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RESEARCH ARTICLE

CH_4 transport in wetland plants under controlled environmental conditions – separating the impacts of phenology from environmental variables

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Abstract

Background and Aims Methane (CH_4) fluxes at peatland plant surfaces are net results of transport of soil-produced CH_4 and within-plant CH_4 production and consumption, yet factors and processes controlling these fluxes remain unclear. We aimed to assess the effects of seasonality, environmental variables, and CH_4 cycling microbes on CH_4 fluxes from characteristic fen species.

Methods Four species (*Carex rostrata*, *Menyanthes trifoliata*, *Betula nana*, *Salix lapponum*) were selected, and their CH_4 fluxes determined in climate-controlled environments with three mesocosms per growing season per species. Microbial genes for CH_4 cycling were analysed to check the potential for within-plant CH_4 production and oxidation. Two extra experiments were conducted: removal of *C. rostrata* leaves to identify how leaves constrain CH_4 transport, and a labelling experiment with *S. lapponum* to distinguish between plant-produced and soil-produced CH_4 in the plant flux.

Results All species showed seasonal variability in CH_4 fluxes. Higher porewater CH_4 concentration

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increased fluxes from *C. rostrata* and *M. trifoliata*, decreased fluxes from *S. lapponum*, and did not affect fluxes from *B. nana*. Air temperature only and negatively affected CH_4 flux from *C. rostrata*. Light level did not impact CH_4 fluxes. Both methanogens and methanotrophs were detected in shoots of *S. lapponum* and *M. trifoliata*, methanotrophs in *B. nana*, and neither in *C. rostrata*.

Conclusion Our study demonstrates that the seasonal phase of the plants regulates the CH_4 fluxes they mediate across species. The detection of methanogens and methanotrophs in herbs and shrubs suggests that microbial processes may contribute to their CH_4 fluxes.

Keywords Controlled environments · Herbs · Shrubs · Microbes · Plant-mediated CH_4 fluxes · Phenology

Introduction

Methane (CH_4) is a powerful greenhouse gas with a global warming potential 28 times larger than that of carbon dioxide over 100 years (Intergovernmental Panel on Climate 2023). Undrained peatlands are an important source of CH_4, accounting for around 12% of global CH_4 emissions (Rydin and Jeglum 2006). These emissions are enabled by the high soil water-table level (WTL), and can be both stimulated and attenuated by members of the plant community. To cope with the anoxic environment, many wetland plants have aerenchymous tissue to transport oxygen from shoots to roots, thereby attenuating the CH_4 flux through increased oxidation in the soil (Heilman and Carlton 2001; Robroek et al. 2015). On the other hand, vascular plants can stimulate CH_4 emissions by transporting soil-produced CH_4 to the atmosphere through the same pathway, and by offering substrates for microbial CH_4 production (Greenup 2000; Joabsson and Christensen 2001; Ström et al. 2003; Barba et al. 2019). The net effect of the plant community on the CH_4 balance of a peatland depends on the interplay between these stimulating and attenuating processes. Gas transport through plants can occur by (i) passive diffusion when CH_4 concentration gradients between the soil, aerenchyma, and air exist, and by (ii) convective transport when partial pressure gradients between the inside and outside of plants are large enough to affect the total pressure in the plant (Ge et al., 2024; Vroom et al. 2022). Plant-mediated CH_4 transport is a crucial mechanism increasing the total ecosystem CH_4 emissions both in open wetlands and woody ecosystems (Shannon et al. 1996; Pangala et al. 2017; Barba et al. 2019; Ge et al. 2023; Machacova et al. 2023) as it allows soil-produced CH_4 to bypass oxidation in oxic surface peat.

Environmental factors, e.g., WTL, temperature, porewater CH_4 concentration, and photosynthetically active radiation (PAR) have been regarded as important controls on ecosystem CH_4 emissions from wetlands (Joabsson and Christensen 2001; Lai et al. 2014a). Yet, the effects of these controls on plant-mediated CH_4 transport remain unclear with contrasting effects reported even for the same species. For example, Noyce et al. (2014) reported that higher WTL led to higher CH_4 emissions by *Carex rostrata*, while Ge et al., (2023) observed no such correlation with the same species. As an indicator of the CH_4 supply to roots, porewater CH_4 concentration can positively affect diffusive transport of CH_4 (Aulakh et al. 2000a, 2000b; Ding et al. 2005), but the effect can disappear when other environmental variables become more limiting (Ge et al. 2023). Plants which affect ecosystem-level CH4 emission mainly by transporting soil-produced CH_4 can lead to high CH_4 emission and a corresponding depletion of the porewater CH_4 concentration (Ge et al. 2023; Van Der Nat and Middelburg 2000). PAR can also positively affect plant-mediated CH_4 fluxes by controlling stomatal conductance when stomata are the main exit of CH_4 from plant into the atmosphere. However, if most of CH_4 escapes into the atmosphere through the stem before reaching the leaves and stomata, no correlation between PAR and plant-mediated CH_4 fluxes can be expected.

Recent studies suggest that environmental controls are less important than species-level differences (Korrensalo et al. 2021) and seasonality (Ge et al. 2023) in determining plant-mediated CH_4 fluxes. It seems that species-specific plant morphology and phenology (the development and senescence over the growing season) can affect the CH_4 transport. Phenology may affect CH_4 transport through changes in plant biomass (Koelbener et al. 2010), root length (Noyce 2009) and permeability (Henneberg et al. 2012), aerenchyma size (Fagerstedt 1992), stomatal conductance (Morrissey et al. 1993), and stimulation of rhizospheric CH_4 production (Lai et al. 2014b) and oxidation (Kankaala et al. 2005). The seasonality of plant-mediated CH_4 fluxes may also differ between plant species (Ge et al. 2023). However, most studies have been conducted in the field, where abiotic conditions typically correlate with phenological changes in plants. Studying plant-mediated CH_4 fluxes under controlled environment would allow investigating the relative importance of both the species identity and species-specific seasonality in regulating the fluxes.

Key traits affecting species-specific variation in CH_4 fluxes include the resistance of tissues to CH_4 transport and release. Studies based on clipping aboveground parts of plants whose gas transport occurs by passive diffusion suggest that leaves and stomata do not restrict CH_4 transport in some species (Nouchi et al. 1990; Kelker and Chanton 1997; MacDonald et al. 1998; Kutzbach et al. 2004; Schimel 1995), while they strongly control the CH_4 release in other species (Morrissey et al. 1993; Schimel 1995; Garnet et al. 2005). For many important wetland genera such as *Carex* spp., different results have been reported for which plant parts and properties determine the resistance to CH_4 transport and release. Some studies suggest that the transport through *Carex* species is controlled by the belowground parts or the root-shoot interface (Kelker and Chanton 1997). If leaves are the transport-restricting part, the release site of CH_4 may be either stomata or intercellular spaces depending on the species (Garnet et al. 2005; Morrissey et al. 1993). Defining how common the stomatal closure is as a controlling mechanism for the CH_4 release in different species would require measuring the diffusive gas transport of aerenchymous plants with varying morphology under different light levels. Such knowledge could be then used for example when choosing the correct drivers for the plant CH_4 transport in process-based ecosystem modelling.

Plants are not merely transporting soil-derived CH_4 to the atmosphere but may also host CH_4 producing and consuming microbes, as verified for *Sphagnum* mosses and tree leaves and stems (Larmola et al. 2010; Putkinen et al. 2014, 2021; Feng et al. 2022). Within-plant microbes and processes, however, are rarely accounted for in ecosystem CH_4 flux studies even though recent studies indicate that both microbial CH_4 production and consumption may occur inside the plants (Jeffrey et al. 2019, 2021; Putkinen et al. 2021). The well-known association between *Sphagnum* mosses and methanotrophs (Larmola et al. 2010; Putkinen et al. 2014) is an exception. Their wider evaluation, especially within plants adapted to CH_4-rich environments like peatlands, is thus warranted. The source of the CH_4 emitted by plants can further be evaluated with labelling experiments. While aerenchymatous herbs have long been regarded as efficient channels for soil-produced CH_4, with shrubs the main source of CH_4 emitted is still poorly studied.

In this study, we investigated the seasonality and species-specific differences in CH_4 fluxes from shoots of common boreal peatland herbs (sedge *Carex rostrata* and forb *Menyanthes trifoliata*) and shrubs (*Betula nana* and *Salix lapponum*) in controlled environmental conditions. The main aims of this study were to (i) evaluate how the phase of the growing season and abiotic factors (temperature, porewater CH_4 concentration and PAR) interact in controlling plant-mediated CH_4 fluxes; (ii) determine whether plant shoots host microbes that may produce or oxidize CH_4. Further, we examined two supplementary aims with more limited material: (iii) identify which plant part contributes most to CH_4 release from *C. rostrata*, one of the most abundant sedge species in northern peatlands; and (iv) identify the source CH_4 emitted by *S. lapponum*. To disentangle the effects of season through changes in both environmental conditions and plant phenology, we collected plant-soil mesocosms containing the target species throughout growing seasons in 2020 and 2021 and placed them in a climate-controlled cabinet for automated measurements under uniform environmental conditions (i.e., constant light–dark cycle, temperature and humidity throughout the growing season) (Kohl et al. 2021). To evaluate the potential for CH_4 production and consumption processes occurring inside of plant shoots, the presence and diversity of methanogens and methanotrophs within shoots of all species were measured via novel probe-capture sequencing. As for our two supplementary aims, a foliage clipping experiment was conducted to reveal whether leaves of *C. rostrata* restrict the transport of CH_4, and a ^{13}C-labelling measurement was conducted with *S. lapponum* to reveal the source of CH_4 emitted from its shoots. We hypothesized that i) phenological stage of the plants modifies the CH_4 flux from the investigated species, ii) CH_4 flux from all investigated species increases with temperature and porewater CH_4 concentration, and is regulated by stomatal conductance, responding to PAR, (ii) CH_4-related processes occurring inside plant shoots include plant-derived and microbial CH_4 production and oxidation, iii) leaves are the main location for CH_4 release from *C. rostrata*, and iv) soil-produced CH_4 is the source of CH_4 emitted from *S. lapponum*.

