

CH₄ transport in wetland plants under controlled environmental conditions – untangling the impacts of phenology

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Abstract

Background and Aims

Methane (CH₄) fluxes at peatland plant surfaces are the net result of transport of soil-produced CH₄ and within-plant CH₄ production and consumption, yet factors and processes controlling these fluxes remain unclear. We aimed to assess the effects of environmental variables, seasonality and CH₄ cycling microbes in CH₄ fluxes from shoots of characteristic fen species.

Methods

We conducted high-frequency automated CH₄ flux measurements from shoots of a sedge (*Carex rostrata*), a forb (*Menyanthes trifoliata*) and two shrubs (*Betula nana*, *Salix lapponum*) in a climate-controlled environment. Measurements were conducted from intact plant-soil samples collected throughout growing seasons 2020 and 2021 from Lompolojänkä fen, northern Finland.

Results

All studied species showed seasonal variability in CH₄ fluxes. The CH₄ fluxes were not impacted by light level, while higher porewater CH₄ concentration 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 CH₄ flux from *C. rostrata*. Both methanogens and methanotrophs were detected in aboveground parts of *S. lapponum* and *M. trifoliata*, methanotrophs in *B. nana*, while neither were detected in *C. rostrata*.

Conclusion

Our study demonstrates that the seasonal phase of the plants regulates CH₄ fluxes they mediate across species, which was not observed in the field. The detection of methanogens and methanotrophs in herbs and shrubs suggests that microbial processes may contribute to their CH₄ fluxes.

INTRODUCTION

Methane (CH₄) is a powerful greenhouse gas with a global warming potential 34 times larger than carbon dioxide over 100 years (Saunio et al., 2016). Undrained peatlands are an important source of CH₄, accounting for around 12.2% of global CH₄ emissions (Rydin & Jeglum, 2006). These emissions are enabled by the high water-table level (WTL) which is a pre-requisite for many peatland plant species to thrive, and can be both stimulated and attenuated by members of the plant community. Vascular plants

can stimulate CH₄ emissions by offering substrates for microbial CH₄ production and by transporting this CH₄ to the atmosphere (Greenup, 2000; Joabsson & Christensen, 2001; Ström et al., 2003; Barba et al., 2019). They can attenuate CH₄ emission by transporting oxygen from shoots to roots, thereby stimulating CH₄ oxidation in the soil (Heilman and Carlton, 2001; Robroek et al., 2015). The net effect of the plant community on the CH₄ balance of a peatland depends on the interplay between these stimulating and attenuating tendencies, which spatiotemporal and interspecific variation is still not well known.

Plant-mediated CH₄ transport allows soil-produced CH₄ to bypass oxidation in oxic surface peat and thus is a crucial mechanism increasing the total ecosystem CH₄ 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). Environmental factors, e.g., soil water table level (WTL), temperature and porewater CH₄ concentration have been regarded as important controls on ecosystem CH₄ emissions from wetlands (Joabsson & Christensen, 2001; Lai et al., 2014a). Yet, the effects of these controls on plant-mediated CH₄ transport remain unclear with contrasting results even for the same species. For example, Noyce et al. (2014) reported that increasing WTL lead to high CH₄ emissions by *Carex rostrata*, while Ge et al., (2023) observed no such correlation with the same species. Similarly, porewater CH₄ concentration has been found to positively influence plant-mediated CH₄ fluxes (Aulakh et al., 2000; Ding et al., 2005), while no such effect was observed in our earlier study where plant-mediated CH₄ fluxes were measured from common boreal peatland plants with different morphological traits (Ge et al. (2023)).

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 CH₄ fluxes. It seems evident that species-specific plant morphology and phenology (the development and senescence over the growing season) can affect the CH₄ transport. Nevertheless, it remains unclear which plant parts and properties determine the resistance to CH₄ transport and release, even for the most well-studied genus, *Carex spp.* Studies based on clipping plant aboveground parts either suggest that shoots do not restrict CH₄ transport (Nouchi et al., 1990; Kelker & Chanton, 1997; MacDonald et al., 1998; Kutzbach et al., 2004; Schimel, 1995), or opposingly, that they strongly control the CH₄ release (Morrissey et al., 1993; Schimel, 1995; Garnet et al., 2005). Phenology may affect CH₄ 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 CH₄ production (Lai et al., 2014b) and oxidation (Kankaala et al., 2005). The seasonal variability of plant-mediated CH₄ fluxes may also differ between plant species (Ge et al., 2023). However, most previous studies have been conducted in the field, where abiotic conditions typically correlate with phenological changes in plants. Studying plant-mediated CH₄ fluxes under controlled environmental conditions would allow investigating the relative importance of both the species identity and species-specific seasonality in regulating the fluxes.

Plants are not merely transporting soil-derived CH₄ to the atmosphere but may also host CH₄ producing and consuming microbes (Larmola et al., 2010; Putkinen et al., 2021). These processes, however, are rarely accounted for in ecosystem CH₄ flux studies even though recent studies indicate that both microbial CH₄ production and consumption (Jeffrey et al., 2019; Jeffrey et al., 2021; Putkinen et al., 2021). While the microbiomes of both herbaceous and shrub species remain poorly examined, the recent detection of CH₄ cycling microbes in tree leaves (Putkinen et al., 2021) and stems (Feng et al., 2022), and the well-known association between *Sphagnum* mosses and methanotrophs (Larmola et al., 2010; Putkinen et al., 2014), support their wider evaluation within other plants, especially those adapted to CH₄-rich environments like peatlands.

In this study, we investigate the seasonality and species-specific differences in CH₄ fluxes from shoots of common boreal peatland herbs (sedge *C. rostrata* and forb *M. trifoliata*) and shrubs (*B. nana* and *S. lapponum*) in controlled environmental conditions. The aims were to (i) evaluate how the phase of the growing season and abiotic factors (temperature, porewater CH₄ concentration and PAR) interact in controlling plant-mediated CH₄ fluxes; (ii) identify which plant part is the main location of CH₄ release from *C. rostrata*, one of the most abundant sedge species in northern peatlands; and (iii) determine whether plant shoots host microbes that produce or oxidize CH₄. To disentangle the effects of season mediated through changes in 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 (Kohl et al., 2021). To reveal whether leaves of *C. rostrata* restrict the transport of CH₄, a foliage clipping experiment was conducted. To evaluate CH₄ production and consumption processes occurring inside of plant shoots, a ¹³C-labelling measurement was conducted, and the presence and diversity of methanogens and methanotrophs within plant shoots were measured. We hypothesized that i) phenological stage of the plants modifies CH₄ flux from the investigated species, ii) CH₄ flux from all investigated species increases with PAR, temperature and porewater CH₄ concentration, iii) leaves are the main location for CH₄ release from *C. rostrata*, and iv) CH₄-related processes occurring inside plant shoots include plant-derived and microbial CH₄ production and oxidation.

MATERIALS AND METHODS

Plant sampling and growth condition

Mesocosms of *Carex rostrata*, *Menyanthes trifoliata*, *Betula nana* and *Salix lapponum* were collected at Lompolojänkä (67.99 °N, 24.21°E), a northern boreal fen (Ge et al., 2023) located in Finnish Lapland. 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 CH₄ fluxes, we collected the mesocosms of each species 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).

