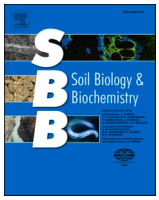
Soil Biology and Biochemistry 199 (2024) 109590

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Reindeer shape soil methanogenic and methanotrophic communities in subarctic fen peatlands, with a minor impact on methane emissions — A field study

Raija Laiho [a,*], Petri Salovaara [a], Päivi Mäkiranta [a], Krista Peltoniemi [a], Timo Penttilä [a], Tuomas Rajala [a], Jenni Hultman [a], Mika Korkiakoski [b], Hannu Fritze [a]

[a] Natural Resources Institute Finland (Luke), P.O. Box 2, FI-00791, Helsinki, Finland
[b] Finnish Meteorological Institute, P.O. Box 503, 00101, Helsinki, Finland

ARTICLE INFO

Keywords:
Peatland
Reindeer
Methane
Methanogens
Methanotrophs

ABSTRACT

Laboratory and field studies with other grazer species suggest that reindeer (*Rangifer tarandus* L.) grazing on northern peatlands could shape the peat soil microbial communities and lead to higher ecosystem methane (CH_4) emissions. We investigated this at two sedge fens in northern Finland, Lompolojänkkä and Halssiaapa, in experiments where reindeer grazing presence or absence was achieved with exclosure fences, and the effects of reindeer droppings were evaluated comparing dropping additions either on peat surface or trampled into the peat to controls with no droppings. Active soil methanogen and methanotroph communities were analyzed by metatranscriptomics. Soil CH_4 fluxes were quantified with manual chambers and portable gas analyzer. Reindeer presence and dropping additions were both connected to differences in the soil communities as compared to controls (no presence or no droppings). The responses differed between the two fens. Activity of rumen microbes in peat could not be detected. Structural equation models indicated that the ecosystem CH_4 flux in both fens depended on measurement year and sedge leaf area. At Halssiaapa trampled droppings, and at Lompolojänkkä both surface and trampled droppings reduced the sedge leaf area. While at Halssiaapa the dropping effect was not altogether statistically significant, in Lompolojänkkä surface droppings reduced the CH_4 flux both directly and through the reduced leaf area. In conclusion, while both reindeer presence and dropping addition were diversely reflected in the active soil communities, reindeer effects on the CH_4 flux were indirect and mediated via vegetation. The results contrast our earlier laboratory findings, and i) caution against liberal generalizations from lab studies to field conditions in peatlands, as well as ii) point to a need for rigorous multivariate analyses for deciphering the complex interactions governing the functions of these ecosystems.

1. Introduction

Large gregarious herbivores exert direct and indirect influences on their environment through grazing, trampling and fertilization (e.g., Sørensen et al., 2009). They may impact for instance on plant biomass (Tanentzap and Coomes, 2012), species composition (Milchunas and Lauenroth, 1993; Herrero-Jáureguie and Oesterheld, 2018) and carbon (C) allocation, e.g. aboveground-belowground ratio (Ylänne et al., 2015), as well as soil communities and respiration (Bardgett and Wardle, 2003; Andriuzzi and Wall, 2017; Ylänne et al., 2021). The strength and direction of the impacts may depend on ecosystem type or climatic region (Milchunas and Lauenroth, 1993; Bardgett and Wardle, 2003; Andriuzzi and Wall, 2017). All these impacts may shape the ecosystem C cycling and balance (Tanentzap and Coomes, 2012; Stark and Ylänne, 2015; Tuomi et al., 2021), including the emissions of greenhouse gases.

Throughout the subarctic region, the extensive peatlands are grazed by *Rangifer tarandus* (L.), in Eurasia reindeer and in North America caribou. These peatlands are a major terrestrial C storage (Yu, 2012), and a source of the potent greenhouse gas methane (CH_4) (Turetsky et al., 2014). Changes in CH_4 emissions have the potential to swiftly modify the global warming potential of peatlands. We found significantly increased CH_4 production potential in peat where reindeer droppings were added in laboratory studies (Laiho et al., 2017; Fritze et al., 2021), but how these results translate into field emissions is not

* Corresponding author.
E-mail addresses: raija.laiho@luke.fi (R. Laiho), timotja@gmail.com (T. Penttilä), mika.korkiakoski@fmi.fi (M. Korkiakoski).

https://doi.org/10.1016/j.soilbio.2024.109590
Received 17 January 2024; Received in revised form 29 August 2024; Accepted 13 September 2024
Available online 22 September 2024

clear. In the few field studies examining grazing in different types of wetlands by other species (sheep, yak, dairy cattle, muskoxen) both increases (Hirota et al., 2005; Sánchez et al., 2017; Hahn et al., 2018) and decreases (Falk et al., 2015) in CH_4 emissions have been reported. As the factors controlling the emissions have complex interactions (Turetsky et al., 2014; Stark and Ylänne, 2015) and the previous studies are not conclusive, potential *in situ* reindeer-induced effects on CH_4 emissions in peatlands need to be quantified.

One direct controlling factor may be through droppings. In our laboratory study we observed that adding reindeer droppings to fen peat increased CH_4 production potential by 40% (Fritze et al., 2021). The increment was explained by increased methanogenic abundance, as methanogens deriving from reindeer rumen through droppings not only survived but also participated in CH_4 production in anoxic peat. Hahn et al. (2018), working on cattle dung, also found rumen methanogen presence in, and increased CH_4 emissions from, peat of non-grazed pristine and restored peatlands following dung addition. Methanogen activity is often highest at depths just below the soil water-table level (WTL) (Shoemaker et al., 2012; Peltoniemi et al., 2016), where methanogenesis may be substrate limited (Bergman et al., 1998). Recent plant photosynthates contribute to CH_4 production (King et al., 2002; Dorodnikov et al., 2011), and droppings could in principle also contribute to the substrate pool. Yet, they may not be a preferred substrate for fermenters as in our laboratory experiment, minor or no CH_4 emission was found from droppings only (Fritze et al., 2021).

Among different types of peatland vegetation, CH_4 emissions are highest in fen sites where graminoids (sedges or grasses) dominate (Saarnio et al., 2007; Turetsky et al., 2014; Abdalla et al., 2016). Methane emission has been observed to correlate positively with sedge leaf area, and to decrease immediately and persistently following sedge clipping and removal (Noyce et al., 2014). Sedges function both as pipelines for CH_4 emissions, bypassing the surface peat where CH_4 oxidation would take place, and as a source of root exudates and litter for decomposers directly into the anoxic zone through the deep-reaching root systems (Dorodnikov et al., 2011). Reindeer grazing impacts on vegetation structure and composition depend on the underlying vegetation type and the intensity of grazing (Bernes et al., 2015; Vowles et al., 2017). In general, in non-forested sites, grazing may to some extent favor graminoids at the expense of deciduous shrubs, whose abundance and/or height decreases (Olofsson et al., 2001; Kitti et al., 2009; Väisänen et al., 2014; Gibson et al., 2021). This can be caused by both grazing as such, and by fertilization through droppings (Barthelemy et al., 2015) and urine, and may affect also the CH_4 emissions. Yet, grazing by free-roaming reindeer may be transient and pulse like, and often only minor impacts on vegetation have been observed, unless grazing intensity has been very high (Olofsson et al., 2004; Kolari et al., 2019). In any case, potential vegetation effects need to be considered when CH_4 emissions are studied because of the big role of sedges as regulators of the emission.

Here, we assessed *in situ* CH_4 emissions of two fen-type peatlands typical in the subarctic region using two approaches: grazing exclusion by exclosure fences, and experimental adding of reindeer droppings. As the droppings may have different impacts if left in oxic conditions on the peatland surface versus anoxic conditions when trampled beneath the surface, simulated trampling was included as a factor. Further, since WTL is a controlling factor for both vegetation composition and CH_4 emissions and varies between the microforms often found in northern fens, we tested whether there was a within-fen difference between drier and wetter microforms. As sedge leaf area plays a role in CH_4 emissions, we also tested the treatment effects on sedge LAI (leaf area index; one-sided leaf surface area/ground area) and analyzed the relationship between LAI and CH_4 emission. Reindeer impacts on vegetation composition were not studied in more detail since that has been done previously (e.g., Kolari et al., 2019). Reindeer prefer as grazing grounds wet fens with an abundance of food plants such as cottongrass (*Eriophorum* L. sp.) and other sedges (*Carex* L. sp.), forbs (e.g., *Menyanthes trifoliata* L.[1]) and horsetails (*Equisetum* L. sp.) (Turunen et al., 2009; Nyström et al., 2013), and the study sites were chosen accordingly. Further, we assessed the impacts of reindeer grazing and droppings on active methanogenic and methanotrophic soil communities and analyzed if the treatments had an impact on the *mcrA* and *pmoA* transcript levels and if these levels were associated with the CH_4 emission.