Materials and methods

Plant sampling and growth conditions

We utilized mesocosms, living pieces of a fen ecosystem in a container, including peat, mosses and vascular plants (Fig. 1, S1). Mesocosms each containing one vascular plant species, *Carex rostrata*, *Menyanthes trifoliata*, *Betula nana* and *Salix lapponum,* were collected at Lompolojänkkä (67.99°N, 24.21°E), a northern boreal fen (Ge et al. 2023) located in Finnish Lapland. All these species use passive diffusion to transport gas, indicated by the small ratio between the maximum and minimum plant CH_4 flux during each day (Ge et al. 2023). For detailed plant traits and transport properties of each species, see Table 2 in Ge et al. (2023).

The plants were collected in growing seasons 2020 (*C. rostrata* and *B. nana*) and 2021 (*M. trifoliata* and *S. lapponum*). To investigate the impact of phenology on plant-mediated CH_4 fluxes, we collected the species for the mesocosms at three time points, which represent phenologically distinct phases. The sampling times were chosen based on earlier studies at the site (Zhang et al. 2020; Ge et al. 2023); early summer leaf out (early-mid June), high summer during the leaf area maximum (July–early August) and autumn senescence (mid-September).

We collected three mesocosms per species per campaign, and placed them in plastic buckets (13 L, height 40 cm, diameter 20 cm). The total number of mesocosms throughout the observations was eventually less than nine due to system malfunctions and data quality control (Table S1). The plant individuals were the same as measured in Ge et al. (2023), enabling the comparison of the results obtained in the field and in the climate-controlled measurements. To reduce the disturbance to the plants, each mesocosm included, intact, the peat surrounding the roots of the target plant individual. We dug the peat down to 40 cm depth to avoid disturbance to the roots, of which the majority are located above the depth of 30 cm in this fen (Mäkiranta et al. 2018). All mesocosms passed careful visual inspection of the success in preserving root systems, with no shrub and herb mesocosms having the end of the rhizome and/or coarse roots sticking out of the sampled peat.

The mesocosms were stored in an environment-controlled room at 4 °C with an 18-h photoperiod before being transported to the climate-controlled cabinet, to ensure uniform growing conditions. We set

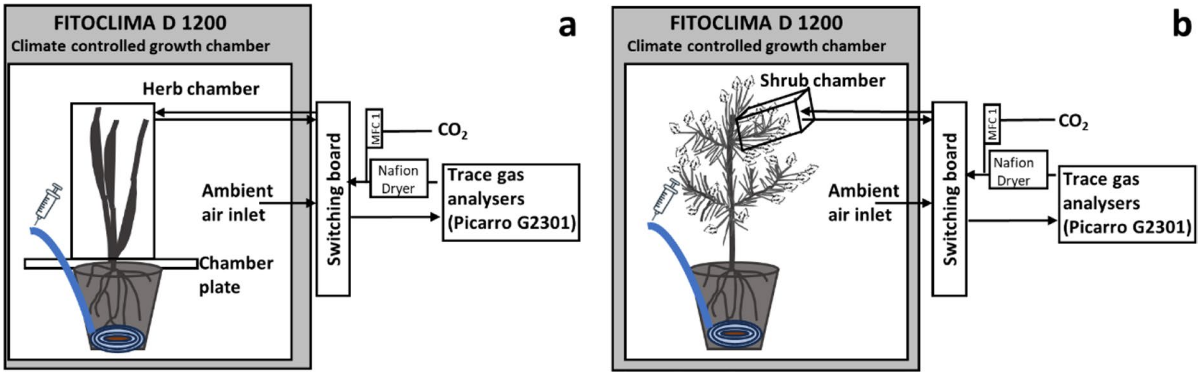

Fig. 1 The controlled-environment chamber system, consisting of two mesocosms (i.e., peat, mosses, and the studied herbaceous or shrub species in 13 L containers), a climate-controlled growth chamber that controlled the conditions of the mesocosms, two different measurement chambers for observing CH_4 fluxes from herbaceous species *Carex rostrata* and *Menyanthes trifoliata* (**a**, herb chamber), and from shrubs *Betula nana* and *Salix lapponum* (**b**, shrub chamber), and a silicone soil air tube for sampling porewater CH_4. Figure modified after Kohl et al. (2021). For a better view of the mesocosm, see Fig. S1

the temperature to 4 °C to slow down the maturation process, to prevent the individuals from progressing to different phenological stages before measurements. The 18-h photoperiod was chosen to mimic the average number of daylight hours at Lompolojänkkä during the three campaigns. The plants did not show any signs of leaf senescence or other adverse impacts during storing in the environment-controlled room. To keep the peat water-saturated, the mesocosms were watered with water collected from the hole left after digging out the specimen and stored in airtight bottles in the same environment-controlled room. The size of the climate-controlled cabinet limited measurements to two mesocosms at a time. We therefore conducted simultaneous measurement of two mesocosms of different species, with the remaining two mesocosms of each target species stored for one or two weeks, respectively, in the environment-controlled room.

Porewater CH_4 concentration

We used passive diffusion sampling technique to take gas samples to estimate porewater CH_4 concentration. Before starting the measurements, a silicone tube attached to a polytetrafluoroethylene (PTFE) tube was placed at the bottom of each bucket (Fig. 1, S2) (Kammann et al. (2001). We used a silicone tube because it excluded water and let gas diffuse through its walls. The outer diameter and wall thickness were 16 and 3 mm for the silicone tube, and 6 mm and 1 mm for the PTFE tube, respectively. The top end of the PTFE tube was sealed with a 3-way stopper through which gas samples were extracted by a syringe (20 ml) and transferred into pre-evacuated vials (12 ml, Labco Limited, UK) daily. CH_4 concentrations were analysed by a gas chromatograph (GC, Agilent 7890A). The measured gas phase concentrations (ppm CH_4) inside the tube were then converted to concentrations (μmol CH_4 l^{-1}) in the surrounding porewater at the bottom of the bucket based on Henry's law:

$$C = HP_{gas} \tag{1}$$

where C is the solubility of a gas at a fixed temperature in a particular solvent (mol l^{-1}), here CH_4 concentration in porewater; P_{gas} is the partial pressure of CH_4 (bar); and H is Henry's law constant (mol l^{-1} bar^{-1}) which for CH_4 at 298 K is 0.0014 mol l^{-1} bar^{-1}.

Experimental set-up

The measurements were conducted in a climate-controlled cabinet (Fitoclima 1200, Aralab, Portugal, Fig. 1, S1), where PAR, temperature and the relative humidity of the air (RH) could be regulated. Each measurement cycle lasted approximately one week and was conducted with two mesocosms hosting two different species at a time. Throughout the observations, regular light cycles (18 h light, 6 h darkness) were applied and RH was held at 50% (Table 1). The PAR was kept at 600 and 100 μmol m^{-2} s^{-1} intensity for the daytime and nighttime, respectively. We did not set PAR to zero as the field site also had light at night over most of the growing season. Temperature was kept at 15 °C for two days and then increased to 20 °C.

The clipping treatment was conducted on *C. rostrata* to reveal whether the CH_4 transport of the plant was restrained by passage through the leaves. Specifically, the foliage of *C. rostrata* was removed and only the stem was left. The clipping treatment was conducted on the fifth day of the measurement cycle, during the 20 °C air temperature phase, and measurements continued for 3 days after the treatment (Table 1). We only conducted the clipping treatment on *C. rostrata* as it is one of the most important and frequent species in northern peatlands and also has

Table 1 Climate-controlled cabinet settings and treatment protocol. The foliage clipping treatment was conducted only on *C. rostrata*, and the ^{13}C labelling on was only conducted on *S. lapponum*

Setting	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
RH (%)	50	50	50	50	50	50	50
T (°C)	15	15	20	20	20	20	20
Light / Dark (h)	18/6	18/6	18/6	18/6	18/6	18/6	18/6
Clipping foliage of *C. rostrata*	no	no	no	no	yes	yes	yes
^{13}C labelling on *S. lapponum*	no	no	no	no	yes	yes	yes

been found to be an efficient CH_4 emitter (Noyce et al. 2014; Ge et al. 2023). Two mesocosms per campaign were treated by clipping. The effect of clipping was assessed by comparing the measurements made during the 20 °C air temperature phase before and after the treatment.

CH_4 flux measurements

For determining CH_4 fluxes, the closed dynamic chamber method (non-steady-state through-flow) was used. Due to the different growth forms of herbs and shrubs, two different measurement chamber designs were used for measuring CH_4 fluxes from their shoots (Fig. 1, S1). We used the chamber design developed by Korrensalo et al. (2021) for the herbs, hereafter called "herb chamber". It consisted of two plexiglass plates and a transparent polymethyl methacrylate (PMMA) chamber (4 L). Measurement of shoot fluxes separate from the rest of the environment was achieved by passing the specimen through the gap between the two plexiglass plates being held together by a hinge and then covering it with the chamber.

Unlike herbs, we measured CH_4 fluxes from parts of shrub shoots by using a 1 L chamber system, hereafter called "shrub chamber" (Toivo Pohja, Juupajoki, Finland, Fig. S1). It consisted of an aluminium bottom and back plate, and a transparent PMMA cover. The measured stem was put through a notch in the back plate and sealed with adhesive putty (Blu Tack, Bostik SA).

