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Aerobic methane emissions from the shoots of
Scots pine

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Academic dissertation

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Plants are recognized sources of methane (CH₄) but plant-mediated CH₄ emissions have mostly been studied on herbaceous species, although also trees are known to emit CH₄. Emissions from tree canopies likely mostly derive from an aerobic, abiotic process. Aerobic CH₄ production from trees has not been thoroughly studied, leaving uncertainties to the global source strength estimates, which vary from 0 to 240 Tg yr⁻¹. Even less is known about how aerobic emissions affect the CH₄ cycles in boreal forest ecosystems, as the environmental and physiological drivers are not fully understood.

In this study, shoot-level CH₄ fluxes of boreal conifer trees were measured outdoors and in the greenhouse, to investigate the environmental and physiological drivers and regulators of shoot-level CH₄ fluxes. Most of the measurements were done from saplings of *Pinus sylvestris* L. (Scots pine), one of the most important tree species of the boreal region of the Eurasian continent, by using chamber enclosure methods with spectral, online greenhouse gas analysers. The measurements were conducted either manually, or with an automated measurement system, developed to overcome issues related to manual measurement techniques.

The shoots of Scots pine showed small but significant emissions of CH₄ in all experimental setups, the emissions were driven by light, enhanced by elevated temperature, and occurred independently from drought and photosynthesis. Solar radiation was a more significant driver of these CH₄ emissions than artificial light with UV-A. These results show that Scots pine canopies have the potential to produce CH₄ in a similar process that has been described before for the foliage of herbaceous plants, but these emissions are smaller than the initial estimates of the aerobic CH₄ source from vegetation. The boreal forest canopies are sources of CH₄ and have the capacity to decrease the CH₄ sink strength of boreal upland forests by ~ 5 %.

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Helsinki, October 2023

Salla Tenhoviirta

LIST OF ORIGINAL ARTICLES

This dissertation is based on the following articles, which are referred to by their Roman numerals in the text:

- I **Tenhovirta S. A. M.**, Kohl L., Koskinen M., Patama M., Lintunen A., Zanetti A., Lilja R., Pihlatie M. (2022). Solar radiation drives methane emissions from the shoots of Scots pine. *New Phytologist* 235: 66–77. <https://doi.org/10.1111/nph.18120>
- II Kohl L., Koskinen M., Polvinen T., **Tenhovirta S. A. M.**, Rissanen K., Patama M., Zanetti A., Pihlatie M. (2021). An automated system for trace gas flux measurements from plant foliage and other plant compartments. *Atmospheric Measurement Techniques* 14: 4445–4460. <https://doi.org/10.5194/amt-14-4445-2021>
- III Kohl L., **Tenhovirta S. A. M.**, Koskinen M., Putkinen A., Haikarainen I., Polvinen T., Patama M., Galeotti L., Mammarella I., Robson T. M., Adamczyk B., Pihlatie M. Radiation and temperature drive diurnal variation of aerobic methane emissions from Scots pine canopy. *In press*.
- IV **Tenhovirta S. A. M.**, Kohl L., Koskinen M., Salmon Y., Paljakka T., Polvinen T., Pihlatie M. Aerobic emissions of methane from the shoots of Scots pine are produced independently of drought or photosynthesis. *Manuscript*.

Author's contributions:

- I The author conducted the gas flux measurements and analysed the data, interpreted the results, wrote the article, and was the corresponding author.
- II The author participated in the building, launching, and troubleshooting of the automated measurement system, conducted the validation measurements of the greenhouse gas fluxes, and contributed to the writing of the article.
- III The author participated in the designing of the experiment, conducted the garden and the greenhouse gas flux measurements, and contributed to the data-analysis, result interpretation and the writing of the article.
- IV The author designed the experiment, conducted the gas flux measurements, analysed the data, interpreted the results, wrote the article and was the corresponding author.

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1 INTRODUCTION

The atmospheric concentrations of methane (CH_4), a powerful greenhouse gas, have increased since preindustrial times, dominantly due to anthropogenic activities such as agriculture, waste management, and the production and use of fossil fuels. CH_4 is also released into the atmosphere from natural sources, e. g. geological activity, permafrost thawing, and wetlands, accounting for $\sim 40\%$ of the global CH_4 budget (Saunio et al., 2020). There are, however, large differences between the top-down and bottom-up estimates of these natural emissions, underlining the uncertainties and incomplete understanding of the CH_4 cycling processes between natural ecosystems and the atmosphere.

Vegetation has recently been recognised as an important component in the global cycles of CH_4 , as plants, both woody and herbaceous have been shown to release CH_4 through several different pathways: Living or dead plants can serve as conduits for CH_4 produced in the soil by transporting it through their tissues into the atmosphere (Pangala et al., 2013, 2015, 2017; Carmichael et al., 2018; Barba et al., 2019), core wood and potentially also other plant tissues may host microbes that produce CH_4 (Yip et al., 2019; Putkinen et al., 2021), and CH_4 can form abiotically and aerobically in plant canopies (Keppler et al., 2006; Brüggemann et al., 2009; Wang et al., 2011).

Despite the new evidence and increasing scientific interest towards vegetation as a source of CH_4 , constructing reliable global upscale estimates still requires more insight to the complex and diverse dynamics behind plan-mediated CH_4 emissions. Considerable uncertainties remain in the global source strength estimates which vary between 5 to 22 % of the total CH_4 budget (Carmichael et al., 2014), thus, although emissions from vegetation are recognised as potentially large, plants are currently not incorporated as a distinct CH_4 source in the global methane budget.

1.1 Forests and trees

According to Food and Agriculture Organization of the United Nations (FAO), $\sim 31\%$ of the global land area is covered by forests, of which $\sim 30\%$ is located in the boreal region (Brandt et al., 2013). Whereas forests are critical components of the global carbon and nutrient cycle, they are also an unignorable potential source of CH_4 .

In terrestrial ecosystems such as boreal forests, the CH_4 flux is the sum of the complex interplay between the CH_4 producing and consuming processes taking place in different ecosystem compartments, both above and below ground. On ecosystem level, these CH_4 dynamics are dominantly determined by the availability of oxygen in the soils: CH_4 is produced by microbial methanogenesis in inundated and anoxic layers, and consumed by microbial methanotrophy when conditions of the soil are oxidised (Le Mer & Roger, 2001). Due to this oxidative CH_4 consumption in the top layers of the soil, well-drained upland forests are normally net sinks of atmospheric (Ito & Inatomi, 2012).

Recently, trees in temperate and boreal upland forest have been shown to emit CH_4 and therefore potentially decrease the sink strength of upland forests (Covey et al., 2012; Machacova et al., 2016; Pitz & Megonigal, 2017; Covey & Megonigal, 2019). The research of trees as components of forest CH_4 cycling has, however, mostly focused on the processes occurring in temperate and tropical wetland forests; in these ecosystems the CH_4 production rates in the soils are high, and trees significantly increase the overall source strength of these

ecosystems by soil-derived CH₄ transport (Terazawa et al., 2007; Pangala et al., 2015, 2017; Jeffrey et al., 2020). Contrarily, in boreal and temperate upland forests the ecosystem CH₄ exchange rates are normally smaller, and result from complex set of interactions between the soil and the trees, many of which are driven by environmental and physiological factors prone to temporal and spatial heterogeneity (Megonigal & Guenther, 2008; Pitz & Megonigal, 2017; Vainio et al., 2021). Consequently, the contribution of CH₄ emissions of trees to the CH₄ exchange of upland forests, especially in the evergreen conifer forests of the boreal region, remains to be fully resolved.

CH₄ emitted from the surfaces of different compartments of trees, such as the stem or shoots, may originate from abiotic or biotic production processes taking place locally within the tissues, or from methanogenesis the soil (Barba et al., 2019; Covey & Megonigal, 2019). Of these source processes, most well-known are the pathways of microbially produced CH₄ emissions from stems: Soil-derived CH₄ can be transported upwards in tree stems along the transpiration stream from which CH₄ diffuses radially through tree stems (Barba et al., 2019). Typically, the emission rates of soil-derived CH₄ are linked to soil water table depth and exhibit a vertical gradient where the emissions decrease along the stem height (Pangala et al., 2013, 2015; Sjögersten et al., 2020; Vainio et al., 2022; Vroom et al., 2022). Stem CH₄ emissions may also originate from production within the stem due to anaerobic conditions and microbial growth, particularly in the heartwood tissue (Covey & Megonigal, 2019; Yip et al., 2019) or from fungal production within or on the surface of the stem (Lenhart et al., 2012).