There were three mesocosms per species per campaign. The total number of mesocosms throughout the observations was less than nine due to system malfunctions and the quality control of closure data (Table S1). The plant individuals were the same ones 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 the peat surrounding the roots of the target plant individual and was placed in a plastic bucket (10 L). We dug the peat up to 40 cm depth to avoid disturbance to the roots, of which the majority are located above the depth of 30 cm in boreal sedge fens (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 a 16-h photoperiod before being transported to the climate-controlled cabinet to ensure uniform growing conditions and to prevent the individuals from progressing to different phenological stages before measurements. 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 an airtight bottle stored 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 CH₄ concentration

Before starting the measurements, a silicone tube attached to a polytetrafluoroethylene (PTFE) tube was placed at the bottom of each bucket (Kammann et al. (2001). 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. CH₄ concentrations were analysed by a gas chromatograph (GC, Agilent 7890A). The measured gas phase concentrations (ppm CH₄) inside the tube were then converted to concentrations (μmol CH₄ l⁻¹) in the surrounding porewater based on Henry's law:

$$C = HP_{gas} \text{ (Eq. 1)}$$

Where C is the solubility of a gas at a fixed temperature in a particular solvent (mol l⁻¹), here CH₄ concentration in porewater; P_{gas} is the partial pressure of CH₄ (bar); and H is Henry's law constant (mol l⁻¹ bar⁻¹) which for CH₄ at 298K 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), 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 CH₄ 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 been found to be an efficient CH₄ 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.

Table 1
Climate-controlled cabinet settings and treatment protocol. The foliage clipping treatment conducted only on *C. rostrata*

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 <i>C. rostrata</i>	no	no	no	no	yes	yes	yes

CH₄ flux measurements

Due to different growth forms of herbs and shrubs, two different measurement chamber designs were used for measuring CH₄ fluxes from their shoots (Fig. 1). 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 CH₄ fluxes from parts of shrub shoots by using a 1 L chamber system, hereafter called “shrub chamber” (Toivo Pohja, Juupajoki, Finland). 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).

CH₄ fluxes were measured in 10-min long closures and there were 72 closures per species per day. During each closure, sample gas in the headspace was circulated in a closed loop between the analyser (Picarro G2301 and G2201i) and the chamber. After each closure, the chamber was flushed with air taken from the cabinet for 10 minutes 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 CH₄ concentrations within the cabinet, which would have had effects on the apparent CH₄ 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.

CH₄ fluxes through plants and the empty chamber were calculated using the least squares methods, defining the best linear fit for the change of concentration over time:

$$F = \frac{dC}{dt} \frac{MPV}{RT} \text{ (Eq. 3)}$$

where dC/dt is the change in CH₄ (ppm) concentration over time (s), M is the molar mass of measured gas (16042 mg for CH₄), P is the atmospheric pressure (101325 Pa), V is the volume of the measurement system (m³), 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 closure. Closures with nonlinear changes in CH₄ were identified to be caused by chamber leak and were excluded. We also discarded closures conducted during system malfunctions, or opening of the cabinet door to dilute cabinet CH₄ concentration. In total, 25% of the closures 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 & 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 CH₄ fluxes from the shoots by plant surface area to estimate area-specific plant CH₄ 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.

¹³C labelling

While aerenchymatous herbs have long been regarded as efficient channels for soil-produced CH₄, with shrubs the main source of CH₄ emitted through them is poorly studied. We therefore conducted a

labelling experiment on *S. lapponum* to reveal whether the CH₄ emitted by its shoots originated from the peat following an approach previously used to confirm peat as the source of CH₄ emitted by *B. nana* (Koskinen et al., 2021). Two ml ¹³C-labelled CH₄ (99% ¹³C, Merck 490229) were mixed with 28 ml dinitrogen (N₂) gas and injected into the silicone tube installed at the bottom of the mesocosms for porewater CH₄ concentration measurements. All three mesocosms of *S. lapponum* from each campaign were labelled once on the fifth measurement day and ¹³CH₄ emissions from shoots were recorded until the end of the measurement cycle. The δ¹³C-CH₄ of shoot-emitted methane was calculated through Keeling plots, i.e., by plotting the measured δ¹³C-CH₄ 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 δ¹³C-CH₄ based on CH₄ emitted from soil to the cabinet air outside the shoot chamber through a single Keeling plot, i.e., by plotting cabinet ¹³C-CH₄ over its inverse concentration 24 h after injection. The proportion of CH₄ emitted by shoots of *S. lapponum* originated from peat was calculated as:

$$f_{\text{peat}} = (\delta^{13}\text{C}_{\text{pot}} - \delta^{13}\text{C}_{\text{shoot}}) / (\delta^{13}\text{C}_{\text{pot}} - \delta^{13}\text{C}_{\text{cabinet}}) \quad (2)$$

where f_{peat} is the proportion of ¹³C-CH₄ emitted by shoots from peat, δ¹³C_{pot}, δ¹³C_{shoot} and δ¹³C_{cabinet} are the value of δ¹³ in pot, shoots, and cabinet, respectively.

Targeted metagenomic analysis of CH₄ cycling microbes

To evaluate the possibility of microbial processes within or on the surfaces of the plants contributing to the plant-derived CH₄ 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). 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 hour. 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

To investigate the effects of seasonality, temperature and clipping treatment on the fluxes under standardized porewater CH₄ concentration conditions, plant CH₄ flux was normalized by porewater CH₄ concentration. This produced a variable hereafter name “apparent plant CH₄ transport efficiency” as we cannot exclude that relevant amounts CH₄ were produced or oxidized within the plants. When evaluating the effect of PAR, we assumed that diurnal changes in porewater CH₄ concentration (measured only once per day) and its impacts on plant CH₄ flux were negligible. Thus, we did not investigate the effect of PAR on apparent plant CH₄ transport efficiency.

The effect of phenology on plant CH₄ flux and apparent plant CH₄ transport efficiency, as indicated by the differences among mesocosms collected at the three different plant phenological phases over the two growing seasons, was tested with one-way ANOVA and post hoc tests. We used linear mixed-effects models (LMMs) to examine the effects of PAR and porewater CH₄ concentration on plant CH₄ flux, as well as the effects of temperature on plant CH₄ flux and apparent plant CH₄ transport efficiency. The LMMs was also used to test the effects of clipping treatment on the plant CH₄ flux and apparent plant CH₄ transport efficiency of *C. rostrata*. These tests were conducted for each species separately. Fixed predictors, analysed one by one, each in a separate LMM, were the environmental variables porewater CH₄ concentration, temperature (15 vs 20°C) and PAR (2 levels of PAR) as well as clipping treatment (intact vs shoot clipping), and the random variable was sample id in all LMMs analyses. The models are presented in Table 2, S2-S4. No statistics was applied for the number of the genes of the microbiology data. All statistic analyses were performed using R v.3.6.1 (Team, 2019).

RESULTS

Seasonality of porewater concentration and plant CH₄ flux

Strong seasonality was observed in the porewater CH₄ concentration of *C. rostrata* and *B. nana* mesocosms, with minimum occurring in high summer (mean 0.02 µmol l⁻¹ for both, Fig. S1). The minimum was over 10000 times lower than that for other growing seasons. Contrastingly, the seasonality in the concentration in *M. trifoliata* and *S. lapponum* mesocosms was small (Fig. S2). The high summer and early autumn mean porewater CH₄ concentration was 17 and 40 µmol l⁻¹ for *M. trifoliata* buckets, and 21 and 23 µmol l⁻¹ for *S. lapponum* mesocosms, respectively.