During the flux measurement, sample gas in the headspace was circulated in a closed loop between the analyser (Picarro G2301 and G2201i) and the closed chamber. The difference between these two analysers is that G2201i can discern between ^{12} and ^{13}C in CO_2 and CH_4. Thus, thus G2201i was used during the ^{13}C labelling measurement which is discussed below. Each measurement lasted for 10 min and there were 72 measurements per species per day. After each measurement, the chamber was flushed with air taken from the cabinet for 10 min, while the other chamber was under measurement. We conducted empty chamber measurements to calculate background fluxes after finishing all campaigns in 2020. Chamber tightness was monitored with daily open-cabinet measurements. Opening the cabinet doors lowered CH_4 concentrations within the cabinet, which would have had effects on the apparent CH_4 fluxes if there was significant leak in the chamber. This was not observed as fluxes measured with the cabinet doors open did not differ from those measured with the doors closed.

CH_4 fluxes through plants and the empty chamber were calculated using the least squares method, defining the best linear fit for the change of concentration over time:

$$F = \frac{dC}{dt}\frac{MPV}{RT} \tag{2}$$

where dC/dt is the change in CH_4 (ppm) concentration over time (s), M is the molar mass of measured gas (16,042 mg for CH_4), P is the atmospheric pressure (101325 Pa), V is the volume of the measurement system (m^3), R is the molar gas constant (8.3144598 J K^{-1} mol^{-1}), and T is the chamber temperature (K).

We used a custom R script for quality control of each measurement. Measurements with nonlinear changes in CH_4 were identified to be caused by chamber leak and were excluded. We also discarded measurements conducted during system malfunctions, or opening of the cabinet door to dilute cabinet CH_4 concentration. In total, 25% of the measurements were rejected. The mean background flux of the empty herb and shrub chambers were very small (-2.08×10^{-6} and -1.41×10^{-6} mg d^{-1}, respectively).

After each campaign, we measured plant surface area (leaf area and stem area for herbs and shrubs, respectively) enclosed in the chambers. The single-sided leaf area was measured by a digital scanner and ImageJ software (Ferreira and Rasband 2012). A digital caliper meter (1150D; Bahco, Eskilstuna, Sweden) was used to measure the stem diameter of shrubs at the point of entry to the shrub chamber, from which we calculated the cross-sectional stem area. After that, we normalized CH_4 fluxes from the shoots by plant surface area to estimate area-specific plant CH_4 flux. We use the stem area instead of leaf area for shrubs because in the autumn campaign, senescence had set in and leaf area was zero and we wanted to use the same parameter for normalizing the fluxes from the shrubs in all campaigns.

^{13}C labelling

The labelling experiment was conducted on *S. lapponum* to reveal whether the CH_4 emitted by its shoots originated from soil-produced CH_4. Two ml ^{13}C-labelled CH_4 (99% ^{13}C, Merck 490,229) were mixed with 28 ml dinitrogen (N_2) gas and injected into the silicone tube installed at the bottom of the mesocosms for porewater CH_4 concentration measurements. All three mesocosms of *S. lapponum* from each campaign were labelled once on the fifth measurement day and $^{13}CH_4$ emissions from shoots were recorded until the end of the measurement cycle. The $\delta^{13}C\text{-}CH_4$ of shoot-emitted methane was calculated through Keeling plots, i.e., by plotting the measured $\delta^{13}C\text{-}CH_4$ over the inverse concentration and estimating the intercept of a linear fit in these plots (Pataki et al. 2003). Similarly, we estimated the initial soil $\delta^{13}C\text{-}CH_4$ based on CH_4 emitted from soil to the cabinet air outside the shoot chamber through a single Keeling plot, i.e., by plotting cabinet $^{13}C\text{-}CH_4$ over its inverse concentration 24 h after injection. The proportion of CH_4 emitted by shoots of *S. lapponum* originated from peat was calculated as:

$$f\_peat = (\delta^{13}C\_pot - \delta^{13}\_shoot)/(\delta^{13}\_pot - \delta^{13}\_cabinet) \tag{3}$$

where f_peat is the proportion of $^{13}C\text{-}CH_4$ emitted by shoots from peat, $\delta^{13}C\_pot$, δ^{13}_shoot and $\delta^{13}\_cabinet$ are the value of δ^{13} in pot, shoots, and cabinet, respectively.

The proportion of label-derived CH_4 to the total CH_4 emitted to the cabinet is calculated as:

$$f_{\_label} = (f\_\delta^{13}C_{emitted} - f\_\delta^{13}C_{background})/(f\_\delta^{13}C_{label} - f\_\delta^{13}C_{background}) \tag{4}$$

where $f_{\_label}$ is the proportion of label-derived CH_4 to the total CH_4 emitted to the cabinet, $F\_\delta^{13}C_{emitted}$, $F\_\delta^{13}C_{background}$, and $F\_\delta^{13}C_{label}$ are the fraction $\delta\ ^{13}C$ of emitted CH_4, cabinet, and the labelling we used, respectively.

Targeted metagenomic analysis of CH_4-cycling microbes

To evaluate the possibility of microbial processes within or on the surfaces of the plants contributing to the plant-derived CH_4 fluxes, we conducted a probe-targeted metagenomic analysis (Siljanen et al. 2022) of *C. rostrata* (whole shoot), *M. trifoliata* (whole shoot, without flowers), *S. lapponum* (stem and leaves separately) and *B. nana* (shoot, i.e. branches with the leaves, and stem separately). The analysis targeted functional genes *mcrA*, coding for the methyl coenzyme-M reductase (methanogens); *pmoA*, coding for the particulate methane monooxygenase (methanotrophs); and *mmoX*, coding for the soluble methane monooxygenase (methanotrophs).

Plant samples (n = 3 per sample type) were collected aseptically during the "high summer" campaign in 2021, except for *B. nana*, which was sampled already in August 2019 (n = 1). All samples were transported on ice and stored in -20 °C until DNA extraction using the Nucleospin Plant II mini kit (Macherey–Nagel, Düren, Germany). The extraction included mortar homogenization in liquid nitrogen and was conducted according to the manufacturer instructions, except of the extension of the lysis step to 1 h from the 10 min suggested as the default option by the manufacturer's manual. This choice was based on our previous testing showing improved extraction with a longer lysis step, which is mentioned as an option also in the manufacturer's manual.

DNA quantity and quality was checked with Qubit fluorometer and Nanodrop One spectrophotometer (both Thermo Fisher Scientific). Targeted sequencing was conducted by Daicel Arbor Biosciences (US) as in Putkinen et al. (2021). Data was analysed as in Putkinen et al. 2021, except for the jplace-file processing and visualization, which was done with Gappa (Czech et al. 2020). Reagent and sample cross-contamination was controlled via parallel sequencing of blank-samples (only DNA-extraction reagents, no plant material). The sequence data produced in this study was deposited to the SRA-NCBI database under the BioProject PRJNA953003.

Statistical analyses

i) Motivation for choosing the independent variables

Utilizing the flux data, we evaluated the variation in two independent variables: the "plant CH_4 flux" as such, and "plant CH_4 transport efficiency" (Ge et al. 2024). Transport efficiency is the plant CH_4 flux divided by daily porewater CH_4 concentration measured each day. Porewater CH_4 concentration determines the supply of soil-produced CH_4 to the roots of plants, and thus can significantly affect the magnitude of the plant CH_4 transport (Ge et al. 2024; Ge et al. 2023). Therefore, the main effects of environmental variables on plant CH_4 flux should preferably be evaluated under standardized porewater CH_4 concentration conditions, i.e., using plant CH_4 transport efficiency. Porewater CH_4 concentration varied over the measurement period for each mesocosm, being affected, e.g., by the increase of temperature and accumulation time. Similarly, the effects of variation in porewater CH_4 concentration should also be removed when investigating the effect of clipping treatment for *C. rostrata*. We assumed that diurnal changes in porewater CH_4 concentration (measured only once per day) and their impacts on plant CH_4 flux were negligible. Since the effects of PAR were investigated within one day, we investigated its effects on plant CH_4 flux only. Notably, the effects of PAR and temperature on of *C. rostrata* and *S. lapponum* is were investigated using only of measurements done before the clipping and labelling, respectively.

ii) Analysis methods

Linear mixed effect models (LMMs) were used to investigate the effects of phenological stages, represented by the different measurement campaigns, and the environmental variables on both plant CH_4 flux and plant CH_4 transport efficiency. These tests were conducted for each species separately. Fixed predictors, analysed one by one, each in a separate LMM, were the phenological stage, and the environmental variables porewater CH_4 concentration, temperature (15 vs 20 °C), and PAR (2 levels of PAR), as well as the clipping treatment of *C. rostrata* (intact vs shoot clipping). The random variable was sample id in all LMMs analyses. The models are presented in Table 2, S2-S3. Differences in estimates of plant CH_4 flux and plant CH_4 transport efficiency between the phenological stages fitted from the LMMs were further evaluated by the Tukey method. No statistics was applied for the number of the genes of the microbiology data. All statistical analyses were performed using R v.3.6.1 (Team 2019).

