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Exploring the mechanisms by which reindeer droppings induce fen peat methane production

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ABSTRACT

Peatlands, especially fens, are known to emit methane. Reindeer (*Rangifer tarandus*) use mires mainly as spring and summer pastures. In this work we observed that adding reindeer droppings to fen peat increased the potential methane production by 40%. This became apparent when droppings originating from reindeer kept in pen or pasture in winter were added to methanogenic fen peat samples. The droppings introduced Methanobacteriaceae (*Methanobrevibacter;* > 90% of the *mcrA* MiSeq reads) to the peat, which was originally populated by Methanosarcinaceae, Methanosaetaceae, Methanoregulaceae, Methanobacteriaceae, Methanomassiliicoccaceae, Methanocellaceae and Methanomicrobiaceae. The original community structure did not explain the induced methane production and neither did the origin of the droppings. Instead, the increment in methane production was explained by the increased methanogenic abundance, measured by *mcrA* qPCR, due to the addition of droppings. The result confirms that methanogens from the reindeer rumen participate in peat methane production. This finding suggests that reindeer grazing may increase methane emissions in northern fens.

1. Introduction

Reindeer (*Rangifer tarandus*) use peatlands mainly as spring and summer pastures (Kolari et al., 2019). As peatlands are known for their capacity to sequester carbon (C) (Nichols and Peteet 2019) and emit methane (CH_4) (Bousquet et al., 2011) we have started a research project evaluating the effect of reindeer grazing on fen peatland methane fluxes in northern Finnish Lapland. We have already shown that reindeer droppings increase the potential methane production in northern fen peat (Laiho et al., 2017) but the mechanisms behind the phenomenon were not identified. As reindeer or caribou (*Rangifer tarandus caribou*) graze everywhere in the Arctic, their diet may impact the quality of the droppings which, in turn, could be reflected in the methane production rate of the peatland pastures. Another explanation is that the droppings may induce a change in the community structure of the methane producing Archaea in the peat. We evaluated these two main hypotheses in an experiment where reindeer droppings collected from animals kept and fed in pens or grazing in natural pastures were added to peat samples in the laboratory. Three research questions guided our work: 1) is the reindeer diet reflected in the methane production potential of the peat, 2) does the addition of droppings either change or increase the size of the peat methanogenic archaeal community and 3) as reindeer graze all year round, does the freezing of the droppings at a typical artic winter temperature influence the results?

2. Material and methods

2.1. Peat and droppings collection

The peat was collected at the Lompolojänkkä fen (Kittilä; 67°60′ N, 24°12′ E) in March 2020 from below the snow and ice cover and transported to the laboratory the following day.

Reindeer droppings were collected on March 23rd, 2020 from the Kutuharju experimental reindeer station (69°15′ N, 26°99’ E). Droppings were collected from female reindeer that were included in a winter-feeding experiment which had two treatments: pen and pasture treatments in which reindeers either fully or only partially depended on artificial feed, respectively. The experiment was initiated on November

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26, 2019 when 60 female reindeer were randomized into the treatments and replicates (2 replicates/treatment and 15 reindeer in each replicate). In the pen treatment, reindeer were kept in 1 ha pens and they were fed with silage (2.2 kg/day/reindeer) and pellet feed (Poron herkku energy; 1.1 kg/day/reindeer). In the other treatment, reindeer were kept in two natural pasture areas (318 ha and 971 ha) in which they were able to crater and forage natural food (ground and arboreal lichen, dwarf shrubs, sedges and grasses). However, during winter 2019–2020 snow and digging conditions were exceptionally difficult (very thick and hard snow layer) which substantially hampered digging and natural foraging of reindeer. Consequently, additional feed (Poron Herkku Energy pellet 1.1 kg/day/reindeer and lichen 0.6 kg/day/reindeer) had to be given also to pasture reindeer since the end of December 2019.

Fresh droppings were collected from clean snow when the experimental reindeer were rounded up into separate pens for the sampling (excrement and blood sample) of the feeding experiment. Droppings from both treatments were collected in separate plastic bags and stored at +4 °C and mailed two days later to the laboratory. The droppings collected from the pen enclosure are called here pen droppings (PED) and the droppings from the more natural pasture treatment are called pasture droppings (PAD). Part of each dropping type were frozen to −20 °C for two days to test the effect of freezing (winter conditions).