We hypothesized that i) reindeer exclosure and ii) reindeer dropping addition is reflected in the microbial community actively participating in the CH_4 fluxes, iii) CH_4 emissions are higher in the area where reindeer may roam than inside the exclosures, iv) reindeer droppings increase CH_4 emissions both inside and outside the exclosures, v) droppings increase CH_4 emissions more when trampled into the anoxic peat than when added on the surface, and vi) CH_4 emission responses to reindeer differ between wetter and drier microforms.

2. Materials and methods

2.1. Study sites

We worked on mesotrophic sites in two fen-type peatlands (Fig. 1), where exclosure fences preventing reindeer grazing split the sites into grazed and non-grazed areas.

The Halssiaapa site (N67°22.117′, E26°39.244′) represented a patterned mesotrophic fen with a string-flark microform structure. Vascular vegetation in the flarks was characterized by sedges *Scheuchzeria palustris* L. and *Eriophorum* x *medium* E. Anders. (pro sp.), with some *E. vaginatum* L., *Carex rostrata* Stokes, *C. chordorrhiza* L.f., *C. limosa* L. and *C. magellanica* Lam., while the strings were characterized by sparse small-sized Scots pine (*Pinus sylvestris* L.) and the shrub *Andromeda polifolia* L. The forb *Menyanthes trifoliata* L. and shrubs *Betula nana* L. and *Vaccinium oxycoccos* L. were found on both microforms. Moss layer in the flarks mostly consisted of *Warnstorfia exannulata* (Schimp.) Loeske and in the strings of various *Sphagnum* L. species. The fence excluding reindeer grazing was installed in the autumn 2001 and had an area of 0.5 ha. Measurement points were installed in flarks and in string margins, which were lawn-like surfaces with continuous *Sphagnum* cover, shrubs, and sedges. String margins were chosen instead of string tops because earlier studies suggest that CH_4 emissions may be at their highest in such locations in wet fens (e.g., Dinsmore et al., 2017). Methane and N_2O fluxes (Dinsmore et al., 2017), vegetation composition (Mörsky et al., 2012; Räsänen et al., 2020) and phenology (Linkosalmi et al., 2022), as well as CO_2 exchange (Haapala et al., 2009) for different subsites in Halssiaapa have been reported earlier.

The Lompolojänkkä site (N67°59.835′, E24°12.546′) represented a slightly drier mesotrophic fen without a clear microform pattern. The main surface type was classified as lawn. Vascular vegetation was characterized by sedges *Carex rostrata* and *C. chordorrhiza*. Additionally, forbs *Menyanthes trifoliata* and *Comarum palustre* L., some horsetail *Equisetum fluviatile* L. and shrubs such as *Betula nana* and *Andromeda polifolia* occurred on the site. Compared to the Halssiaapa site the vascular vegetation was clearly more abundant and taller. Moss layer consisted mostly of *Sphagnum* species, *S. fallax* (Klinggr.) Klinggr. being the most abundant. The fence was installed in the autumn 2017 and had an area of 0.2 ha. All measurement points were installed in the lawn surface, concentrating on the typical sedge-dominated vegetation with high CH_4 emission potential and avoiding the occasional shrub-dominated spots. The wider Lompolojänkkä peatland is an ICOS Class II ecosystem station (https://www.icos-finland.fi/stations). Methane fluxes (Zhang et al., 2020), vegetation composition (Räsänen et al., 2020; Mäkiranta et al., 2018), biomass production (Mäkiranta et al., 2018) and phenology (Mäkiranta et al., 2018; Linkosalmi et al., 2022), as well as N_2O fluxes (Lohila et al., 2010), and CO_2 exchange (Aurela et al., 2009; Laine et al., 2019) of the fen have been reported earlier.

[1] Plant names follow WFO (2022).

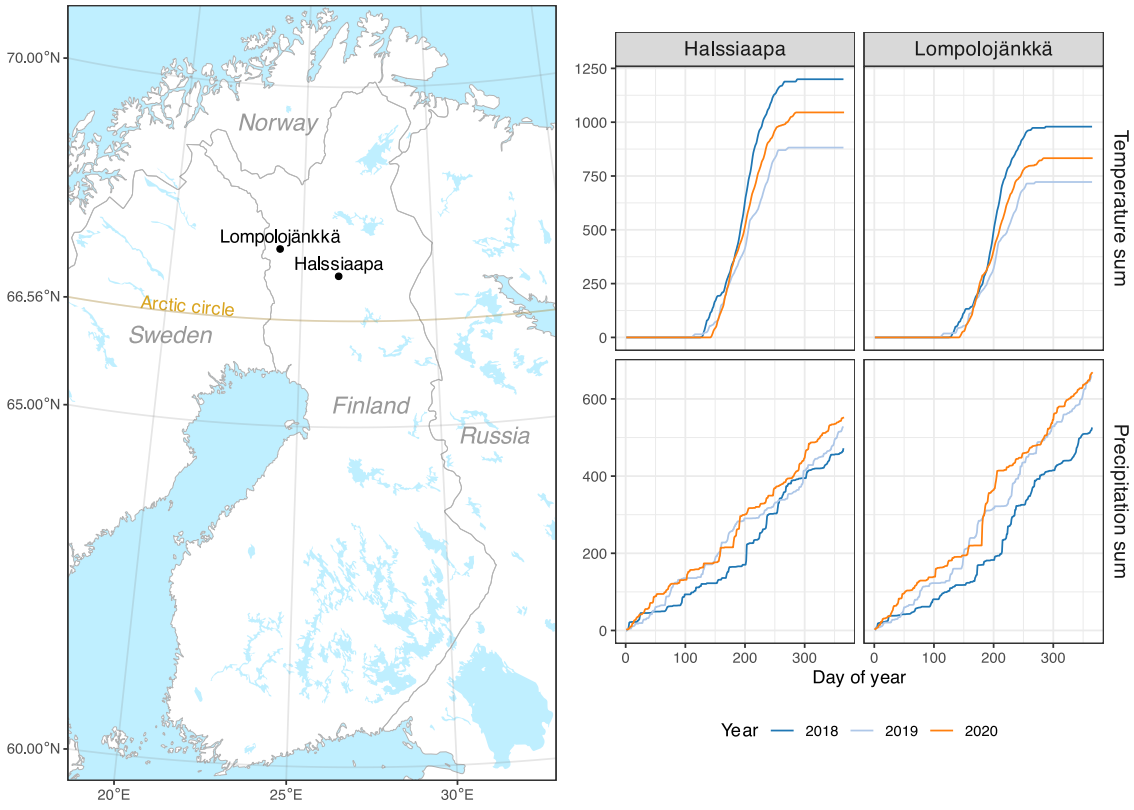


Fig. 1. Location of the two study sites, Halssiaapa and Lompolojänkkä fens, and their cumulative air temperature (with +5 °C threshold) and precipitation sums during the study period (2019–2020) and the preceding year. Weather data are from the Finnish Meteorological Institute measurement stations next to the study sites, except for Lompolojänkkä precipitation series that is from Kenttärova station 1.8 km from the site.

Surface peat in both sites consisted mostly of sedge and moss remains in early stages of decomposition (H2 to H3 at the von Post scale). Our research focused on the dominant surface types of the study sites: in Halssiaapa moss-covered flarks, and in Lompolojänkkä the lawn surface. Both may be considered "intermediate surfaces" in the context of the sites, as moss-covered flarks are somewhat more elevated than moss-free flarks with open water surface.

Of the two study years, 2019 was cooler than 2020, and both years were cooler than 2018 (Fig. 1, Fig. S1). The year 2018 preceding the measurements was drier than the study years.

2.2. Experimental setup

The study followed a split-plot design with the grazed (outside the exclosure fence) and non-grazed (inside the exclosure fence) areas as whole-plots that were not replicated within the fen sites. The following dropping treatments formed the basic subplots that were installed in the dominant surface types of the sites: 1) control with no droppings or trampling outside and inside the exclosure fence (three replicates within each whole-plot); 2) droppings added to the peatland surface inside the fence (three replicates where droppings were added in spring and another three where droppings were added in summer within each whole plot); 3) droppings artificially trampled down to a depth of 5–10 cm inside the fence, the depth depending on the resistance of the vegetation present in the plot (three replicates where droppings were added in spring and another three where droppings were added in summer within each whole plot). In Halssiaapa, there were additionally three control subplots installed in string margins within each whole plot, and three subplots in string margins inside the exclosure where droppings were added to the surface in summer. Since trampling as such (without dropping addition) may also affect the CH_4 emission we further controlled this effect in Lompolojänkkä by installing three subplots with trampling only.

There were thus altogether 33 subplots for the CH_4 flux and LAI measurements in Halssiaapa and 27 in Lompolojänkkä. The subplots were prepared at the end of May 2019, except for the controls with trampling only that were prepared in the beginning of July 2019. Their locations were chosen, visually judged, to represent the typical surface types of the sites, and to be comparable between treatments. The CH_4 flux measurement locations were framed with 30-cm diameter metal collars, 4 cm deep, with a groove for the measurement chamber (Fig. S2). A 10-cm diameter area in the center was then delineated for estimation of LAI. Each subplot was located next to boardwalks that were installed before the onset of the experiment to avoid disturbance-induced fluxes.