Whereas the processes involved in the CH₄ emissions of tree stems are relatively well known, release of CH₄ from tree shoots remains the least understood and the most enigmatic process of tree-mediated CH₄ fluxes. Microbial methanogenesis has been suggested as one possible production process of CH₄ emitted from tree foliage, as advances in microbial research methods have revealed new evidence of methanogenic archaea inhabiting conifer needles (Putkinen et al., 2021). Despite the possibility of at least some level of microbial involvement of in canopy level CH₄ fluxes, emissions of CH₄ from tree canopies are thought to originate mostly from an aerobic, abiotic production process. Isolating the aerobic production from the potential microbial sources of CH₄ – both transported and locally produced – in living trees is challenging, and the pathways or quantities of aerobic CH₄ emissions from tree canopies have not been thoroughly investigated.

1.2 Aerobic production of methane in vegetation foliage

Until relatively recently, production of CH₄ in biological systems has been thought to occur by microbial methanogenesis in strictly anaerobic conditions. Due the highly oxic conditions of leaves, resulting from photosynthesis, vegetation foliage was not previously considered a possible site of CH₄ production. This conception was challenged by the discovery of aerobically and non-enzymatically produced CH₄ emissions from terrestrial plants (Keppler et al., 2006), a topic subject a scientific debate for some time after its release and not to the least due to the massive scale of the initial estimates on the strength of this potential source (Kirschbaum et al., 2006, 2007; Dueck et al., 2007; Evans, 2007; Beerling et al., 2008; Kirschbaum & Walcroft, 2008; Nisbet et al., 2009). The plant scientific community has, since then, reached a consensus on the existence of aerobic CH₄ release from plants, as the phenomenon has been confirmed by experiments of plant biomass and living plants

(Wang et al., 2008, 2009; Brüggemann et al., 2009; Qaderi & Reid, 2009, 2011), but the extent of the emissions of aerobically produced CH₄ is yet to be determined.

Several plant compounds have been identified as possible chemical precursors for CH₄ in aerobic conditions. Laboratory experiments have shown that CH₄ may originate from structural or non-structural plant compounds such as pectin (Keppler et al., 2008; McLeod et al., 2008; Bruhn et al., 2009), lignin, cellulose (Vigano et al., 2008), methionine (Althoff et al., 2014; Lenhart et al., 2015), or surface wax (Bruhn et al., 2014), under UV-radiation and elevated temperatures. In addition to such environmental stressors, in living plants, also drought and wounding of the tissues have been shown to induce CH₄ emissions from plant foliage (Qaderi & Reid, 2011; Wang et al., 2011; Lenhart et al., 2015).

The findings from both purified plant compounds and fresh plant materials link aerobic CH₄ production to the increased activity of reactive oxygen species (ROS), especially OH radicals (Messenger et al., 2009a,b), which are produced in plant tissues during abiotic stresses like drought and heat (Xiong et al., 2002). Although the prevailing perception is that ROS, related to stress, is in fact the proximal driver of aerobic CH₄ production in plants, light might be an important component in the ROS-induced release of CH₄ as indicated by the studies reporting increased CH₄ emissions when exposing plants to UV-B (Bruhn et al., 2009), solar radiation (Keppler et al., 2006) or blue light (Martel & Qaderi, 2019).

Although the chemical pathways, potential precursors, and drivers of aerobically produced CH₄ have been extensively studied in laboratory settings, research of aerobic CH₄ emissions from living, intact plants in ambient outdoor conditions remains sparse, and even more so from the trees. Because of this, it has not been established whether tree canopies are a source of aerobically produced CH₄ in the boreal regions, to which extent aerobic CH₄ emissions from conifer trees are induced *in situ* by environmental factors within the ambient range, or how these emissions and their source processes are affected by tree physiological processes and functions, such as for example stress-tolerance or stomatal conductance. This lack of knowledge currently prevents upscaling canopy emissions to global scale and forecasting the future development the CH₄ exchange of boreal forests in the changing climate.

Only a few previous publications have reported *in situ* measurements of CH₄ from canopies of conifer trees, some showing small CH₄ emissions (Machacova et al., 2016; Vainio et al., 2022), while others show considerably large uptake (Sundqvist et al., 2012; Gorgolewski et al., 2023), or no detected fluxes (Takahashi et al., 2012). Although to some extent the differenced may explained by the highly dynamic nature of CH₄ fluxes of upland forests (Covey & Megonigal, 2019), it is possible that the largest of these discrepancies between the reported uptake and emission rates derive from measurement uncertainties of shoot-level trace-gas fluxes (Kohl et al., 2019).

1.3 Technical aspects of quantifying methane emissions from shoots

One of the main reasons to the delays in the defining of the aerobic CH₄ emissions of vegetation or tree canopies are the technical difficulties related to the detection of trace-level gas fluxes from vegetation foliage *in situ*, as the exchange rates of aerobically produced CH₄ at leaf surfaces are small in respect to the atmospheric background mixing ratio of CH₄. With the precision of current gas analysis methods, these emissions can only be detected by enclosing the studied shoot inside a closed chamber and repeatedly measuring the CH₄ mixing ratios of the chamber headspace. In the past, the mixing ratios were quantified by gas

chromatography from repeatedly drawn samples of the chamber headspace. In order to reliably detect the changes in the mixing ratios of CH₄, the shoots chamber incubations lasted for several hours (Machacova et al., 2014; Vainio et al., 2022). During these long incubations the conditions inside the chamber become significantly altered in respect to the ambient surroundings, due to the warming of the chamber air, depletion of CO₂ by photosynthesis, and increased humidity due to transpiration. The altered chamber conditions are likely to affect the physiological functions of the measurement shoot, and thus add uncertainties to conclusions made from these measurements.

Nowadays, CH₄ exchange of the shoot enclosed in the measurement chamber can be measured with optical, high-frequency online analysers, allowing significantly shorter (ca. 10 min) incubation times and higher measurement precision as the older methods that rely on repeated manual gas sampling. Online chamber measurement techniques can be further improved by replacing the photosynthesised CO₂ in the shoot chambers, drying the headspace air from excess humidity, and preventing overheating of the chamber headspace air. Conducting the shoot-level gas-flux measurements in setups where these functions and the switching between measurement shoots are automated, allows large numbers of replicate measurements and improves the method detection limit, when compared to manual measurements. Establishing such automated measurements in the field to meet the need for continuous, long-term canopy measurements, to characterise the seasonality of canopy-level CH₄ fluxes, and finally determine their part in the ecosystem level CH₄ exchange.

2 AIMS OF THE STUDY

The aim of this study was to provide new insight to the shoot-level CH₄ emissions of Scots pine – one of the most common conifer species in the Nordic boreal forests – and to define the environmental drivers, temporal patterns, and tree-physiological regulators of these emissions under both outdoor ambient and semi-controlled greenhouse conditions.

To increase the mechanistic understanding of the processes involved in the shoot-level CH₄ fluxes of boreal trees and to estimate the contribution of aerobic canopy emissions to the boreal forest CH₄ cycles, this synthesis will answer the following research questions:

- 1) Are the shoots of Scots pine a source of CH₄ (I, III)
- 2) What are the environmental and tree-physiological determinants of the shoot-level CH₄ emissions (I, III and IV)
- 3) What do they reveal about the source process? (I, III and IV)
- 4) How do these emissions compare to the global estimates of the aerobic CH₄ source (III)

Furthermore, the dissertation introduces the novel, automated chamber measurement system for trace gas fluxes, providing technical solutions for establishing urgently needed continuous field measurements of canopy CH₄ fluxes (II).

