Brief Communication: The reliability of gas extraction techniques for analysing CH_4 and N_2O compositions in gas trapped in permafrost ice-wedges

Ji-Woong Yang[1*], Jinho Ahn[1], Go Iwahana[2], Sangyoung Han[1], Kyungmin Kim[1] and Alexander Fedorov[3,4]**

[1]School of Earth and Environmental Sciences, Seoul National University, Seoul, South Korea
[2]International Arctic Research Center, University of Alaska, Fairbanks, USA
[3]Melnikov Permafrost Institute, Russian Academy of Science, Yakutsk, Russia
[4]North-Eastern Federal University, Yakutsk, Russia
*Now at: Laboratoire des Sciences du Climat et de l'Environnement, LSCE/IPSL, CEA-CNRS-UVSQ, Université Paris-Saclay, Gif-sur-Yvette, France
**Now at: Division of Earth and Planetary Materials Science, Department of Earth Science, Graduate School of Science, Tohoku University, Sendai, Japan

Correspondence: Jinho Ahn (jinhoahn@snu.ac.kr)

Abstract. Methane (CH_4) and nitrous oxide (N_2O) compositions in ground ice may provide information on their production mechanisms in permafrost. However, existing gas extraction methods has not been well tested. We test conventional wet and dry gas extraction methods using ice-wedges from Alaska and Siberia. We find that both methods extract gas from the easily extractable parts of the ice (e.g., gas bubbles), and yield similar results for CH_4 and N_2O mixing ratios. We also find insignificant effects of microbial activity during wet extraction. However, both techniques are unable to fully extract gas from the ice, presumably because gas molecules adsorbed onto or enclosed in soil aggregates are not easily extractable. Estimation of gas production in subfreezing environment of permafrost should consider the incomplete gas extraction.

1. Introduction

Permafrost preserves large amounts of soil carbon (C) and nitrogen (N) in a frozen state

(e.g., Hugelius et al., 2014; Salmon et al., 2018), temporarily removing this frozen carbon and nitrogen from active global cycles. Therefore, future projections of permafrost stability are of great interest, particularly because thawing permafrost may lead to decomposition and/or remineralization of the buried soil C and N and their abrupt emission into the atmosphere in the form of greenhouse gases (GHGs) – carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which in turn can trigger positive feedbacks (e.g., Salmon et al., 2018). In addition, the projected polar amplification (e.g., Masson-Delmotte et al., 2013) may strengthen these positive feedbacks. However, the processes responsible for in-situ C and N remineralization and GHG production in ground ice are poorly understood, despite the fact that ground ice accounts for a substantial portion (up to approximately 40–90% by volume) of Pleistocene ice-rich permafrost, or Yedoma (e.g., Kanevskiy et al., 2013; Jorgenson et al., 2015).

The gases trapped in ground ice allow unique insights into the origin of ground ice and evidence for in-situ microbial aerobic and anaerobic respirations (Boereboom et al., 2013; Kim et al., 2019; Lacelle et al., 2011). Among others, the GHGs in ground ice may provide detailed information on in-situ biogeochemical processes responsible for GHG production (i.e., methanogenesis, nitrification, and denitrification) (e.g., Boereboom et al., 2013; Kim et al., 2019). However, analytical methods remain poorly scrutinized. Boereboom et al. (2013) utilized the conventional melting-refreezing method (wet extraction) used in polar ice core analyses. In this technique, the ice samples were melted under a vacuum to liberate the enclosed gases, then refrozen to expel the dissolved gases present in the meltwater. Other studies conducted by Russian scientists used an on-site melting method in which a large (1–3 kg) block of ground ice sample was melted in a saturated sodium chloride (NaCl) solution, in order to minimize gas dissolution (Arkhangelov and Novgorodova, 1991). A recent study instead used a dry extraction technique to prevent the microbial activity during wet extraction (Kim et al., 2019), which employed a needle-crusher in a vacuum to crush approximately 10 g of ice sample

without melting (Shin, 2014).

In this study, for the first time we test the reliability of both wet and dry extraction methods for CH_4 and N_2O mixing ratios and contents (volume or moles of gas in a unit mass at standard temperature and pressure conditions (STP)) using permafrost ground ice samples. Ice-wedge samples from Alaskan and Siberian permafrost were used because ice wedges are one of the most abundant morphological features of massive ground ice, consisting of approximately 5 to 50% by volume of the upper permafrost (Kanevskiy et al., 2013; Jorgenson et al., 2015). More specifically, this study aims to address the following scientific questions: 1) Do wet and dry extraction methods yield different results? 2) Are the melting-refreezing results affected by microbial activity during gas extraction? 3) How effectively does the wet/dry extraction extract gases from ice wedges? To address the first question, CH_4 and N_2O results from dry and wet extractions were compared. For the second question, we applied the wet extraction method to both biocide-treated and control samples. Finally, for the third question we carried out tests with and without extended number of hitting ice with a needle system in a crushing chamber, as well as additional dry extraction from ice samples that had been degassed by our wet extraction method.

2. Materials and Methods

2.1.Ice samples and sample preparation

The ice-wedge samples used in this study were collected from Churapcha, Cyuie (central Yakutia), and Zyryanka (north-eastern Yakutia) in Siberia, as well as from northern Alaska (Supplementary Figure 1). The Churapcha site (61.97°N, 132.61°E) is located approximately 180 km east of Yakutsk. The Cyuie site (61.73°N, 130.42°E) is located approximately 30 km southeast of Yakutsk. The Cyuie samples were collected from two outcrops (CYB and CYC) (Kim et al., 2019). At each site, 30 cm long ice-wedge cores were

drilled perpendicular to the outcrop surface (Supplementary Figures 2 and 3).

Zyryanka is located in the southern boreal region of the Kolyma River, at the junction of the Chersky and Yukaghir Ranges, in a region affected by thermokarst development (Fedorov et al., 1991). Site A (Zy-A) is located on a tributary of the Kolyma River, approximately 22 km north of Zyryanka. Site B (Zy-B) is approximately 14 km west of the start of the Kolyma tributary, which begins ~11 km north of Zyryanka. Site F (Zy-F) is located approximately 4 km west of the tributary that leads to site B. The ground ice samples were collected from riverbank walls exposed by lateral erosion using a chainsaw (Supplementary Figure 4). Most of the outcrops that were sampled for ground ice were on the first (lowest) terrace of the river.

For the Alaskan sampling locations, Bluff03 (69.40°N, 150.95°W) and Bluff06 (69.14°N, 150.61°W) are located in the Alaska North Slope region, approximately 120 and 150 km from the Arctic Ocean, or 100 and 70 km northwest of the Toolik Field Station (68.63°N, 149.59°W), respectively. Samples from Bluff03 were collected from the bluff walls that had developed by gully formations on a gentle slope of the Yedoma using a chainsaw. Samples of Bluff06 were collected from outcrops within eroded frozen peatland in a thaw lake basin (Supplementary Figure 5). All the ice-wedge samples used in this study were stored in a chest freezer at < -18°C before analysis.

The ice-wedge ice is most different from polar ice cores, in that their gas mixing ratios are not homogeneous (e.g., Kim et al., 2019), which may hinder exact comparison with results from adjacent ice samples. We therefore randomly mixed sub-samples to reduce the effect of the heterogeneous gas composition distribution (random cube method hereafter). Approximately 100–200 g of an ice-wedge sample was cut into 25 to 50 cubes of 3–4 g each, and for each experiment, ~10 to 12 cubes were randomly chosen so that the total weight of the sub-sample was ~40 g.