In each fen the experimental set-up for the microbial sampling involved 12 subplots (treatment n = 3) having the following four treatments: Controls in the whole-plots on both side of the fens are called Fence inside (no reindeer) and outside (reindeers present). The dropping treatments in the fenced whole-plot are called surface (droppings added on the fen surface) and trampled (droppings pushed into the surface peat through simulated trampling). To measure the short-term effects of dropping, additional droppings were added in August 2020 to the surface two weeks before sampling in the whole-plot area outside the fence.

The reindeer droppings used were collected on drier mineral-soil areas around the fens in autumn 2018 and spring 2019. In the spring collection, the average mass of a single dropping heap was determined to be 100 g (fresh mass) based on weighing twenty heaps. The droppings were stored frozen before addition that was done as 100 g fresh mass per subplot with defrosted droppings. According to our laboratory study the freezing of droppings does not influence the potential to raise peat soil CH_4 flux (Fritze et al., 2021). Dropping addition in Halssiaapa took place in May 22 (spring) and July 16 (summer), 2019, and in Lompolojänkkä in May 24 and July 2, 2019.

The trampling treatment aimed to simulate a situation where several reindeer walk single file, making the typical reindeer paths found in peatlands, and trample on the droppings of the reindeer in front. It was done with a perforated potato masher.

2.3. Analyses of active methanogens and methanotrophs

Peat samples for methanogenic and methanotrophic metatranscriptomes were cored at the end of August 2020 with a soil corer sterilized with 70% ethanol and cut into 10 cm sections from the surface down to 30 cm on site. Sub-samples for the analyses were immediately transferred with sterilized forceps to Whirl-packs (Nasco, Fort Atkinson, WI, USA) and frozen in a liquid nitrogen shipping container. The samples were transported frozen to the laboratory at Luke and kept at - 80 °C until analyses.

RNA was extracted and metatranscriptomic libraries were prepared as previously described in Viitamäki et al. (2022). Briefly, 0.5 g of soil was used in the extraction with CTAB and phenol-chloroform-isoamylalchol. Nucleic acids were purified with Qiagen AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) and quality of the resulting DNase treated RNA was checked with Bioanalyzer RNA 2100 Nano/Pico Chip with Total RNA Assay (Agilent, Santa Clara, CA, USA) and by running 16S rRNA PCR to verify that there were no DNA traces left in the sample. RNA extractions were more successful for Lompolojänkkä samples with 36 out of 41 samples having enough RNA for library preparation while in Halssiaapa the corresponding value was 15 out of 39. NEB Ultra II RNA library prep kit for Illumina was used in constructing complementary DNA (cDNA) libraries, which were sequenced with Illumina NovaSeq with PE 150 cycles at the Institute of Biotechnology, University of Helsinki, Finland.

Sequence quality was assessed with FastQC v. 0.11. (https://www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC v. 1.3 (Ewels et al., 2016). Trimming and quality filtering were performed with Cutadapt v. 1.10 (Martin, 2011), applying a quality cut-off of 25 and a minimum adapter overlap of 10 bp. Metaxa2 v. 2.1.3 (Bengtsson-Palme et al., 2015) was used to identify reads mapping to the small subunit (SSU) rRNA. These were then classified against the SILVA database release 132 (Quast et al., 2013) using the mothur v. 1.40.5 classify.seqs command with a confidence cut-off of 60% (Schloss et al., 2009). Protein-coding genes, *mcrA* and *pmoA*, were annotated by mapping the reads to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Prokaryote database release 86 (Kanehisa and Goto, 2000) using DIAMOND blastx v. 2.1.3 (Buchfink et al., 2015) with an E-value cut-off of 0.001. Putative *pmoA* and *amoA* genes were further annotated as in Viitamäki et al. (2022). Relative abundances of the reads were calculated using the total read counts in the sample.

2.4. Gas flux measurements

CH_4 flux measurements were carried out during May 20 – August 31, 2019, and June 2 – August 25, 2020. Each site was visited at about weekly intervals, and each subplot was measured several times during one visit. In the trampled subplots, one measurement event followed immediately after the trampling. Different subplots were measured in random order. The dropping-addition subplots were also measured 1–16 times before addition to explore the variation in the inherent/pre-experiment flux level.

Measurements were done using LI-7810 portable gas analyzer (LI-COR Biosciences, USA) and a closed, opaque steel chamber (diameter 30 cm, height 30 cm). The chamber was equipped with a fan and a thermometer. Fan speed was set slow as to not flush out CH_4 from the moss layer or surface soil. Air was circulating in a closed loop between the chamber and analyzer, i.e., the sampled air was returned to the chamber after sampling. Each measurement event lasted 3 min, during which the gas concentration in the chamber headspace was analyzed at intervals of 1 s.

Fluxes were calculated based on the concentration change within the sealed chamber over time:

$$F = \left(\frac{dC(t)}{dt}\right)_{t=0} \frac{MV}{V_m A} \frac{273.15}{273.15 + T} \quad (1)$$

Where $\left(\frac{dC(t)}{dt}\right)_{t=0}$ is the concentration change at the start of the measurement (i.e. the slope of the fit), V is the volume of the chamber, M is the molar mass of CH_4 (16.04 g mol^{-1}), A is the surface area of the chamber, V_m is the ideal gas molar volume (0.0224 m^3 mol^{-1}), and T is the air temperature inside the chamber. $\left(\frac{dC(t)}{dt}\right)_{t=0}$ was estimated from a linear model (e.g. Korkiakoski et al., 2017). The starting and ending times of the measurements were identified visually, so that the initial disturbance caused by the chamber placement was removed. Typically, the initial disturbance lasted around 30 s. The fitting period was allowed to vary within 2–2.5 min. If the fitting period was longer, then datapoints were discarded from the end of the closure to reach a fitting period length of 2.5 min. None of the measurements had fitting periods shorter than 2 min.

Data quality was inspected visually. Obvious cases of chamber leakage, ebullition, and uneven concentration development were discarded from further analysis. However, the measurements made immediately after trampling were kept for further analysis even if issues mentioned above, apart from chamber leakage, were observed.

2.5. Covariate measurements

In each subplot, the species composition of vascular plants and mosses was identified (Table 1). For estimating the leaf area index (LAI) of vascular plants, the number of leaves per species was measured in the 10 cm diameter area in the center of the subplot (Fig. S2). For *Vaccinium oxycoccos* that has tiny leaves, the length of leaf-bearing stems was measured instead. Leaf and stem samples for measuring leaf areas were then collected from separate LAI plots. The leaf areas were estimated from photographs of the sample leaves against white A4 sheets. In 2019,

Table 1
Vascular plant species present at the gas flux measurement subplots.

Halssiaapa	Lompolojänkkä
Sedges	
Carex limosa	*Carex chordorrhiza*
C. magellanica	*C. rostrata*
Carex chordorrhiza[a]	*Carex limosa*[a]
C. rostrata[a]	
Eriophorum vaginatum	
Scheuchzeria palustris	
Forbs	
Menyanthes trifoliata	*Comarum palustre*
Equisetum fluviatile[a]	*M. trifoliata*
	Equisetum fluviatile
Shrubs	
Andromeda polifolia	*A. polifolia*
Betula nana	*B. nana*
Vaccinium oxycoccos	*V. oxycoccos*

[a] In subplots established in 2020.

GIMP software (the GIMP Team, version 2.10.10) was used for the estimation, and in 2020 the CANOPEO mobile phone application (Patrignani and Ochsner, 2015). The average leaf size was then calculated from the proportion of the green area and the area of the paper, and the number of leaves. The measurements and sampling were done in three campaigns in 2019 (28 May, 15 July, 21 August in Halssiaapa; 23 May, 1 July, 16 August in Lompolojänkkä) and 3–5 campaigns in 2020 (24 June, 4 August, 10 August in Halssiaapa; 5 June, 26 June, 9 July, 29 July, 7 August in Lompolojänkkä). The sedge LAI that was used in the data analyses described below was interpolated to the gas-measurement dates with log-Gaussian-shaped curves. To help estimate realistic LAI curve shapes with a sharp rise and gradual decay over the growing season, the LAI measurements were augmented with near-0 values at spring (10 May) and autumn (5 October), and, assuming that the growth curve shape parameters were locally correlated across plots of the same site, informative curve parameter priors were set per site and year.

Soil WTL was manually measured from dipwells (perforated plastic tubes, one per treatment) each day when gas flux measurements were done. Soil temperature at 5 and 15 cm depths was monitored with one sensor (iButton, Maxim Integrated, USA) per treatment. Additionally, soil temperatures were measured (CEM DT-612, CEM Industries, India) during each gas flux measurement at each subplot.

