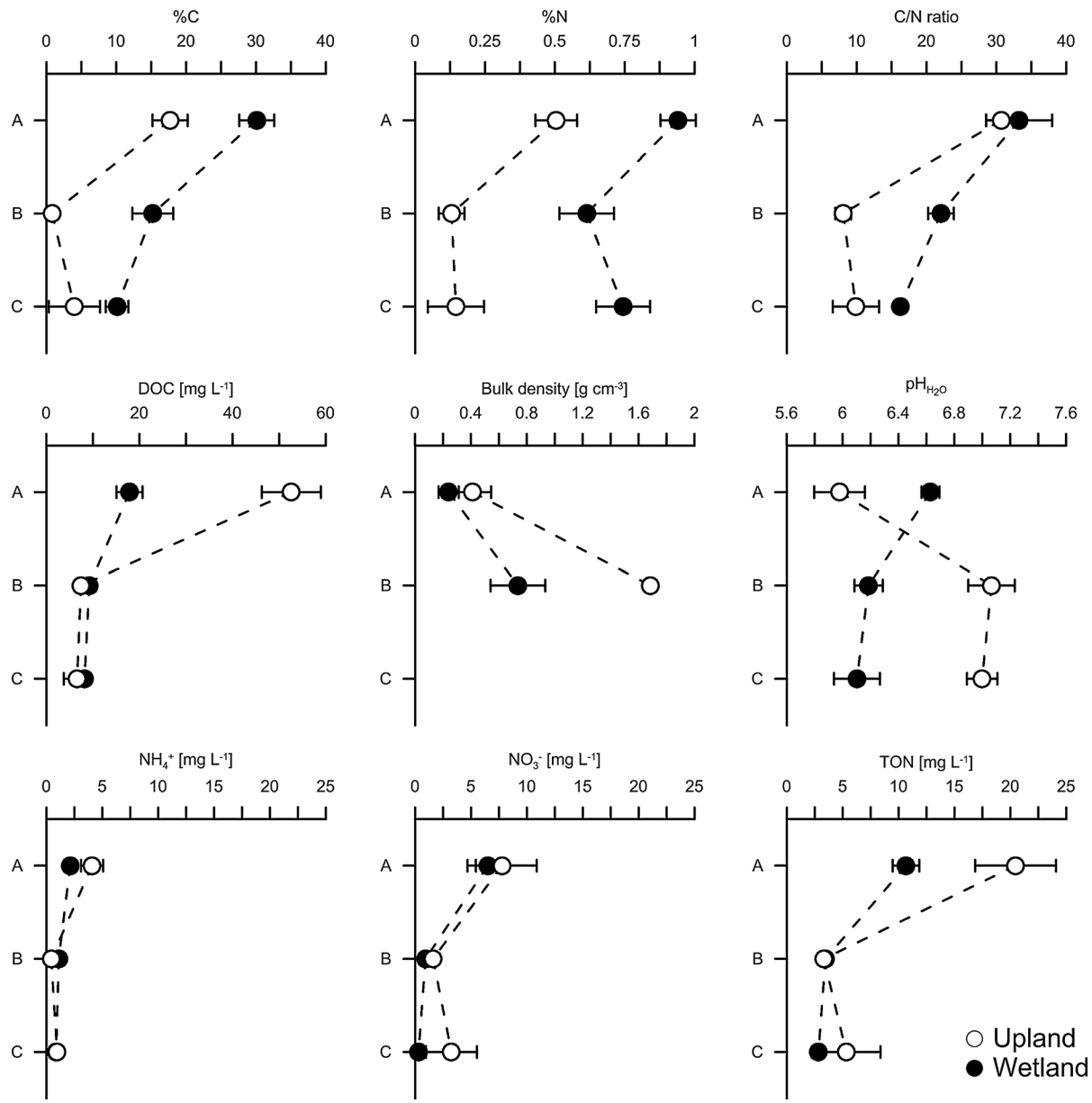

Fig. 2 Mean values (±SE of the mean) of soil chemical and physical properties for upland (*open circle*) and wetland (*filled circle*) soils at the chamber sampling site, Flakkerhuk, Disko Island, Greenland for the A, B and C horizons within the active layer

was no significant difference in bulk density between upland and wetland soils (Fig. 2). Soil pH values were in the neutral range (pH of 5.9–7.1) for both soils. A slight increase in soil pH with depth was observed for the upland soil, whereas a minor decrease in pH occurred in the wetland soils (Fig. 2). Concentrations of NH_4^+ and NO_3^- varied from 0 to 5 and 0 to 10 mg L^{-1}, respectively, for both soil types and the highest concentrations were found in the A (Fig. 2). Concentrations of total dissolved organic nitrogen (TON) were also similar between soil types and highest in the A horizon for both soil types, ranging from an average of 10 mg L^{-1} in the wetland soil to an average of 20 mg L^{-1} in the upland soils (Fig. 2).