105 **2.2.Gas extraction procedures**

106 ***Dry extraction (needle crusher)***

107 For dry extraction, we used a needle-crusher system at the Seoul National University
108 (SNU, Seoul, South Korea) (Shin, 2014). In brief, 8~13 g of ice sample were crushed in a cold
109 vacuum chamber (extraction chamber). The ice samples were usually hit five times by the
110 needle set. The temperature within the extraction chamber was maintained at -37°C by using a
111 cold ethanol-circulating chiller. The extracted gas was dried by passing it through a water vapor
112 trap at -85°C and cryogenically trapping it in a stainless-steel tube (sample tube) at
113 approximately -257 °C using a helium closed-cycle refrigerator (He-CCR). Since the extraction
114 chamber cannot accommodate ~40 g of ice at once, the ~40 g of random cube sub-samples
115 were extracted using three sequential extractions and the gas liberated from each extraction
116 was trapped in a sample tube.

117 Following extraction, the sample tubes were detached from the He-CCR, warmed to
118 room temperature (~20°C), and attached to a gas chromatograph (GC) equipped with an
119 electron capture detector (ECD) and a flame ionization detector (FID) to determine the mixing
120 ratios of CH_4 and N_2O. Details of the GC system are given in Ryu et al. (2018). The daily
121 calibration curves were established using working standards of 15.6 ± 0.2 ppm CH_4, 10000 ±
122 30 ppm CH_4, 2960 ± 89 ppb N_2O, 29600 ± 888 ppb N_2O, and a modern air sample from a
123 surface firn at Styx Glacier, Antarctica (obtained in November 2016), which was calibrated as
124 1758.6 ± 0.6 ppb CH_4 and 324.7 ± 0.3 ppb N_2O by the National Oceanic and Atmospheric
125 Administration (NOAA).

126

127 ***Wet extraction (melt-refreeze)***

128 For the control and $HgCl_2$-treated wet extraction experiments, a melting-refreezing wet
129 extraction system at SNU was employed (Yang et al., 2017; Ryu et al., 2018). The gas

130 extraction procedure is identical to the procedure described in Yang et al. (2017) and Ryu et al.
131 (2018), except for the sample gas trapping procedure (see below). Ice-wedge sub-samples of
132 ~40 g (composed of 10–12 ice cubes for each) were placed in a glass container welded to a
133 stainless-steel flange (sample flask), and the laboratory air inside the sample flasks was
134 evacuated for 40 min. The sample flasks were then submerged in a warm (~50°C) tap water
135 bath to melt the ice samples. After melting was complete, the meltwater was refrozen by
136 chilling the sample flasks with cold ethanol (below -70°C). The sample gas in the headspace
137 of each sample flask was then expanded to the volume-calibrated vacuum line to estimate the
138 volume of extracted gas, and trapped in a stainless-steel sample tube by the He-CCR device.
139 In this study, we attached the He-CCR device to our wet extraction line and the gas samples in
140 the flasks were cryogenically trapped. The reasons for using He-CCR instead of direct
141 expansion to a GC are twofold: 1) to better compare the dry and wet extraction methods by
142 applying the same trapping procedure, and 2) to maximize the amount of sample gas for GC
143 analysis, because the gas expansion from a large flask allows only a small fraction of gas to be
144 measured by the GC.

145 For biocide-treated tests, 1.84 mmol of mercuric chloride ($HgCl_2$) was applied per unit
146 kilogram of soil, following established procedures for soil sterilization (Fletcher and Kaufman,
147 1980). We obtained the average dry soil mass (0.33 g) from the leftover meltwater samples of
148 the previous wet extractions, which were carried out for comparison between dry- and wet
149 extractions. Taking the average dry soil mass (0.33 g) into account, we added 24 μL of saturated
150 $HgCl_2$ solution (at 20°C) to the sample flasks. The flasks with $HgCl_2$ solution were then frozen
151 in a deep freezer at < -45°C to prevent the dissolution of ambient air into the solution during
152 ice sample loading. After the wet extraction procedure was complete, the extracted gas was
153 trapped in a sample tube and the CH_4 and N_2O mixing ratios were determined using the same
154 GC-ECD-FID system as the dry-extracted gas. The resulting CH_4 and N_2O mixing ratios have

not been corrected for partial dissolution in ice melt in the flasks, because CH_4 and N_2O trapped in refrozen ice are negligible compared to the ranges of the systematic blanks (see Appendix).

2.3.Gas content

The analytical methods described previously are for determining the mixing ratios of CH_4 and N_2O in the extracted gas. To convert these mixing ratios into moles of CH_4 and N_2O per unit mass of ice-wedge sample (CH_4 and N_2O content, respectively, hereafter) requires data regarding the amount of gas extracted. The gas content is a measure of gas volume enclosed in a unit mass of ice sample at STP (in mL kg_{ice}^{-1}). Thus, the CH_4 and N_2O contents can be calculated using the gas content, the total mass of the random cube ice, and the gas mixing ratio. The gas content in the control and $HgCl_2$-treated wet extraction experiments was calculated from the temperature and pressure of the extracted gas and the internal volume of the vacuum line. The details of the extraction system and correction methods used for estimating gas content are described in Yang (2019). Similarly, the gas content of the dry extraction samples was also inferred from the volume and pressure of gas inside the vacuum line once the sample tube was attached to the line for GC analysis. The uncertainties of the calculated CH_4 and N_2O contents were calculated by using error propagation of the blanks and gas content uncertainties (see Appendix for uncertainty estimation of the blank corrections and gas contents).

2.4.Dry soil content

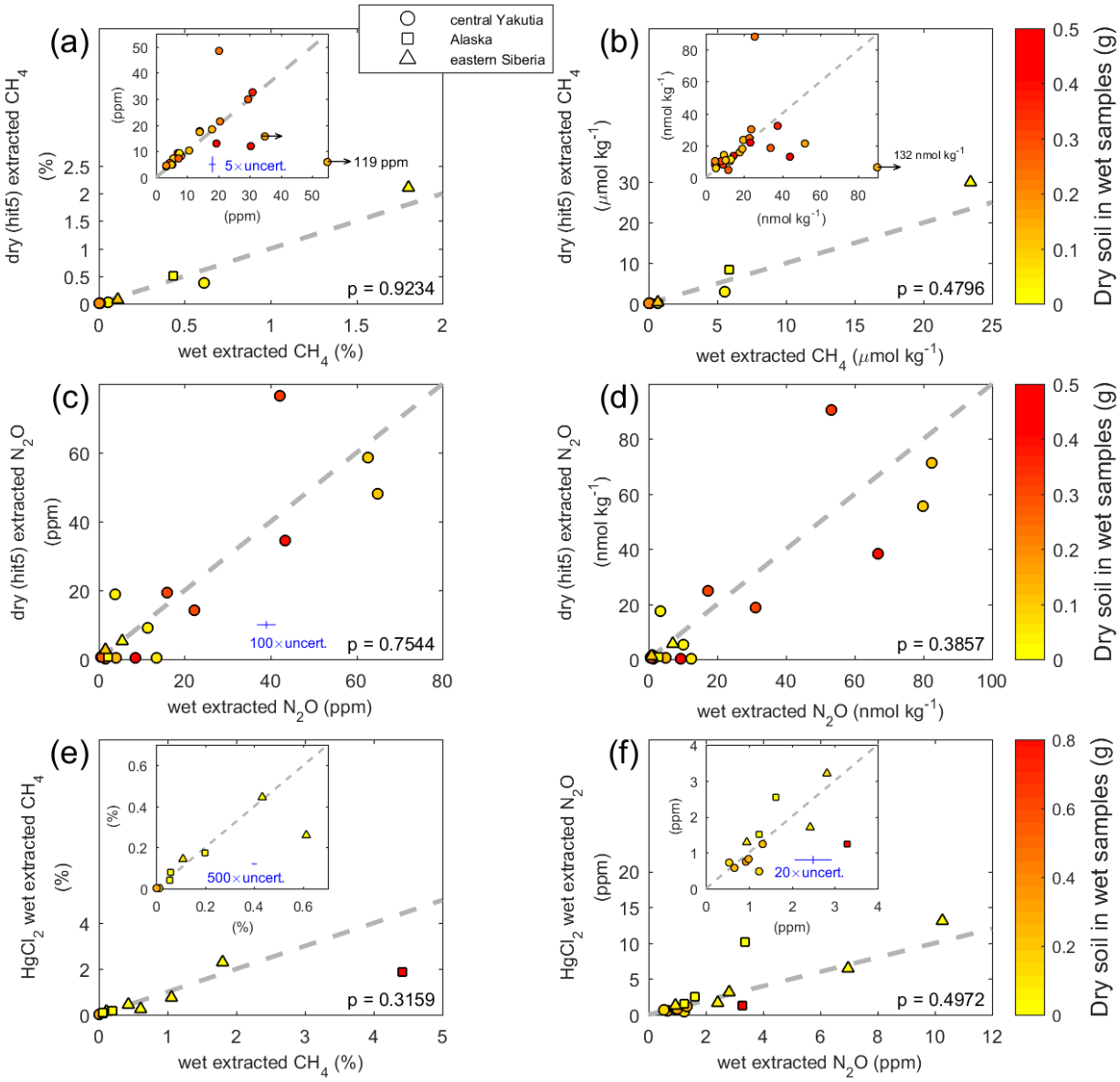
Dry soil content was measured using the leftover meltwater from the control-wet extraction tests. After the control-wet extractions were complete, the sample flasks were shaken thoroughly and the meltwater samples were each poured into a 50 mL conical tube. The meltwater and soils were separated by a centrifugal separator at 3000 rpm for 10 min. The