2.2. C:N ratios, microcosms, methane production and pH

The fresh peat was homogenized and live roots, mainly *Carex* species, were removed by hand. We took 15 ml samples, corresponding to 15.2 g fresh mass and 0.8 g dry mass (C:N ratio 25.7) on average, with a syringe and inserted them into 125 ml serum bottles together with 30 ml sterilized ultrapure H_2O (Milli-Q). These samples were divided between nine microcosm types while one without reindeer droppings served as the peat control (C). Two microcosm sets received the pen droppings stored at either +4 °C or at −20 °C, respectively CPED+4 and CPED-20. The same set-up was prepared for the pasture droppings (CPAD+4 and CPAD-20). Two to three droppings, 1.86 g fresh mass and 0.44 g dry mass (C:N ratio for PED and PAD 21.6 and 26.3, respectively) on average, were added to the peat. Additionally, two respective microcosm sets with the same amounts of droppings were set up without peat (PED+4, PED-20, PAD+4, PAD-20). Each microcosm type had five replicates totalling to 45 serum bottles. The suspensions were mixed and flushed with N_2 for 2 min and capped with rubber stoppers. The bottles were left to adjust for 3 days at 14 °C and were again flushed with N_2 for 1 min before the first measurement. Production of CH_4 was followed from the headspace over 91 h, during which five measurements were taken and analysed with gas chromatography as in Jaatinen et al. (2005). The bottles were kept at 14 °C between measurements. pH was measured directly from the bottles at the end of the measurement period. Carbon and nitrogen were determined from oven-dried samples with a LECO TRUMAC CN analyser (ISO 10694).

2.3. DNA extraction, qPCR and sequencing

All the microcosm serum bottles were frozen to −20 °C for several months due to closure of the laboratory (Covid-19). Thereafter, materials in the bottles were melted, homogenized for 30 s with an Ultra Turrax and the suspensions were placed into 50 ml Falcon centrifuge tubes and centrifuged (10 000 g; 20 min; 4 °C). Between samples the Ultra Turrax blade was sterilized always for 1 min in a 3% hypochlorite solution, followed by rinsing in sterile water and 70% alcohol, respectively. The pellets were collected for DNA extraction and a new round of dry mass estimation (105 °C; 12 h). The DNA was extracted using a Qiagen DNeasy PowerSoil Pro kit according to the manufacturer's instructions. DNA concentration and purity were determined with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific).

To detect the *mcrA* copy number of methanogenic Archaea we used the primers *mcrA*-F (5′- GGT GGT GTM GGD TTY ACH CAR TA -3′; Petersen et al., 2014) based on Steinberg and Regan (2008) and *mcrA*-R (5′- CGT TCA TBG CGT AGT TVG GRT AGT -3′; Steinberg and Regan, 2008). The qPCR was performed in duplicate using a Mic4 (bio molecular systems, Australia) qPCR machine with Maxima™ SYBR Green qPCR Master Mix (2 ×) (Thermo Fisher Scientific, Germany) in a 6 μl final reaction volume containing 2 μl template, 0.3 μM of each primer and 1 × qPCR master mix. The qPCR reaction mixtures were prepared with a Myra liquid handling system (bio molecular systems, Australia). During qPCR fluorescence was measured at the end of each extension step. Each qPCR run was carried out under the following conditions: initial denaturation at 95 °C for 10 min; 40 cycles denaturation at 95 °C for 30 s; annealing at 55 °C for 45 s; and extension at 72 °C for 30 s followed by the melt analysis from 55 °C to 95 °C. The copy numbers in samples were calculated based on comparison to threshold cycle values of the standard curve. Samples with the lowest amplification efficiency from each treatment were subjected to inhibition testing as in Goebel et al. (2010) by spiking the template DNA with 10^6 copies of standard plasmid.

Amplicon sequencing was done at the Institute of Genomics Core Facility, University of Tartu. DNA quality control and quantification were performed with Qubit® 2.0 Fluorometer (Invitrogen, Grand Island, USA). The methanogenic archaea targeting partial the *mcrA* gene was amplified from total DNA using primers *mcrA*-F (5′- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG - GGT GGT GTM GGD TTY ACH CAR TA -3′) and *mcrA*-R (5′- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G - CGT TCA TBG CGT AGT TVG GRT AGT -3′) with overhangs as described in Zhu et al. (2017). Amplicon libraries for Illumina (Illumina, San Diego, USA) next-generation sequencing were generated by a two-step PCR. First, the *mcrA* gene region was amplified with 32 cycles and then Illumina adapter and index sequences were added by 7 cycles of PCR. The quality control of amplicon libraries was performed by Agilent 2200 TapeStation analysis (Agilent Technologies, Santa Clara, USA) and with a Kapa Library Quantification Kit (Kapa Biosystems, Woburn, USA). Amplicon libraries were pooled in equimolar concentrations and the sequencing was carried out with an Illumina MiSeq System (Illumina) using MiSeq Reagent Kit v2 Nano in paired end 2 × 250 bp mode producing ca. 0.75 M reads per flow cell.