Results

Seasonality of porewater concentration and plant CH_4 flux

Strong seasonality was observed in the porewater CH_4 concentration of *C. rostrata* and *B. nana* mesocosms, with minimum occurring in high summer (mean 0.02 μmol l^{-1} for both, Fig. S3). The minimum was over 10,000 times lower than that in early summer and autumn. Contrastingly, the seasonality

Table 2 Summary statistics for the linear mixed-effects model describing the effects of porewater CH_4 concentration ($[CH_4]_{pw}$) on the plant CH_4 flux of *C. rostrata*, *M. trifoliata*, *B. nana* and *S. lapponum*. The observations used here are before the clipping and labelling experiments. Asterisks denote statistical significance: **, 0.01; ***, 0.001

C. rostrata				*M. trifoliata*			
Fixed part	Estimates	SE	*P* value	Fixed part	Estimates	SE	*P* value
Intercept	1.45889	1.95450	0.48500	Intercept	0.11557	0.11557	0.29800
$[CH_4]_{pw}$	0.03266	0.00422	< 0.001 ***	$[CH_4]_{pw}$	0.00085	0.00018	< 0.001 ***
Random part				Random part			
SD (Sample ID)	19.13100	4.37400		SD (Sample ID)	0.03371	0.18361	
Residual SD	3.52400	1.87700		Residual SD	0.00355	0.05957	
S. lapponum				*B. nana*			
Fixed part	Estimates	SE	*P* value	Fixed part	Estimates	SE	*P* value
Intercept	-0.00307	0.00146	0.12385	Intercept	0.13380	0.09446	0.21800
$[CH_4]_{pw}$	0.00003	0.00001	< 0.01 **	$[CH_4]_{pw}$	0.00009	0.00033	0.79500
Random part				Random part			
SD (Sample ID)	0.00001	0.00289		SD (Sample ID)	0.03906	0.19763	
Residual SD	0.00000	0.00148		Residual SD	0.00094	0.03069	

in the porewater CH_4 concentration in *M. trifoliata* and *S. lapponum* mesocosms was small (Fig. S4). For *M. trifoliata* mesocosms, the high summer and early autumn mean porewater CH_4 concentrations were 17 and 40 µmol l^{-1}, with SD 12 and 24, respectively. The high summer and early autumn mean porewater CH_4 concentrations for *S. lapponum* mesocosms were similar, with the mean and SD around 21 µmol l^{-1} and 10, respectively.

The porewater CH_4 concentration had a strong positive correlation with plant CH_4 flux of *C. rostrata*, *M. trifoliata*, and *S. lapponum* (all $P<0.01$, Table 2). In contrast, no significant relationship between CH_4 concentration and plant CH_4 flux was observed for *B. nana*.

To focus on the seasonality unrelated to porewater CH_4 concentration, we investigated the variability in the transport efficiency. The seasonal pattern in the transport efficiency and in the plant CH_4 flux appeared to differ between the four studied species. The transport efficiency of *C. rostrata* was higher (mean 234 mg m^{-2} h^{-1} (µmol $l^{-1})^{-1}$ in high summer than in early summer and autumn (0.02 and 0.06 mg m^{-2} h^{-1} (µmol $l^{-1})^{-1}$, respectively) (both $P<0.001$, Fig. 2). However, the mean plant CH_4 flux of *C. rostrata* was at its minimum in early summer (3.04 mg m^{-2} h^{-1}), increased to 4.86 mg m^{-2} h^{-1} in high summer, and reached its maximum in early autumn (10.70 mg m^{-2} h^{-1}). Similar to *C. rostrata*, the maximum transport efficiency of *B. nana* occurred in high summer (mean 4.08 mg m^{-2} h^{-1} (µmol $l^{-1})^{-1}$) but the difference to the early summer minimum (0.001 mg m^{-2} h^{-1} (µmol $l^{-1})^{-1}$) for *B. nana* was smaller in magnitude than in *C. rostrata*. The maximum mean plant CH_4 flux of *B. nana* occurred in early summer (0.63 mg m^{-2} h^{-1}) and the minimum in early autumn (0.001 mg m^{-2} h^{-1}), respectively. For *M. trifoliata*, the transport efficiency was significantly higher in high summer than in early autumn ($P<0.05$). Somewhat higher plant CH_4 flux of *M. trifoliata* was observed in early and high summer than in early autumn, but they did not differ significantly. Both plant transport efficiency and CH_4 flux of *S. lapponum* were higher in early autumn than in summer ($P<0.001$ and $P<0.01$, respectively).

Effects of temperature and PAR

The response to increase in temperature from 15 °C to 20 °C, differed between the plant species and also

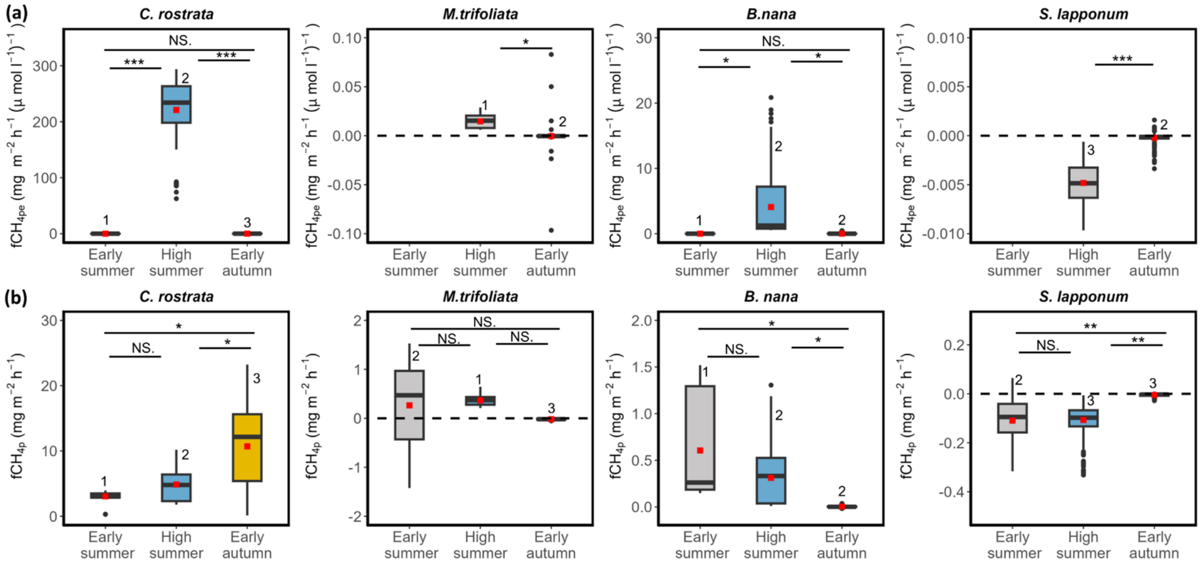

Fig. 2 Characteristics of the **a**) seasonal CH_4 transport efficiency (fCH_{4pe}, mg CH_4 m^{-2} leaf or stem area h^{-1} (µmol CH_4 l^{-1} porewater$)^{-1}$), and **b**) plant CH_4 flux (fCH_{4p}, mg CH_4 m^{-2} leaf or stem area h^{-1}, of herbs (*C. rostrata* and *M. trifoliata*) and shrubs (*B. nana* and *S. lapponum*). Red dots are mean fCH_{4pe} and fCH_{4p} of each season. Note the different y-axis scales. The number above the box denotes the number of specimens observed during each campaign. NS denotes no significant difference and asterisks denote statistical significance: *, 0.05 **, 0.01; ***, 0.001

between plant CH_4 transport efficiency (Fig. 3) and flux (Fig. S5). The transport efficiency of *C. rostrata* significantly decreased after the temperature was increased ($P < 0.001$) while in the other three species there was no response. However, with the plant flux calculated per unit of leaf area, higher temperature led to higher plant CH_4 flux of *C. rostrata* and *S. lapponum* (both $P < 0.001$), while it reduced the flux of *M. trifoliata* ($P < 0.001$). No significant temperature effect was observed in the flux of *B. nana*. As for the effects of PAR, it did not significantly affect plant CH_4 flux of any investigated species (Fig. 4).

Impact of *Carex rostrata* clipping

Clipping treatment where leaves were removed but the stem was left untouched did not significantly affect plant CH_4 transport efficiency of *C. rostrata* (Fig. 5). However, higher plant CH_4 flux was observed after conducting the clipping treatment.