Since soil pH is known to affect CH_4 fluxes (e.g., Bergman et al., 1998), it was measured in the laboratory from suspensions where 15 ml of peat was added to 45 ml of water and left standing over night before the measurement.

2.6. Data analysis

2.6.1. Microbial data

Putative methanogenic archaeal and methanotrophic bacterial sequences were extracted using the comprehensive latest published knowledge reviewed in Knief (2019). Sequences from the same family (if possible) were combined and data was organized into data matrices. The abundances of the most dominant (0.5%) putative methanogenic archaeal families and of all observed known and putative methanotrophic bacterial taxa at the family level (if possible) were statistically tested between treatments. A negative binomial generalized linear model was used for analyzing abundances. For testing hypotheses i and ii, the read counts of the studied family were the response variable, fence (inside and outside the fence without droppings) or treatment (inside the fence: control without droppings, droppings added in the surface, and droppings added and trampled) as the explanatory variable, and log-scale total read counts of all archaea for methanogenic or bacteria for methanotrophic sequences per sample as the offset. Modeling of abundances from Halssiaapa and Lompolojänkkä data sets were conducted separately.

The *mcrA* and *pmoA* abundances in treatment groups were analyzed with a negative binomial regression model. Additive plot-wise random effects were added to the mean function to remove clustering due to the split-plot-design, and the log-scale total read counts of the sequenced samples were used as offset terms.

The *mcrA*, *pmoA* and *mcrA/pmoA* associations with CH_4 fluxes of the preceding 9 days (9 days was the minimum for at least 1 measurement/sample) were analyzed using linear regression. Each flux was independently regressed on the logarithm of each gene-read fraction, with and without treatment factors. The model without treatment factors included plot-wise random effects, the model with treatment factors did not due to insufficient number of replicates.

For modeling we used the functions lm, glm.nb and emmeans of the packages 'MASS' and 'emmeans' and for data wrangling and illustrations we used 'ggpubr', 'dplyr' and 'ggplot2' (Wickham, 2016) packages in R studio environment version 2022.07.1 (RStudio Team, 2022) and in R version 4.2.1 (R Core Team, 2022).

2.6.2. CH_4 flux and covariate data

Statistical differences between groups related to the hypotheses iii-vii (treatment, fence, position and/or site) were estimated using generalized mixed additive models. The group variables and their interactions, with the additional year grouping, were modelled as linear fixed effects, the environmental covariates LAI, WTL and T were modelled as smooth splines, and the incomplete split-plot design were accounted for by plot-wise random intercept terms.

Exploration of the simultaneous dependency on treatment and grouping variables of LAI and CH_4 flux, a set of structural equation models (SEMs) was first hypothesized (Fig. S3) and then estimated. No interactions were included. Based on posterior predictive checks non-linear likelihoods were chosen for LAI (gamma-distribution) and fluxes (skew-normal). An additional linear (Gaussian) SEM model was estimated after square-root-transforming fluxes and log transforming LAIs.

When the sedge LAI was involved in the group comparisons, the posterior mean interpolation values were used. In the multivariate analysis we included the interpolation uncertainty by sampling LAI predictions from the posterior estimates of the LAI interpolation curves and running analysis on each set separately. The resulting set of analysis were then combined.

The group comparisons were conducted using the R-library 'gamm4', the LAI interpolation models were estimated with 'rstan', and multivariate analysis were done with 'brms'.

The variation of soil pH between the study sites and treatments was tested by ANOVA.

3. Results

3.1. Sedge LAI, WTL, soil T and pH

Sedge leaf area index (LAI) was higher in Lompolojänkkä than in Halssiaapa, and higher in 2020 than in 2019 (Fig. S4). In Halssiaapa, sedge LAI was similar inside and outside of the fence. In Lompolojänkkä in 2020, sedge LAI was higher inside the fence at the end of the measurement season; this difference emerged somewhat earlier under surface-addition droppings than in the control plots. In Halssiaapa, there were no statistically significant differences between control plots and the dropping additions. In Lompolojänkkä in 2020, control plots had higher sedge LAI than the dropping addition plots at the end of the measurement season, indicating earlier decline under droppings.

The soil surface (−5 cm) temperatures during the measurement periods varied more widely in 2019 than 2020, reaching up to 30 °C (Fig. S5). Soil WTL varied between 0 and -15 cm during the measurement periods in the dominant surface of both sites, the string edges having 5–10 cm lower WTLs (Fig. S5). The surface peat (0–10 cm) pH differed between the two fen sites, being on average 4.5 (S.E. 0.7) at Halssiaapa and 5.1 (S.E. 0.05) at Lompolojänkkä. The treatments had no significant effects (Table S1).

3.2. Active soil methanogenic and methanotrophic communities

Due to low RNA recovery from the deeper peat samples, we present here only the results from the 0–10 cm depth. From all transcriptomic sample libraries at 0–10 cm depth, on average 96% of the archaeal reads were methanogenic while only 6% of the bacterial reads were methanotrophic (Table S2).

Twenty-one different known or putative methanogenic taxa were obtained from the archaeal read-based metatranscriptomic data (Table S3). The abundance of Rice Cluster II and Methanosarciniales in Halssiaapa and the abundances of Methanosaetaceae, Methanosarcinacea, Metahanoregulaceae, Methanobacteriaceae, Methanomicrobiales and Methanocellaceae in Lompolojänkkä showed differences inside and outside the fence (Fig. 2), supporting hypothesis i (reindeer exclosure is reflected in the microbial community).

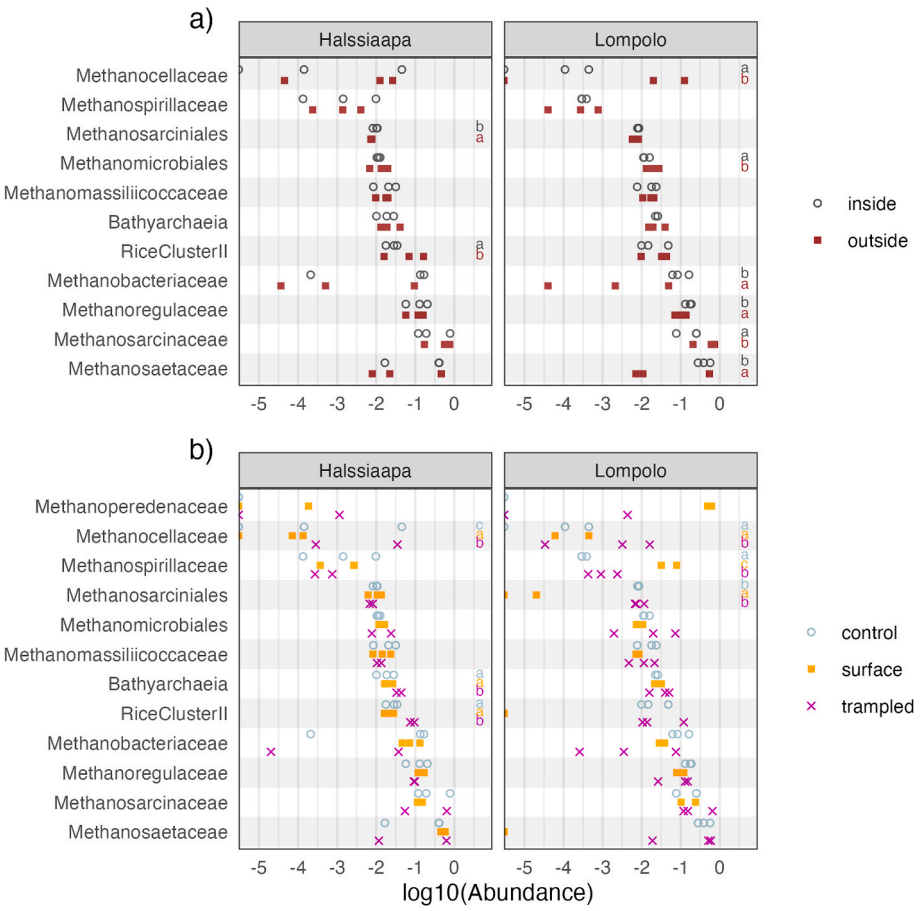


Fig. 2. Relative abundances (log transformed) of the most dominant known or putative methanogens at the family level at the 0–10 cm depth a) inside and outside the fence (no droppings) and b) inside the fence from the control treatment (no droppings) and with added surface or trampled droppings. Statistical differences are shown by letters if they were detected.

RiceCluster II was more abundant outside the fence compared to inside and Methanosarciniales vice versa. Methanosaetaceae, Methanoregulaceae and Methanobacteriaceae were more abundant inside the fence and Methanosarcinacea, Methanomicrobiales and Methanocellaceae outside the fence. Trampled droppings increased the abundances of Bathyarchaeia, Rice Cluster II and Methanocellaceae in Halssiaapa, and Methanosarciniales and Methanocellaceae in Lompolojänkkä, supporting hypothesis ii (reindeer dropping addition is reflected in the microbial community). The abundance of Methanospirillaceae increased after addition of surface droppings.