separated wet soils were wind-dried in evaporating dishes at approximately 100°C for 24 hours. The weight of each individual evaporating dish was pre-measured before use. The dry soil content was calculated by subtracting the weight of the evaporating dish from the total weight of the dried soil sample plus the evaporating dish.

3. Results and Discussion

3.1.Comparison between wet and dry extraction methods

The results from the wet and dry extractions were compared using 23 ice-wedge samples (21 for N_2O) from Alaska and Siberia. In both the CH_4 and N_2O mixing ratio analyses, we found that the wet and dry extraction results did not differ significantly ($p > 0.1$), regardless of sampling site or soil content (Figure 1, a to d). We note that the heterogeneous distribution of gas mixing ratios of in centimetre scales (Kim et al., 2019) may not have been completely smoothed out by our sub-sample selection, although we randomly chose 8–12 ice cubes for each measurement. Some previous studies have avoided using the wet extraction method because of potential reactivation of microbial CH_4 and/or N_2O production in ice melt (e.g., Cherbunina et al., 2018; Kim et al., 2019). Assuming that activation of microbial metabolism is unlikely during dry extraction at a temperature of -37°C in the extraction chamber for < 1 h, our findings may imply that wet extraction does not stimulate microbial reactivation to a measurable extent.



199

Figure 1. Comparison of CH_4 and N_2O mixing ratios and contents obtained by different extraction methods. Shown are scatter plots between wet- and dry (hit5) extraction results of CH_4 (a and b) and N_2O (c and d), and between control- and biocide-treated wet extraction results for CH_4 (e) and N_2O (f). The 'hit5' denotes the dry extraction with five times hitting (see Section 3.3). Left panels (a, c, and e) and (f) present in mixing ratios of gas in bubbles, while right (b) and (d) panels in moles of gas in a unit mass of ice (gas content). The sampling locations are indicated by different symbols. The color of each data point indicates the dry soil weight in the subsamples used in control wet extraction. The 1-sigma uncertainties of the mixing ratios (a, c, e, and f) are magnified by 5x, 20x, 100x, and 500x as denoted as blue error bars (see Appendix). The error bars are not visible where the error bars are smaller than markers. The grey dashed lines are 1:1 reference line. Note that the units of the axes of the insets in (e) and (f) are identical to the original plots. The p-value of two-sided Students' t-test of each comparison is denoted at the bottom right corner of each plot.

200

3.2.Testing microbial alteration during wet extraction

To test the microbial production of CH_4 and N_2O during wet extraction more accurately, we conducted wet extraction experiments on samples treated with $HgCl_2$, a commonly used effective biocide (e.g., Torres et al., 2005), and compared the results with those of untreated (control) wet extractions. We prepared 12 additional ice-wedge samples using the random cube method for these tests (see Materials and Methods section). We found no significant differences between the control and $HgCl_2$-treated wet extraction results for both CH_4 and N_2O mixing ratios (Figures 1e and 1f), indicating that the bias due to microbial activity during approximately an hour of the melting-refreezing procedure is not significant. This is further supported by tests on an additional 12 ice-wedge samples (using the random cube protocol) treated with 2-bromo-ethane-sulfonate (BES), a specific methanogenesis inhibitor (e.g., Nollet et al., 1997) (Figure A3). Similar to the $HgCl_2$-treated experiments, 25 μL of a saturated BES solution was added to each sample flask. These additional tests were carried out only for CH_4. The two-sided t-test for the CH_4 data indicates an insignificant difference between the two results ($p > 0.9$). Data from individual sampling sites also do not show significant differences ($p > 0.9$ for the Alaskan samples and $p > 0.5$ for the central Yakutian samples).

According to microbial sequencing studies that have shown the presence of viable microbes in permafrost and ground ice (e.g., Katayama et al., 2007), it is likely that culturable microbes exist in the ice-wedge samples used in this study. However, considering that at least 14 days and up to 3 months of culturing was required to identify microbe colonies extracted from ground ice (Katayama et al., 2007; Lacelle et al., 2011), our melt-refreeze time of an hour was insufficient for microbial activity to resume and produce CH_4 and N_2O.

3.3.Dry extraction efficiency and gas mixing ratios

One limitation of our needle crushing dry-extraction technique is the inability to

completely extract gas from ice samples, because small ice particles and/or flakes placed in the space between the needles are not fully crushed. The gas extraction efficiency of the SNU needle crusher system has been reported as ~80–90% for polar ice core samples (Shin, 2014). However, the gas extraction efficiency has not been tested for ice-wedge samples. Depending on the extraction efficiency, the needle crushing method could underestimate the gas contents if the gas is not completely extracted. Another possible bias in the gas mixing ratios arises if the CH_4 and N_2O compositions are different between the crushed and uncrushed portions of the ice-wedge samples.

To estimate the biases arising from incomplete gas extraction, we designed a series of tests to identify the differences of the CH_4 and N_2O mixing ratios and contents between the crushed and uncrushed sample portions. Each ice-wedge sample that was randomly collected was first crushed by the regular dry extraction procedure (by hitting it five times with the needle system, 'hit5'), and the gas liberated from the sample was trapped in a sample tube. Then we performed an additional 100 hits on the leftover ice ('hit100'), monitored the amount of additional gas liberated, and trapped the additional gas in a separate sample tube. Comparisons between the hit5 and hit100 results are summarized in Table 1.