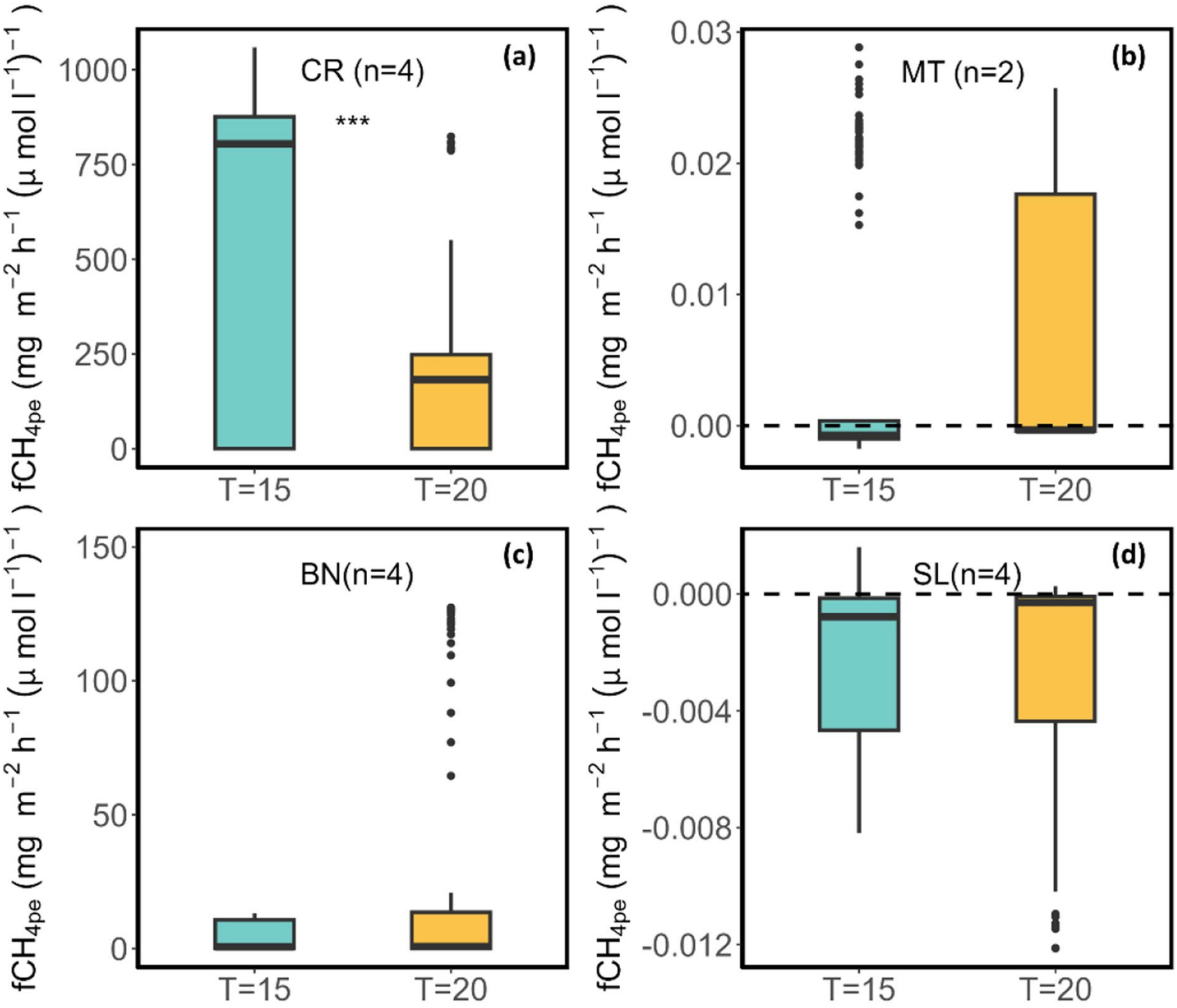

Fig. 3 Temperature effects on plant CH_4 transport efficiency (fCH_{4pe}, mg CH_4 m^{-2} leaf area h^{-1} (μmol l^{-1})$^{-1}$ of *C. rostrata* (CR, **a**), *M. trifoliata* (MT, **b**), *B. nana* (BN, **b**) and *S. lapponum* (SL, **d**). Summary statistics for the linear mixed-effects model describing the effects of temperature on fCH_{4pe} shown in Table S3. The number (n) of total mesocosms per species is less than 9 because of system and GC malfunctions, and data quality control. Dashed horizontal line in panels with negative fCH_{4pe} denotes zero flux level. Note the different y-axis scales. Asterisks denote statistical significance: ***, 0.001

Source of plant CH_4 flux from *Salix lapponum*

The isotope labelling revealed that methane injected into the root space is rapidly transported through *S. lapponum* and emitted from its shoot (Fig. 6). After the injection, the $\delta^{13}C$-CH_4 value of mesocosm CH_4 (inferred from the CH_4 emitted into the cabinet air) was *c.* + 60‰ (red horizontal line in Fig. 6). Assuming a background $\delta^{13}C$-CH_4 value of -50‰, our injection has therefore changed porewater CH_4 concentration less than 0.15% of the concentration prior labelling, i.e., the label application had minimal effects of the environment of *S. lapponum* roots. During the measurements, the maximum $\delta^{13}C$-CH_4 in the cabinet was *c.* + 26‰, which was lower than that from the mesocosm due to mixing with ambient air. $\delta^{13}C$-CH_4 emitted into shrub chambers was *c.* + 48‰, which was higher than that of the cabinet air (*c.* + 26‰) and closer to $\delta^{13}C$-CH_4 in the mesocosm (*c.* + 60‰). Labelling also showed that a major part of CH_4 (*c.* two thirds) emitted from *S. lapponum* was transported from the mesocosm through plant and emitted through the shoot, rather than from leakage of cabinet air into the shrub chamber or oxidized to carbon dioxide.

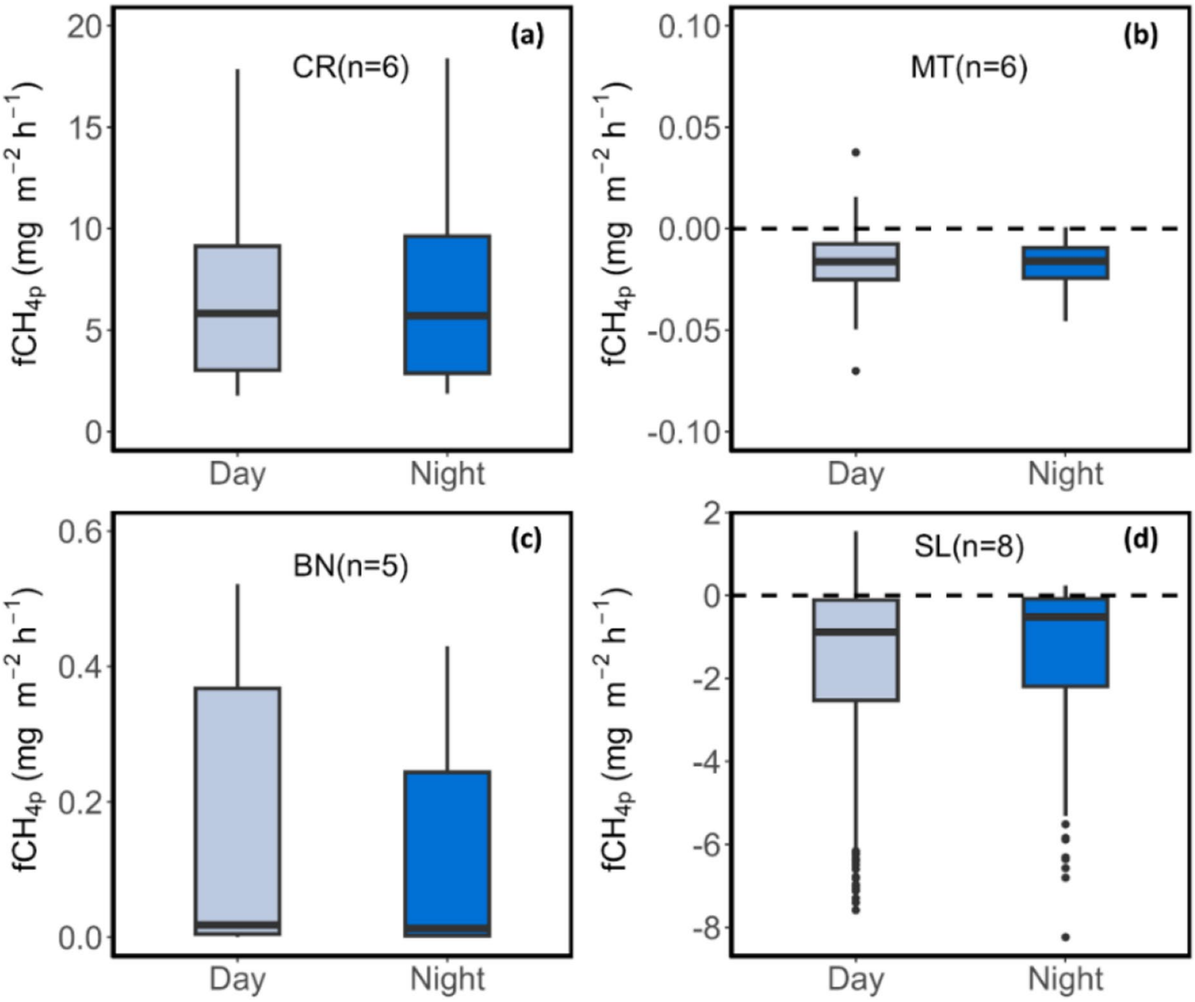

Fig. 4 PAR effects on plant CH_4 flux (fCH_{4p}, mg CH_4 m^{-2} leaf area h^{-1}) of *C. rostrata* (CR, **a**), *M. trifoliata* (MT, **b**), *B. nana* (BN, **c**) and *S. lapponum* (SL, **d**). The number (n) of total mesocosms per species is less than 9 because of system and GC malfunctions, and data quality control. Dashed horizontal line in panels with negative fCH_{4p} denotes zero flux level. Note the different y-axis scales

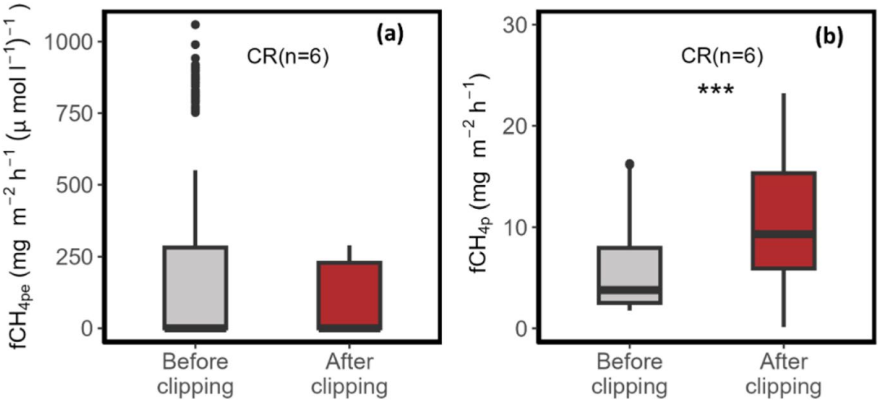

Fig. 5 Clipping effects on plant CH_4 transport efficiency (fCH_{4pe}, mg CH_4 m^{-2} leaf area h^{-1} (μmol $l^{-1})^{-1}$, **a**) and plant CH_4 flux (fCH_{4p}, mg CH_4 m^{-2} leaf area h^{-1}, **b**) of *C. rostrata* (CR). The number (n) of total mesocosms per species is less than 9 because of system and GC malfunctions, and data quality control. Same mesocosms before and after the clipping treatment. Asterisks denote statistical significance: ***, 0.001