Study I focused on the shoot-level CH₄ emissions and their environmental and physiological drivers in the spring, to investigate whether these emissions are light and temperature-driven or if they increase along the increased physiological activity during the spring-awakening of the trees. In study III, the diurnal cycles and the responses to light and temperature of shoot-level CH₄ fluxes were defined from outdoor and greenhouse measurements; to quantify and compare the CH₄ fluxes in different settings, these fluxes were also investigated along with field data from mature trees, and finally, to compile a global upscale estimate of the CH₄ aerobic emission. In study IV, to constrain the chemical source of the shoot-level CH₄ emissions from plant-physiological processes, the CH₄ production process of was isolated from photosynthesis-associated biochemical processes and transpiration in a drought-manipulation experiment conducted in the greenhouse.

3 MATERIALS AND METHODS

3.1 Plant material and measurement sites

3.1.1 Measurement saplings

All measurements apart from the field measurements of study III were conducted on 2-3-yr-old nursery saplings of *Pinus sylvestris* L. (Scots pine) or *Picea abies* (L.) Karst. (Norway spruce). The saplings were obtained from commercial nurseries (Huutokoski Ltd and Harviala Ltd) in the fall before the measurements campaigns. They were delivered either in 15 L pots (II, IV), or as balled and burlapped specimens in which case they were planted into 20 L pots with mineral soil collected from the Hyytiälä research forest (I/2019 and III, garden measurements), or commercial peat and humus mixture (I/2020). All saplings overwintered outdoors in their pots until the start of the experiments, planted into a sand bed. A new set of saplings was used for each of the experiments, except for the garden measurements of study III.

3.1.2 Garden measurements

Study I and the garden measurements of study III were conducted outdoors in the Viikki Plant Growth Facility (lat. 60°13'40"N, long. 25°01'05"E), in the garden of a sheltered courtyard between the glasshouses, where the saplings remained in the sand bed. Four Scots pine and three Norway spruce were measured each year in study I. For III garden measurements, the measured Scots pine saplings were the same for I/2019.

3.1.3 Greenhouse measurements

The greenhouse experiments were conducted indoors in the Viikki Plant Growth Facility in a greenhouse growth compartment. In study II the measurements were made on two branches of one Scots pine sapling, whereas studies III and IV each had six Scots pine saplings that were placed in groups of three onto two growing benches. Before setting up the measurement system for the experiments, the saplings were let to acclimatise to conditions for three or four weeks (III and IV, respectively). After the acclimation, period the saplings were watered manually 1 L per week (II and III), or automatedly irrigated 300 mL every morning (IV). For studies II and III, natural light was let into the compartment through the glass ceiling and artificial lighting was provided 15 hours per day by LED lamps (B100/AP67; Valoya Oy) at ca. 15 cm above the measured shoots and UV-A lamps (QUV© UVA-340 fluorescent tube; Q-lab Corporation) at ca. 20 cm above the measured shoots. For study IV, the ceiling was covered with a blackout curtain, and a 9/15hour light-dark cycle was controlled by the LED lamps together with HPS lamps hung from the ceiling.

3.1.4 Field measurements

The field measurements in the study III were conducted in a Scots pine dominated forest stand around the SMEAR II measurement station in Hyytiälä (lat. 61°51'52"N, long. 24°17'43"E). The site is located on a mineral soil, 181 m above sea level, with the mean annual temperature of 3.5 °C and precipitation 711 mm in 1980-2010 (Pirinen et al., 2012). The forest was established by sowing after a clear-cut in 1962, and by the time of the

measurements, the trees had reached the height of 18-20 m. Thus, measurements were set up on a scaffold tower that allowed access to the canopies of three mature Scots pine trees.

3.2 Gas flux measurements

Two types of chamber measurement setups were used in quantifying the greenhouse gas exchange of the tree shoots, and the background emissions potentially produced by chamber materials: the outdoor measurements in the garden (I and III) and in the field (III) were done manually, whereas the greenhouse measurements (II-IV) were conducted with an automated chamber system (Fig. 2 and Fig. 3). In addition to the shoot-level chamber measurements, for study III, above-canopy eddy covariance (EC) measurement data was used to determine the ecosystem-level CH_4 flux.

All the chamber measurements of CH_4 fluxes, as well as the manual measurements of CO_2 , were done in a non-steady-state setting, where the gas sample is circulated between the measurement chamber and a greenhouse gas (GHG) analyser in a closed loop. In this setting the gas fluxes are directly estimated from the development of the mixing ratios in the sample gas. In the automated greenhouse measurements, CO_2 and H_2O were also measured in a steady-state flow-through setup, where the gas flux is quantified by measuring the difference in their mixing ratios in air entering and leaving the measurement chamber. In both measurement setups, the chamber is ventilated with ambient air between the measurements.

3.2.1 Manual shoot flux measurements

The manual measurement system used in the garden and field measurements in studies I and III consisted of transparent cylindrical shoot chambers and a portable GHG analyser (UGGA; ABB Ultraportable Greenhouse Gas Analyzer; Los Gatos Research, San Jose, CA, USA). The shoot chambers (Fig. 1 A) were constructed of a circular opaque bottom and top pieces made of polytetrafluoroethylene (PTFE) held together by four metal bars, which formed a permanently installed frame around the measured shoot. The bottom piece was equipped with a fan to mix the headspace air during the measurement and adhesive putty (Blu Tack; Bostik



Figure 1. Manual (A) and automated (B) shoot chambers used in the greenhouse gas flux measurements. In (B), the PAR sensor is placed onto the lid of the measurement chamber.

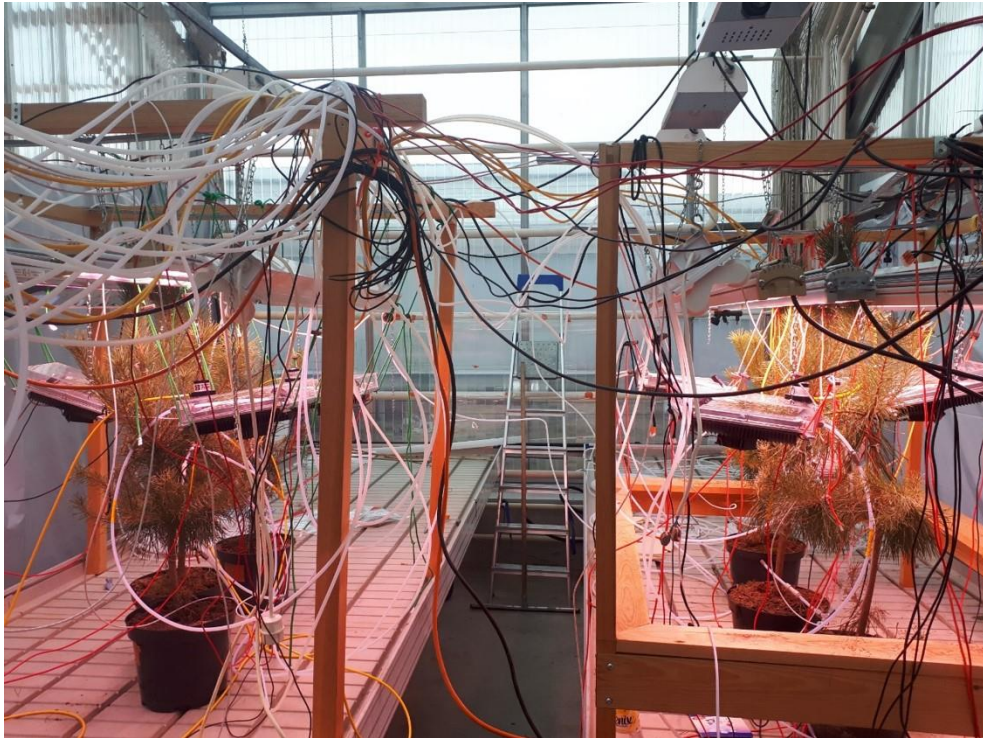


Figure 2. The chamber setup of the automated gas flux measurements in the greenhouse. LED panels and fixtures for the UV-A fluorescent tubes illuminate the measurement chambers (III). In study IV, sunlight was blocked by a blackout curtain covering the ceiling and walls, and the high pressure sodium (HPS) lamps above the wooden measurement frames were switched on.