Volumetric soil moisture content was significantly lower for upland soils (31.3 ± 1.7 %) compared to wetland soils (95.1 ± 0.9 %) (Fig. 3a). However, in the upland soils, the Betula site (22.8 ± 1.4 %) had significantly lower soil moisture content than the Salix site (36.4 ± 1.3 %) (Fig. 3A), whereas no site-specific differences were observed for the two wetland sites. Soil temperature at 5 cm depth did not differ between soil types and was 8.2 ± 0.3 °C and 9.9 ± 0.2 °C for upland and wetland soil, respectively (Fig. 3b). Within both groups of soil type sites, soil temperature differed significantly. Thus, Betula was significantly colder (6.6 ± 0.2 °C) than Salix (9.2 ± 0.3 °C) and Fen1 (11.2 ± 0.2 °C) was significantly warmer than Fen2 (9.2 ± 0.2 °C). The spatiotemporal variability of soil moisture and soil temperature as indicated by the maximum-minimum range was highest for wetland soils (Fig. 3a, b).

In situ measurements of CH_4 fluxes

Upland soils (Betula and Salix) were, as expected, net consumers of atmospheric CH_4 in situ (mean rate ± SE of −4 ± 0.5 µmol m^{-2} h^{-1}) whereas wetland soils were net emitters in situ (14 ± 1.5 µmol m^{-2} h^{-1}) during the studied period (Fig. 4). Thus, the dry and more oxic soils at the upland site, relative to the wetter and anoxic wetland soils, had a significant influence on the in situ CH_4 fluxes. The spatio-temporal variability of CH_4 fluxes within soil types was higher for wetland than upland soils (Fig. 4). No significant differences in fluxes were found for uplands soil types (Betula and Salix) or wetland types (Fens 1 and 2). Soil temperatures for the four soils in the study period are too similar to access the importance of temperature on CH_4 fluxes.

Compared to other Arctic wetlands, observed emissions at Flakkerhuk are low, as compared to wetlands in Siberia (in situ rates from 12 to 139 µmol CH_4 m^{-2} h^{-1}) (Wagner et al. 2003) or the wetlands in Zackenberg, East Greenland (average in situ rates of