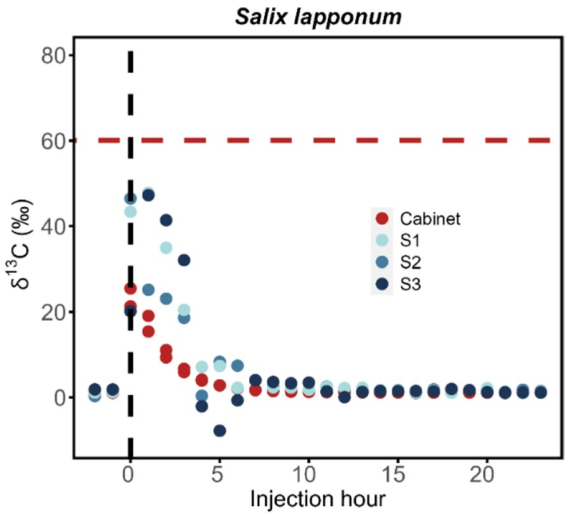

Fig. 6 ^{13}C-CH_4 labelling result of CH_4 fluxes through *S. lapponum*. Red horizontal line is the intercept of keeling plot, representing $\delta^{13}C$-CH_4*S. lapponum* mesocosms (S1, S2 and S3). Dashed vertical black line represents the moment of injection. Red dots are the $\delta^{13}C$-CH_4 of the cabinet where mesocosms were placed. Blue dots with different shades are the $\delta^{13}C$-CH_4 of fluxes emitted by shoots of *S. lapponum*

Plant-associated methanogens and methanotrophs

We found some of the plant shoots to host microbes that produce or oxidize CH_4 but not all species. Targeted metagenomic sequencing and the following phylogenetic placement analysis revealed methanogenic and methanotrophic functional genes in the shoots of *M. trifoliata* and in the leaves and stem of *S. lapponum* (Fig. 7, Figs. S6–S8). None of the genes were detected in *C. rostrata*. In the separately screened *B. nana* samples (shoot and stem, n = 1), methanotrophs, but not methanogens, were found (Fig. 8). Sequence read numbers (after the hmmer profile screening) were low with mean counts in different sample types varying from 0 to 378 reads in *mcrA*, from 48 to 393 in *pmoA* (including *amoA*), and from 7 to 10,782 in *mmoX* (including non-methane mono-oxygenases; Table S4).

In both *M. trifoliata* and *S. lapponum,* sequences of the *mcrA* gene were mainly related to the genera *Methanobacterium (order Methanobacteriales)* and *Methanoregula (Methanomicrobiales)*, accompanied by *Methanotrichales* and *Methanocellales*. The highest diversity of different genera was detected in the stems of *S. lapponum.*

In *M. trifoliata,* methanotrophic *pmoA* genes were related to both alpha- and gammaproteobacteria, with *Methylocystis* & *Methylocapsa* and *Methylobacter* & *Methylomagnum* as the most abundant genera within those classes, respectively. In *S. lapponum, Methylocystis* and *Methylobacter* were the largest groups. *B. nana* harboured alpha- (in shoot & stem) and gammaproteobacterial (in stem) *pmoA* genes. All sample types, except *C. rostrata*, showed presence of the still poorly known *pxmABC* gene cluster (Tavormina et al. 2011) and also of alphaproteobacterial. Upland soil cluster α, common in habitats with low/atmospheric CH_4 concentrations (i.e. capable of high-affinity

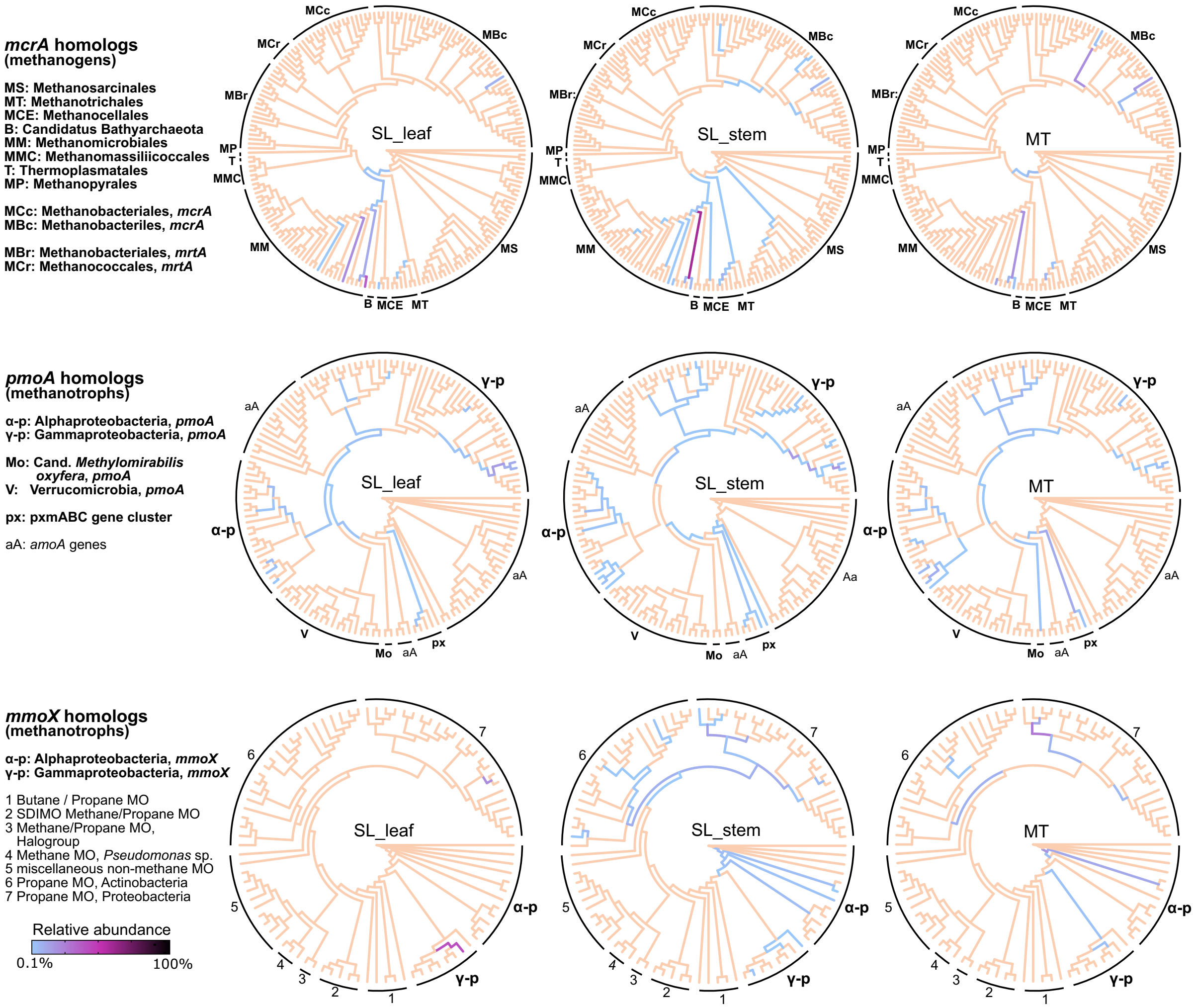

Fig. 7 Diversity of CH_4 cycling genes *mcrA* (for methanogens), *pmoA* (for methanotrophs) and *mmoX* (for methanotrophs) in *S. lapponum* (SL) leaf and stem samples, and in *M. trifoliata* (MT), combined stem and leaf (n = 3). Taxonomy of the gene sequences was analysed via phylogenetic placement on the reference trees of genes from known strains (placements accumulated to the inner branches based on the likelihood-weight threshold of 0.8). Figure shows these placements as heat-trees, where relative abundances of placements are visualized as indicated by the colour bar. Reference branches with no placements or < 0.1% relative abundance are shown with light orange. Composition of the reference strains reflects the probes included in our analysis = coverage of the analysis. Detailed information of each of the reference strains can be found in Figs. S6-S8. Results for *B. nana* are in Fig. 8. No sequences were detected in *C. rostrata*

oxidization) (Tveit et al. 2019; Fig. S6). Based on the *mmoX* gene (found only in some of the methanotrophic taxa) *M. trifoliata* and *S. lapponum* stem contained both alpha- and gammaproteobacterial methanotrophs (especially *Methylocella* and family *Methylococcaceae*). *S. lapponum* leaf contained only gammaproteobacterial *mmoX* (*Methylomagnum*), and no *mmoX* was present in the *B. nana* samples, only genes for other related mono-oxygenases.