Sequencing primers, low-quality reads (average quality scores less than 25) and reads shorter than 150 bp were removed from the paired-end reads using Cutadapt v. 3.2 (Martin 2011). Raw sequences were processed by the mothur software package v.1.44.3 (Schloss et al., 2009; Schloss 2020), following the standard MiSeq SOP operating procedure (https://mothur.org/wiki/miseq_sop/) (Kozich et al., 2013). The subsequent filtering removed the sequences with homopolymers longer than 6 nucleotides and sequences shorter than 300 nucleotides or longer than 500 nucleotides. The unique sequences were then aligned in mothur with default settings while referring to the pre-aligned *mcrA* sequences provided by the Fungene Pipeline database (http://fungene.cme.msu.edu) (Fish et al., 2013). Chimeras were detected to be 0.9% of all the reads with the UCHIME algorithm by using the mothur software platform. Using the default opti clustering method OTUs were assembled using metrics to determine the quality of clustering in mothur. The valid sequences were classified with the default Bayesian method by Wang et al. (2007) and assigned into operational taxonomic units (OTUs) at 84% identity of *mcrA* gene sequences by using a previously generated database (Yang et al., 2014). Since many of the representative sequences with the Bayesian approach remained unclassified, we did an extra search with online MegaBLAST (Morgulis et al., 2008) according to default settings to find the closest similarities with sequences deposited in the databases of GenBank/NCBI. Raw sequence data is deposited to the sequence read archive (SRA) of NCBI/EMBL database under the BioProject id PRJNA707381 with the accession numbers SAMN18203477-SAMN18203521 for methanogenic archaeal partial *mcrA* gene.

2.4. Statistics

The methane production potential and the *mcrA* copy number means (±SD) are reported per g dry mass. Analyses of variance (ANOVA) followed by Tukey's test at the $p < 0.05$ level on square root (CH_4) and natural log (qPCR) transformed data was used to test the difference between treatments. All statistical analyses for *mcrA* amplicon sequencing data were conducted in R studio version February 1, 5042 and R version 4.0.2 (R Core Team, 2020). OTU data from the amplicon sequencing was normalized using the geometric mean of pairwise ratios (GMPR) method (Chen et al., 2018). The differences between average library sizes and OTU numbers were tested with ANOVA with Tukey's HDS test or Kruskal-Wallis and Dunn's test. We performed permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distance matrices with function adonis2 from vegan (Oksanen et al., 2019) to test the effect of temperature (+4 °C and −20 °C), incubated material (peat, droppings, and peat with droppings) and feeding habitat for droppings (pen or pasture) on methanogenic archaeal community composition. We conducted 2-D nonmetric multidimensional scaling (NMDS) with stable solution from random starts, axis scaling and species scores with function metaMDS from vegan using the Bray-Curtis dissimilarity index. Methanogenic archaeal OTUs indicative for material, temperature and origin of the droppings were obtained by differential abundance analysis (DESeq2) which identified significant groups (>|1.4| log2 fold change with adjusted $p < 0.05$) (Love et al., 2014).

3. Results

The addition of reindeer droppings significantly increased the peat pH from 5.9 to a mean of 6.7 and droppings alone without peat had the highest pH with a mean of 7.3. The freezing of the droppings had no effect on the pH. The peat alone (C) produced methane at a rate of 0.267 (±0.028) nmol CH_4 h^{-1} g^{-1} peat. The addition of +4 °C treated droppings significantly increased the rate to 0.449 (±0.087) and 0.461 (±0.113) nmol CH_4 h^{-1} g^{-1} for the CPED+4 and CPAD+4 treatments, respectively. The addition of −20 °C treated droppings to the peat generated 92 and 84% of the methane production potential compared to their +4 °C treated counterparts, respectively (Fig. 1) but the difference was insignificant. The droppings of the pen fed reindeer alone without peat produced almost no methane, regardless of whether the droppings had been kept at +4 °C or −20 °C, whereas the droppings of reindeer kept in natural pastures had a methane production rate of 0.0296 (±0.034) nmol CH_4 h^{-1} g^{-1} when kept at +4 °C and the freezing of the droppings resulted in a 10-fold decrease of that rate (Fig. 1) which was significant.