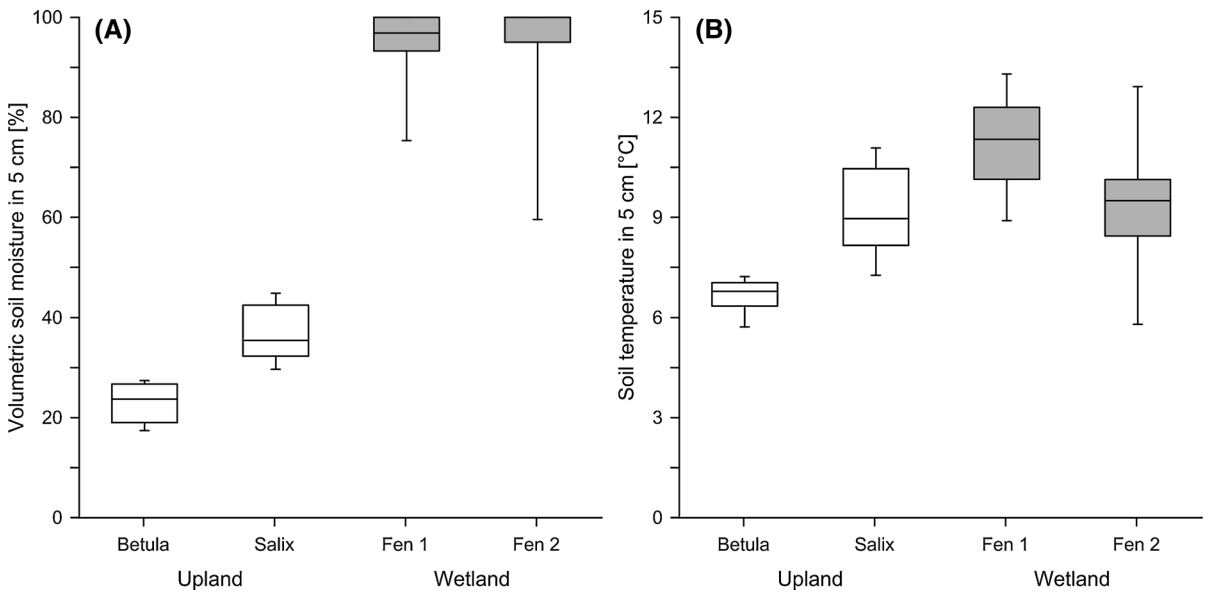

Fig. 3 Boxplots of **a** Volumetric soil moisture between 0 and 5 cm and **b** Soil temperature at 5 cm depth for the four different investigated plots at Flakkerhuk, Disko Island, Greenland. The lower side of the boxplot represents the 1st quartile, the middle line the median and the upper side of the box the 3rd quartile. The bars represent maximum and minimum observations

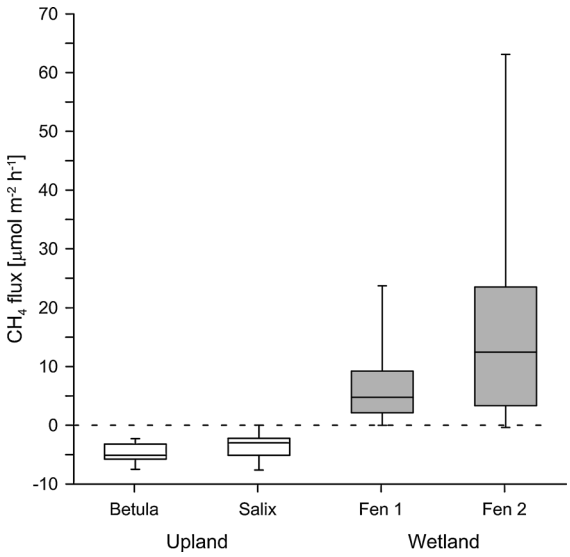

Fig. 4 Boxplots of CH_4 fluxes for the four different investigated plots at Flakkerhuk, Disko Island, Greenland. The dashed line represents a zero flux of CH_4. The lower side of the boxplot represents the 1st quartile, the middle line the median and the upper side of the box the 3rd quartile. The bars represent maximum and minimum observations

119 µmol CH_4 m^{-2} h^{-1}) (Christensen et al. 2000). On the other hand, CH_4 uptake rates measured for the upland soils at Flakkerhuk are comparable to temperate forest soils (−7 µmol m^{-2} h^{-1}; e.g. Smith et al. 2000; Skiba et al. 2009) as well as to in situ fluxes observed in glacier-forefield soils in Sweden (−0.36 to −2.89 µmol CH_4 m^{-2} h^{-1}) (Nauer et al. 2012) and markedly higher than in situ fluxes (−0.01 to −0.05 µmol m^{-2} h^{-1}) measured in recently exposed glacier fore-field soils of Southeast Greenland (Bárcena et al. 2010).

Laboratory studies of CH_4 oxidation and production

High-affinity CH_4 oxidation was only observed in active layer samples, but there was a large difference between uplands and wetlands (Fig. 5a). The high-affinity CH_4 oxidation reported here for the upland soils are comparable to other incubation studies of Arctic upland soils (0.29–0.5 nmol g $peat^{-1}$ day^{-1} in Graef et al. 2011; 1.24-2.14 nmol g $soil^{-1}$ d^{-1} in Bárcena et al. 2011).

Low-affinity CH_4 oxidation rates in the active layer were 50 ± 14 and 66 ± 36 nmol CH_4 g dw^{-1} day^{-1} in the wetland and upland soils, respectively, not significantly different (Fig. 5b). Relative to the active layer, the low-affinity oxidation rates were two to three-fold lower in the permafrost layers for wetland and upland, respectively. The observed low-affinity CH_4 oxidation rates were lower than rates (0–19.2 µmol CH_4 g dw^{-1} h^{-1}) measured in typical peat tundra soils in the Lena Delta, Siberia using a similar headspace concentration of 1 % CH_4 (Knoblauch et al. 2008). However, low-affinity rates based on Flakkerhuk samples were comparable to CH_4 oxidation rates (2.4–6.5 nmol g $soil^{-1}$ d^{-1}) from a more recent study of the less developed minerogenic soils in Eureka, Ellesmere (Martineau et al. 2010), which reflects the soil conditions at Flakkerhuk to a higher degree compared to more developed peat soils. Also, in that study a similar CH_4 headspace concentration of 0.83 % CH_4 was applied. Low-affinity CH_4 oxidation rates have been measured for a range of Arctic soils in Alaska (Mackelprang et al. 2011), Canada (Barbier et al. 2012), Svalbard (Graef et al. 2011) and Siberia (e.g. Wagner et al. 2003, 2005), but headspace CH_4 concentrations in these studies differ greatly, from 0.1 to 2.5 %, hampering comparison of rates between studies. It has been shown that laboratory based CH_4 oxidation rates appear to be proportional to headspace concentration of CH_4 for both oxic soils and rice paddies (Bender and Conrad 1995).