The porewater CH₄ concentration was a strong controller for plant CH₄ flux of *C. rostrata*, *M. trifoliata*, and *S. lapponum* (all P < 0.01, Table 2). Higher concentration significantly increased the plant CH₄ flux of *C. rostrata* and *M. trifoliata* and decreased the flux of *S. lapponum* (all P < 0.01, Table 2). In contrast, no significant relationship between CH₄ concentration and plant CH₄ flux was observed for *B. nana*.

To focus on the seasonality unrelated to porewater CH₄ concentration we investigated the variability in the apparent transport efficiency. The seasonal pattern in the apparent transport efficiency and in the

plant CH₄ flux appeared to differ between the four studied species. The apparent 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 CH₄ 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 apparent 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 CH₄ 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*, both transport efficiency and plant CH₄ flux were significantly higher in high summer than in early autumn (both P < 0.001), while the opposite was true for *S. lapponum* (both P < 0.001).

Table 2

Summary statistics for the linear mixed-effects model describing the effects of porewater CH₄ concentration ([CH₄]_{pw}) on the plant CH₄ 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.

<i>C. rostrata</i>				<i>M. trifoliata</i>			
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	
<i>S. lapponum</i>				<i>B. nana</i>			
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

Effects temperature and PAR

The response to increase in temperature from 15°C to 20°C, differed between the plant species and also between apparent CH₄ transport efficiency (Fig. 3) and plant CH₄ flux (Fig. S3). 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 CH₄ 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 CH₄ 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 affected the apparent CH₄ transport efficiency of *C. rostrata* (Fig. 5). However, higher plant CH₄ flux was observed after conducting the clipping treatment.

Source of plant CH₄ 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 $\delta^{13}\text{C-CH}_4$ value of mesocosm CH₄ (inferred from the CH₄ emitted into the cabinet air) was *c.* +60‰ (red line in Fig. 6). Assuming a background $\delta^{13}\text{C-CH}_4$ value of -50‰, our injection has therefore changed porewater CH₄ 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 $\delta^{13}\text{C-CH}_4$ in the cabinet was *c.* +26‰, which was lower than that from the mesocosm due to mixing with ambient air. $\delta^{13}\text{C-CH}_4$ emitted into shrub chambers was *c.* +48‰, which was higher than that of the cabinet air (*c.* +26‰) and closer to $\delta^{13}\text{C-CH}_4$ in the mesocosm (*c.* +60‰). Labelling also showed that major part of CH₄ (*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.

Plant-associated methanogens and methanotrophs

We found some of the plant shoots to host microbes that produce or oxidize CH₄ but not from 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. S4–S6). None of the genes were detected in *C. rostrata*. In the separately screened *B. nana* samples (shoot and stem, *n* = 1), methanotrophs, but no 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 10782 in *mmoX* (including non-methane mono-oxygenases; Table S3).

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 CH₄ concentrations (i.e. capable of high-affinity oxidization) (Tveit et al., 2019; Fig. S4). 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 CH₄ fluxes from shoots of four common boreal peatland plants, and the responses of the fluxes to temperature, PAR and porewater CH₄ concentration using a high-frequency, automated, climate-controlled measurement system. The species represented different plant functional types, including a sedge (*Carex 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 CH₄. This is to our knowledge the first study to disentangle the importance of phenology and abiotic factors in regulating CH₄ fluxes through peatland plants and to examine the presence of CH₄ cycling microbes in a range of peatland plants. We found that phenology is an overriding factor in controlling plant-mediated CH₄ fluxes, but that the magnitude and seasonal course of phenology effects is species-specific. Out of the studied abiotic factors, temperature and porewater CH₄ concentration were also found to regulate plant-mediated CH₄ fluxes, but 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 CH₄ fluxes. Based on the results of our clipping experiment with *C. rostrata*, CH₄ release was not regulated by leaves. The plant transported CH₄ 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 CH₄ emission through some plants may also be modulated by CH₄ production and oxidation by microorganisms inside the plant.

Plant phenology controls plant-mediated CH₄ flux

Our measurements were conducted in a climate-controlled environment, and therefore the strong seasonality observed in plant CH₄ flux was ascribed to phenology rather than seasonally different weather conditions. The plant CH₄ flux of *C. rostrata*, a widely studied species and the most efficient CH₄ emitter, was significantly higher in high summer than that in early summer (Fig. 2), matching our earlier field measurement results (Ge et al., 2023). With this species, the variations in the transport between seasonal growth stages may be linked to the known seasonal changes in plant morphology. In early summer, newly-merged shoots do not have roots (Hultgren, 1989), and the aerenchyma size is rather

small (Fagerstedt, 1992), which could explain the small plant CH₄ flux in early summer. In contrast, the plant CH₄ flux of *M. trifoliata* and *S. lapponum* did not change between early and high summer, which suggests that the aboveground leaf-out during this phase would not affect their CH₄ transport. With *B. nana*, plant CH₄ flux was higher in early summer than that in high summer.

The smaller amount of shoot biomass of *C. rostrata* in early summer than that in high summer might also cause the higher plant CH₄ flux, yet the possibility is small due to following reasons. Firstly, no correlation between leaf area and CH₄ flux through *C. rostrata* was observed in this study (Fig. S7) or the field measurement (Ge et al., 2023). Secondly, clipping the shoots did not significantly change the plant CH₄ flux of *C. rostrata* (Fig. 5). Thirdly, stomatal control in aerenchymatous plants is poorly developed due to living in high-moisture environments (Lange et al., 1971). This echoes with the poor correlation between PAR and the plant CH₄ flux of *C. rostrata* and other investigated species and also the fact that CH₄ can be released from stems or leaf sheaths, as found in many species like rice plants (Nouchi et al., 1990) and forbs (Shannon et al., 1996). All these lead us to believe that seasonal changes in belowground parts of *C. rostrata*, instead of leaves, control CH₄ transport of *C. rostrata*, which is against our hypothesis. Unlike *C. rostrata*, the plant CH₄ flux of *B. nana* was higher in early summer than that in high summer. However, due to the lack of morphological data, it is unknown what happens for them in this part of the growing season leading the changes in the flux of *B. nana*.

The plant CH₄ flux of *C. rostrata* was higher in early autumn when it was senescencing than that in high summer. This indicates the increasing proportion of brown leaves does not affect the transport of *C. rostrata*, which is again support the speculation that the belowground parts of plant regulate the transport. The root permeability, a key parameter controlling plant CH₄ transport capacity (Beckett et al., 2001; Henneberg et al., 2012), could decrease in early autumn (Nouchi et al., 1994; Wassmann & Aulakh, 2000) and, thus, reduce the plant CH₄ flux of *C. rostrata*. Yet, the significantly higher porewater CH₄ concentration in early autumn than that in high summer might compensate for the effect of root permeability on the transport and lead to the maximum plant CH₄ flux.