The abundance of methane producing *Archaea* was measured via quantitation of *mcrA*. The equation for the qPCR standard curve was $y = -3.319\,x + 32.73$ (R^2 0.9979) and showed a linear response from 2.38×10^2 to 2.38×10^7 copies per reaction with 1.001 reaction efficiency. No inhibition was detected in samples with spiked standard plasmid DNA. The peat (C) had 2.14×10^8 ($\pm 5.21 \times 10^7$) g^{-1} *mcrA* copy numbers. The addition of +4 °C treated droppings significantly increased the *mcrA* copy number to 2.40×10^9 ($\pm 1.07 \times 10^9$) and 3.27×10^9 ($\pm 2.04 \times 10^9$) g^{-1} for the CPED+4 and CPAD+4 treatments, respectively. The addition of −20 °C treated droppings to the peat also increased the the *mcrA* copy numbers, but only by 39% and 37% compared to their +4 °C treated counterparts, respectively (Fig. 2). The difference was significant between the CPAD+4 and CPAD-20 treatments. The CPED-20 and CPAD-20 treatments were also significantly different from the peat (C) control. The droppings of the pen fed reindeer had *mcrA* copies of 5.09×10^9 ($\pm 4.62 \times 10^8$) g^{-1} and of the natural pasture reindeer had 1.17×10^{10} (7.37×10^9) for the PED+4 and PAD+4 treatments, respectively. Freezing of the droppings resulted in a 16% and 68% decrease in the copy numbers for the PED-20 and PAD-20 treatments, respectively (Fig. 2) and was significant for the PAD-20 treatment.

Addition of reindeer droppings introduced rumen specific methanogens into the peat and this was reflected in the NMDS (Fig. 3) where peat with reindeer droppings (CD) showed a distinct methanogenic community from the one inhabiting the peat alone (C). Stripping the CD treatments from all rumen specific *mcrA* sequences reversed the situation, since there was no difference between the C and CD treatments (data not shown). Thus, the separation of the C and CD treatments is only due to rumen specific *mcrA* sequences and therefore the methanogenic community of the dropping treatments (D) separated along the

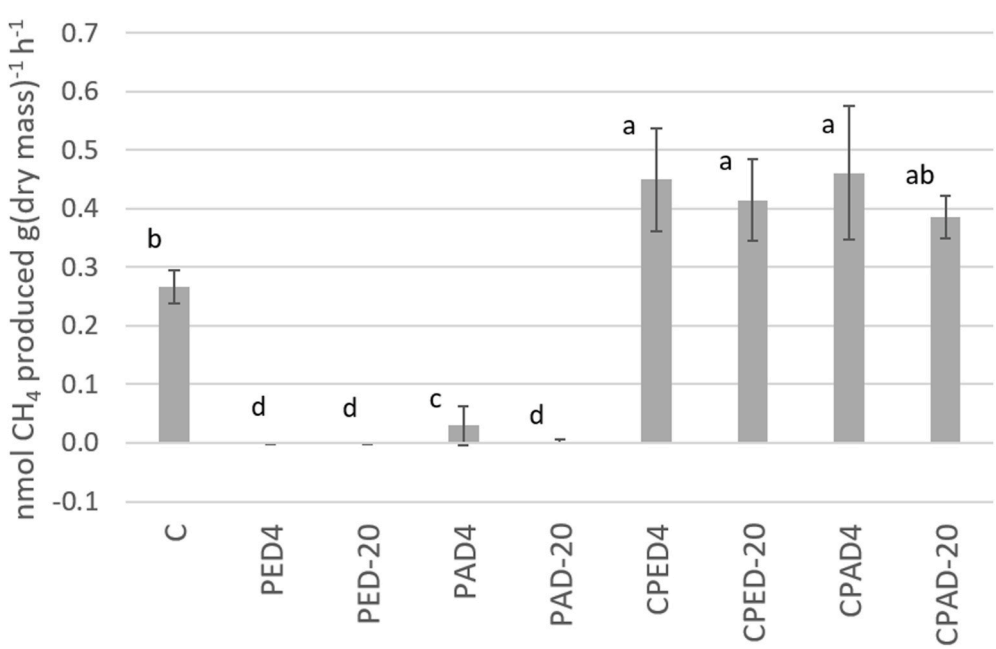

Fig. 1. CH_4 production potential from the incubation bottles including the control with only peat (C), only droppings from the pen (PED) or pasture (PAD) kept at +4 °C or −20 °C, and droppings added to peat. Abbreviations: C; control peat; D, droppings; PE, pen; PA, pasture; numerical values refer to the dropping temperature when added to the peat. Bars represent the standard deviations. Means indicated with the same letter are not significantly different ($p < 0.05$).