A potential CH_4 production (after saturation of the sample) was observed in the active layer of upland soils at both sites, but were absent in the permafrost samples (Fig. 5c). The highest CH_4 production (67 ± 7 nmol CH_4 g dw^{-1} d^{-1}) was observed for the active layer in the wetland and decreased with depth, reaching 0.78 ± 0.6 and 0.42 ± 0.16 nmol CH_4 g dw^{-1} d^{-1} in the top and deep permafrost, respectively (Fig. 5c). Similar to the in situ CH_4 emission, potential CH_4 production rates for the wetland soils at Flakkerhuk were lower than published rates using similar protocols from Siberia (112.8–934 nmol CH_4 g dw^{-1} day^{-1}, Wagner et al. 2003) or Western Canada (34–924 nmol CH_4 g dw^{-1} day^{-1}, Barbier et al. 2012). Surprisingly, the maximum rate of CH_4 production for one upland soil sample was comparable to the rates that observed for the wetland (not shown). There was a considerable time lag (weeks to months) before the upland or wetland soil samples started producing CH_4, however wetland samples produced more CH_4 over a shorter period indicating differences in the population abundance of methanogens between the two soil types.

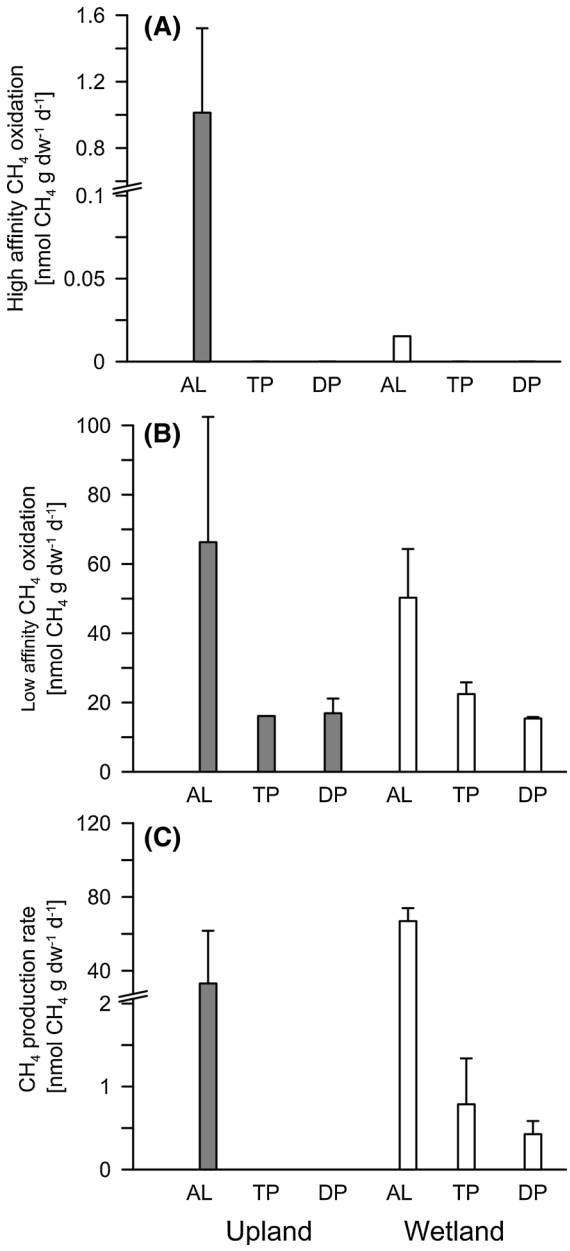

Fig. 5 Mean (±SE) **a** high affinity CH_4 oxidation, **b** low affinity CH_4 oxidation and **c** CH_4 production rates (nmol CH_4 g dw^{-1} day^{-1}) for the active layer (AL), top permafrost (TP) and deep permafrost (DP) in the upland (*grey bars*) and the wetland sites (*white bars*) at Flakkerhuk, Disko Island, Greenland. Symbols without an error bar indicate only one measurement of the rate