In early autumn, *M. trifoliata* was senescencing and shrubs *B. nana* and *S. lapponum* had already dropped all their leaves. For *M. trifoliata* and *B. nana* also the plant CH₄ flux decreased from high summer to early autumn. As for *S. lapponum* who constantly showed an uptake of CH₄, and the remarkable increase of plant CH₄ flux in early autumn means the decrease of absorption rate. The changes in the flux of these species in early autumn might indicate that physiological activity, or lack of leaves (for shrubs only), might be the key behind the phenology-driven CH₄ flux in these species. The importance of physiology and leaves in regulating CH₄ transport of herbs and woody species has also been reported (Garnet et al., 2005; Pangala et al., 2014; Pangala et al., 2015). Yet, plant CH₄ flux of these species did not respond to PAR, and the effects of foliage removal to the flux are still unknown. Also, the seasonal changes in the belowground parts of these species are unknown, making us unable to identify the main control behind phenology in regulating CH₄ flux.

Porewater CH₄ concentration affects plant CH₄ flux of most investigated species

We found that porewater CH₄ concentration increased the plant CH₄ 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. S1-S2). As an indicator of CH₄ supply to the roots, porewater CH₄ concentration has been reported to control CH₄ flux from both herbs (Schimel, 1995; Aulakh et al., 2000), and woody species (Pangala et al., 2014). Yet, in our earlier field study we did not observe any relationships between the CH₄ flux and CH₄ concentration for any of the studied species (Ge et al., 2023). However, in this field study, porewater CH₄ concentration varied less (from 3.33 to 537 $\mu\text{mol l}^{-1}$, Ge et al., 2023) than the concentrations in the current laboratory study (from 0.0018 to 511 $\mu\text{mol l}^{-1}$). Notably, CH₄ flux from *C. rostrata* did not show any signs of saturation even though the concentration (Fig. S1) was high in early summer and autumn (mean 105 and 284 $\mu\text{mol l}^{-1}$, respectively). In contrast, CH₄ flux through rice reached a saturation point when the concentration reached merely 14 $\mu\text{mol l}^{-1}$ (Aulakh et al., 2000). This indicates that the ability of plants to increase their transport with higher porewater CH₄ concentration is species-specific. Species reaching the saturation point faster could then potentially limit the total CH₄ flux into the atmosphere.

The close relationship between porewater CH₄ concentration and the plant CH₄ flux of *M. trifoliata* (Table 2) suggest that CH₄ concentration controls the CH₄ flux of *M. trifoliata*. This result implies that the high plant CH₄ flux of *M. trifoliata* observed in the field (Ge et al. 2023) was probably caused by the constantly and significantly high porewater CH₄ concentration where it grew. In contrast, the CH₄ flux of *M. trifoliata* was small in the climate-controlled measurement in the present study where CH₄ 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 CH₄ concentration.

Temperature decreases apparent plant CH₄ transport efficiency, but only of *C. rostrata*

The apparent CH₄ transport efficiency of *C. rostrata* significantly decreased after increasing temperature. This reflects that a higher temperature stimulated both porewater CH₄ production (increasing the CH₄ concentration) and plant CH₄ flux, but the increase of CH₄ concentration was relatively higher than the increase of the flux, leading to a lower apparent CH₄ transport efficiency. Thus, potentially, the ability of *C. rostrata* to transport CH₄ could be a limiting factor for ecosystem CH₄ emissions when rising temperatures and higher substrate availability stimulates methanogenesis. As the apparent CH₄ 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. S8), 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 CH₄ flux measured in the field (-5 to 25 mg CH₄ 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.

CH₄ exchange processes of plants include within-plant CH₄ production and oxidation

Role of plant-associated microbes in peatland CH₄ 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 CH₄ 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 CH₄ 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 & 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 Lompolojänkka 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 CH₄ oxidation within these plants that have long been merely regarded only as CH₄ 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 CH₄ sink (negative plant CH₄ flux) throughout the growing seasons (Fig. 2).

Although *B. nana* mostly emitted CH₄ in this study, our earlier field measurements of *B. nana* showed consumption of CH₄ in early summer (Ge et al., 2023), and Riutta et al. (2020) also reported the attenuating effects of *B. nana* on CH₄ 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) CH₄ 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 CH₄ balance not only by consuming soil-derived CH₄ but also as a sink for the atmospheric CH₄. Especially *Methylocystis* methanotrophs have been detected in other plant types as well (Putkinen et al., 2021), and like with methanogens, most methanotrophs living in Lompolojännkä 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 CH₄ 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 CH₄ fluxes. Our results show that plant CH₄ emission and uptake varies between species and with plant growth stages over the growing seasons. Our study further shows that the responses of plant CH₄ flux to temperature and porewater CH₄ concentration vary between species. We detected methanogens and methanotrophs in the shoots of both herbs and shrubs, suggesting that

besides transportation of soil-produced CH₄, the plant-mediated CH₄ fluxes could also involve within-plant CH₄ oxidation and/or production. Further studies are needed to assess their roles and activities in different plant-soil environments, conditions and seasons.

Declarations

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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, HS. MG, MK, AK, AP, HS analysed data. MG, MK, AK and AP wrote the manuscript with contributions from all other authors.

DATA AVAILABILITY

The original data are available at <https://doi.org/10.5281/zenodo.7812992>. Additional data related to this article can be obtained from the corresponding author (MG) on request.

COMPETING INTERESTS

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

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Figures

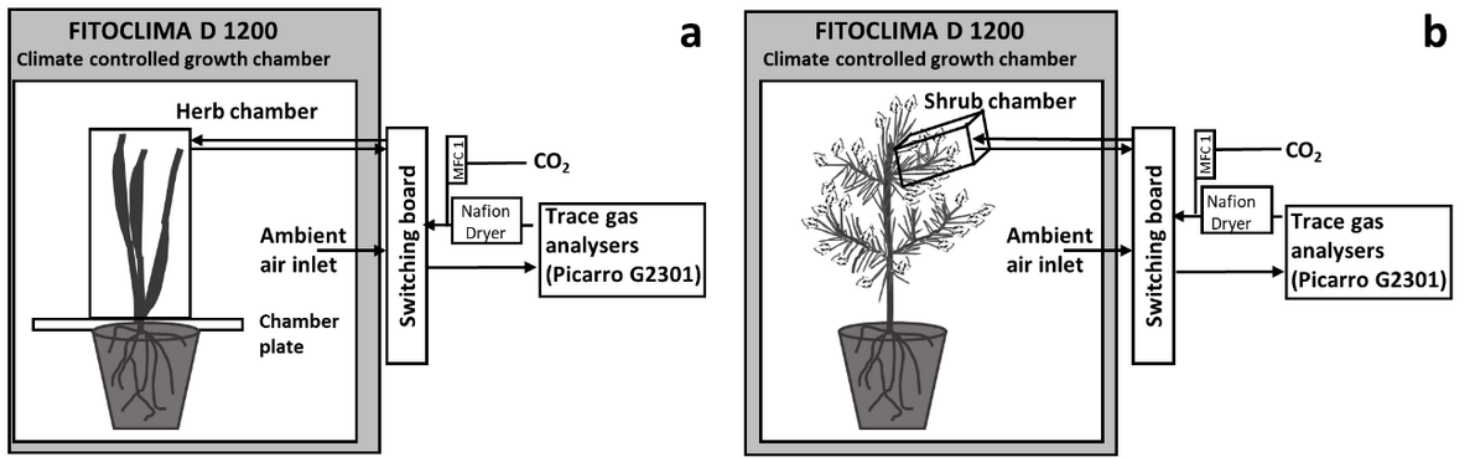


Figure 1

The controlled-environment chamber system, consisting of a climate-controlled growth chamber, and two different measurement chambers for observing CH₄ fluxes from herbaceous species *Carex rostrata* and *Menyanthes trifoliata* (a, herb chamber), and from shrubs *Betula nana* and *Salix lapponum* (b, shrub chamber). Figure modified after Kohl et al. (2021)