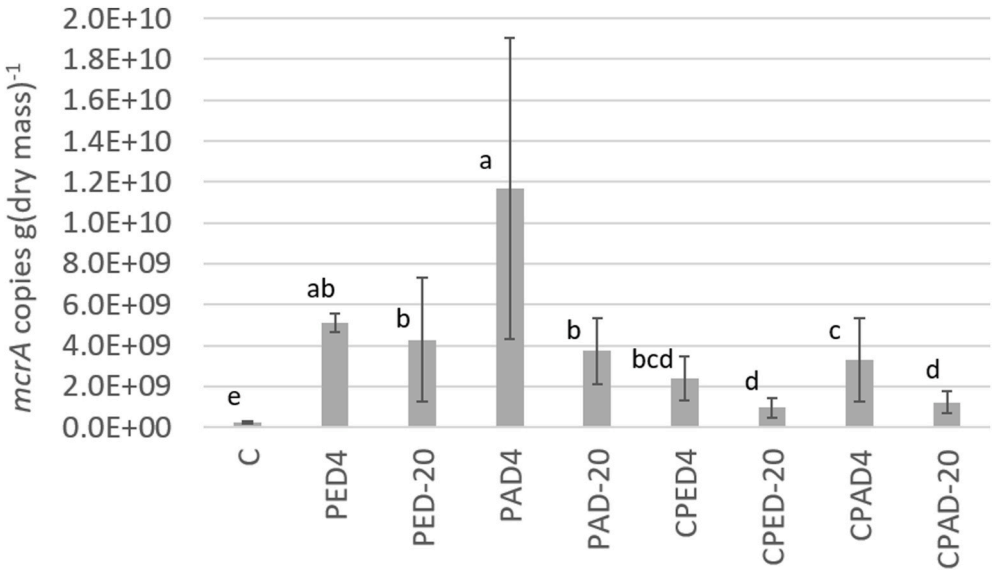

Fig. 2. Methanogenic *mcrA* gene copies from the incubation bottles including the control with only peat, droppings with or without peat from the pen or pasture reindeers. See abbreviations in Fig. 1. Bars represent the standard deviations. Means indicated with the same letter are not significantly different ($p < 0.05$).