The lower CH_4 emission and potential production at Flakkerhuk can be attributed to the relatively young age of the wetland soils (less than 2,000 years old) and consequent lack of accumulated soil organic carbon (SOC) in the active layer and in the permafrost, particularly when compared to other sites. SOC accumulation rates for the wetland and upland soils at Flakkerhuk (Jensen et al. 2006) were 4 and 0.4 g C m^{-2} $year^{-1}$, respectively. Compared to carbon accumulation estimates for Canadian (14.9–22.6 g C m^{-2} $year^{-1}$; van Bellen et al. 2011), Siberian (12.1 to 23.7 g C m^{-2} $year^{-1}$; Turunen et al. 2001) and Finnish (18.5 g C m^{-2} $year^{-1}$; Turunen et al. 2002) wetlands, the SOC accumulation rates in these young Arctic wetlands are notably smaller. Thus, CH_4 emitted in situ at Flakkerhuk likely originates from the top 20 cm and not deeper from the profile. For all permafrost samples studied, the potential low-affinity CH_4 oxidation was on average 31-times higher than potential CH_4 production capacity (Fig. 5b, c). Also, it is noted that potential CH_4 oxidation rates surpassed CH_4 production on most occasions except for a few samples in the wetland and one upland sample (data not shown) similar to Barbier et al. (2012). Our data thus point to minimal potential to increase CH_4 emission from the soils if they are thawed because CH_4 emission originates from the active layer and oxidation offsets the production. Moosavi and Crill (1998) also found that up to 78 % of gross CH_4 production was oxidized before reaching the atmosphere, and Wagner et al. (2005) showed that increasing low-affinity CH_4 oxidation in wet tundra soils over the growing season most likely decreased net CH_4 emission.

Microbial activity

Activity of the methanogenic and methanotrophic organisms suggests that active microbial populations were abundant in the different soils layers. This was confirmed by the leucine incorporation approach (proxy for microbial cell production in soils (Michel and Bloem 1993)). Thus, incubation of soils from the active layers at 1–21 °C demonstrated production rates from 3.6 × 10^7 to 114 × 10^7 cells h^{-1} g dw^{-1} (using published conversion factors for leucine incorporation into bacterial cells; see Supplementary Material for details). In the permafrost soils, actively growing cells were present immediately after thaw but the production rates were about 10^2-fold lower than in the active soils. In the both the active layers and the permafrost soils, Q_{10} values between 2.7 and 4.7 were found. These values are close to Q_{10} values of about 4 observed for microbial leucine incorporation in forest

soils during a simulated temperature increase (Rousk et al. 2012) and suggest that the arctic microbes do not have specific temperature adaptations.

Microbial community composition and diversity

The microbial diversity and potential activity in the soil samples used for the CH_4 oxidation and production experiment was characterized by pyrosequencing of 16S rRNA genes from DNA and cDNA, respectively. A total of 496,225 high-quality, chimera-free sequences were successfully generated from 24 samples and taxonomy was assigned to 14,415 operational taxonomic units (OTUs) clustered at 97 % identity and represented by at least two sequences using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (http://qiime.org/).

Overall, the two soil types showed only minor differences in diversity at the phylum level. The phyla *Actinobacteria*, *Proteobacteria* and *Acidobacteria* comprised 70 and 80 % of the microbial community in wetland and upland soils, respectively (Table 1). The wetland soil displayed a significantly higher relative abundance of archaeal phyla. Especially, the phylum *Euryarchaeota* was tenfold more abundant in wetland samples, consistent with this phylum containing obligate anaerobic *Archaea* producing CH_4 from degraded organic matter. Rarefaction analysis of DNA sequences suggested slightly higher species richness in the active layer of wetland soils compared to upland soils (Fig. S2). For the potentially active microbial community (16S cDNA sequences), species richness at both sites and at all three sampled depths was similar. An exception was the top permafrost sample from wetlands, which had a significantly less diverse active community, likely because these samples were dominated by a single archaeal methanogenic genus (Table 1).

Abundance and diversity of methanogens and methanotrophs

The archaeal methanogens comprised a large part of the detected 16S cDNA sequences in wetland soils, compared to upland soils. The methanogens were almost exclusively assigned to the genus *Methanosaeta* of the order *Methanosarcinales*, as well as to the orders *Methanobacteriales* and *Methanomicrobiales* (Table 2) all of which have previously been detected in Arctic wetland active layers and permafrost in Canada (Barbier et al. 2012), Svalbard (Høj et al. 2005; Tveit et al. 2013) and Eastern Siberia (Ganzert et al. 2007).