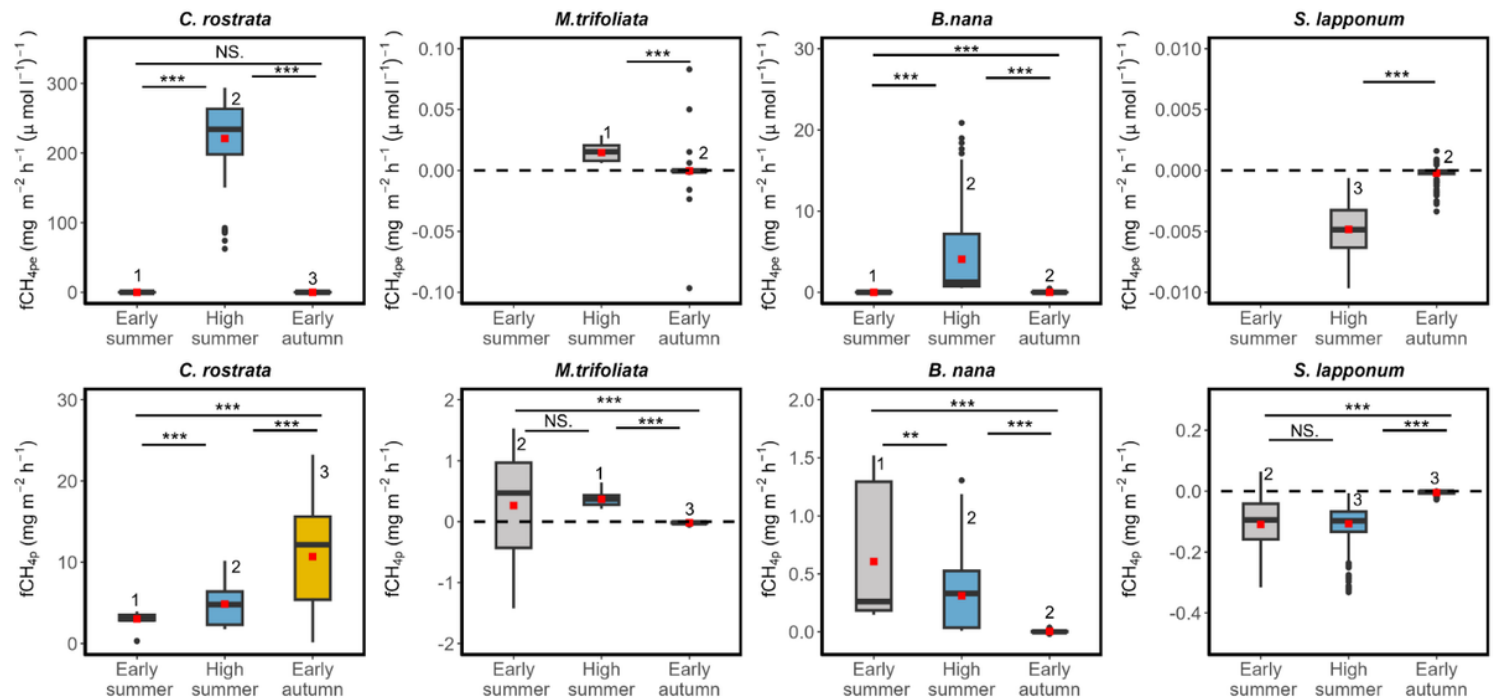


Figure 2

Characteristics of the seasonal apparent CH₄ transport efficiency (fCH_{4pe} , mg CH₄ m⁻² leaf or stem area h⁻¹ (μmol CH₄ l⁻¹ porewater)⁻¹) and plant CH₄ flux (fCH_{4p} , mg CH₄ 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: **, 0.01; ***, 0.001.