SA, Colombes, France) to seal the opening for the stem. During each flux measurement, the chamber frame was covered with a UV-transparent fluorinated ethylene propylene-foil (FEP), to enclose the shoot into a 5.2 L chamber space. The chamber closures were 15 min in the field measurements of study III and 7-10 min in the garden measurements of studies I and III.

3.2.2 Automated shoot flux measurements

The automated chamber system used in the greenhouse measurements (II-IV) consisted of a custom-built switching board to control the measurements together with GHG analysers for the CH₄ and CO₂ flux measurements in the closed-loop (Picarro G2301; Picarro Inc. Santa Clara, CA, USA) and for the CO₂ and H₂O flux measurements in the flow-through (Li-850; Li-cor Biosciences, Lincoln, NE, USA) setups. During the closed-loop measurement, the CO₂ removed by photosynthesis was replaced by injections of pure CO₂ at 400 ppm, and the build-up of transpired water was prevented with a membrane dryer (Nafion MD-050-12S-2; PermaPure LTD, Lakewood, NJ, USA). Here, the measurement closures of the closed-loop setup were 20 min long each, while the flow-through measurements lasted for 10 min.

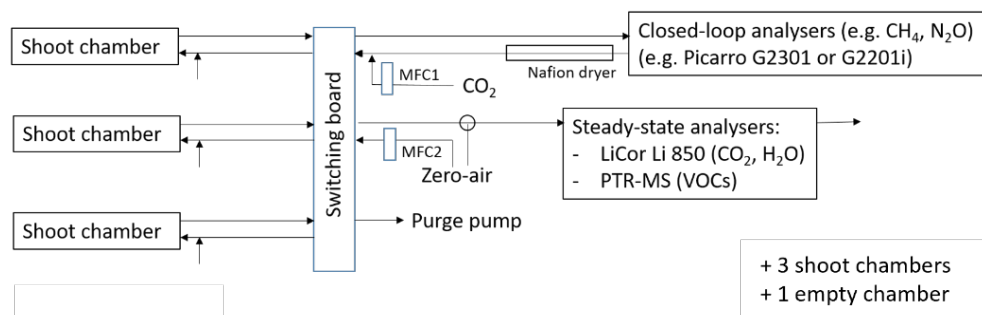


Figure 3. The instrumental setup of the automated chamber system used in greenhouse gas flux measurements from the shoots in studies II-IV

The box-shaped shoot chambers of the automated chamber system (Fig. 1 B, custom built by Toivo Pohja Tmi, Juupajoki, Finland), consisted of an aluminium bottom and a rear plate, and a removable, UV-transparent acrylic glass cover which was attached to the base with screws. As with the manual chambers, inside each automated chamber was a fan to circulate headspace air, and the shoot entry was sealed with adhesive putty. In addition, the chamber bottoms were equipped with Peltier cooling elements, which were used for lowering the chamber temperature during the measurements in studies II and III.

3.3 Ancillary measurements

3.3.1 Temperature and light

For the garden measurements (I and III), the ambient air temperature ($^{\circ}\text{C}$), global radiation (W m^{-2}), and photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) were obtained from the weather station of the Viikki Plant Growth Facility, which is positioned on the roof at ca. 7 m height. There, these variables are continuously measured with an outside temperature sensor, a CM3P thermopile radiation sensor (both manufactured by Priva Agro, De Lier, The Netherlands) and an LI-190 PAR sensor (Li-Cor Biosciences)

In the greenhouse measurement system (II-IV), the temperature of the air inside each of the measurement chambers, as well as the room temperature of the compartment, were continuously measured with Pt 100 temperature probes (SKS Automaatio Oy, Vantaa, Finland). PAR was measured with sensors (Kipp&Zonen PQS1; OTT HydroMet, Delft, The Netherlands) placed on top of each measurement chamber.

3.3.2 Soil moisture (IV)

The water status of the soil in the growing pots were followed in study IV, where saplings were exposed to drought. The volumetric soil water content (m^3/m^3) was determined from the soil dielectric number, continuously from four points in each growing pot with 5TE sensors (METER Group AG, München, Germany), using an equation that defines the soils water content of cultivated peatland soils (Myllys & Simojoki, 1996):

$$\theta_v = -7.33 \cdot 10^{-2} + 4.17 \cdot 10^{-2} K_a - 8.01 \cdot 10^{-4} K_a^2 + 5.56 \cdot 10^{-6} K_a^3, \quad (1)$$

where θ_v is the volumetric water content of the soil, K_a is the dielectric number, and the parameters are pooled from different peat types.

Soil water potential (SWP) was measured with a with an electronic pressure meter (Soil Measurement Systems, Huntington Beach, CA, USA) ca. twice a week from 30 cm long tensiometers (Soil Measurement Systems) placed in each of the growing pots. The SWP was determined as the measured pressure (hPa) subtracted by the height of the water column (30 cm).

3.3.3 Shoot sample processing and growth estimation

To obtain the biomasses and needle leaf areas of the foliage, the measured shoots were collected immediately after each measurement campaign, after which the 1-yr-old (Y_1) and Y_0 needles were separated from the branches. The needle leaf areas were quantified by scanning either all the needles (IV) or a subset of needles which was then scaled to the whole branch by weight (II and III). The needles were then oven dried (72 h at 65 °C in I-III and 24 h in IV) and weighed.

The measurements of studies I and IV begun before the onset of new growth and lasted through the bud burst and shoot elongation, thus the biomasses of the measured shoots significantly increased during the campaigns. To consider this effect of Y_0 growth in the biomass-scaled gas flux calculations, in these studies, the biomasses were estimated for each measuring day based on the relative growth of the Y_0 shoots. For this, needle biomass was assumed to increase proportionally with the elongation of the Y_0 shoot. The Y_0 growth was either manually measured weekly (I/2020, IV), or estimated (I) with a temperature-based growth model (Schiestl-Aalto et al., 2013).

3.3.4 Tree-physiological measurements

Foliar water potential of the needles (NWP) was measured in studies I and IV to assess the water status of the tree saplings. For study I, the measurement was done in the morning of each of the measurement days, and for IV in five afternoons every 2-3 days during the drought- and rewatering period. The measurements were prepared by first cutting a pair of needles from each of the sapling, which were sealed into small plastic bags to avoid evaporation. Before the measurement, a fresh cut was applied to the needle sample, after which the NWP was measured with a pressure chamber (I505D-EXP; PMS Instrument Co, Albany, OR, USA).

The photosynthetic potential of the saplings were defined as a measure of spring recovery (I) by measuring the Chlorophyll *a* (Chl*a*) fluorescence to analyse the maximum efficiency of photosystem II (PSII, F_v/F_m). The measurement was done on the same days as the NWP measurements but on different saplings than those measured for their greenhouse gas exchange. First, the photosynthetic reactions of the measured needles were stopped by enclosed in lightproof clips for an hour. The Chl*a* fluorescence was then measured using the Field Fluorescence Monitoring System (FMS2+; Hansatech, King's Lynn, UK), from which the F_v/F_m is then calculated as

$$F_v/F_m = \frac{F_m - F_0}{F_m}, \quad (2)$$

where F_0 is the minimum fluorescence measured in low-intensity light, and F_m is the maximum fluorescence measured during brief (< 1 s) flashes of saturating light.

3.3.5 Drought treatment and control measurements (IV)

To investigate the effect of drought to the CH₄ fluxes from Scots pine, the greenhouse gas fluxes of Scots pine saplings were continuously measured with the automated chamber system under drought. For this, three saplings were treated with drought and three remained as watered controls throughout the measurements. The drought was initiated by removing the automated irrigation from the treatment saplings. The progression of the drought was monitored based on the drying of the growing soil (SWP and soil water content), the eventual decline in the fluxes of CO₂ and H₂O, and water status of the needles (NWP). The drought treatment was stopped when the net CO₂ uptake and transpiration (H₂O flux) rates of the treatment saplings had decreased to near zero, by first watering all the saplings to field capacity and then restoring the automated irrigation to the treatments saplings.

3.4 Data analyses

3.4.1 Data preparation

Before the calculating the change of gas mixing ratios over time (dC/dt) to be used in flux calculations, each of the measurement closures were examined graphically. For this, the mixing ratios of the gases were plotted as the as a function of time. The start and end points of the closures were then adjusted on demand to omit values clearly caused by mixing of the headspace gases at the beginning of the closure, leakage of the chambers (manual measurements in studies I and III), or inaccuracies in the recorded start and end times.