As expected, the methanogenic community diversity and abundance differed between the two soil types. No methanogens were detected in the upland soil active layer whereas all three orders of methanogens as indicated by the 16S rRNA sequences were present in the active layer of the wetland soil (Table 2). The dominant types of methanogens in both soils at Flakkerhuk, based on 16S cDNA sequencing, belonged to the acetoclastic group of the

Table 1 Diversity and relative abundance (% ±SD) based on 16 s rDNA for all archaeal phyla and the bacterial phyla comprising 95 % of the community for the active layer of wetland and upland soils at Flakkerhuk on Disko Island, Greenland

Kingdom	Phylum	Wetland	±SD	Upland	±SD
Archaea	*Crenarchaeota*	0.10	0.01	0.03	0.00
	Euryarchaeota	0.30	0.03	0.01	0.00
	Parvarchaeota	0.05	0.01	0.01	0.00
Bacteria	*Actinobacteria*	29.5	0.6	27.7	0.7
	Proteobacteria	27.9	0.4	31.6	0.7
	Acidobacteria	12.9	0.2	21.0	0.4
	Chloroflexi	9.5	0.2	4.2	0.1
	Bacteroidetes	4.1	0.2	2.6	0.2
	Gemmatimonadetes	3.8	0.1	3.5	0.1
	Verrucomicrobia	2.8	0.1	2.4	0.1
	TM7	2.2	0.2	1.1	0.1
	Nitrospirae	1.6	0.0	0.3	0.0
	Firmicutes	1.2	0.1	0.2	0.0
	Other	4.1	0.1	5.3	0.1

Table 2 Relative abundance (% ± standard deviation) of DNA and cDNA of methanogens and methanotrophs at the order/family/genus level in the active layer (AL), top permafrost (TP), deep permafrost (DP) for wetland and upland soils at Flakkerhuk, Disko Island, Greenland

Order	Family	Genus	Wetland								Upland							
			DNA		cDNA						DNA		cDNA					
			AL	±SD	AL	±SD	TP	±SD	DP	±SD	AL	±SD	AL	±SD	TP	±SD	DP	±SD
Methanogens																		
Methanobacteriales	*Methanobacteriaceae*	*Methanobacterium*	0.07	0.03	0.18	0.03	0.51	0.02	0.82	0.06	0	0	0	0	0.01	0.00	0.02	0.00
Methanocellales	Unknown[a]	Unknown	0.03	0	0	0	2.85	0.07	0.90	0.05	0	0	0	0	0.01	0.00	0.02	0.00
Methanomicrobiales	*Methanoregulaceae*	*Candidatus Methanoregula*	0.01	0	0	0	0	0	0.06	0.01	0	0	0	0	0	0	0	0
	Methanospirillaceae	*Methanospirillum*	0.01	0	0	0	0	0	0.02	0.000	0	0	0	0	0	0	0	0
Methanosarcinales	*Methanosaetaceae*	*Methanosaeta*	0.12	0.58	4.07	0.58	43.9	3.83	11.1	1.14	0	0	0	0	0.09	0.01	0.61	0.01
	Methanosarcinaceae	*Methanosarcina*	0.05	0.21	1.50	0.21	0.48	0.02	0.42	0.03	0	0	0	0	0	0	0.03	0.000
Methanotrophs																		
Methanosarcinales	*ANME-2D*[b]	Unknown	0.02	0.00	0.06	0.01	0	0	0	0	0.06	0	0.008	0	0	0	0	0
Methylococcales[c]	*Crenotrichaceae*	*Crenothrix*	0.12	0.01	0.28	0.03	0.10	0.01	0.16	0.02	0	0	0	0	1.91	0.27	1.15	0.18
	Methylococcaceae	*Methylocaldum*	0.004	0.00	0.006	0.00	0	0	0.04	0.00	0	0	0	0	0	0	0	0
		Methylomicrobium	0	0	0	0	0	0	0	0	0	0	0	0	0.001	0.00	0	0
		Methylomonas	0.007	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Unknown	0	0	0	0	0.013	0.000	0	0	0	0	0	0	0	0	0.008	0.00
Rhizobiales[d]	*Methylobacteriaceae*	*Methylobacterium*	0	0	0	0	0	0	0.009	0.00	0	0	0	0	0.08	0.01	0.001	0.00
	Methylocystaceae	*Methylosinus*	0.006	0.00	0	0	0	0	0	0	0.005	0.00	0	0	0	0	0	0
		Unknown	0.008	0.00	0	0	0	0	0.002	0.0000	0.32	0.03	0.20	0.000	0.14	0.02	0.23	0.04

[a] Unknown designates families or genera that could not be specifically assigned to known taxonomic groups

[b] Represents a family containing anaerobic methanotrophic (ANME) archaea (Haroon et al. 2013)