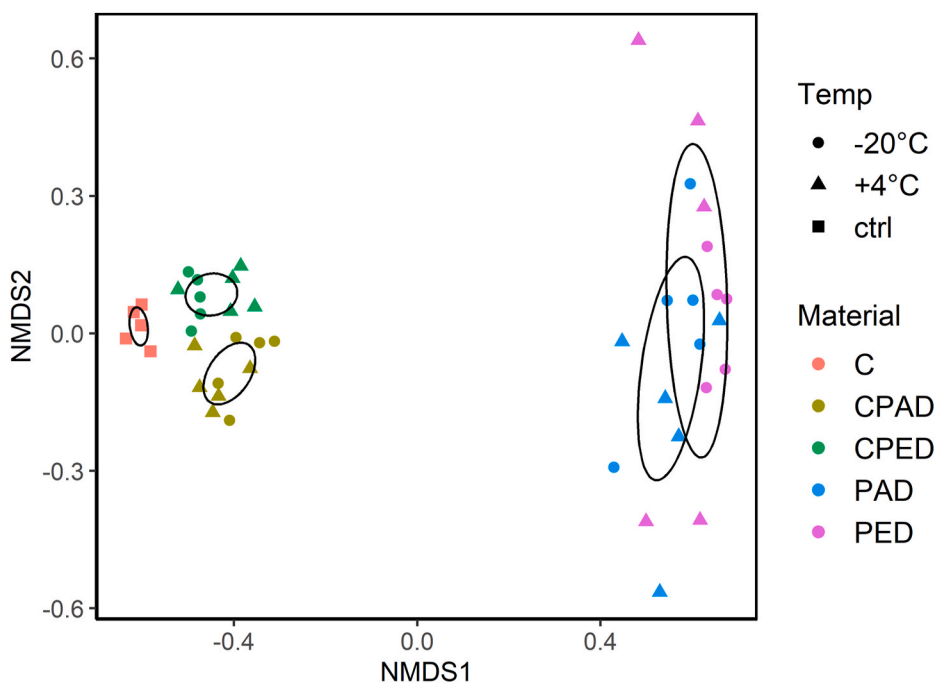

Fig. 3. Non-metric multidimensional scaling (NMDS) analysis for GMPR transformed methanogenic archaeal *mcrA* data. Ellipses are 95% confidence ellipses for each material based on standard errors of sample scores. See abbreviations in Fig. 1. Temperatures −20 °C and +4 °C refer to the two dropping storage temperatures, while ctrl refers to incubation temperature +14 °C.

first NMDS axis opposite to the C treatment community.

About 77% of the variation in the methanogenic community composition was attributed to the material in the incubation bottle (peat, droppings or peat with droppings), according to the PERMANOVA (Table 1). Temperature of the added droppings (+4 °C or −20 °C) did not explain any of the variation, whereas origin of droppings (pen or pasture) explained a minor part, 2%, of the variation in methanogenic community composition.

Average library size of GMPR normalized reads in sample types varied between 11 478 and 18 874. OTU numbers were significantly lower in droppings compared to peat and peat with droppings (Supplementary Table S1). In general, most of the reads (≥90%) from the

Table 1
Results of PERMANOVA analysis to test the effect of the incubation bottle (droppings, peat or peat with droppings), dropping temperature (+4 °C or −20 °C) and the origin of reindeer droppings (from pen or pasture) on *mcrA* originated OTU composition. Differences are considered significant if $p \leq 0.05$.

Source	F	R^2	P
Material	74.881	0.76516	0.001
Temperature	2.036	0.01040	0.138
Origin of droppings	3.928	0.02007	0.046
Residuals		0.20437	

droppings affiliated to the genus *Methanobrevibacter* of the family Methanobacteriaceae, whereas the majority of the peat derived reads (75%) affiliated to Methanoregulaceae (closest similarity to genera *Methanoregula),* Methanobacteriaceae (closest similarity to genus *Methanobacterium)* and Methanosaetaceae (Fig. 4). None of the OTUs were indicative for reindeer dropping addition temperatures (+4 °C or −20 °C; Supplementary Table S2). An OTU that showed the closest similarity to the species *Methanobrevibacter smithii* was specific for droppings that originated from reindeer kept in pens. Whereas two OTUs with closest similarity to two other *Methanobrevibacter* species *(M. woesei* and *M. wolinii)* were specific for droppings that originated from reindeer feeding in pastures. Most of the dropping OTUs were classified or showed closest similarity to several *Methanobrevibacter* species and to genera *Methanocorpusculum* and *Methanosphaera*. The methanogenic community in the peat was more diverse and had representatives classified or showing closest similarity to families of Methanosarcinaceae (closest similarity to genera *Methanosarcina* and *Methanimicrococcus*), Methanosaetaceae, Methanoregulaceae (closest similarity to genus *Methanoregula),* Methanobacteriaceae (closest similarity to *Methanobacterium*), Methanomassiliicoccaceae (closest similarity to *Methanomassiliicoccus*), Methanocellaceae (closest similarity to *Methanocella)* and Methanomicrobiaceae (closest similarity to genera *Methanosphaerula)*. However, in some cases the classification and similarities found for representative OTU sequences were weak (below 80%) which leaves their methanogenic origin uncertain. For example, two different OTU sequences affiliating to Methylocellaceae showed only 76 and 77% similarity to database matches.