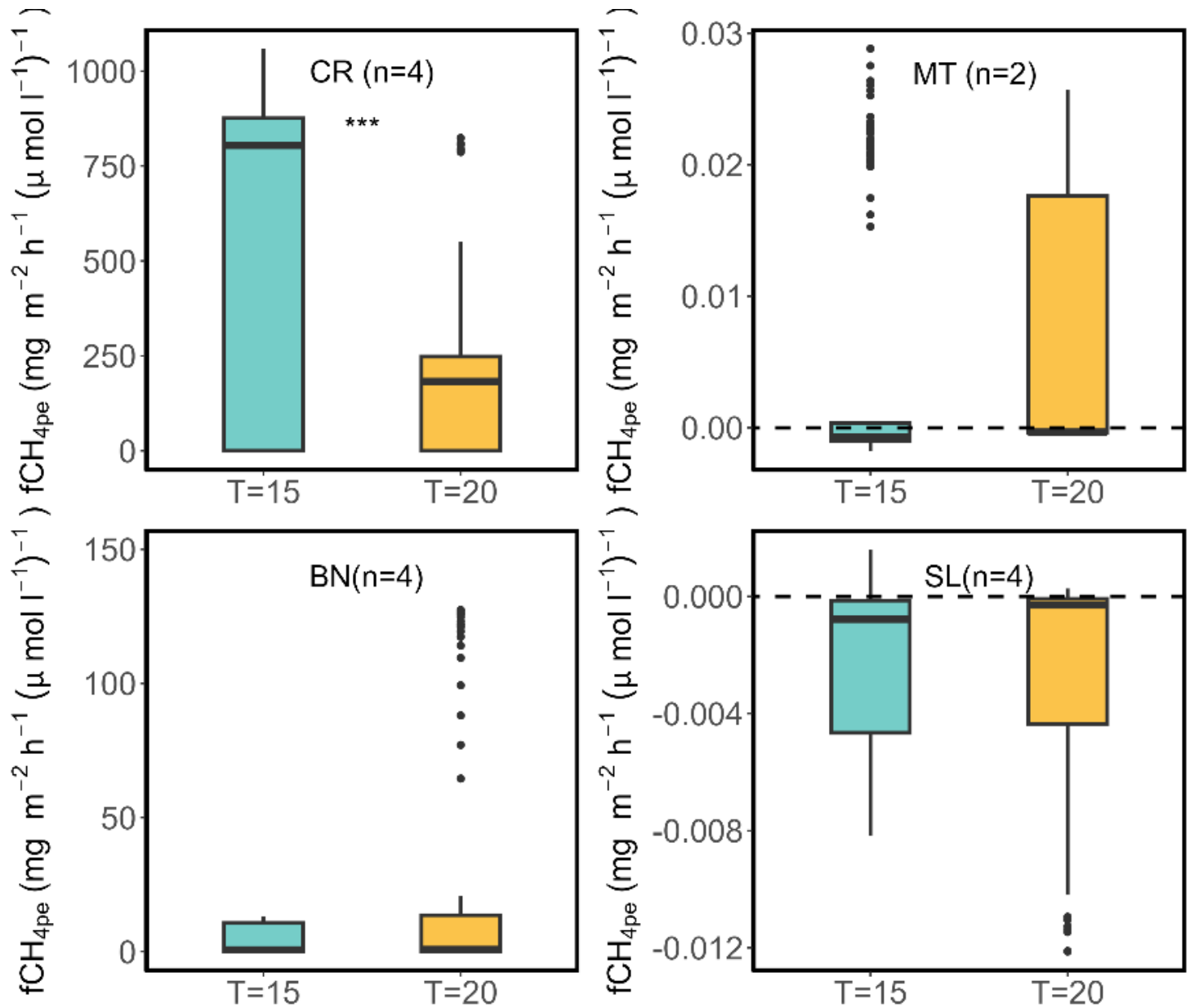


Figure 3

Temperature effects on apparent CH_4 transport efficiency ($\text{fCH}_{4\text{pe}}$, mg CH_4 m^{-2} leaf area h^{-1} ($\mu\text{mol l}^{-1}$) $^{-1}$) of *C. rostrata* (CR), shrubs *B. nana* (BN) and *S. lapponum* (SL). Summary statistics for the linear mixed-effects model describing the effects of temperature on $\text{fCH}_{4\text{pe}}$ shown in Table S2. 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 $\text{fCH}_{4\text{pe}}$ denotes zero flux level. Note the different y-axis scales. Asterisks denote statistical significance: ***, 0.001.

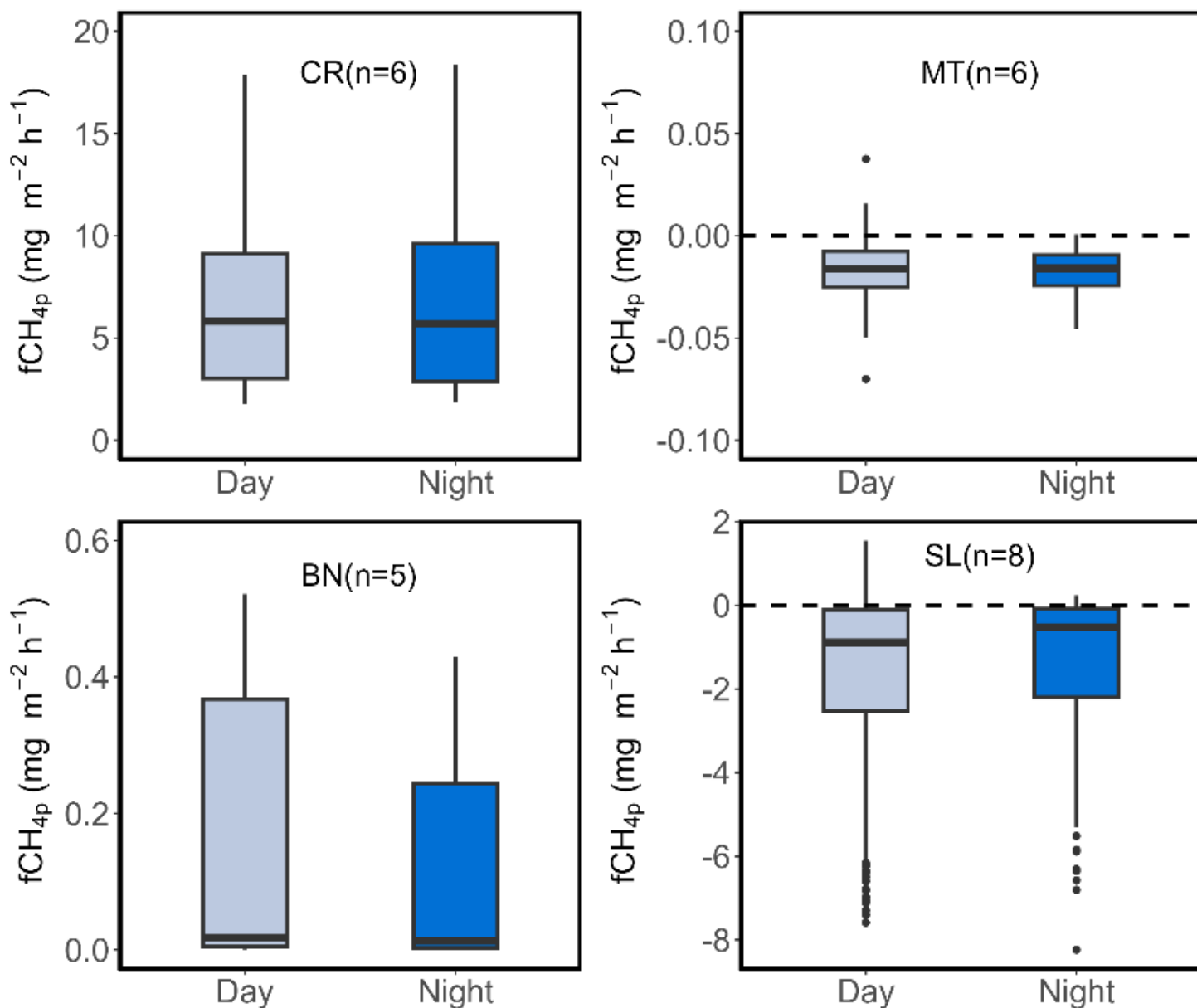


Figure 4

PAR effects on plant CH₄ flux (fCH_{4p} , mg CH₄ m⁻² leaf area h⁻¹) of *C. rostrata* (CR), *M. trifoliata* (MT), and *B. nana* (BN) and *S. lapponum* (SL). 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.