Eleven known or putative methanotrophic taxa were obtained from the bacterial read-based metatranscriptomic data (Table S4). Methylophilacea was more abundant inside the fence in Halssiaapa, whereas methanotrophic genera of Beijerinckiaceae (*Methylovirgula*, *Methylosinus*, *Methyloferula*, *Methylobacterium*, *Methylorubrum*, *Methylorosula*, *Methylocystis*, *Methylocapsa*, *Methylocella*) and Rhizobiales (including only genus *Rhodomicrobium*) were more abundant outside the fence in Lompolojänkkä (Fig. 3), further supporting hypothesis i. Methylococcaceae and Methylophilaceae were more abundant in control without droppings compared to both dropping treatments (surface and trampled) in Halssiaapa, supporting hypothesis ii. In turn Methyloligellaceae were more abundant and Methylophilaceae less abundant in treatment with trampled droppings compared to control without droppings in Lompolojänkkä.

The relative *mcrA* abundance differed significantly between the two fens, with Lompolojänkkä having higher values (Table 2). The reindeer absence or presence (samples from inside or outside the fence) had no influence on the results. Both surface and trampled droppings treatments had significantly higher relative abundances than the control. The two fens did not differ in their *pmoA* relative abundance values. The *pmoA* values were lower outside the fence where reindeers were present, and the trampled droppings treatment also induced lower *pmoA* presence.

3.3. CH_4 fluxes

Both sites acted as CH_4 emitters, the emission being higher at Lompolojänkkä than at Halssiaapa, especially in 2019 (Fig. 4, 6 and 7). At Halssiaapa, the emission was higher in 2020 than in 2019, while at Lompolojänkkä, the difference between years was smaller. The emission was lower at string edges than at flarks at the Halssiaapa site, where these microforms were present. Further at Halssiaapa, the emission inside and outside of the fence was similar in both years and in both microforms (Table S5). At Lompolojänkkä, the emission was somewhat (50 mg m^{-2} d^{-1}) higher inside the fence in 2020 ($p < 0.001$). Thus, our hypothesis iii (CH_4 emissions are higher in the area where reindeer may roam) was not supported.

The mixed model analysis indicated that dropping addition did not affect the CH_4 emission from the dominant surfaces at Lompolojänkkä and Halssiaapa (Fig. 5, Table S6), thus no immediate support was found for hypotheses iv and v (droppings increase CH_4 emissions iv: both inside and outside the exclosures and v: more when trampled into the anoxic peat than when added on the surface). At Halssiaapa in 2020, the surface addition on string edges had significantly higher emissions (Fig. 5, Fig. S6). This effect was, however, inflated by very high emissions in the treatment plots on one particular day. This specific date

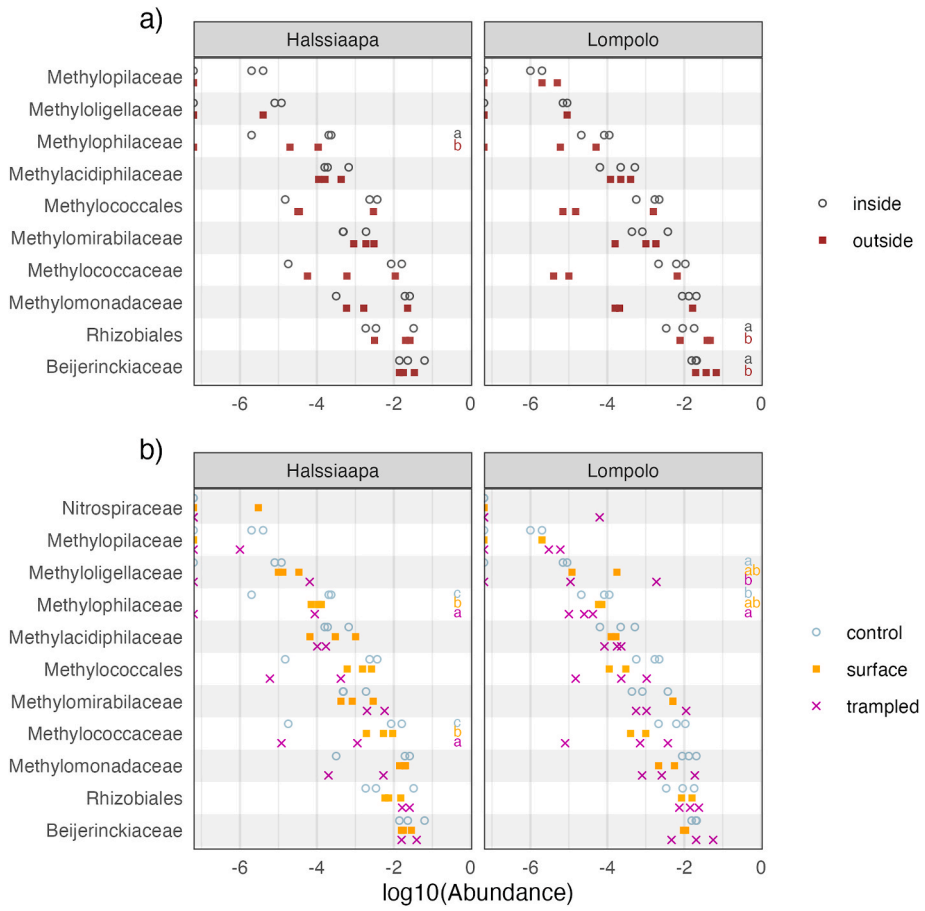


Fig. 3. Relative abundances (log transformed) of the observed known or putative methanotrophs at family level at the 0–10 cm depth a) inside and outside the fence (no droppings) and b) inside the fence from the control treatment (no droppings) and with added surface or trampled droppings. Statistical differences are shown by letters if they were detected.

Table 2
Site and treatment effects on relative mcrA and pmoA abundance values; effect sizes are in logarithmic scale. Reference categories: Halssiaapa, inside the fence, no droppings.

Effect	*mcrA*				*pmoA*			
	Estimate	S.E.	Statistic	P	Estimate	S.E.	Statistic	P
Site	0.629	0.256	2.454	0.014	−0.771	0.474	−1.626	0.104
Fence	0.642	0.353	1.819	0.069	−1.347	0.592	−2.275	0.023
Surface droppings	0.882	0.354	2.494	0.013	−1.001	0.581	−1.722	0.085
Trampled droppings	1.171	0.370	3.168	0.002	−1.492	0.633	−2.356	0.018

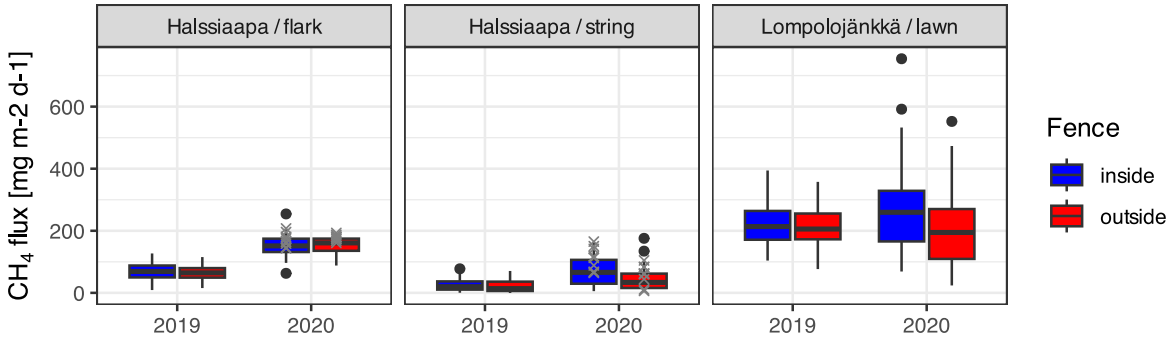


Fig. 4. Boxplot visualization of CH_4 fluxes from control plots (no dropping addition) inside and outside the reindeer exclusion fences.