For the CH₄ flux measurements conducted with the automated chamber system in the greenhouse (II-IV), the mixing ratios of CH₄ were corrected for the dilution by the CO₂ injections. For study III this was done by an injection model (described in full detail in study II) in which the corrected CH₄ mixing ratios were determined as

$$[CH_4]_{corr}(t) = \frac{[CH_4]_{raw}(t)}{1 - [CO_2]_{inj}(t)}, \quad (3)$$

where $[CH_4]_{corr}(t)$ is the corrected dry CH₄ mixing ratio at time point (t), $[CH_4]_{raw}(t)$ is the measured dry CH₄ mixing ratio, and $[CO_2]_{inj}(t)$ is the mixing ratio of the injected CO₂ at time point t .

In study IV the dilution by CO₂ injections was considered by discarding approx. 250-300 seconds of data from around each CO₂ injection and calculating the change in the dC/dt of CH₄ and CO₂ based on the remaining data segments. This was done by using the least squares method to define the best linear fit for the dC/dt as

$$C_t = \beta_0 t - t_0 + \beta_1 \cdot segment_1 + \dots + \beta_n \cdot segment_n, \quad (4)$$

where C_t is the mixing ratio of the sample gas at time t , $t - t_0$ is the time from the beginning of the measurement (seconds), $segment_{1..n}$ are dummy variables signifying which

observations belong to each separate segment of the measurement, and β_0 is the overall average dC/dt during the measurement.

3.4.2 Flux calculations

The automated correction of H₂O spectral interference by the GHG analyser (UGGA) was inaccurate during the measurements for study I. To correct this error in the shoot flux measurement data, the effect of increased air humidity to the apparent CH₄ mixing ratio measured by the analyser was empirically quantified: First, an empty measurement chamber was flushed with dry air, after which ca. 1 ml of water was injected into the measurement loop. This measurement was repeated six times. A correction factor f was determined as the regression slope between the measured mixing ratios of dry CH₄ and H₂O:

$$[CH_4]_{dry,corr}(t) = [CH_4]_{dry,raw}(t) - [H_2O](t) \cdot f, \quad (5)$$

resulting in a value $-9.122 \cdot 10^{-7}$ ppm CH₄ ppm⁻¹ H₂O. This value was used to correct for the CH₄ mixing ratios in the analyser data of study II before calculating the dC/dt .

For all chamber measurements of CH₄ and the non-steady-state closed-loop measurements of CO₂ in study IV, the flux rates were calculated using the least squares method that defines the best linear fit for the dC/dt , after which for the shoot measurements, the flux rates were calculated and scaled to foliar dry mass as

$$F = \frac{dC}{dt} \cdot \frac{V}{m_{needles}} \cdot \frac{p}{R \cdot T}, \quad (6)$$

where F is the gas flux, m is the dry foliar needle mass in g, V is the chamber volume in L, p is the atmospheric pressure (assumed 10132.5 Pa), R is the ideal gas constant (8.31446 J mol⁻¹ K⁻¹) and T is the temperature in K.

The CO₂ flux in study I was estimated using an exponential function fitted to the mixing ratio over time (t):

$$C(t) = C_{lim} + (C_0 - C_{lim}) \cdot e^{-k \cdot t}, \quad (7)$$

where C_0 is the initial mixing ratio, C_{lim} is an asymptote, e is Euler's number (2.71828), and k is a rate constant. The initial dC/dt was calculated as the initial slope of

$$\frac{dC}{dt} = -k \cdot (C_0 - C_{lim}). \quad (8)$$

For the steady-state flow-through measurements of H₂O and CO₂ the dC/dt was used to calculate the flux rate as

$$F = \frac{\text{flow rate}}{m} \cdot (C_{out} - C_{in}), \quad (9)$$

where the C_{in} and C_{out} are the gas mixing ratios of air going in and out of the measurement chamber, and flowrate is 850 ml min⁻¹. In study III the fluxes (F_a) were also scaled to foliar needle area LA , in which case the equation was

$$F_a = \frac{\text{flow rate}}{LA} \cdot (C_{out} - C_{in}). \quad (10)$$

To calculate the stomatal conductance for study III, saturation partial pressure water vapour ($p_{w,s}$) was first calculated as

$$p_{w,s} = 0.61365 \cdot e^{17.502 \frac{T}{240.97+T}} \cdot 10^3, \quad (11)$$

after which the vapour pressure deficit (VPD) was calculated as

$$VPD = \frac{(p_{w,s} - p_w)}{p_a}, \quad (12)$$

where p_a is the ambient pressure (assumed 10132.5 Pa) and p_w is the measured partial pressure of water vapour in p_a . Stomatal conductance g_s was then calculated from the VPD and F_a as

$$g_s = \frac{F_{A(H_2O)}}{VPD}. \quad (13)$$

Finally, the shoot CH_4 emissions were corrected for background CH_4 emitted by chamber materials by subtracting the background fluxes from the shoot chamber measurements before scaling to $m_{needles}$ or LA. This correction was based on the CH_4 flux measurements of the empty chambers: for the manual measurements (I and III), the empty chamber flux was determined by testing for sensitivity to environmental drivers by linear analyses of CH_4 flux as functions of global radiation and temperature and using this linear model to predict a background flux for each of the shoot chamber closures (I/2019). When such sensitivity was not detected (I/2020 and III), the subtracted chamber background was determined as the mean CH_4 of the empty chamber fluxes measured over the campaigns.

For automatic measurements, the empty chamber background was either determined as the CH_4 flux of the empty chamber measurement done closest in time to the shoot chamber measurement (IV), or as the mean empty chamber CH_4 flux (II and III).

3.4.3 Detection limits

Method detection limits (MDL) for single chamber closures in studies I, III and IV were determined as three times the standard deviation (SD, I and IV) or the Allan deviation (III) of the CH_4 flux of the empty chamber measurements. Basing the MDLs on the measurements of empty chambers instead of the measurement accuracy of the analysers alone, allowed to account for also the possible unidentified measurement uncertainties, such as instrument drift or small leakages in the measurement systems (Werle et al., 1993). To be used in respect to the shoot CH_4 flux measurements, each campaign's MDL was scaled to the respective average shoot dry masses of each campaign. These detection limits decrease with \sqrt{n} of the repeated measurements.

3.5 Statistical analyses

The CH_4 fluxes of different chronological and treatment-control subgroups in studies I and IV were compared by testing the mean CH_4 fluxes between these subgroups with linear mixed-effects analyses using the individual measurement days (IV) and individual trees (I and IV) as random intercepts. For study IV, the temperature inside the measurement chamber was used as a fixed effect to separate its effect from the drought treatment. The significances

of the differences between the subgroups in study I was tested using multiple comparisons of the means.

The effects of environmental variables or the uptake rate of CO₂ to the CH₄ fluxes (I and IV) were tested with a linear mixed-effects analyses, where the models were used to express the correlations of the CH₄ fluxes as a function of global radiation (I), CO₂ fluxes, PAR, and temperature (I and IV). In study IV the correlation of CH₄ flux and temperature was done separately on day and night measurements. As with the temporal subgroups, a random effect was added for individual trees to solve the non-independency of data.

Data analyses were conducted using the R software version 4.2 (R Development Core Team 2015), with the additional packages ‘nlme’ v. 3.1-157 (Pinheiro et al., 2021), ‘multcomp’ v. 1.4-20 (Hothorn et al., 2008) and ‘lme4’ v. 1.1-31 (Bates et al., 2015).

4 RESULTS

4.1 Shoot-level methane fluxes

The shoots of Scots pine emitted CH₄ in all the experimental setups, outdoors as well as in the greenhouse (Fig 4). The highest emissions ($8.34 \pm 0.64 \text{ ng CH}_4 \text{ g}^{-1} \text{ DW h}^{-1}$, mean \pm SE) were observed during sunlit conditions in the garden (I), whereas the night-time fluxes in both garden and greenhouse (I, III, IV) were not significantly different from zero when all greenhouse night-time fluxes were grouped together. In contrast, in study IV, where the night-time fluxes of the drought experiment were assessed alone, there was a very small ($0.23 \pm 0.015 \text{ ng CH}_4 \text{ g}^{-1} \text{ DW h}^{-1}$) but statistically significant CH₄ night-time emission.