Discussion

We quantified the magnitude and seasonality of the CH_4 fluxes from shoots of four common boreal peatland plants, and the responses of the fluxes to temperature, PAR and porewater CH_4 concentration using a high-frequency, automated, climate-controlled measurement system. The species represented different plant functional types, including a sedge (*Carex*

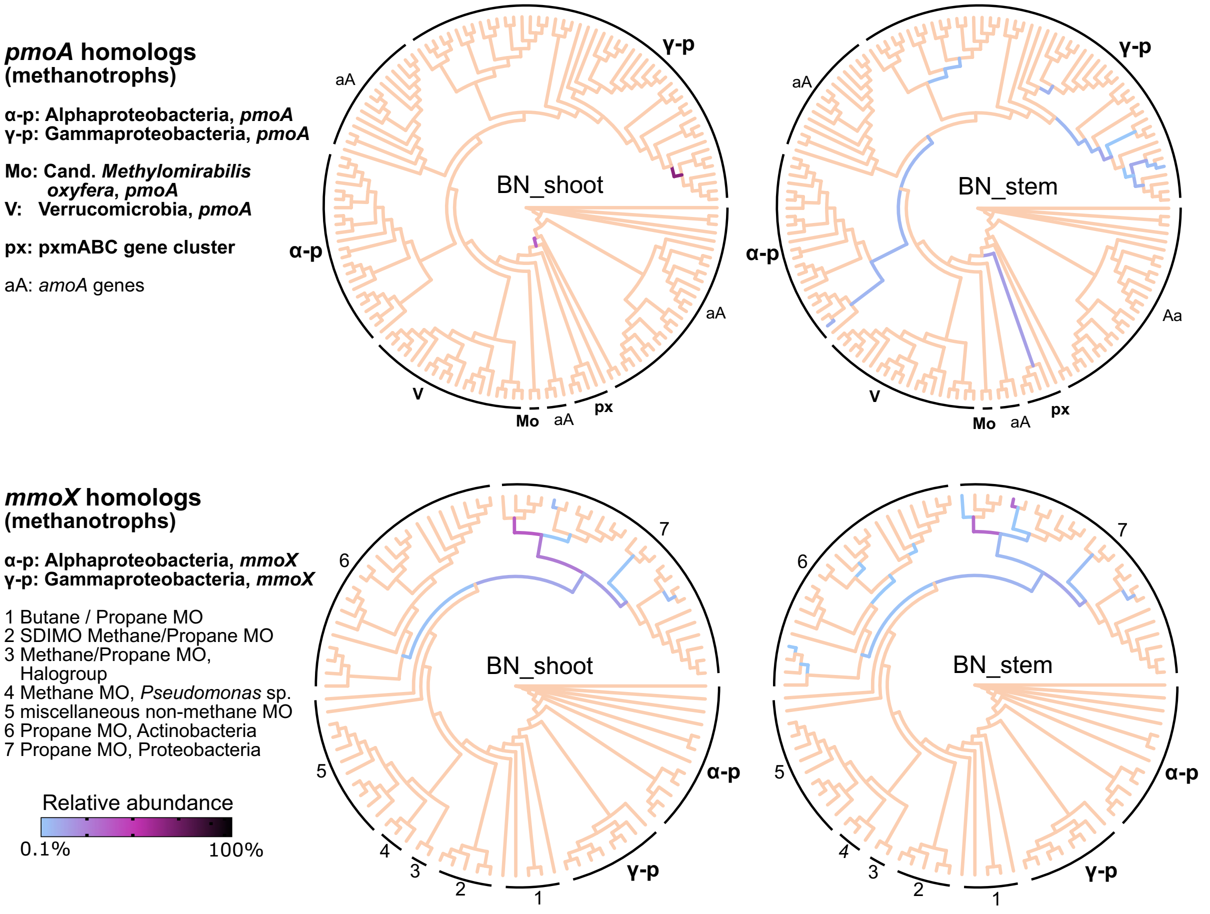

Fig. 8 Diversity of methanotrophic genes *pmoA* and *mmoX* in *B. nana* (BN) shoot and stem samples (n = 1). Taxonomy of the gene sequences was analysed via phylogenetic placement on the reference trees of genes from known strains (placements accumulated to the inner branches based on the likelihood-weight threshold of 0.8). Figure shows these placements as heat-trees, where relative abundances of placements are visualized as indicated by the colour bar. Reference branches with no placements or < 0.1% relative abundance are shown with light orange. Composition of the reference strains reflects the probes included in our analysis = coverage of the analysis. Detailed information of each of the reference strains can be found in Figs S7 and S8. No *mcrA* sequences were detected in *B. nana*

rostrata), a forb (*Menyanthes trifoliata*), and two shrubs (*Salix lapponum* and *Betula nana*). Further, we used the clipping treatment to reveal the role of leaves in restricting the emission from *C. rostrata,* and labelling and microbial analyses to evaluate the source of the plant-emitted CH_4. This is to our knowledge the first study to disentangle the relative importance of phenology and abiotic environmental factors in regulating CH_4 fluxes through peatland plants and to examine the presence of CH_4 cycling microbes in a range of peatland plants. We found that phenology is an overriding factor in controlling plant-mediated CH_4 fluxes, but that the magnitude and seasonal course of phenology effects is species-specific. Out of the studied abiotic factors, temperature and porewater CH_4 concentration were also found to correlate with plant-mediated CH_4 fluxes, but again the magnitude and direction of the effect of these variables differed between species. Hence, besides phenology, our results highlight the substantial role of species-specific differences in regulating the plant-mediated CH_4 fluxes. Based on the results of our clipping experiment with *C. rostrata*, CH_4 release was not regulated by leaves. The plant transported CH_4 in the tested species, *Salix lapponum*, appeared to be mainly soil-derived. However, the discovery of methanogens and methanotrophs in the plant shoots suggests that the CH_4 emission through some plants may also be modulated by CH_4 production and/or oxidation by microorganisms inside the plant.

Plant phenology controls plant-mediated CH_4 flux

Our measurements were conducted in a climate-controlled environment, and therefore the strong seasonality observed in plant CH_4 flux could be ascribed to phenology rather than seasonally different temperature and light conditions. The plant CH_4 flux of *C. rostrata*, a widely studied species and an efficient CH_4 emitter, was similar between early summer and high summer, mismatching our field measurements, which observed a high-summer peak. In high summer, the well-developed roots (Hultgren 1989) and the large aerenchyma size (Fagerstedt 1992) could lead to the high plant CH_4 flux. Yet, the significantly lower CH_4 concentration in high summer (Fig. S3), probably caused by higher CH_4 oxidation when plants are active and can transport more oxygen into the roots, restrained plant CH_4 flux. Nevertheless, the high transport capacity as well as the low porewater CH_4 concentration, led to the significantly higher transport efficiency of *C. rostrata* in high summer. Similarly, the plant CH_4 flux of *M. trifoliata* and shrubs *S. lapponum and B. nana* did not change between early and high summer, suggesting that the aboveground leaf-out during this phase would not affect their CH_4 transport.

The higher plant CH_4 flux of *C. rostrata* in early autumn when it was senescencing indicates that increasing proportion of brown leaves does not affect the transport of this species. Gas transport through senescing leaves was also observed in Korrensalo et al. (2021), although with decreased rate. These results may suggest that stomata are not the main releasing site of CH_4 from *C. rostrata* since they are known to decrease as leaves age (Locke and Ort 2014). This speculation can also explain the poor correlation between PAR and plant CH_4 flux of *C. rostrata*.

Instead of stomata, the CH_4 might mainly be released from stems or leaf sheaths, as suggested also for *C. rostrata* before by Noyce et al. (2014) and for other species like rice plants (Nouchi et al. 1990) and forbs (Shannon et al. 1996). This speculation can be supported by the following results. Firstly, no correlation between leaf area and CH_4 flux through *C. rostrata* was observed in this study (Fig. S9) or the field measurement (Ge et al. 2023). Further, clipping the shoots did not significantly change the transport efficiency of *C. rostrata* (Fig. 5). The higher CH_4 flux of *C. rostrata* after clipping the shoots than before was most likely because on average, the porewater concentration was higher after clipping than before clipping, as the clipping was conducted at the end of each measurement round and thus more CH_4 was accumulated in the bucket.

Besides stems or leaf sheaths, the seasonal changes in belowground parts of *C. rostrata* may be linked to the variations in the transport between seasonal growth stages. Root permeability, a key parameter controlling plant CH_4 transport capacity (Beckett et al. 2001; Henneberg et al. 2012), could decrease in early autumn when plants age (Nouchi et al. 1994; Wassmann and Aulakh 2000) and, thus, reduce the plant CH_4 flux. Yet, in early autumn, CH_4 production can be enhanced by the increasing amounts of substrates due to the presence of more dead organic material, while simultaneously CH_4 oxidation could be lower as less active plants led to less oxygen transport to the roots. Taken together, these factors result in a significantly higher porewater CH_4 concentration in early autumn than during the other seasons, which might compensate for the effect of root permeability on the transport and lead to the high plant CH_4 flux we observed.

Most of CH_4 might not be released from the stomata of *M. trifoliata* either, since a close relationship between PAR and plant CH_4 flux might be observed otherwise. Instead, most of CH_4 could be released from stem before it reached the leaves. MacDonald et al. (1998) also reached the same conclusion for gas transport through *M. trifoliata*. Thus, the significant decrease of flux in early autumn might not be due to the decreasing stomatal conductance in senescencing. Instead, like *C. rostrata*, changes in belowground parts might be the key for the small flux in early autumn, and the small porewater CH_4 concentration cannot compensate such changes (around 7 times smaller than that in *C. rostrata* buckets).

Shrubs *B. nana* and *S. lapponum* had already dropped almost all their leaves in early autumn. For *B. nana* also the plant CH_4 flux decreased from summer to early autumn. As for *S. lapponum,* there was an increase of plant CH_4 flux from uptake of CH_4 in the summer to the flux being close to zero in early autumn. The changes in the flux of these species in early autumn might further indicate that physiological activity, or lack of leaves, might be the key behind the phenology-driven CH_4 flux in these shrubs, both for the release and uptake of CH_4 through the plant. The importance of physiology and leaves in regulating CH_4 transport of woody species has also been reported (Pangala et al. 2014, 2015). Yet, plant CH_4 flux of these species did not respond to PAR, which was probably because most of CH_4 was not release from stoma, but from the stem, as demonstrated in many woody species (Feng et al. 2022; Halmeenmäki et al. 2017).

Apart from variations in the CH_4 transport driven by plant morphology and physiology, the autumnal change in the fluxes through the investigated species could also be influenced by the ceasing photosynthesis leading to a decrease in oxygen release into the rhizosphere (Lai et al. 2012). Further, photosynthesis is also linked to the amount of root exudates released by the plants (Joabsson et al. 1999; Ström et al. 2003) and a decrease in root exudation towards the autumn (Edwards et al. 2018) could alter the functioning of the rhizosphere microbial community and thus play a role in the seasonality of plant CH_4 flux. However, further work is required to identify the relative importance of the different mechanisms involved.