4. Discussion

Peatlands cover ca. 2.84% of the world's land area yet account for a significant proportion of terrestrial carbon (Xu et al., 2018). Estimates of the carbon store in northern peatlands have ranged from 500 to 1055 gigatons (Nichols and Peteet 2019). Peatlands act as a carbon sink due to the accretion of partially decayed plant matter in the anoxic conditions found below their generally high-water tables. Due to their ability to sequester carbon dioxide, peatlands play an important role in mitigating global climate warming. However, peatlands are also the largest natural source of atmospheric methane (Neef et al., 2010; Bousquet et al., 2011). Thus, any land use change concerning peatlands must be evaluated due to their importance for the earth's climate. Fen-type peatlands are important spring and summer pastures for both the semi-domesticated and wild reindeer (*Rangifer tarandus*) in Northern Eurasia (Kumpula et al., 2008; Forbes and Kumpula 2009, Nystöm et al., 2013), as well as caribou (*Rangifer tarandus caribou*) in North America (Stuart-Smith et al., 1997; Rettie and Messier 2000; Hornseth and Rempel 2016). These are regions where anomalously high methane emissions have been measured (Turetsky et al., 2014) and thus could potentially be attributed to reindeer presence. Reindeer droppings fall on fen surfaces but as reindeer paths show hoof penetration down to 15–20 cm, parts of their droppings are also introduced through trampling directly into the wet, anoxic subsurface zone, where methane production activity is high (Peltoniemi et al., 2016). Mimicking this effect, we have shown that addition of reindeer droppings increased methane production in the laboratory (Laiho et al., 2017) and verified this result here again. In this study, we wanted to determine the mechanisms behind the increase. Next to our experiment, reindeer dropping addition has been investigated by Hayashi et al. (2014). They followed with a similar set up the addition of winter droppings to tundra ecosystem mineral soil but reported no significant effect on the methane flux. Their experiment was performed under aerobic conditions and mineral soil is not known to harbour high populations of methanogenic Archaea even though they are present (Angel et al., 2012).

By repeating the experimental layout of Laiho et al. (2017), we tested three new hypotheses. We hypothesized that 1) the reindeer diet is reflected in the droppings and thus in the peat methane production potential, 2) the addition of droppings either changes or increases the size of the peat methanogenic community and 3) as reindeer graze all year round we also hypothesized that freezing of the droppings to a typical artic winter temperature influences the results. Hypothesis 1 had to be rejected. The different feed of the two reindeer populations did not affect the potential methane production of the peat. Both diets increased the potential peat methane production to the same extent. The diet was, however, reflected in the methane production of the droppings themselves. The pasture reindeer droppings exhibited low methane production whereas the droppings from the pen reindeer produced no methane. Hypothesis 2 had to be accepted in both aspects. The addition of droppings changed the methanogenic community structure by introducing rumen specific methanogens into the peat and increased the *mcrA* copy number significantly, increasing the methanogenic Archaea abundance. This was reflected in the potential methane production. The higher methanogenic Archaea abundance in pasture reindeer droppings as compared to the pen droppings explains the observed methane production of the samples kept in +4 °C. Hypothesis 3 had to be partly

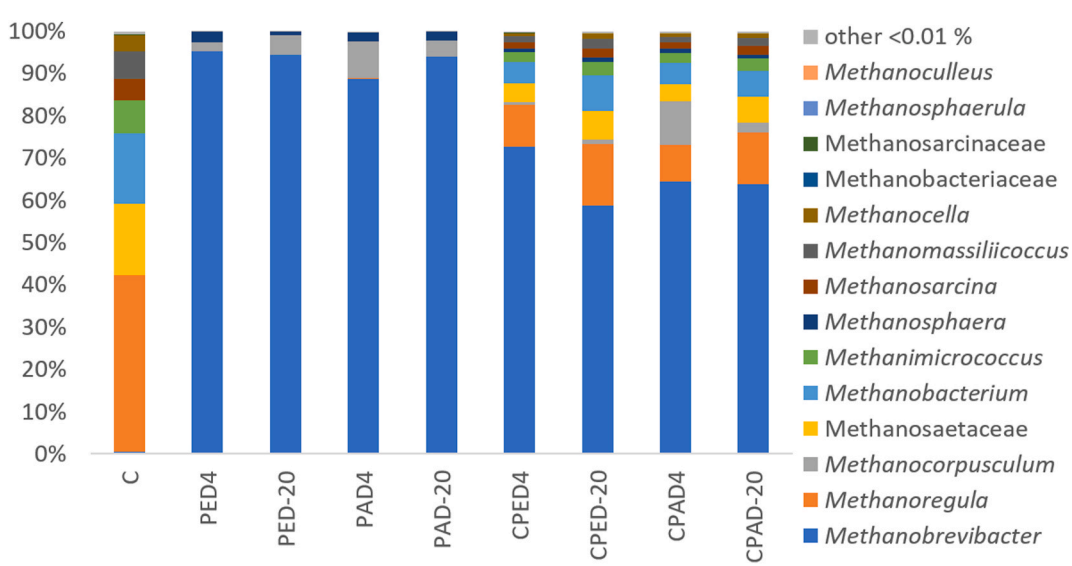

Fig. 4. Relative proportions of methanogenic *mcrA* derived reads from samples at genus level or at family level when OTUs could not be identified at the genus level. Reads that had relative proportion below 0.01% of the total reads are shown as a separate group. See abbreviations in Fig. 1.