Here we regard the ratio of gas content of hit100 to that of hit5 (hit100/hit5 ratio hereafter) as a measure of the gas extraction efficiency of the needle crusher system. The results demonstrate an average hit100/hit5 ratio of gas content of 0.40 ± 0.07 for the Zyryanka samples, 0.24 ± 0.07 for the Bluff samples, and 0.14 ± 0.11 for the Cyuie samples (Table 1). Despite the fact that the number of samples was limited, the ice-wedge samples from the different sites show distinct hit100/hit5 ratios of the amount of extracted gas. However, we observed that the leftover ice from the Bluff and Zyryanka samples were not well-crushed, even after 100 hits with the needle crusher. This was especially true if the ice sub-samples contained soil aggregates: the frozen soil aggregates were barely crushed. In contrast, the Cyuie samples were

relatively well-crushed, and the leftover samples were apparently finer-sized ice flakes. We also observed that the hit100/hit5 ratios of gas content are highly variable within samples from a particular site, implying that the extraction efficiency of the needle crusher not only depends on site characteristics, but also on the individual ice sample hardness. When compared with the dry soil content measured from the sub-samples used for wet extraction, no relationship was observed between the dry soil content and the extraction efficiency (Figures 1 and A3). In addition, in the case of samples uncrushed by the hit100 test, it is difficult to estimate the extraction efficiency using the hit100/hit5 ratio of gas content, as the hit100 tests liberated only a marginal portion of gas from these samples. This is because the large-sized uncrushed soil aggregates or particles may have prohibited the needle crusher from crushing the small-sized ice flakes or grains. The needles move up and down together, as they are fixed to a pneumatic linear motion feedthrough device, thus if there is a sizable soil clod that cannot be crushed, it blocks the needle crusher from moving further down. Therefore, we do not recommend using a needle crusher system to measure gas contents in ice-wedge samples.

The hardness of the ice samples may also affect the gas mixing ratio analysis in the hit5 and hit100 procedures. The hit100/hit5 ratio of CH_4 mixing ratio of Bluff and Zyryanka samples are less than 1 in four out of six samples, yielding an average of 0.9 ± 0.5. However, all five samples from the Cyuie ice-wedges have ratios greater than 1, with an average of 4.7 ± 2.6 (Table 1). The higher hit100/hit5 ratio of CH_4 mixing ratios of Cyuie samples indicates that the gases extracted via the hit100 procedure have higher CH_4 mixing ratios than the gases extracted via the hit5 procedure. Considering these results with those discussed previously, we speculate that there are three ways gas can be trapped in ice-wedge ice: enclosed in bubbles, adsorbed on soil particles, and entrapped in soil aggregates. The better-crushed leftover ice flakes in the Cyuie samples may have allowed most of the gas in bubbles and part of the CH_4 molecules adsorbed on soil particles and/or trapped in microsites within soil aggregates to be

liberated. Thus, the hit5 CH_4 mixing ratios of the Cyuie samples may more reflect the gas mixing ratios in bubbles, while the hit100 results reflect more of the contribution from gas adsorbed on soil and trapped within soil aggregates than the hit5 results because the ice sample containing larger-sized aggregates has greater hardness than those with smaller aggregates or fine particles. If this is the case for the Cyuie samples, we can infer that CH_4 is more concentrated in soil particles and in microsites within soil aggregates, compared to in bubbles in the ice. This is partly supported by evidence that ice-wedge layers exhibit relatively trace amounts of CH_4 compared to the surrounding permafrost soil layers (Rivkina et al., 2007); however, this needs to be further evaluated by detailed microbial and chemical analyses. In the meanwhile, in the Bluff and Zyryanka samples, the hit5 results reflect the mixing ratios of the gases from the crushed portions, regardless of their origin: bubbles, particle adsorption, or microsites in aggregates (Table 1). Given that some of the Bluff and Zyryanka ice-wedge samples were not fully crushed by the hit100 tests, it may require additional hits or another extraction technique. Unlike CH_4, the N_2O mixing ratios from the hit100 extractions are higher than the hit5 in ten out of eleven samples, regardless of the sampling site. The hit100/hit5 ratios of N_2O mixing ratios of the Bluff and Zyryanka samples (1.9 ± 0.8 on average) are not significantly different ($p = 0.32$) from those of the Cyuie samples (2.9 ± 1.8 on average). This can probably be explained by the fact that the N_2O mixing ratio is not necessarily higher in soil-rich ice because N_2O is an intermediate product of denitrification, while CH_4 is produced as the final product of methanogenesis.

One may expect that a different crushing technique might be more suitable for ice-wedge samples. However, none of the existing dry extraction techniques - centrifugal ice microtome (Bereiter et al., 2013), mechanical grater (Etheridge et al., 1988), or ball-mill crusher (Schaefer et al., 2011) is more advantageous for ice-wedge analysis compared to the needle crusher system used in this study. The hard portion of ice wedges (e.g., frozen soil

aggregates, large soil particles) could easily damage the metal blades of the centrifugal ice microtome and mechanical grater devices, or block the space within the ball-mill chamber, limiting the movement of the milling balls.

It is worth noting that friction between stainless steels could produce CH_4 with carbon from the damaged stainless-steel surface and hydrogen gas (Higaki et al., 2006). If needle crushing causes contamination in this way, the dry extraction results should be affected by the number of hits. To check the impact of the needle crushing procedure on ice-wedge CH_4 and N_2O measurements, we carried out blank tests by changing the numbers of hits from 5 to 100. The results of these tests show no systematic offset among the experiments with different numbers of hits (Figure A2), which implies that the crushing procedure does not affect the dry extraction results for CH_4 and N_2O. Even though a small of contamination does exist, its effects have already been subtracted via blank correction and taken into account in the overall error estimation (see Appendix). Therefore, we consider that our findings are not artefacts of metal friction during crushing.

To summarize, from the hit5 and hit100 comparison tests, we found that 1) the needle crusher method is not able to fully crush the ice-wedge ice samples and thus is unsuitable for measuring gas contents in a unit mass of ice, and that 2) weak crushing (e.g., a small number of hits by the needle crusher system) may better reflect gas mixing ratios of the soft parts of the samples (such as air bubbles) than strong crushing (e.g., a greater number of hits).

Table 1. Results of dry extraction tests with 5- and additional 100 times hitting ice-wedge samples, denoted as 'hit5' and 'hit100', respectively. 'hit100/hit5' is the ratio in extracted gas content or gas mixing ratio of 'hit100' to 'hit5' cases. Also shown are gas content results from both experiments, where the hit100 values are given both in the unit of ml kg^{-1} at STP conditions and µmol/kg (in parenthesis). It should be noted that the 'hit100' gas content results indicate the additional amount of gas extracted after 'hit5' crushing and evacuation.

Site Location	Sample	soil content	gas content				CH_4 mixing ratio				N_2O mixing ratio			
			Wet control	Dry hit5	Dry hit100	hit100/hit5	Wet control	Dry hit5	Dry hit100	hit100/hit5	Wet control	Dry hit5	Dry hit100	hit100/hit5
		wt. %	ml/kg	ml/kg	ml/kg		ppm	ppm	ppm		ppm	ppm	ppm	
Zyryanka, Northeastern Siberia	Zy-A-W1-D	0.155	20.2	13.1	6.3	0.48	6138	3713	2721	0.7329	11.37	9.10	10.15	1.12
	Zy-F-1	0.618	13.5	8.1	3.4	0.42	1080	655.6	173.5	0.2646	1.57	2.81	2.65	0.942
	Zy-A-W1-Low	0.049	30.6	27.8	8.0	0.29	4309	5073	4818	0.9497	2.07	0.69	2.02	2.9
	Zy-B-Low-B	0.107	29.1	23.9	10.0	0.418	18030	21010	35290	1.680	5.37	5.32	15.36	2.89
Northern Alaska	Bluff03-IW1	2.07	13.2	12.2	2.6	0.21	44160	25230	12240	0.4851	5.58	2.36	4.93	2.09
	Bluff06-B3	0.078	20.1	20.9	5.6	0.27	558.7	164.2	219.5	1.337	3.74	18.78	30.14	1.605
Cyuie, Central Yakutia	CYC-01-B	0.252	18.0	21.7	7.1	0.33	18.0	18.3	25.4	1.39	1.55	1.60	2.59	1.62
	CYB-04-C	0.498	20.9	30.7	1.5	0.049	20.2	48.4	165.6	3.42	0.71	0.65	2.96	4.5
	CYB-03-A	0.420	19.7	23.7	1.0	0.041	20.5	21.5	67.1	3.12	0.91	1.01	1.06	1.05
	CYB-02-A	0.403	32.0	25.5	1.9	0.073	29.1	18.7	159.8	8.55	1.00	0.58	3.19	5.5
	CYC-03-B	0.830	22.6	15.7	3.3	0.21	20.3	13.9	94.5	6.80	1.40	0.65	1.08	1.7