Porewater CH_4 concentration affects plant CH_4 flux of most investigated species

We found that porewater CH_4 concentration increased the plant CH_4 flux of *C. rostrata* and *M. trifoliata*, decreased the flux of *S. lapponum*, but had no effect on *B. nana* (Table 2), even though the concentration varied in all mesocosms (Figs. S3-S4). As an indicator of CH_4 supply to the roots, porewater CH_4 concentration has been reported to control CH_4 flux from both herbs (Schimel 1995; Aulakh et al. 2000a, 2000b), and woody species (Pangala et al. 2014). Yet, in our earlier field study we did not observe any relationships between the CH_4 flux and CH_4 concentration for any of the studied species (Ge et al. 2023). However, in the previous field study, porewater CH_4 concentration varied less (from 3.33 to 537 µmol l^{-1}, Ge et al. 2023) than the concentrations in the current laboratory study (from 0.0018 to 511 µmol l^{-1}). Notably, plant CH_4 flux of *C. rostrata* increased from high summer to early autumn, together with a large increase in the porewater concentration (mean 0.02 and 284 µmol l^{-1} in high summer and early autumn, respectively, Fig. S3). In contrast, CH_4 flux through rice reached a saturation point when the concentration reached merely 14 µmol l^{-1} (Aulakh et al. 2000a, 2000b). This indicates that the ability of plants to increase their transport with higher porewater CH_4 concentration is species-specific. Species reaching the saturation point faster could then potentially limit the total CH_4 flux into the atmosphere.

The close relationship between porewater CH_4 concentration and the plant CH_4 flux of *M. trifoliata* (Table 2) suggest that CH_4 concentration controls the CH_4 flux of *M. trifoliata*. This result implies that the high plant CH_4 flux of *M. trifoliata* observed in the field (Ge et al. 2023) was probably caused by the constantly and significantly high porewater CH_4 concentration where it grew. In contrast, the CH_4 flux of *M. trifoliata* was small in the climate-controlled measurement in the present study where CH_4 concentration in *M. trifoliata* mesocosms were small, up to 27 times lower than that in the field. Thus, instead of a high transport efficiency, the high flux of *M. trifoliata* observed in the field could be mainly due to the high CH_4 concentration.

Temperature decreases plant CH_4 transport efficiency, but only of *C. rostrata*

Within campaigns, the CH_4 transport efficiency of *C. rostrata* significantly decreased after increasing temperature. even though a rising porewater CH_4 concentration alone did not affect the transport efficiency. Thus, potentially, the ability of *C. rostrata* to transport CH_4 could be a limiting factor for ecosystem CH_4 emissions when rising temperatures and higher substrate availability stimulates methanogenesis. As the CH_4 transport efficiency of the other studied species did not respond to warmer temperature, it appears that the temperature effect on plant transport is species-specific. The negative impacts of peat temperature on the transport of *C. rostrata* were also observed in our field measurement conducted in the high summer when peat temperature was at a similar range as in this climate cabinet study (11 °C to 17 °C) (Ge et al. 2023).

It is evident that an experiment in controlled climate cabinet cannot, and by definition should not, directly mimic natural conditions and hence the interpretation of the temperature dependency should be considered with care. The decreasing transport of *C. rostrata* after the increase of temperature could also be due to decreased activity or increased stress of the plants inside the cabinet. However, we did not observe a significant decrease of net ecosystem exchange, an indicator of plant growth conditions, after the increase of temperature (Fig. S10), which suggests that the plants would not have experienced at least a severe stress. We also acknowledge that the field sampling, transport and setting up of the experiment can have caused disturbance to the plants. However, the comparable plant CH_4 flux measured in the field (-5 to 25 mg CH_4 m^{-2} leaf area h^{-1}) and in the climate-controlled cabinet (-0.4 to 21 mg m^{-2} h^{-1}) suggest that the plants displayed rather realistic transport rates in the laboratory conditions. Further, the temperature and light conditions were chosen to represent typical field conditions to which the plants have adapted to.

CH_4 exchange processes of plants include within-plant CH_4 production and oxidation

Role of plant-associated microbes in peatland CH_4 cycle is still poorly understood, apart from the association between methanotrophs and *Sphagnum* mosses (e.g. Larmola et al. 2010; Putkinen et al. 2014). Especially, to our knowledge, markers of microbial CH_4 production within plant tissues has never been reported in this context. Based on our results, methanogenic archaea can be a part of the microbiome of both forbs (*M. trifoliata)* and shrubs (*S. lapponum*, in both stem and leaf parts), which suggests that microbial CH_4 production could occur in the shoots of these plants.

The detected methanogens included both hydrogenotrophic (*Methanocellales*, *Methanomicrobiales, Methanobacteriales)* and acetoclastic (*Methanotrichales*) orders (Knief 2019). Interestingly, most of them (*Methanocellales*, *Methanomicrobiales* and *Methanotrichales*) are proposed to possess features allowing adaptation to oxidative environments (Lyu and Lu 2018) – such as above-ground plant parts. Our findings corroborate previous plant microbiome studies: *Methanomicrobiales* and *Methanotrichales* were found in spruce needles (Putkinen et al. 2021) and *Methanomicrobiales, Methanocellales and Methanobacteriales* as part of poplar stem wood (Yip et al. 2019; Feng et al. 2022). Yet, all these taxa are commonly found also in the anaerobic peat, including Lompolojänkkä where the plant-soil mesocosms were collected (unpublished, Putkinen et al.).

Similar to methanogens, methanotrophs were discovered in forb (*M. trifoliata*) and in both studied shrubs (*S. lapponum* and *B. nana*). This result implies potential for CH_4 oxidation within these plants that have long been merely regarded only as CH_4 transporters (Shannon et al. 1996; Ding et al. 2005; Ge et al. 2023). The detection of methanotrophs inside shrub *S. lapponum* fits well with the plant as a whole acting as a constant CH_4 sink (negative plant CH_4 flux*)* throughout the growing seasons (Fig. 2). Although *B. nana* mostly emitted CH_4 in this study, our earlier field measurements of *B. nana* showed consumption of CH_4 in early summer (Ge et al. 2023), and Riutta et al. (2020) also reported the attenuating effects of *B. nana* on CH_4 flux.

The methanotrophs we detected entailed several taxonomic groups, representing both obligate and facultative methanotrophs (latter found mainly in the alphaproteobacteria (Knief 2015)). Diversity was demonstrated also in the presence of variable forms of methane mono-oxygenases: in addition to common particulate (pMMO, coded by *pmoA*) and soluble (sMMO, coded by *mmoX*) forms, all plants, except *Carex*, contained genes for the pxmABC cluster, thought to code a novel type of particulate MMO, the pXMO (Tavormina et al. 2011). Although the function of the pXMO is still poorly understood, it has been suggested to support methanotrophs under hypoxia (Kits et al. 2015). On the species-level, *Methylocystis bryophila*, able to use multiple C sources and entailing pMMO variants for both low- (pMMO1) and high-affinity (pMMO2) CH_4 oxidation (Han et al. 2018), highlights the versatility of the detected methanotrophs. In addition, sequences of the USCa, including of the first cultivated organism from this group, *Methylocapsa gorgona* (Tveit et al. 2019), further demonstrated that plant-associated methanotrophs may affect peatland CH_4 balance not only by consuming soil-derived CH_4 but also as a sink for the atmospheric CH_4. Especially *Methylocystis* methanotrophs have been detected in other plant types as well (Putkinen et al.

2021), and like with methanogens, most methanotrophs living in Lompolojänkkä plants have close relatives in the surrounding peat (unpublished, Putkinen et al.) and in other boreal peatlands.

While our results indicate that within-plant microbial mechanisms could play a role in modulating plant-derived CH_4 flux, quantification of these microbes and the related processes is challenging. This stems from the methodological difficulties in extracting the endo- and epiphytic microbes from among the vast amounts of plant-derived genetic material. Novel tools, like probe-targeting, do aid in this task, but still leave room for uncertainties (e.g., regarding sensitivity of the analysis in different plant material types). This, together with the generally low number of recovered sequences, limits the quantitative comparison of microbial communities in differing samples. Moreover, for the evaluation of their actual activity, analyses need to go beyond the DNA level and require additional analysis, e.g., visualisation of microbes within the plant structures or at least analysing samples where surface microbes would have been rinsed away).

Conclusion

The number of high-frequency observations (around 4500 flux measurements in total), make our data unique and give a way forward to disentangling the roles of plant phenological stages and environmental drivers in plant-mediated CH_4 fluxes. The high frequency of the measurements allows us to state that there are no rapidly occurring effects on CH_4 transport by changes in PAR intensity in the species we studied. Our results suggest that plant CH_4 emission and uptake varies between species and with plant growth stages over the growing seasons. Our study further indicates that the responses of plant CH_4 flux to temperature and porewater CH_4 concentration vary between species. We detected methanogens and methanotrophs in the shoots of both herbs and shrubs, suggesting that besides transportation of soil-produced CH_4, the plant-mediated CH_4 fluxes could also involve within-plant CH_4 oxidation and/or production. Further studies are needed to assess their roles and activities in different plant-soil environments, conditions and seasons.

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Author contribution AL, PM, MP, and MK designed the field study. MG conducted the field and laboratory measurements with methodological help from AP, LK, and HS. MG, MK, AK, AP, and HS analysed the data. MG, MK, AK and AP wrote the manuscript with contributions from all other authors.

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Declarations

Competing interests The authors declare no conflicts of interest associated with this manuscript.

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