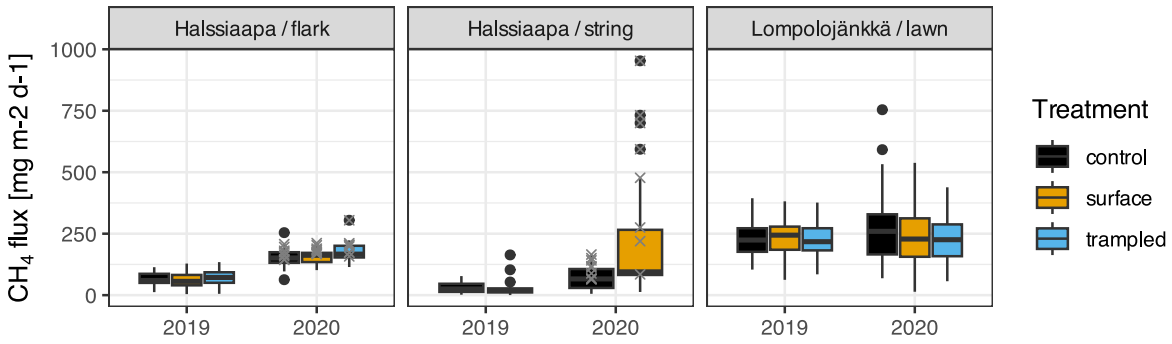


Fig. 5. Boxplot visualization of direct dropping treatment effects on the soil CH_4 flux. The values on a particularly hot day are highlighted with grey x's.

showed quite high temperature compared to others, but no clear abnormalities (e.g., no signs of ebullition, no abnormal rainfall or drought events). Thus, only very partial support was provided for hypothesis vi (CH_4 emission responses to reindeer differ between wetter and drier microforms). Including the covariates sedge LAI, WTL, and soil temperature improved model fitness (measured by Akaike information criterion), indicating significant covariation with emission, LAI most of all.

We then combined the different factors in a multivariate structural equation model (SEM) and estimated the model parameters with the anomalously hot date in 2020 excluded. The lawn CH_4 flux in Halssiaapa depended significantly on LAI and year (Fig. 6, Table 3). Based on untransformed data, the measurement year had both a direct effect and an indirect effect through sedge LAI on the CH_4 flux. After log-transformation of sedge LAI and flux data, the LAI-mediated effect seemed to depend more on trampled addition of droppings reducing LAI than the yearly variation in LAI. The total effect of trampled droppings at Halssiaapa was not significant, however (Table 4). In Lompolojänkkä, there was similarly a LAI effect and a year effect, but the year effect was opposite to that in Halssiaapa (Fig. 6, Table 3). Additionally, in Lompolojänkkä, both surface and trampled addition of reindeer droppings had a negative effect on LAI, and thus an indirect negative effect on the CH_4 flux. Transformation of the LAI and flux data showed up also a direct negative effect of surface addition of droppings on the CH_4 flux. Altogether, the surface dropping addition thus had a significant negative

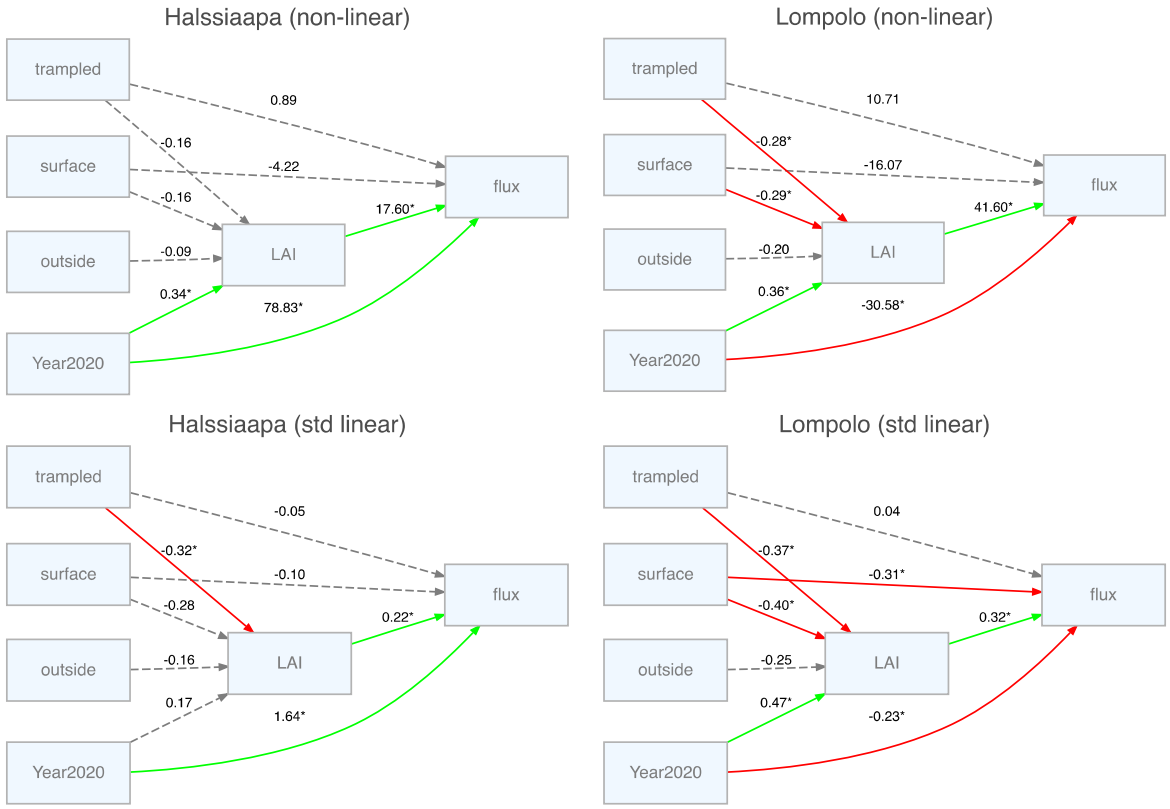


Fig. 6. Structural equation modeling results for CH_4 flux at the two study sites. Upper panels (non-linear) show the estimated path-coefficients from non-transformed data and non-Gaussian likelihoods for the naturally positively skewed sedge leaf area index (LAI) and flux values; these effects are not linear. Lower panels (std linear) show standardized coefficient estimates from log/square-root transformed LAI/flux data, where Gaussianity approximately holds, and approximately linear effect sizes can be quantified. Green arrows indicate statistically significant positive effects, and red arrows statistically significant negative effects. Dashed lines indicate non-significant effects with the sign showing the direction. trampled and surface refer to dropping addition treatments that are contrasted to controls with no droppings; outside refers to reindeer grazing presence outside the exclosure fences that is contrasted to measurements inside the fence with no reindeer presence, and Year 2020 refers to the 2nd measurement year that is contrasted to the 1st measurement year 2019. Results are for the dominant surfaces of the sites: moss-covered flarks at Halssiaapa and lawns at Lompolojänkkä.

Table 3
Pathway effect estimates, estimation errors (standard error), and lower and higher quartiles, from treatment to CH_4 flux, estimated (top panel) using untransformed data when the effects are nonlinear, and (bottom panel) using log/square-root transformed LAI/flux data and assuming the effects to be linear. Reference categories: year 2019, inside the fence, untreated control (no dropping addition). Abbreviations: LAI = leaf area index, dropp = dropping addition.

Response	Halssiaapa					Lompolojänkkä			
	Covariate	Estimate	Error	Q2.5	Q97.5	Estimate	Error	Q2.5	Q97.5
Untransformed data, nonlinear model									
LAI	intercept	−1.229	0.095	−1.417	−1.041	−0.224	0.115	−0.450	0.005
LAI	year-2020	0.342	0.135	0.083	0.589	0.358	0.095	0.179	0.529
LAI	fence-outside	−0.091	0.138	−0.362	0.182	−0.197	0.136	−0.475	0.067
LAI	dropp-surface	−0.165	0.089	−0.347	0.010	−0.288	0.098	−0.489	−0.101
LAI	dropp-trampled	−0.163	0.088	−0.327	0.022	−0.281	0.099	−0.478	−0.083
CH_4 flux	intercept	62.98	3.65	55.88	70.19	204.13	9.688	184.89	222.93
CH_4 flux	LAI	17.60	7.306	3.176	32.26	41.60	7.34	27.17	55.97
CH_4 flux	year-2020	78.83	3.587	71.51	85.55	−30.58	7.285	−44.70	−16.18
CH_4 flux	dropp-surface	−4.224	3.290	−10.64	2.267	−16.07	9.578	−34.66	2.838
CH_4 flux	dropp-trampled	0.889	3.254	−5.497	7.239	10.71	9.806	−8.306	30.27
Transformed data, linear model									
LAI	intercept	−1.241	0.101	−1.445	−1.046	−0.269	0.114	−0.501	−0.051
LAI	year-2020	0.111	0.153	−0.209	0.380	0.361	0.091	0.196	0.536
LAI	fence-outside	−0.103	0.144	−0.384	0.187	−0.197	0.137	−0.467	0.071
LAI	surface dropp	−0.183	0.109	−0.383	0.054	−0.311	0.101	−0.520	−0.117
LAI	trampled dropp	−0.210	0.092	−0.383	−0.022	−0.284	0.103	−0.473	−0.069
CH_4 flux	intercept	9.159	0.263	8.646	9.679	15.69	0.316	15.06	16.30
CH_4 flux	LAI	0.830	0.124	0.597	1.085	1.190	0.149	0.891	1.477
CH_4 flux	year-2020	4.060	0.178	3.711	4.410	−0.676	0.237	−1.136	−0.209
CH_4 flux	surface dropp	−0.237	0.196	−0.620	0.148	−0.903	0.339	−1.563	−0.238
CH_4 flux	trampled dropp	−0.131	0.190	−0.505	0.239	0.124	0.358	−0.572	0.831

Table 4
Mediator effects of the treatments via the sedge leaf area index (LAI) on the methane flux, based on the transformed data and linear models (see Table 3).