3.4.Residual gas mixing ratios and contents after wet extraction

To examine how well the gas is extracted by wet extraction, we applied the dry extraction method to refrozen ice-wedge samples after wet extraction. We first prepared degassed ice-wedge samples that had undergone repetitive wet extractions (wet-degassed ice hereafter). Once the wet extraction experiments were completed, we repeated two cycles of melting-refreezing and evacuation procedures to degas the ice melt. After degassing by a total of three cycles of wet extraction and evacuation, the outermost surfaces (~2 mm) of the wet-degassed ice were trimmed away in the walk-in freezer at SNU on the morning of experiments. The wet-degassed ice was then inserted into the needle crusher and the crusher chamber was evacuated. A specific amount of standard air was injected. Then, the wet-degassed ice samples were hit 20 or 60 times by the needle crusher. The amount of gas and gas mixing ratio of the additionally extracted gas from the wet-degassed ice are shown in Figure 2 and Table A1.

The tests using the wet-degassed ice show an additional gas extraction of ~12 to 20 ml kg_{ice}^{-1}, which is ~43 to 88% of the amount of gas extracted during the initial wet extraction. The additionally extracted gas from the dry extraction is referred to as residual gas hereafter. This is remarkably in contrast to the less than 1% residual fraction of the SNU wet extraction system for ice from polar ice sheets. If such a considerable amount of gas is left intact by repeated wet extractions, the composition of the additional gas is important to understand how much the conventional wet extraction results are biased.

Figure 2 and Table A1 show the mixing ratios and contents of CH_4 and N_2O in the residual gas. The mixing ratios of the residual gas were estimated using mass balance calculations with observed mixing ratios and the amounts of the injected standard and extracted residual gas. The CH_4 mixing ratios of the residual gas range from 10.37 to 23.78 ppm, which is similar to the range of the wet extracted gas. This evidence indicates that CH_4 in ice-wedges cannot be fully extracted by a melting-refreezing procedure. We suspect two possible reasons

for this: (1) During wet extraction, the ice-wedge samples melted and the soil particles settled at the bottom of the sample flask without any physical impact to the soil particles, causing the adsorbed CH_4 molecules on the soil particles to remain adsorbed. (2) During refreezing, the soils accumulated at the bottom of the flasks are crumpled around the centre of the refrozen ice, because the sample flasks are chilled from outside, which facilitated gas entrapment within the frozen soil aggregate. In contrast, the N_2O mixing ratios of the residual gas exhibit very low values compared to those from the initial wet extraction (Figure 2 and Table A1). These results imply that most of the N_2O in ice wedges is extracted by three melting-refreezing cycles, such that only a small amount of N_2O is left adsorbed or entrapped in ice-wedge soils. The authors posit that this might be attributed to the high solubility of N_2O to water compared to CH_4. However, it needs further investigation to better understand this.

In this section, we found that a certain amount of gas remained in ice wedges, even after three cycles of wet extraction, which is extractable instead by needle crushing. This implies that, unlike polar ice cores, wet extraction of ice-wedges does not guarantee near-complete gas extraction, and therefore, precise measurements of the gas content of ice wedges are difficult to obtain. The difficulty in measuring gas content imposes a large uncertainty in estimating CH_4 and N_2O contents. Furthermore, we found that the residual gas has a similar order CH_4 mixing ratio as the gas extracted by initial melting-refreezing, indicating that a comparable amount of CH_4 still remains unextracted in ice-wedges. Hence, a novel extraction method is required to produce reliable gas content and gas mixing ratios in ice wedges. In contrast, our results show that the N_2O content of the residual gas is at trace levels, which may suggest that most of the N_2O in ice-wedges is extractable during initial melting-refreezing. Therefore, wet extraction could be applicable for estimating the N_2O content of ice wedges. However, given that the above evidence resulted from three consecutive cycles of melting-refreezing and evacuation, it is unclear how many melting-refreezing cycles are required to extract most of

the N_2O from ice wedges. It should be noted that combination of repetitive wet extractions with dry extraction does not guarantee reliable estimation of N_2O mixing ratio, because extraction efficiency of the other gas components may be different from that of N_2O. Our findings imply that previous estimates of CH_4 budget in ground ice based on wet extraction principle (e.g., Boereboom et al., 2013; Cherbunina et al., 2018) might have been underestimated, and that the CH_4 production within subfreezing permafrost environment could be larger than previously estimated. Future study should be devoted to a novel extraction method which is easy to extract gas molecules from ice effectively.

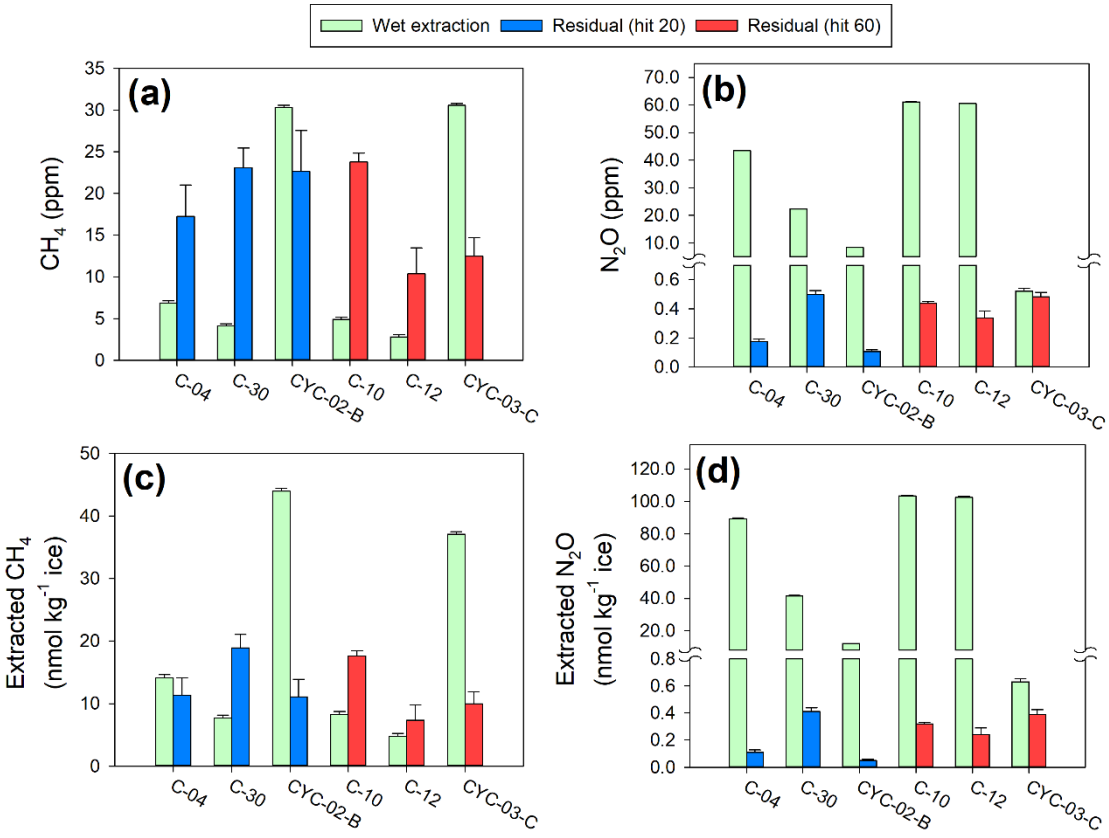


Figure 2. Comparison of wet-extracted gas and residual gas for CH_4 and N_2O mixing ratios (a and b) and contents (c and d). The residual gas was extracted from the dry extraction method using the wet-degassed ice samples. The light green bars show the results of initial wet extraction, and the blue and red bars indicate the dry extraction of wet-degassed ice with 20- and 60-times hitting, respectively. The Cyuie samples are denoted as 'CYC', while 'C' indicates the Churapcha samples.