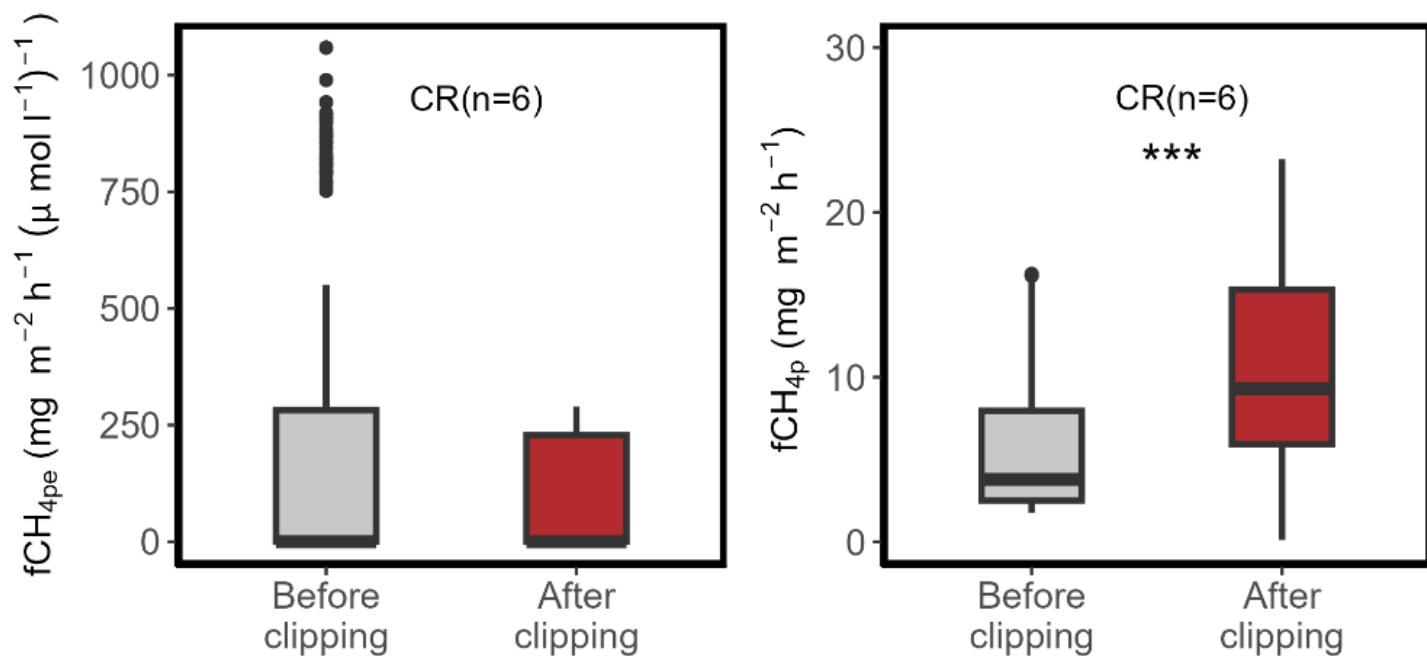


Figure 5

Clipping effects on apparent CH_4 transport efficiency (fCH_{4pe} , $mg\ CH_4\ m^{-2}\ leaf\ area\ h^{-1}\ (\mu mol\ l^{-1})^{-1}$) and plant CH_4 flux (fCH_{4p} , $mg\ CH_4\ m^{-2}\ leaf\ area\ h^{-1}$) 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.