Effect	Halssiaapa				Lompolojänkkä			
	Estimate	Error	Q2.5	Q97.5	Estimate	Error	Q2.5	Q97.5
Direct, surface droppings	−0.24	0.20	−0.62	0.15	−0.90	0.34	−1.56	−0.24
Direct, trampled droppings	−0.13	0.19	−0.51	0.24	0.12	0.36	−0.57	0.83
Indirect, surface droppings	−0.15	0.09	−0.32	0.04	−0.37	0.14	−0.67	−0.13
Indirect, trampled droppings	−0.17	0.08	−0.33	−0.02	−0.34	0.13	−0.59	−0.08
Total, surface droppings	−0.39	0.19	−0.77	−0.01	−1.28	0.34	−1.94	−0.60
Total, trampled droppings	−0.30	0.19	−0.68	0.07	−0.21	0.36	−0.92	0.50

total effect on the CH_4 flux, contrasting the hypotheses.

The relative abundances of the *mcrA* and *pmoA* genes or their ratio showed no statistically significant patterns relative to the CH_4 fluxes measured preceding the sampling for the active community (not shown).

Methane emission generally increased with increasing sedge leaf area, was highest when the soil water-table level was 5–7 cm below the fen surface and increased with increasing soil temperature at 5 cm depth until ca. 15 °C (Lompolojänkkä) or 23 °C (Halssiaapa) (Fig. 7.).

4. Discussion

The first two hypotheses that i) reindeer exclosure and ii) reindeer dropping addition are reflected in the microbial community actively participating in the CH_4 flux were supported. Both reindeer presence and their droppings affected the community, but the two fens did not show identical reactions. For instance, the numerical response of methanogenic families reacting significantly to fencing was higher, six out of eleven tested, in the Lompolojänkkä fen, which was fenced 2.5 years before sampling. Only two methanogenic families reacted in the Halssiaapa fen, which was fenced 16.5 years ago. Neither the methanogenic nor the methanotrophic families reacting to the fencing treatment were the same in the two fens. This could be a time since fencing effect, but we suspect that this result is rather due to the spatial variation of microorganisms, which is large both within and between functionally similar peatlands (Juottonen et al., 2015). Therefore, when replicating a field trial at different peatlands, one can expect to test the experimental factors on different microbial communities and as such peatland microbiomes often show individual responses (e.g., Peltoniemi et al., 2016).

Of the methanotrophic families, Methylophiliaceae increased significantly due to reindeer exclosure by fencing in the Halssiaapa fen. A similar pattern has also been reported by Aggerbeck et al. (2022) studying fencing exclosure of muskoxen (*Ovibos moschatus*). In the Lompolojänkkä fen, two families of the Order Rhizobiales (Beijerickiaceae and unknown) showed increased presence due to reindeer presence. A changed methanotrophic community in similar exclosure experiments has also been reported by Rainer et al. (2020, 2021) in arctic peat soil due to geese grazing and in grassland soil grazed by sheep (Li et al., 2020; Wang et al., 2022).

Of the methanogenic species *Methanocella* has earlier been reported to increase due to grazing (Mutschlechner et al., 2018) and we could see the same trend for the Methanocellaceae family in the Lompolojänkkä fen. In general, however, our results are hard to compare to other grazing studies because there are not many, and the resolution of identification differs. A good example is that order Methanobacteriales was reported to increase due to grazing by Mutschlechner et al. (2018) but in our study we observed the opposite with the Methanobacteriales family Methanobacteriaceae at the Lompolojänkkä fen. These differences can result from different ecosystems like forest (Mutschlechner et al., 2018) versus peatlands (our study) and/or the use of different sequencing technologies, DNA based amplicon sequencing versus RNA based shot-gun sequencing, which was performed in our study.

While reindeer presence/absence achieved by the fencing treatment gives a summarized effect of grazing, droppings and urine, our dropping

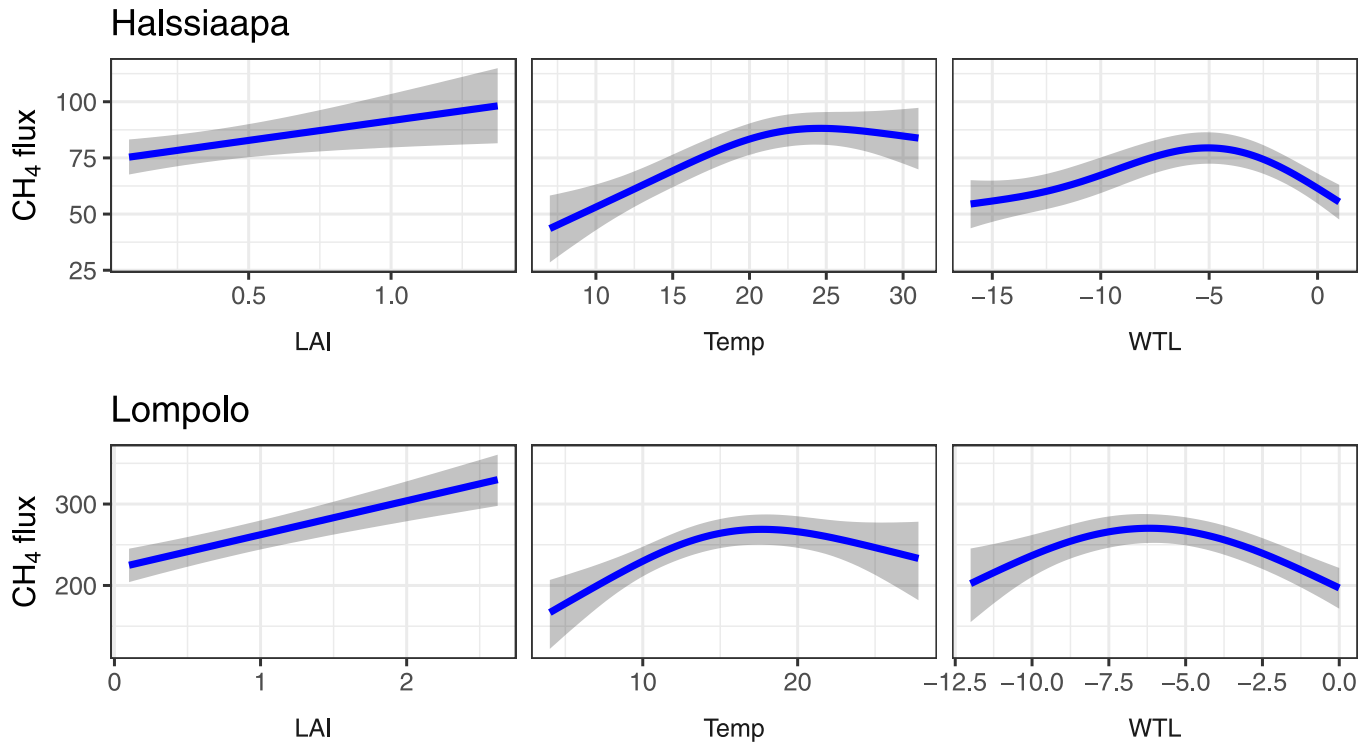


Fig. 7. Responses of the CH_4 flux [mg m^{-2} day^{-1}] from the two fen sites to sedge leaf area (LAI), soil temperature at 5 cm depth (Temp), and soil water-table level (WTL): smoothed responses from the non-linear SEM model, averaged over fence, year and treatment groups. Results are from the dominant surfaces of the sites: moss-covered flarks at Halssiaapa and lawns at Lompolojänkkä.

(feces) addition is measuring an individual effect and thus the response may differ from the overall effect. The addition of droppings also significantly affected the presence of different methanotrophic and methanogenic families, and again, the fens differed in respect to which family reacted. Not much is known about ruminant methanotrophs which could have been added with feces to the environment and certainly nothing about the ones inhabiting reindeer. Rumen methanotrophs are reported to exist (Khatri et al., 2021) but at this stage of knowledge we are not able to relate our results to the possibility if some of them were added by the droppings. As no methanotrophic family increased due to either surface or trampled dropping addition this seems not likely.