4. Conclusions

In this study we carried out comparisons between wet and dry extractions, between untreated and biocide-treated wet extractions, and gas extraction from the easily to extract and difficult to extract parts of ice-wedge ice to better understand the characteristics of each extraction method, in order to adequately analyse CH_4 and N_2O mixing ratios and gas contents from permafrost ice wedges. Based on these comparisons, our major findings are summarized as follows:

1) Existing wet and dry extraction methods allow gas extraction from the soft parts of ice (e.g., ice bubbles) and show insignificant differences in CH_4 and N_2O mixing ratios.
2) Wet extraction results are unlikely to be affected by microbial production of CH_4 and N_2O during the melting-refreeze procedure.
3) Both dry and wet extraction methods are not able to fully extract gas from ice wedge samples, presumably due to gas adsorbed on soil particles or enclosed within soil aggregates, which may have different gas mixing ratios compared to the gas in bubbles. Further research is required to develop a proper method to quantify and extract adsorbed and enclosed gases. In the meantime, we propose that both existing techniques may be suitable for gas mixing ratio measurements for bubbles in relatively soft ice wedges (i.e., easily crushed ice wedges by hit5 extraction, e.g., Cyuie ice wedges in this study). Exceptionally, the N_2O content in ice wedges may be measured by using repeated wet extractions, but this is not the case for determining the N_2O mixing ratio.
4) Our findings indicate that previous estimates of ground ice CH_4 and N_2O budget might be underestimated, implying that the greenhouse gas production in subfreezing environment of permafrost is larger than our current understanding.

5) Our finding indicates that the saturated NaCl solution is unnecessary to prevent microbial activity during melting, as employed by, e.g., Cherbunina et al. (2018). However, it remains as an open question how effectively the adsorbed gas molecules can be extracted by the method.

Appendix. Systematic blank correction and uncertainty estimation

Since the SNU dry extraction systems, including the sample tubes, were originally designed for CO_2 measurements from polar ice cores, these systems have not been tested for CH_4 and N_2O analysis. We therefore carried out a series of tests to estimate the systematic blank, which is defined here as blanks.

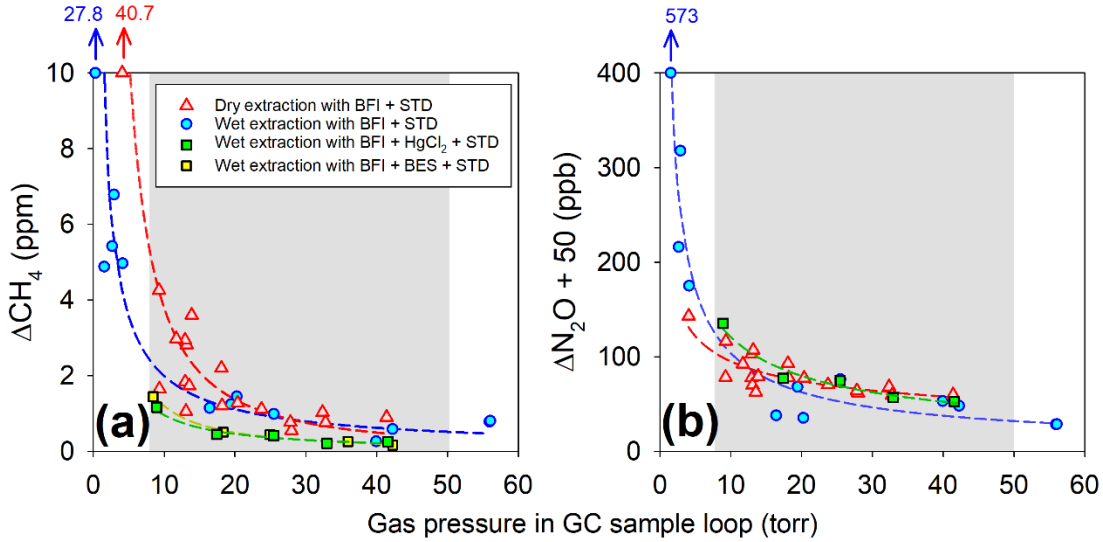
The systematic blanks were tested with bubble-free ice (BFI) and standard air in a cylinder calibrated by NOAA. The BFIs were prepared as described in Yang et al. (2017). A major difference is that the BFI block was cut into small BFI pieces of 3–4 g, to mimic the random cube sampling protocol (see Materials and Methods section in the main text). The systematic blanks for the dry extraction method were tested as follows. A total of ~45 g of BFI cubes was placed into the crushing chamber, sealed with a copper gasket, and evacuated until the gas pressure inside the chamber dropped lower than ~60 mTorr, because of the vapor pressure formed by sublimation of the BFI. After evacuation was completed, standard gas was injected into the crushing chamber. The amount of standard injected was controlled by a volume calibrated vacuum line in the dry extraction system. Then the BFI samples were hit with the needle system 5 to 100 times, and the gases in the chamber were passed through a water trap and cryogenically pumped into the sample tubes, using the He-CCR. The number of hits did not significantly affect the systematic blank (Figure A2) and the regression curve for blank correction was fitted to the entire set of data points (red dashed curve in Figure A1).

For the wet extraction, a total of ~45 g of BFI cubes was placed into each sample flask. The flasks were connected to the wet extraction line and sealed with a copper gasket, then evacuated. Once a vacuum was established, a known amount of standard gas was injected into each flask and the flasks were submerged into a warm water bath for ~40 min to melt completely. The flasks were then submerged into the cold ethanol bath, which was chilled to -80°C, to refreeze. For the $HgCl_2$ and Sodium 2-bromo-ethane-sulfonate (BES) treated

experiments, we first prepared the saturated solutions of $HgCl_2$ and BES at room temperature (20°C) and added 24 μL of $HgCl_2$ or 20 μL of BES solution into the empty flasks in a fume hood. Then we placed the flasks in a deep freezer, maintained at -45°C for 20 min, to freeze the solutions before the BFI pieces were placed.

The results of the blank experiments are shown in Figure A1. The systematic blanks appear to be inversely correlated with the gas pressure in the sample tube. The systematic blank test results were fitted using exponential regression curves (dashed lines in Figure A1), and these regression curves were then used for systematic blank correction in our ice-wedge sample analyses.

To calculate uncertainties of the blank corrections, the blank test data were fitted with exponential regression curves (Figure A1). The root-mean-square-deviations (RMSD) of the data from the regression curves are taken as the uncertainties of blank corrections (Figure 1). Since the ice-wedge data used in this study showed the pressure in GC sample loop of about 8 ~ 50 torr, the RMSD were estimated from the blank test data within this pressure range. The uncertainty of the gas content measurement is calculated by error propagation from those of pressure, line volume, and mass of ice samples.



457

Figure A1. Systematic blank of the needle crushing (dry extraction) and melting-refreezing (wet extraction) methods for (a) CH_4 and (b) N_2O measurements in control and biocide ($HgCl_2$) treated experiments. Also plotted are the CH_4 blanks of BES-treated wet extractions. The dashed lines represent exponential regression curve fittings. Note that all data are plotted against the amount of gas trapped in the sample tube, presented here as the pressure in the GC sample loop when the sample gas is expanded. The grey shaded areas indicate the range of ice-wedge samples used in this study (see main text). The big-delta (Δ) notion in the y-axes indicate the offset from the values of the standard used.