The emissions measured during overcast sky in the garden were smaller ($0.55 \pm 0.44 \text{ ng CH}_4 \text{ g}^{-1} \text{ DW h}^{-1}$) than those measured in the forest in varying cloud conditions or in the greenhouse under UV-A (2.49 ± 0.56 and $2.15 \pm 0.04 \text{ ng CH}_4 \text{ g}^{-1} \text{ DW h}^{-1}$, respectively). The lowest CH₄ fluxes were measured in the greenhouse in steady lighting conditions, during the pre- and post-drought periods of study IV, when the shoots showed CH₄ uptake in some of the measurement days (min. daytime mean flux $-0.83 \pm 0.39 \text{ ng CH}_4 \text{ g}^{-1} \text{ DW h}^{-1}$, see also Fig. 7). Apparent CH₄ uptake was also observed in individual measurements of the in study I on both Scots pine and Norway spruce.

4.2 Light and temperature

CH₄ fluxes were affected by the light in both ambient conditions and under artificial lighting, however, the emissions measured under full sunlight were up to six times larger than those measured under artificially provided PAR in the greenhouse. In the outdoor measurements under natural light the CH₄ emissions correlated positively with global radiation and PAR (II), and in the greenhouse (III) the CH₄ fluxes followed the diurnal pattern of the steady night-day-cycles, showing emissions during light hours and no fluxes during dark hours.

In outdoor ambient conditions the CH₄ emissions correlated with temperature in direct sunlight, whereas during cloudy conditions (PAR ≤ 500) there was no correlation in ambient temperatures between 0 and 15 °C (I). In the higher temperatures of the greenhouse (between 15 and 40 °C), CH₄ emissions correlated with temperature even at lower light levels (PAR $\sim 500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ + UV-A, and PAR $800 \mu\text{mol m}^{-2} \text{ s}^{-1}$, III and IV, respectively). Moreover, in study IV where the shoots were a source of small emissions of CH₄ also in the dark, both day and night-time fluxes of CH₄ correlated positively with temperature.

In the outdoor measurements the slope of the PAR:CH₄ correlation increased with temperature (I; Fig. 5b), while in the greenhouse at a given PAR the CH₄ emissions increased with temperature (III; Fig. 6), demonstrating an interaction effect of temperature and light to the CH₄ emissions.

4.3 Temporal variation

Shoot-level CH₄ fluxes varied between days (I, IV), and the daily variation of the daytime shoot-level CH₄ fluxes was biggest in the outdoor ambient conditions (I). In the greenhouse measurements (III, IV), under constant, artificial lighting and freely fluctuating temperature, the daily variance of the daytime fluxes was smaller, but still proportionally prominent to the

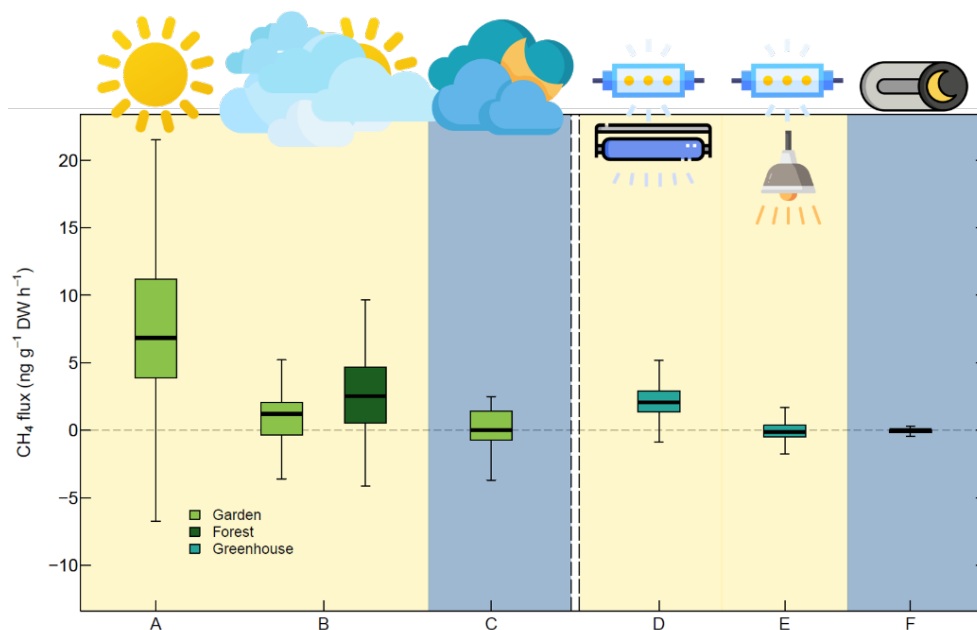


Figure 4. Variability of methane (CH_4) fluxes (ng g^{-1} dry weight (DW) h^{-1}) of Scots pine shoots in different lighting and growing conditions in the garden, forest field site and greenhouse. (A) Fluxes in sunlight (photosynthetically active radiation; PAR 500-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), (B) overcast weather (PAR 50-500) in the garden or varying cloud conditions in the forest, (C and F) in the night-time (PAR < 50), under LED and UV-A lighting (PAR ~ 500), and under LED and high-pressure sodium lamp (HPS) lighting (PAR ~ 600). Vector graphics are designed by Freepik.

overall average fluxes. For the night-time CH_4 fluxes, the variance between days was notably smaller during both continuous measurement campaigns in the greenhouse (III, IV), along with the relatively stable temperatures during the dark periods. No clear connection between the onset of growth and spring awakening of the trees could be observed (I) over a 3-month period in the spring.

The shoot-level CH_4 fluxes of all around-the-clock measurements followed pronounced diurnal cycles (III), where an apparent shift in the flux dynamics occurred along the increased physiological activity in the morning, the highest emissions were timed to the afternoon hours, and a fast decline of emissions occurred after the dying of the light in the evening. In the outdoor garden measurements (Fig. 6 A-D) the CH_4 emissions followed a bell-shaped curve similar to those of PAR and temperature in the daytime, whereas in the greenhouse (Fig. 6 E-H) the CH_4 emission pattern was more box-shaped as the onset and decline of the emissions followed the bimodal temporal distribution of the artificial lighting and UV-radiation. Moreover, despite the relatively steady PAR (Fig. 6 F) and UV-radiation throughout the daytime, the highest CH_4 emissions were measured in the afternoon. Interestingly, the emission pattern was distinct from the triangular pattern of the temperature.