[c] The methanotrophs within the *Methylococcales* order belong to the Type I methanotrophs (Trotsenko and Murrell 2008)

[d] The methanotrophs within the *Rhizobiales* order belong to the Type II methanotrophs (Trotsenko and Murrell 2008)

Methanosarcina and *Methanosaeta* genera (Thauer 1998). Although identification of 16S cDNA sequences alone cannot be taken as direct evidence of activity, this is in line with a previous observation that mainly the acetoclastic pathway of methanogenesis is active under cold soil temperatures (Barbier et al. 2012). Similar to Høj et al. (2005), methanogens of the order *Methanocellales* of the Rice cluster I lineage was notable in comprising almost 3 % of all 16S cDNA sequences (Table 2).

A tenfold increase in relative abundance of 16S cDNA for the *Methanosaeta* genus was observed in the top permafrost layer as compared to the active layer as well as a minor increase of the cDNA abundance of an unknown genera in the order *Methanocellales*, which was absent from the active layer (Table 2). The relative abundance of 16S cDNA from the *Methanosarcina* genus decreased slightly in permafrost layers compared to the active layer for the wetland (Table 2). In the deep permafrost layer all detected genera of methanogens were represented by 16S cDNA sequences, with the *Methanosaeta* genus representing the majority. Notably, the average fraction of total archaeal 16S cDNA originating from methanogens in wetlands was 47 and 13 % in the top and deep permafrost layers, respectively, suggesting that methanogens dominated the microbial activity in some of the permafrost soils.

Similar to the wetland, but at much lower abundance, methanogenic 16S cDNA occurred in permafrost layers of the upland soils and was constricted to *Methanobacterium* and *Methanosaeta*, as well as to unknown genera within the *Methanocellales* family. In conclusion, the methanogenic community composition of especially the wetland soil, but also the uplands soil at Flakkerhuk, resembled other Arctic soils.

Although no methanogenic DNA or cDNA was detected in the active layer of the upland soil CH_4 production in all three samples from the upland soil could still be observed in the laboratory (Fig. 5). We cannot rule out that abiotic degassing could cause the observed increase in headspace CH_4 concentration and hence interpreted as a flux, but this increase was slow and did not occur until after a few weeks indicating slow growth of methanogens rather than rapid degassing following thaw in the laboratory.

Several genera of methanotrophs of the orders *Methylococcales* (Type I) and *Rhizobiales* (Type II) were identified from the cDNA sequences in both wetland and upland soils, although with a somewhat higher occurrence in uplands (Table 2), caused primarily by the genus *Crenothrix* within the order *Methylococcales* (Stoecker et al. 2006), which was, however, absent in the active layer of the upland soils but present in all other investigated layers (Table 2). Several OTUs that could not be assigned to a specific genus were classified as members of families known to contain methanotrophic genera. These could potentially represent unknown methane oxidizing species (Table 2). This could potentially represent unknown methane oxidizing species (Table 2). The methanotrophic community at the DNA level was more diverse in the active layer of the wetland compared to upland soil considering the relative abundance of sequences (Table 2).

In the active layer of the wetland, 16S cDNA was detected predominantly from the Type I MOBs *Chrenothrix* and *Methylocaldum*, but not Type II, suggesting that the Type I MOBs dominate in the wetland environment. This finding is in accordance with other studies of Arctic wetlands that found a predominance of Type I MOBs in wet soil environments. Contrary to this, only 16S cDNA of Type II MOBs within the *Rhizobiales* order was detected in the active layer of the upland soil. Although cDNA of the *Methylosinus* genus was observed at Flakkerhuk, the relative abundance was very low (Table 2). This genus has previously been found in Siberian upland soil (Knoblauch et al. 2008). It is possible that the majority of MOB 16S cDNA sequences in the active layer are related to an unknown genera that are capable of oxidizing atmospheric CH_4. It has been shown that certain Type II MOBs of the *Methylocystis* genus within the *Methylocystaceae* family (Kolb 2009) or closely related aerobic methanotrophs within the upland-soil-cluster α or γ (USCα/USCγ), oxidize atmospheric CH_4 in soils (Shrestha et al. 2012; Nauer et al. 2012). The relatively short length of the 16S sequences generated by pyrosequencing constrained deeper probing at genus and species level within the *Methylocystaceae* family Thus, it was not possible to determine whether the relatively large population of an unknown genus within the *Methylocystaceae* family contains high-affinity MOBs. Recently, Martineau et al. (2014) detected active high-affinity MOBs in the active layer of permafrost soils in Canada confirming the presence of this group of MOBs in similar Arctic soils.