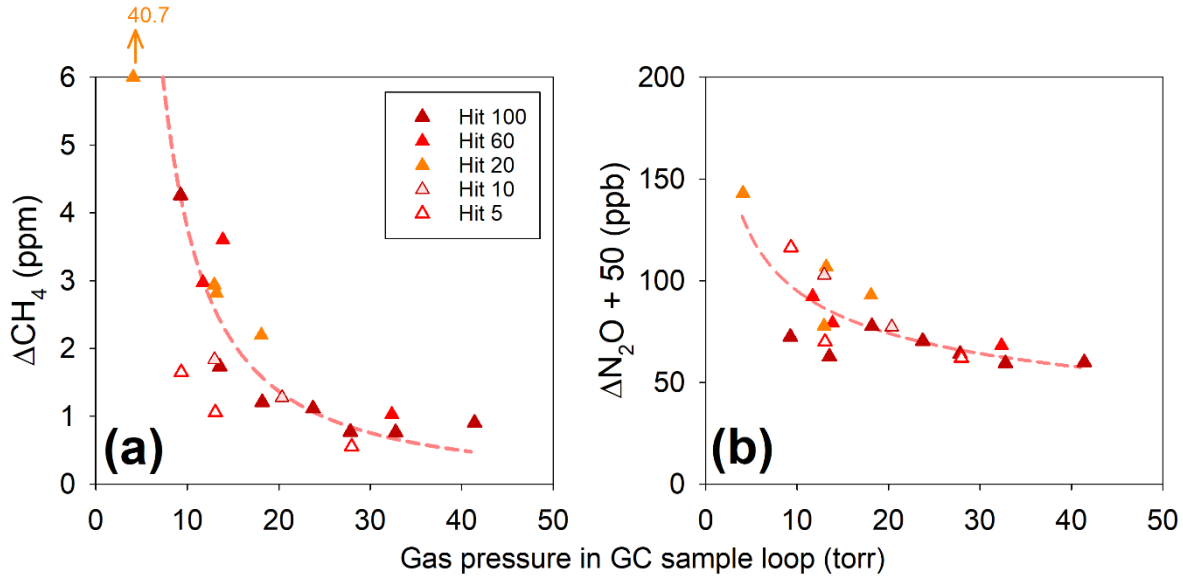


Figure A2. Influence of different number of hitting on the systematic blank of the needle crushing (dry extraction) system for (a) CH_4 and (b) N_2O measurements. Note that all data are plotted against the amount of gas trapped in the sample tube, presented here as the pressure in the GC sample loop when the sample gas is expanded (see main text). The big-delta (Δ) notion in the y-axes indicate the offset from the values of the standard used.

458

459

460

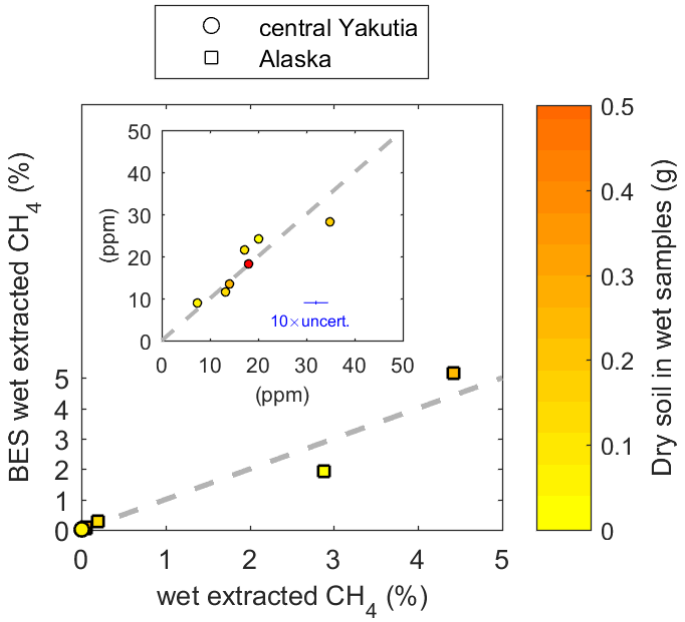


Figure A3. Comparison between control- and BES-treated wet extraction results for CH_4. The sampling area is indicated by different symbols. The color of each data point indicates the dry soil weight in the subsamples used in control wet extraction. The grey dashed lines are 1:1 reference line. The blue error bar indicates the 1-sigma uncertainty of mixing ratios magnified by 10x.

461

462

Table A1. Comparison of results from extracted gas from the conventional wet extraction method and the residual gas in ice after 3-times wet extraction. The residual gas was extracted by a needle crusher (see section 3.4 for details of the mehtods)

Site location	Sample	soil content	Wet extraction					Residual gas				
			gas content	CH_4 mixing ratio	N_2O mixing ratio	CH_4 content	N_2O content	gas content	CH_4 mixing ratio	N_2O mixing ratio	CH_4 content	N_2O content
		wt. %	ml/kg	ppm	ppm	nmol/kg	nmol/kg	ml/kg	ppm	ppm	nmol/kg	nmol/kg
Churapcha, central Yakutia	C-10	0.524	37.9	4.9	61.13	8.3	103	16.6	23.8	0.437	17.6	0.324
Churapcha, central Yakutia	C-30	1.03	41.7	4.1	22.28	7.7	41.5	18.4	23	0.50	19	0.41
Cyuie, central Yakutia	CYC-03-C	1.09	27.2	30.5	0.52	37.1	0.63	17.9	12.5	0.48	10.0	0.39
Churapcha, central Yakutia	C-04	1.38	46.0	6.9	43.46	14	89.2	14.7	17	0.17	11	0.11
Cyuie, central Yakutia	CYC-02-B	1.12	32.5	30.3	8.34	44.0	12.1	11.0	23	0.11	11	0.053
Churapcha, central Yakutia	C-12	0.370	38.0	2.8	60.47	4.8	103	15.9	10	0.34	7.3	0.24

Data availability

The data will be uploaded on the public data repository of Pangaea after publication.

Author contributions

JWY and JA conceived the research and designed the experiments. GI, JA, KK, and AF drilled the ice-wedge ice samples from Alaska and Siberia. JWY, JA, SH, and KK conducted the laboratory experiments. JWY and JA led the manuscript preparation with inputs from all other co-authors.

Competing interests

The authors declare no conflict interest.

Acknowledgements

The authors greatly acknowledge those who contributed to collect ice-wedge ice samples. We thank Gwangjin Lim and Jaeyoung Park for their help in sample preparations and gas extraction experiments, and Min Sub Sim for kind advice on inhibition experiment for methanogen.

Financial support

This project was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) (NRF-2018R1A2B3003256 and NRF-2018R1A5A1024958) and the NASA ABoVE (Arctic Boreal and Vulnerability Experiment; grant no. NNX17AC57A).

References

Arkhangelov, A. A., and Novgorodova, E. V.: Genesis of massive ice at 'Ice Mountains', Yenesei River, Western Siberia, according to results of gas analyses, Permafrost Periglac. Proc., 2, 167-170, http://doi.org/10.1002/ppp.3430020210, 1991.

Bereiter, B., Stocker, T. F., and Fischer, H.: A centrifugal ice microtome for measurements of atmospheric CO_2 on air trapped in polar ice cores, Atmos. Meas. Tech., 6, 251-262, http://doi.org/10.5194/amt-6-251-2013, 2013.