Salix lapponum

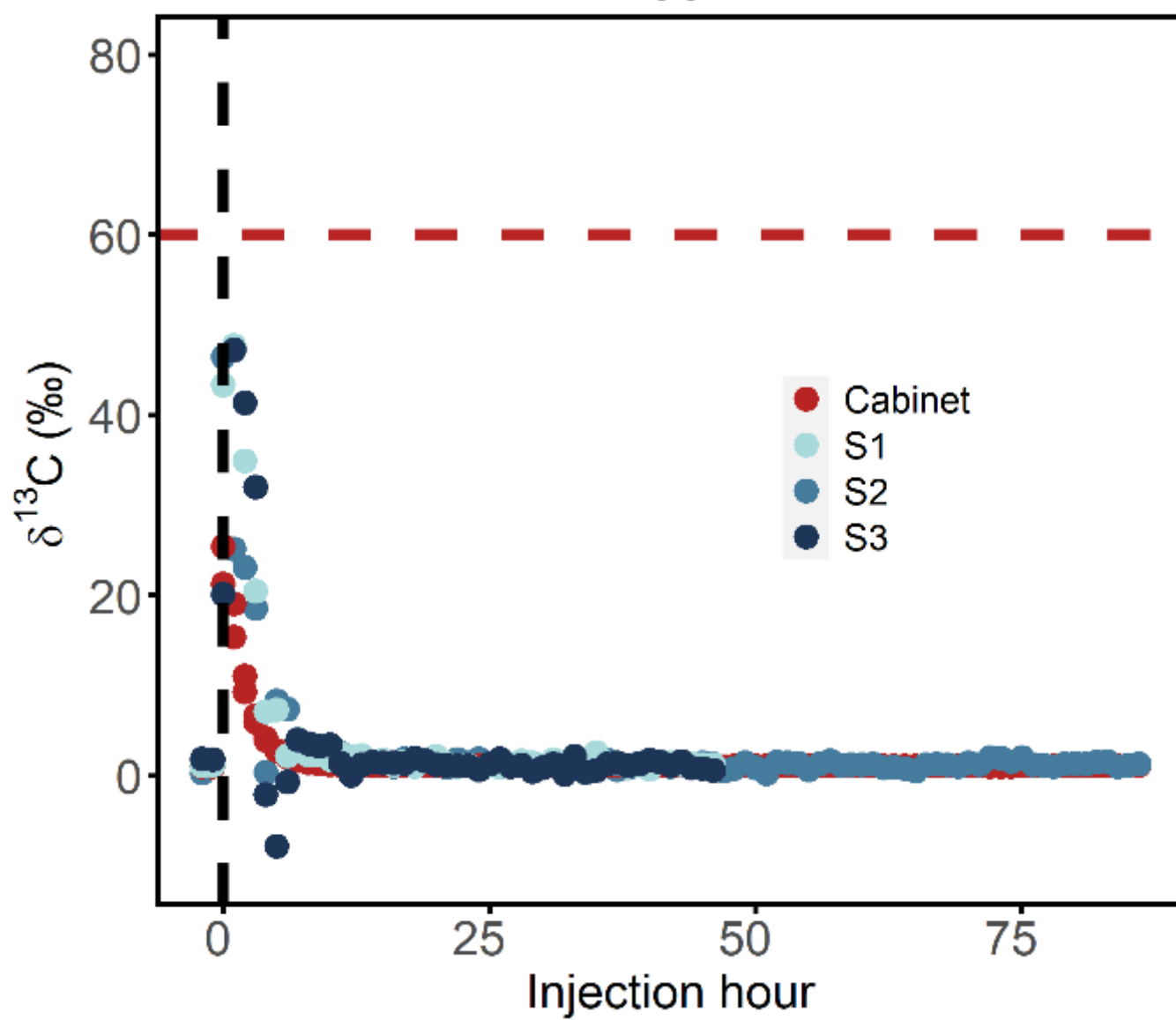


Figure 6

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

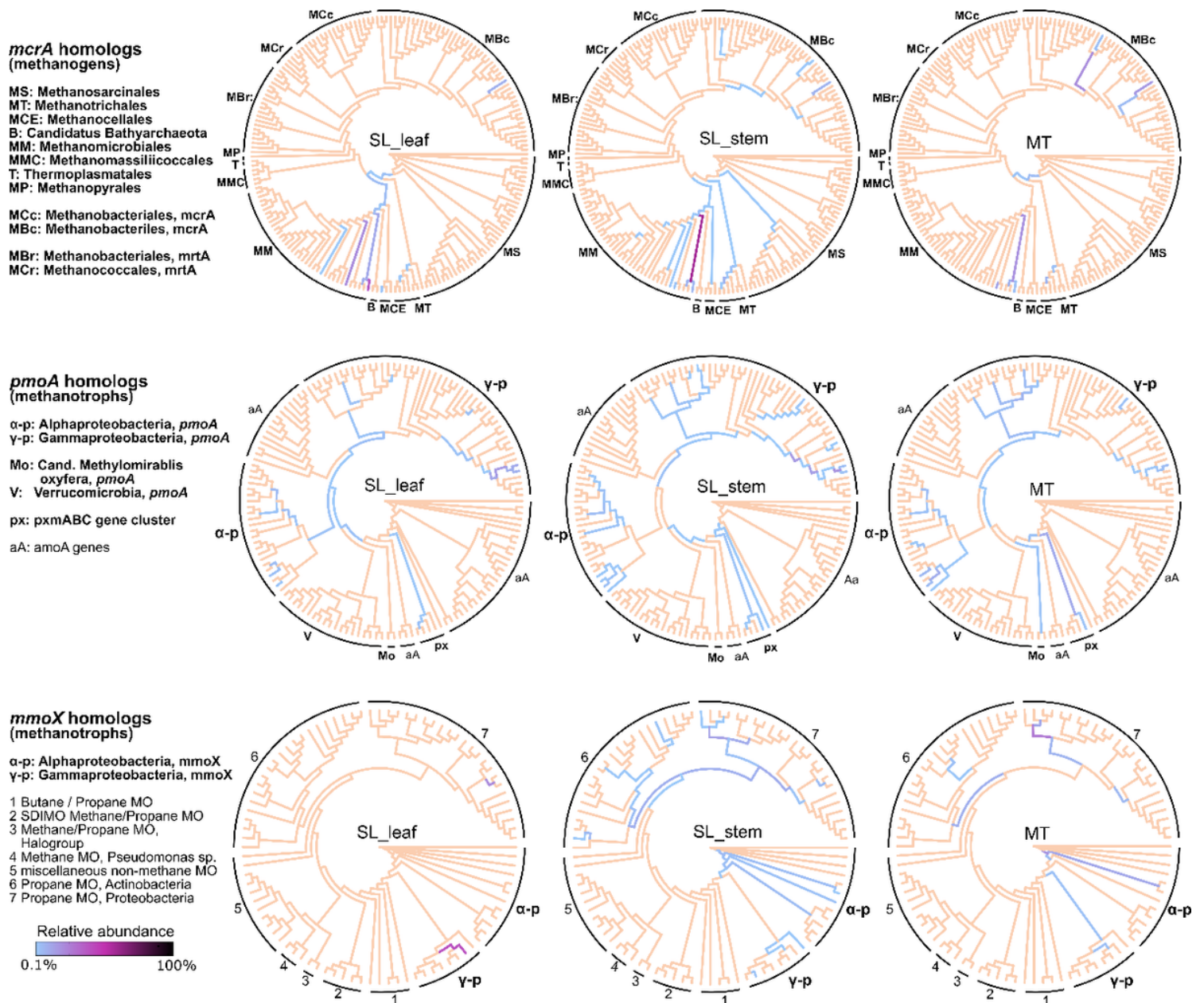


Figure 7

Diversity of CH₄ 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. S4-S6. Results for *B. nana* are in Fig. 8. No sequences were detected in *C. rostrata*.

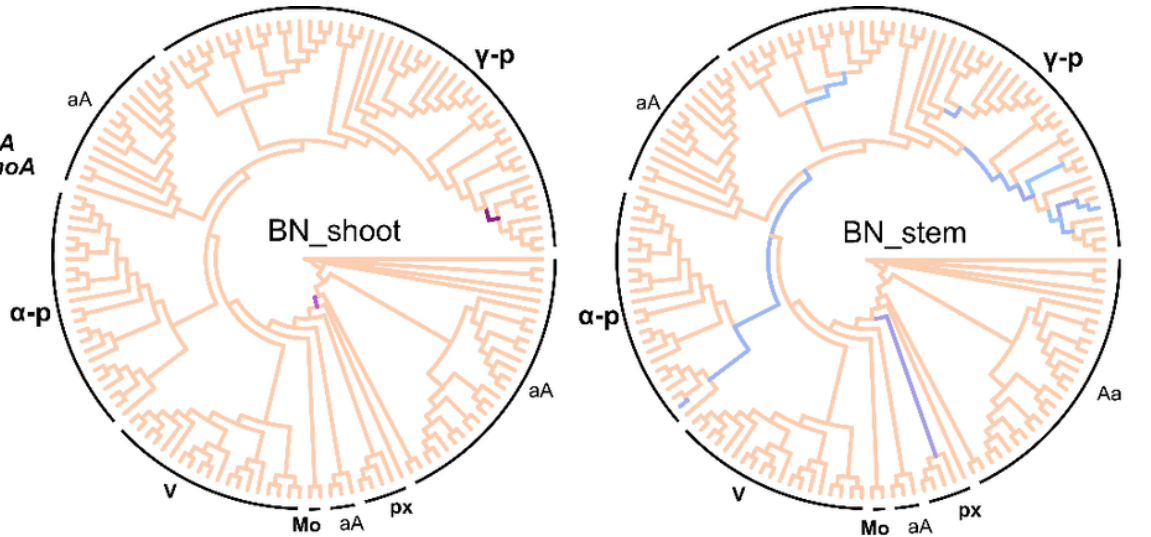
pmoA homologs (methanotrophs)

α-p: Alphaproteobacteria, *pmoA*
γ-p: Gammaproteobacteria, *pmoA*

Mo: *Cand. Methylophilus*
oxyfera, *pmoA*
V: *Verrucomicrobia*, *pmoA*

px: pxmABC gene cluster

aA: amoA genes



mmoX homologs (methanotrophs)

α-p: Alphaproteobacteria, *mmoX*
γ-p: Gammaproteobacteria, *mmoX*

- 1 Butane / Propane MO
- 2 SDIMO Methane/Propane MO
- 3 Methane/Propane MO, Halogroup
- 4 Methane MO, *Pseudomonas* sp.
- 5 miscellaneous non-methane MO
- 6 Propane MO, Actinobacteria
- 7 Propane MO, Proteobacteria

Relative abundance
0.1% 100%

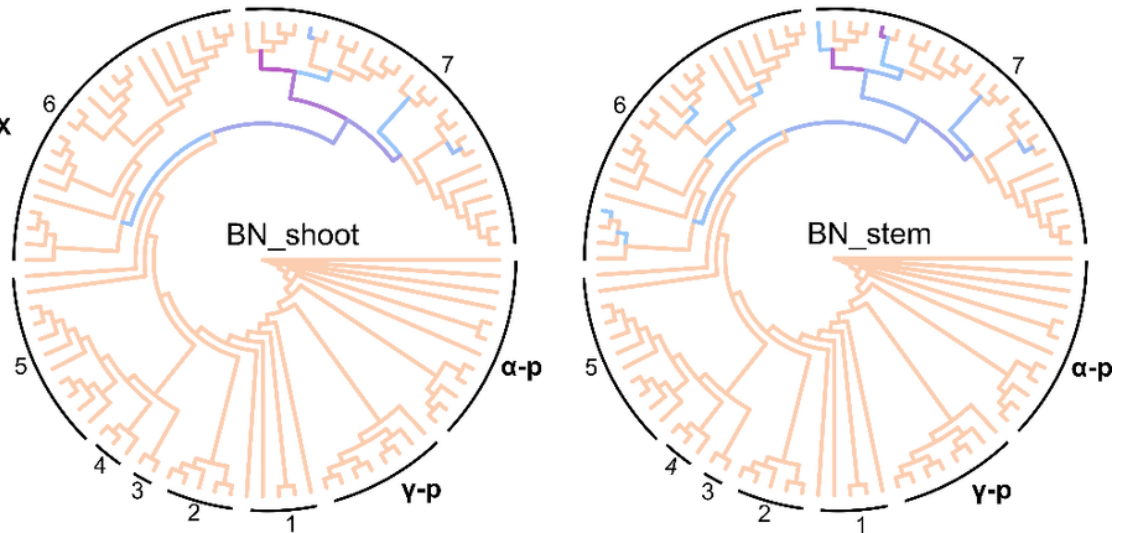


Figure 8

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 S5 and S6. No *mcrA* sequences were detected in *B. nana*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigS4mcrArefDBtree.pdf](#)

- [FigS5pmoArefDBtree.pdf](#)
- [FigS6mmoXrefDBtree.pdf](#)
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