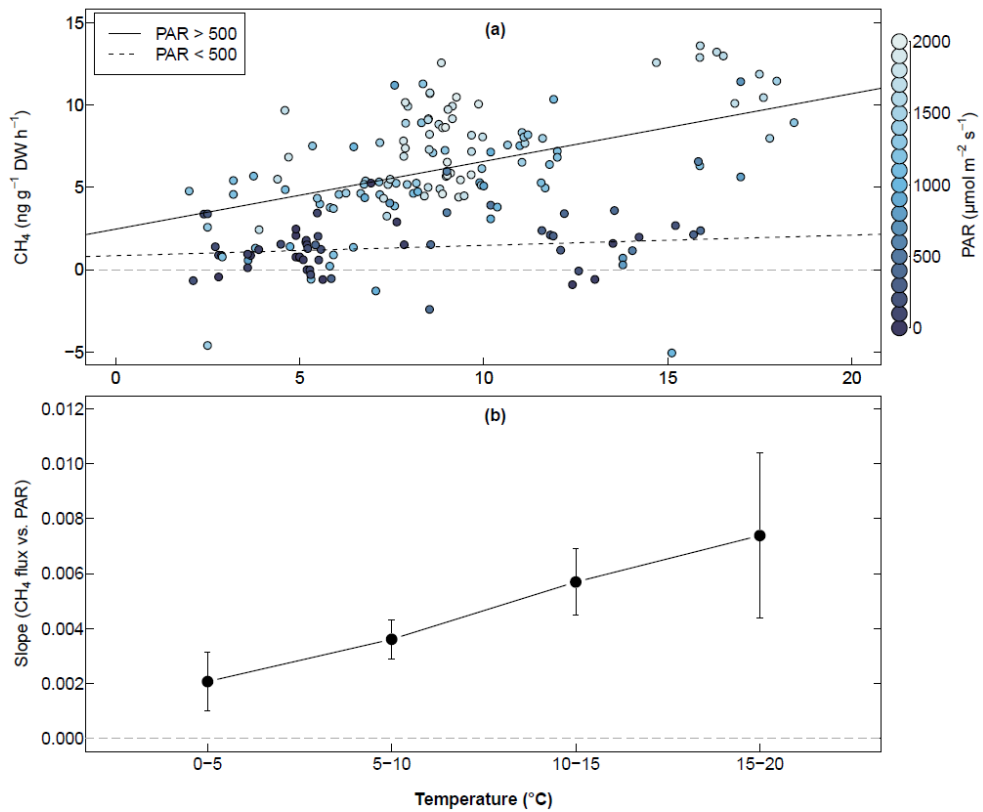


Figure 5. Linear model fits showing the relationship of ambient air temperature ($^{\circ}\text{C}$) and methane (CH_4) emissions (ng g^{-1} dry weight (DW) h^{-1}) from the shoots of Scots pine, in groups of low (dashed line, $y=0.063x+0.847$, $p=0.19$, $R^2=0.032$) and high (solid line, $y=0.412x+2.46$, $p<0.001$, $R^2=0.19$) photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) (a). The slopes (\pm standard error) of the mixed effects linear model fits for the CH_4 and PAR correlation, divided into four temperature bins (b). The grey horizontal lines indicate the 0-line for CH_4 emissions and slopes.

The diurnal pattern of the shoot CH_4 fluxes observed during the drought-experiment (IV), without a UV-light source or sunlight reaching in the compartment, was different from those observed in the garden and in the greenhouse under UV-light. In contrast to the no net flux of CH_4 detected in the night-time and to the emissions observed throughout the day in other studies, in study IV the shoots showed small but consistent CH_4 emissions when the lights were off, and a rapid onset of CH_4 uptake in the morning. As in the garden and in the greenhouse under UV-light (I and III), the shoots emitted CH_4 in the afternoon.

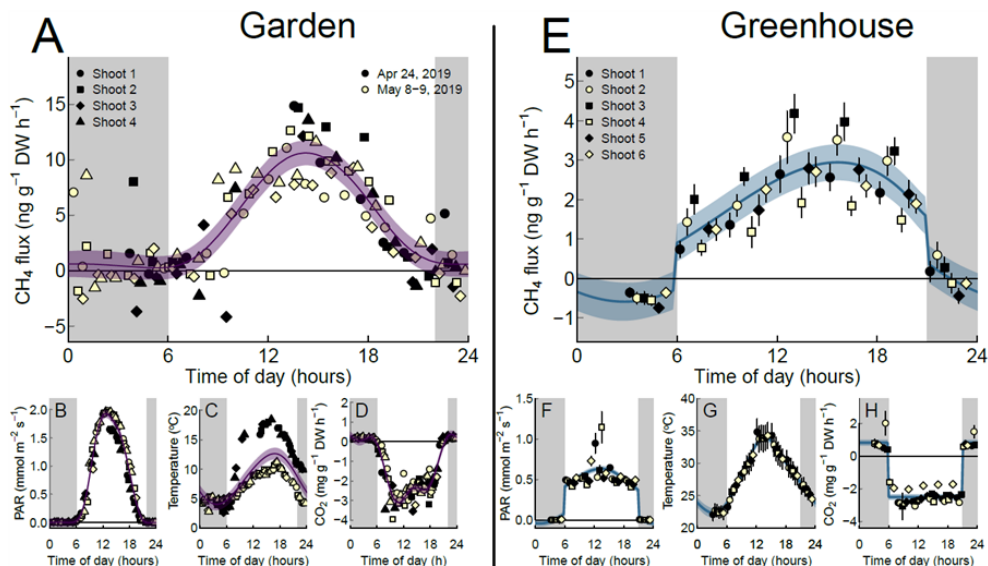


Figure 6. Diurnal patterns of shoot-level methane (CH_4) fluxes of Scots pine saplings together with photosynthetically active radiation (PAR, $\text{mmol m}^{-2} \text{s}^{-1}$), air temperature ($^{\circ}\text{C}$), leaf carbon dioxide (CO_2) exchange in the garden (A-D) and greenhouse (E-H) experiments. Points indicate the individual measurements (chamber closures) (A-D), and the mean of 33 measurement (one per day) (E-H). Error bars indicate 95% confidence intervals (2 standard errors) (E-H). Purple and blue lines represent a smoothing function (Wood, 2004) that accounts for random effects (individual shoot). The time of day is shown on the x-axes, and the light grey panels represent dark hours.

4.4 Methane emissions and tree-physiological processes

The CH_4 emissions of Scots pine correlated positively with the net uptake of CO_2 in the ambient outdoor measurements (I) and showed a similar but not identical diurnal pattern as CO_2 net uptake in the greenhouse (III). During the drought-manipulation experiment, the net CO_2 uptake correlated negatively (drought-treated saplings) or had no correlation (irrigated control saplings) with the CH_4 fluxes. Moreover, the CH_4 emission rates showed no short-time response to the inhibition of CO_2 uptake at the end of the measurement closure when the CO_2 mixing ratio had decreased due to photosynthesis (I). Finally, no clear connection was observed between the CH_4 emissions and the increase of photosynthetic activity and changes in the water status in the spring (I) nor in the greenhouse during the decline of net CO_2 assimilation (Fig. 7) in drought-manipulation experiment (IV).

Stomatal conductance, transpiration and the CH_4 emissions exhibited diurnal patterns under non-water-stressed conditions (III: Fig. 3), where the gas exchange increased after the beginning of the light-period (Fig. 6), but whereas the CH_4 emissions during the dark hours were no different from zero, the transpiration and stomatal conductance only decreased partially in the night. During the drought experiment (IV), the shoot-level CH_4 emissions of