Boereboom, T., Samyn, D., Meyer, H., and Tison, J. -L.: Stable isotope and gas properties of two climatically contrasting (Pleistocene and Holocene) ice wedges from Cape Mamontov Klyk, Laptev Sea, northern Siberia, The Cryosphere, 7, 31-46, http://doi.org/10.5194/tc-7-31-2013, 2013.

Brown, J., Ferrians Jr., O. J., Heginbottom, J. A., and Melnikov, E.: Circum-Arctic map of permafrost and ground-ice conditions, version 2, National Snow and Ice Data Center, Boulder, CO, 2002.

Cherbunina, M. Y., Shmelev, D. G., Brouchkov, A. V., Kazancev, V. S., and Argunov, R. N.: Patterns of spatial methane distribution in the upper layers of the permafrost in central Yakutia, Mosc. Univ. Geol. Bull., 73, 100-108, 2018.

Fedorov, A. N., Botulu, T. A., Vasiliev, I. S., Varlamov, S. P., Gribanova, S. P., Dorofeev, I. V.: Permafrost-landscape map of the Yakut ASSR, Gosgeodezia, Moscow, Russia, Map, 1991 (In Russian).

Higaki, S., Oya, Y., and Makide, Y.: Emission of methane from stainless steel surface investigated by using tritium as a radioactive tracer, Chem. Lett., 35, 292-293, https://doi.org/10.1246/cl.2006.292, 2006.

Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J. W., Schuur, E. A. G., Ping, C. -L., Schirrmeister, L., Grosse, G., Michaelson, G. J., Koven, C. D., O'Donnell, J. A., Elberling, B., Mishra, U., Camill, P., Yu, Z., Palmtag, J., and Kuhry, P.: Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps, Biogeosciences, 11, 6573-6593, https://doi.org/10.5194/bg-11-6573-2014, 2014.

Jorgenson, M. T., Kanevskiy, M., Shur Y, Moskalenko, N., Brown, D. R. N., Wickland, K., Striegl, R., and Koch, J.: Role of ground ice dynamics and ecological feedbacks in recent ice wedge

degradation and stabilization, J. Geophys. Res., 120, 2280-2297, https://doi.org/10.1002/2015JF003602, 2015.

Kanevskiy, M., Shur, Y., Jorgenson, M. T., Ping, C. -L., Michaelson, G. J., Fortier, D., Stephani, E., Dillon, M., and Tumskoy, V.: Ground ice in the upper permafrost of the Beaufort Sea coast of Alaska, Cold Reg. Sci. Technol., 85, 56-70, https://doi.org/10.1016/j.coldregions.2012.08.002, 2013.

Katayama, T., Tanaka, M., Moriizumi, J., Nakamura, T., Brouchkov, A., Douglas, T. A., Fukuda, M., Tomita, F., and Asano, K.: Phylogenetic analysis of bacteria preserved in a permafrost ice wedge for 25,000 years, Appl. Environ. Microbiol., 73, 2360-2363, https://doi.org/10.1128/AEM.01715-06, 2007.

Kim, K., Yang, J. -W., Yoon, H., Byun, E., Fedorov, A., Ryu, Y., and Ahn, J.: Greenhouse gas formation in ice wedges at Cyuie, central Yakutia, Permafrost Periglac. Process., 30, 48-57, http://doi.org/10.1002/ppp.1994, 2019.

Lacelle, D., Radtke, K., Clark, I. D., Fisher, D., Lauriol, B., Utting, N., and Whyte, L. G.: Geomicrobiology and occluded O_2-CO_2-Ar gas analyses provide evidence of microbial respiration in ancient terrestrial ground ice, Earth Planet. Sci. Lett., 306, 46-54, https://doi.org/10.1016/j.epsl.2011.03.023, 2011.

Masson-Delmotte, V., Schulz, M., Abe-Ouchi, A., Beer, J., Ganopolski, A., Rouco, J. F. G., Jansen, E., Lambeck, K., Luterbacher, J., Naish, T., Osborn, T., Otto-Bliesner, B., Quinn, T., Ramesh, R., Rojas, M., Shao, X., and Timmerman, A.: Information from paleoclimatic archives, Climate change 2013: The Physical science basis, Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change, 383-464, https://doi.org/10.1017/CBO9781107415324.013, 2013.

Nollet, L., Demeyer, D., and Verstraete, W.: Effect of 2-bromoethanesulfonic acid and Peptostreptococcus products ATCC 35244 addition on stimulation of reductive acetogenesis in the ruminal ecosystem by selective inhibition of methanogenesis, Appl. Environ. Microbiol., 63, 194-200, 1997.

Rivkina, E., Shcherbakova, V., Laurinavichius, K., Petrovskaya, L., Krivushin, K., Kraev, G., Pecheritsina, S., and Gilichinsky, D.: Biogeochemistry of methane and methanogenic archaea in permafrost, FEMS Microbiol. Ecol., 61, 1-15, https://doi.org/10.1111/j.1574-6941.2007.00315.x, 2007.

548 Ryu, Y., Ahn, J., and Yang, J. -W.: High-precision measurement of N_2O concentration in ice cores,
549 Environ. Sci. Technol., 52, 731-738, https://doi.org/10.1021/acs.est.7b05250, 2018.
550 Salmon, V. G., Schadel, C., Bracho, R., Pegoraro, E., Celis, G., Mauritz, M., Mack, M. C., and
551 Schuur, E. A. G.: Adding depth to our understanding of nitrogen dynamics in permafrost
552 soils, J. Geophys. Res., 123, 2497-2512, https://doi.org/10.1029/2018JG004518, 2018.
553 Schaefer, H., Lourantou, A., Chappellaz, J., Luthi, D., Bereiter, B., and Barnola, J. -M.: On the
554 stability of partially clathrated ice for analysis of concentration and $\delta^{13}C$ of palaeo-
555 atmospheric CO_2, Earth Planet. Sci. Lett., 307, 334-340,
556 https://doi.org/10.1016/j.epsl.2011.05.007, 2011.
557 Shin, J.: Atmospheric CO_2 variations on millennial time scales during the early Holocene, Master
558 thesis, School of Earth and Environmental Sciences, Seoul National University, South
559 Korea, 58 pp., 2014.
560 Strauss, J., Laboor, S., Fedorov, A. N., Fortier, D., Froese, D., Fuchs, M., Grosse, G., Günther, F.,
561 Harden, J. W., Hugelius, G., Kanevskiy, M. Z., Kholodov, A. L., Kunitsky, V. V., Kraev, G.,
562 Lapointe-Elmrabti, L., Lozhkin, A. V., Rivkina, E., Robinson, J., Schirrmeister, L.,.,
563 Shmelev, D., Shur, Y., Siegert, C., Spektor, V., Ulrich, M., Vartanyan, S. L., Veremeeva, A.,
564 Walter Anthony, K. M., and Zimov, S. A.: Database of Ice-Rich Yedoma Permafrost (IRYP),
565 PANGAEA, https://doi.org/10.1594/PANGAEA.861733, 2016.
566 Torres, M. E., Mix, A. C., and Rugh, W. D.: Precise $\delta^{13}C$ analysis of dissolved inorganic carbon in
567 natural waters using automated headspace sampling and continuous-flow mass
568 spectrometry, Limnol. Oceanogr.: Methods, 3, 349-360, 2005.
569 Yang, J. -W., Ahn, J., Brook, E. J., and Ryu, Y.: Atmospheric methane control mechanisms during
570 the early Holocene, Clim. Past, 13, 1227-1242, https://doi.org/10.5194/cp-13-1227-2017,
571 2017.
572 Yang, J. -W.: Paleoclimate reconstructions from greenhouse gas and borehole temperature of polar
573 ice cores, and study on the origin of greenhouse gas in permafrost ice wedges, Ph.D. thesis,
574 School of Earth and Environmental Sciences, Seoul National University, Seoul, 188 pp.,
575 2019.
576