We expected to detect rumen methanogens in the RNA pool since Fritze et al. (2021) showed in a laboratory experiment that dropping addition to peat introduced *Methanobrevibacter,* a rumen methanogen belonging to the family Methanobacteriaceae, to strongly participate in the increased methane production. *Methanobrevibacter* is known to be the dominant member, having as high as 95% of archaeal reads, of the reindeer rumen microflora (Salgado-Flores et al., 2016). In this field study, Methanobacteriaceae represented only 5% of the total archaeal reads (Table S3) and could not be linked to our dropping treatments. Thus, it seems unlikely that the rumen *Methanobrevibacter* participated in the CH_4 production. This finding does not yet rule out that rumen-inhabiting methanogens overall could have been present or participating in peat CH_4 production. Reindeer rumen methanogens also include members, for instance, of the family Methanosarcinaceae (Sundset et al., 2009), which made up 30% of our peat soil archaeal reads. But as also the Methanosarcinaceae did not respond to our dropping treatments we are not able to make such a conclusion. Still further, Methanomethylophilaceae, a core rumen methanogen family (Anderson et al., 2021), made up only 0.01% of our archaeal reads and could not be modelled. With this low presence this family cannot be held responsible for the field CH_4 fluxes measured.

Participation of rumen methanogens to the CH_4 flux has, apart from our laboratory study (Fritze et al., 2021), been speculated by Hahn et al. (2018) and Aggerbeck et al. (2022), who both analyzed peat soil DNA pools. Hahn et al. observed a transfer of rumen methanogen sequences (*Methanobrevibacter* spp.) to pristine and rewetted fens from cattle dung, while Aggerbeck et al. found fingerprints of muskoxen rumen microbiota in the fen microbiome of a fencing exclosure study. But in contrast to the RNA pool analyses that we did, the DNA pool analyses detect also resting and dead microbes and could thus be the reason as to why rumen microbiota were detected. We could also not detect active presence of known rumen-origin methanogens in test samples taken in shorter term (two weeks) after dropping addition (data not shown). Put together, these results give the impression that rumen methanogens are not long lived when excreted into nature.

We also could not detect a connection between the *mcrA* and *pmoA* transcript levels and the field CH_4 flux. We measured a higher *mcrA* level in the dropping treatments, and a lower *pmoA* level outside the fence and in the trampled dropping treatment but could not relate this to the CH_4 flux. This may be at least partly due to difficulties in fitting together data from single (active microbes) versus repeated (CH_4 flux) measurements. Ideally, the microbial analyses should also be repeated, but this is still often constrained by available resources.

With the CH_4 cycle community analyses at hand, against our hypotheses (iii, iv, v) and unlike the laboratory results (Laiho et al., 2017; Fritze et al., 2021), reindeer presence and reindeer droppings did not increase the CH_4 fluxes of the two fens. This study is thus an important reminder that patterns that are clearly observed under laboratory conditions do not necessarily translate into measurable outcomes at the field scale. This even when the laboratory conditions are carefully designed to mimic field conditions in small scale. Any reindeer effects that we observed on site were minor, mostly indirect through sedge leaf area, and opposite to the laboratory findings. The response of the flux to surface dropping addition was negative in both sites, but significant only

at Lompolojänkkä.

We wish to specifically point out the importance of choosing the data analysis methods in case of several correlated variables, such as ours. While the multivariate mixed models, which are widely used, indicated no significant effects, the SEM analysis identified both indirect and direct effects significantly affecting the CH_4 flux at Lompolojänkkä. Fen type peatlands may thus show an individual response to grazing pressure which can either increase CH_4 fluxes (Falk et al., 2015) or not (this study). The SEM analysis allowed us to construct a model based on soil biochemistry that captures the soil process pathways better than a mixed model. Within the SEM framework, linearization of (presumably) non-linear pathways affected the statistical results. Especially with the skewed LAI values of Halssiaapa, with high variability and measurement uncertainty during 2020, the linearization significantly equalised the years and increased the treatment differences. It is difficult to specify the best model here: On one hand, soil process understanding and raw data support the non-linear model, but on the other hand the linearization (and assumed Gaussianity) enables (an approximate) mediator analysis.

In general, we were not too surprised to see differing responses in the two sites, in spite that they both represented the same vegetation type, open fen (intermediate fen to be somewhat more precise) in the same climatic zone. The same has actually been found in a previous experimental study that was replicated across two fens; earlier concerning climate change impacts (Peltoniemi et al., 2016; Mäkiranta et al., 2018; Laine et al., 2019). Even though fens are generally wet ecosystems supported by groundwater or surface water inputs, subtle differences in their soil water-table regime and vegetation composition may obviously lead to differing responses. While *in situ* impact studies typically involve just one site, future syntheses or meta analyses will hopefully reveal the strongest general patterns.

In the absence of a clear general impact, it was intriguing, however, to find the strong dropping effect at the string edges in the one warm day, supporting in a very minor way hypothesis vii. We wonder if this could reflect some reindeer rumen microbes yet surviving in the peat, as observed in the laboratory (Fritze et al., 2021), but remaining inactive and activating only at high temperature. This remains speculative and does not currently have any practical implications, and probably neither in the warmer future since reindeer fate in clearly warmer climate is uncertain.

The slight direct negative impact of the surface addition of reindeer droppings on the soil CH_4 flux was unexpected. Perhaps the droppings acted as a temporary physical barrier for CH_4 flux through diffusion. Overall, our results concerning the CH_4 fluxes and their dependence on sedge leaf area, soil WTL and soil temperature are well in line with earlier research (Turetsky et al., 2014). The CH_4 flux increased with increasing sedge leaf area, and droppings decreased the flux indirectly by decreasing the leaf area. This may also be a transient physical disturbance effect, and we cannot exclude a later fertilizing effect that would increase the leaf area and thus the flux. Yet, sedge leaf area tended to be higher inside the fence, indicating that reindeer grazing on sedges is likely to override any potential fertilization effect. The close linkage between sedge leaf area and CH_4 flux is well known, caused by both sedges acting as a pipeline for emission, allowing CH_4 to bypass oxidation in the oxic surface layer (Ge et al., 2023), and production of suitable substrate for methanogenesis through exudation and litter production by sedge roots growing deep into the anoxic soil (Saarinen, 1996; Dorodnikov et al., 2011).

In general terms, vegetation composition is known to be an important regulator of peatland CH_4 fluxes (Joabsson et al., 1999; Bastviken et al., 2023). Plants not only produce substrate for methanogenesis, but they may, e.g., shape the soil oxygen gradient by releasing oxygen through the aerenchyma to the rhizosphere, and form conduits for CH_4 produced in anoxic soil layers to move to the atmosphere without passing the oxic surface soil where CH_4 oxidation could take place. Sedges, especially, are linked to high peatland CH_4 fluxes (Ge et al., 2023, 2024), which is why we focused on sedges in this study. The magnitudes of both the direct and indirect (fertilization, physical impacts) grazing effects on sedge abundance would generally depend on grazing intensity, which we could not control for the reindeer presence treatment. While we observed minor negative reindeer impacts on sedge leaf area in one site, the vegetation study by Kolari et al. (2019) with ten sites observed no significant impacts of grazing on *Carex* spp. sedge abundance. This seems to imply that the reindeer impact through sedge leaf area remains generally modest at most, with the current grazing intensity. Reindeer may have a stronger impact on shrub abundance and morphology (Kolari et al., 2019; Verma et al., 2020), which we did not consider as shrubs have not been linked to high CH_4 fluxes; rather, they may have a minor attenuating impact on the flux (Ge et al., 2023, 2024).

In high Arctic peatlands, grazing has been shown to influence the carbon cycle, e.g., by reducing the ecosystem CH_4 emission, likely through a reduction in the abundance and biomass of sedge tillers (Falk et al., 2015). However, the more northern ecosystems are likely to be more sensitive to such disturbance than our subarctic sites, due to lower plant productivity and shorter warm season. Overall, grazing impacts appear to be habitat specific (Sjögersten et al., 2008), and the story concerning subarctic fens may not be finished yet as the impacts on the C cycle apart from CH_4 fluxes still remain unknown. There may be implications, e.g., through reduced bryophyte abundance as observed by Kolari et al. (2019). Megaherbivore impacts on peatland carbon fluxes and greenhouse gas balance still merit further research as small shifts in their ecosystem processes may elicit large changes at the global scale.

In conclusion, even though the grazing reindeer shape to some extent the composition of the microbial communities participating in the CH_4 cycle, the practical consequences of this through the realized ecosystem CH_4 emissions appear to be minor or non-existent.

CRediT authorship contribution statement

Raija Laiho: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Petri Salovaara:** Writing – review & editing, Methodology, Investigation, Data curation. **Päivi Mäkiranta:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Krista Peltoniemi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Timo Penttilä:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Tuomas Rajala:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Jenni Hultman:** Writing – review & editing, Methodology, Investigation, Data curation. **Mika Korkiakoski:** Writing – review & editing, Methodology, Investigation. **Hannu Fritze:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Flux and covariate data are available at Zenodo, https://doi.org/10.5281/zenodo.10406403.

Acknowledgements

We thank Elli-Noora Kamppuri for field work in 2020, Juha-Matti Pitkänen for sampling and RNA extraction, and Rauna Lilja (University of Helsinki) and Hanna Finne (Finnish Meteorological Institute) for assistance in field work. This work was supported by the Research Council of Finland (315415).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109590.

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