In contrast to the active layer, the relative abundances of all detected 16S cDNA from MOBs were higher in upland permafrost layers compared to wetlands. For the wetland soil the *Chrenothrix* genus was the most abundant MOB in both top and deep permafrost with minor proportions of the other Type I MOBs in the *Methylocystaceae* family, *Methylocaldum* and an unknown genus, as well as the Type II MOBs *Methylobacterium* and unknown genera of the *Methylocystaceae* family. For the upland soils cDNA from *Chrenothrix* was likewise detected in both permafrost layers and at a markedly higher abundance than for the corresponding layers of the wetland. The Type I MOB, *Methylomicrobium*, was also present in the top permafrost of the upland albeit in low abundance. Similar to the active layer, the dominant fraction of the MOB community in the upper and deeper permafrost layers belonged to unknown genera of the *Methylocystaceae* family and only a very small proportion of the Type II MOB, *Methylobacterium*. Putative methane oxidizers of the order *Methylacidiphilales* in the phylum *Verrucomicrobia* were detected at low levels in all three soil layers, but since these OTUs could not be assigned to any known families or genera it is unclear whether they could be involved in methane oxidation in these soils (data not shown).

Upland soils displayed a markedly higher relative abundance of MOBs than wetland soils, both in the active layer as well as the permafrost layers. Although the presence of anaerobic archaeal CH_4 oxidizers were indicated, their importance in oxidizing CH_4 in these soils may be limited as their abundance is lower compared to the aerobic MOB (Table 2). The abundance of MOBs in wetland soils was likely constrained by the lack of oxygen that can only be supplied effectively by the aerenchyma tissue of specially adapted vascular plants that also grow in the wetlands at Flakkerhuk. However, for the upland soils the active layer was presumably aerated due to well-drained conditions during the growing season and it is likely that the MOBs living in the active layer could have colonized the permafrost and thrive better than the same MOBs in the wetland because of higher oxygen availability.

Conclusions

We measured in situ CH_4 fluxes during a three week investigation period in the summer of 2011, potential rates of CH_4 oxidation and production, and metagenomic data for the functional groups of methanotrophs and methanogens for active and permafrost layers were to improve the understanding on CH_4 dynamics in typical upland and wetland soils for a young Arctic landscape on Disko Island, West Greenland.

Generally, there was limited soil development and upland soils had only shallow organic-rich A-horizon with minimal soil development below 20 cm. As expected wetland soils were net emitters of CH_4, while drier upland soils were net sinks of atmospheric CH_4 comparable to uptake rates for temperate forest soils. However, soil organic carbon accumulation rates and in situ CH_4 emission were markedly less than in comparable wetlands across the Arctic, which is attributed to the relative young age of the landscape.

Also, our data shows that high rates of low-affinity CH_4 oxidation exceed potential CH_4 production in active and permafrost layers of wetland soils which could limit in situ CH_4 emission from wetlands. In upland soils high-affinity oxidation of atmospheric most likely CH_4 drives the net uptake in upland soils. Thus, the observed in situ and laboratory CH_4 dynamics indicate a profoundly different regulation of soil-atmosphere exchange of CH_4 in upland and wetland soils..

The potential biological growth and functional diversity of the methanotrophic and methanogenic community by pyrosequencing analysis of 16 s rRNA genes was also investigated. The microbial growth was highest in active layers and responded similarly to temperature increase. Once thawed, all permafrost samples showed microbial activity, which is in line with our assumption of both CH_4 oxidation and production being of biological origin. Methanogens were detected in all soils except in the active layer of the upland soils. After thaw CH_4 produced in active or permafrost layers in upland and wetland was offset by CH_4 oxidation in the same layers. In the active layer of upland soils only activity of Type II MOBs was detected, whereas the active layer of the wetland soils possessed both Type I and Type II, although only Type I were active. This could indicate a higher proportion of high-affinity MOBs in upland soils which is in line with in situ uptake of CH_4 as well as detection of high-affinity CH_4 oxidation in the laboratory. The upland and wetland soils harbored different assemblies of methanogens and MOBs indicating a niche adaption of methanotrophs governed by soil moisture that

indicate differential microbial regulation of CH_4 fluxes in upland and wetland soils.

We conclude that the soils in the young Arctic landscape at Flakkerhuk show similar potential to oxidize CH_4, but not production of CH_4 compared to older landscapes elsewhere in the Arctic. This is important to take into consideration when evaluating the impact of permafrost thaw on Arctic CH_4 budgets. Future work should center on how the functional diversity of methanotrophic communities in both upland and wetland soils respond to changes in soil hydrology and temperature.

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