accepted. Droppings that were frozen at -20 °C tended to produce less methane when added to peat, through this was not significant. On the other hand, the pasture droppings had lower methane production when frozen. Freezing diminished the *mcrA* copy numbers in the droppings themselves and in the peat samples receiving the frozen droppings. This was statistically significant in the case of the reindeer feeding in the pastures. The freezing was not reflected in the methanogenic community structure of the droppings or the peat receiving them.

Feeding reindeer with either a standard pellet diet or with lichens has earlier been shown to change the archaeal community, including the methanogenic, rumen and cecum populations (Saldago-Flores et al., 2016). It has also been shown that diet is reflected in the dropping microbiome of reindeer (Zielinska et al., 2016) and red deer (*Cervus elaphus)* (Menke et al., 2019). Different diets are also reflected in the level of the archaeal biomass indicator, archaeol, when analysed from cattle faeces (Gill et al., 2011). The diet used in this experiment was reflected in the methanogenic community composition through the origin of the droppings but only explained 2% of the variation. This was probably not enough to induce a statistically significant difference related to diet in the increased methane production after dropping addition. The methane production of different peat origins is generally pH dependent having an optimum at ca. 6–6.5 (Dunfield et al., 1993) but the pH-related changes in methane production are not as strong as those observed here after dropping addition. Also, the reported optimum pH value is close to the pH measured in the peat both with and without dropping addition and thus does not explain the methane production increase of the dropping addition. Furthermore, freezing the droppings did not affect the pH, but the potential methane production decreased as did the methanogen presence indicated by *mcrA* qPCR. Also, the C:N ratio of the material was not changed due to dropping addition and cannot explain the increased methane production upon dropping addition. Peat methane production is C-limited (Bergman et al., 1998) and is dependent on the fermentation activity of microorganisms fuelling the methanogens with mainly acetate and hydrogen. There must be a shortage of this fuelling process in the droppings which is reflected in their low to non-existent methane production potential. When the methanogens inhabiting the droppings are introduced into an environment supporting methane production, the observed increase in potential methane production may be due to the biomass increment of the methanogenic archaea which the droppings add to the peat. The methanogen biomass, indicated as *mcrA* qPCR, increased upon addition of rumen specific methanogens to the peat. This is because the droppings have a significantly higher methanogenic biomass content on a dry mass basis than peat (Fig. 2). The final dry mass mixing ratio of droppings and peat was on average 1:2 in the PD treatments, which explains their methanogen abundance increase compared to the peat alone. The frozen droppings added fewer methanogens to the system and this is reflected in the abundance and activity. It is probably not the proliferation of the peat inhabiting methanogens during the experiment which explains the results since the differences in potential methane production were documented already after 24 h (data not shown) and did not change during the incubation period. In addition, we could not detect significant changes in the original peat inhabiting methanogens after dropping additions. This became apparent when stripping the peat with dropping additions from all rumen specific *mcrA* sequences. Then the methanogenic community structure was not different from the peat control (data not shown). We therefore conclude that the increased potential methane production rate due to reindeer dropping addition originates from the added biomass of rumen methanogens to the peat.

These results show that the methanogenic rumen microbiota contribute to the peat methane production, but final verification must be performed in a similar experiment that uses RNA transcripts to evaluate the active community, since DNA, as used here, also includes the gene copies in dormant and dead microbial cells. The contribution of rumen methanogens to methane emission has been speculated about but not shown before. In field studies where grazing was excluded by fencing Mutschlechner et al. (2018) could show that in upland alpine forest soils grazed by cattle the ecosystem turned from a methane sink into a source. The result was strongly linked with the increased presence of methanogenic Archaea, which could originate from the cattle dung (Mutschlechner et al., 2018; Radl et al., 2007). Cattle dung application also changed the peat methanogenic Archaea community by introducing *Methanobrevibacter* species and this was linked to increased peatland methane production (Hahn et al., 2018). Therefore, dung, next to the observed vegetation change, could also explain why Muskoxen (*Ovibos moschatus*) grazing in an artic mire increased the peatland methane flux (Falk et al., 2015).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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