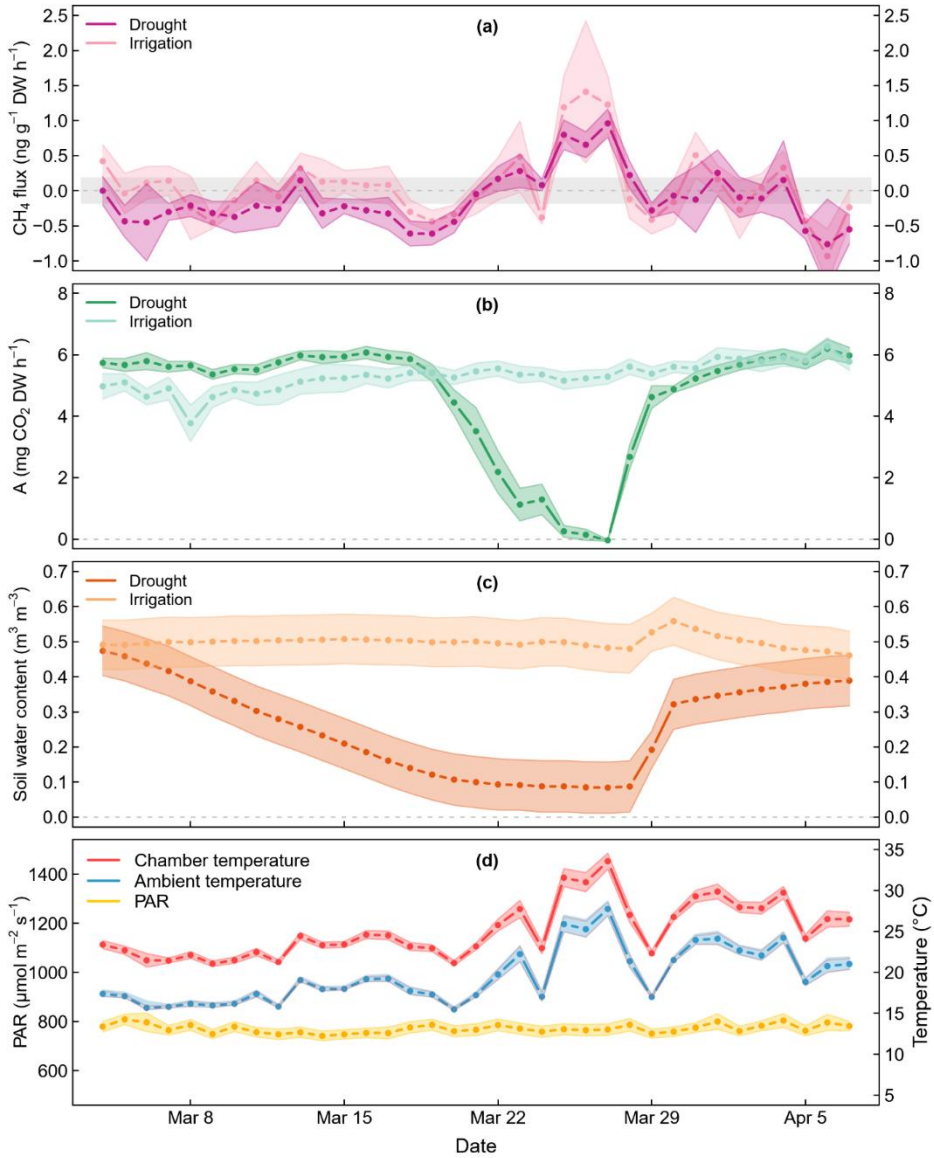


Figure 7. Daily daytime means (\pm standard error) of (a) shoot-level methane (CH_4) fluxes (ng g^{-1} dry weight (DW) h^{-1}), (b) net uptake of carbon dioxide (CO_2) (A, mg g^{-1} DW h^{-1}) and (c) soil water content ($\text{m}^3 \text{m}^{-3}$) of drought-treated and irrigated saplings, and (d) chamber (blue) and ambient (red) air temperatures ($^{\circ}\text{C}$) and photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$). The grey area in (a) is the detection limit for daily daytime measurements. The dark colours in (a-c) represent the drought-treated saplings and the light ones represent the irrigated controls. The dots are the mean values of daytime measurement whereas the shaded, coloured areas the corresponding standard errors.

the drought-treated saplings were statistically no different from those measured from the shoots of the irrigated saplings, despite the decline of the transpiration rate and stomatal conductance to almost zero.

Drought did not have a statistically significant effect on the net CH₄ fluxes of the shoots of Scots pine saplings in the greenhouse (IV), as the CH₄ fluxes of the drought-treated saplings did not differ at any time from those of the irrigated control saplings (Fig. 7). The CH₄ fluxes of the drought-treated saplings, however, appeared to vary less during days and decline to zero during nights, timing together with the decline of the stomatal conductance and transpiration, as well as the highest daytime temperature, but this difference could not be verified by statistical testing in respect to the irrigated saplings (IV).

DISCUSSION

4.5 Shoots of Scots pine are a source of aerobically produced methane

The CH₄ flux measurements conducted in several independent settings in the garden, greenhouse, and field conditions verify that Scots pine shoots are a source of CH₄. These emissions originated from an aerobic production process taking place locally in the canopies, as transport of soil-derived CH₄, another possible source for these emissions, was prevented by maintaining well-drained soil conditions in the growing pots and finally ruled out by a drought treatment (IV). For field measurements done on mature trees (III), aerobic production in the canopies is supported by the argument that soil-derived CH₄ is not transported all the way up to tree canopies (Vroom et al., 2022).

The CH₄ emissions from the shoots varied depending on the experimental setups and prevailing environmental conditions (Fig. 4). Outdoors, the CH₄ emissions were highest in the garden under sunlit conditions, whereas on average no emissions were detected in the night. In the greenhouse, the CH₄ emission rates were comparable to outdoor-measurements when UV-radiation was provided, but when it was not, the fluxes were very small, varying between CH₄ uptake in the mornings and emissions in the afternoons. The observations of CH₄ uptake (I, IV) could result from microbial methanotrophy which may occur on sites of CH₄ emission on different surfaces of trees (Putkinen et al., 2021; Jeffrey et al., 2021), but the presence of methanotrophic microbes was not screened in these studies. In future studies, the contribution of methanotrophic (as well as methanogenic) microbes to the canopy-level fluxes from boreal trees should be addressed by combining gas flux measurements with metagenomic tools for analysis of the canopy microbiota.

Examining the CH₄ fluxes shown in this study in respect to earlier research is challenging, because aerobic CH₄ emissions from plant biomass have mostly been studied in laboratories under temperature and light conditions exceeding realistic ambient ranges. Moreover, only few published papers have reported CH₄ flux measurements from conifer shoots before, and those that do show contradicting results. While the CH₄ emissions presented here, measured during overcast sky in the garden and in the field, were comparable to earlier results from shoot measurements of Scots pine in similar light conditions (Machacova et al., 2016), others have also reported CH₄ uptake at rates on average 2-3 orders of magnitude larger than those observed in the measurements of this study (Sundqvist et al., 2012; Gorgolewski et al., 2023). In study I similar, apparent CH₄ uptake rates were proven to result from measurement error by the GHG analyser due to spectral interference by H₂O, highlighting the uncertainties associated with spectral measurement methods of trace-level gas fluxes (Kohl et al., 2019). In this study these measurement uncertainties were solved by cross-checking results with different GHG analysers.

4.6 Aerobic methane emissions and their temporal patterns are driven by light and enhanced by temperature

The emission rates of aerobic CH₄ were, to a large extent, characterised by the intensity and spectral composition of light during each of the experiments (Fig. 4). In ambient conditions outdoors, the emission rates correlated with global radiation (I), while under artificially provided PAR and UV-radiation in the greenhouse (III), the emissions followed the diurnal pattern of the bimodular day/night-cycles. As these emissions are shown to occur

independently from photosynthesis (IV) the results support the conception that the CH₄ emissions of tree canopies are produced by an abiotic, photochemical reaction similar as described for the foliage several intact plants of herbaceous species and compounds (Keppler et al., 2006, 2008; Messenger et al., 2009a; Qaderi & Reid, 2009; Wang et al., 2009).

Interestingly, the CH₄ emissions were highest outdoors in the spring when the UV levels in Finland are low, although previous studies suggest that high levels of UV-radiation, especially in the spectral region of UV-B, are the key driver of aerobic CH₄ emissions from plant materials (Vigano et al., 2008; Brüggemann et al., 2009; Abdulmajeed et al., 2017; Martel & Qaderi, 2017). Despite the correlation of PAR and CH₄ emissions outdoors (I), in the greenhouse similar levels of PAR and temperature did not induce emissions from the shoots without additionally provided UV-A (IV), and even when UV-A was provided at higher levels than present outdoors in the spring (III), the CH₄ emissions were smaller than in full sunlight (I).

These apparent discrepancies in the effects of PAR and UV radiation to the CH₄ emissions can be explained by a closer inspection of the different lighting setups: in the greenhouse the artificially provided light was mostly depleted of the short wavelengths (blue light), while blue light is abundant in full sunlight. Therefore, while it is evident that UV-A can induce the production of CH₄ from the shoots of Scots pine (III) and would do so also as a component of solar radiation, light in the shorter regions of the visible spectrum, such as blue light, may be a previously underestimated driver for the aerobic CH₄ production (Martel & Qaderi, 2019). One of the possible precursors of CH₄, lignin (Vigano et al., 2008) is highly absorbent to blue light which is also a significant driver of lignin photodegradation in litter (Wang et al., 2021). Hence, it may be that in the highly lignified conifer needles, similar photodegradation process could drive CH₄ from lignin also in living tissues.

The observations of small but consistent night-time CH₄ emissions (IV) indicate that not all production of CH₄ in the canopies is strictly light-dependent. This is supported by the findings that in the absence of light, aerobic CH₄ emissions of CH₄ from plants are induced by stress and increase of temperature (Bruhn et al., 2009; Lenhart et al., 2015). The observations of the night-time CH₄ emissions, however, were not consistent with study III where there was no CH₄ flux detected during the dark hours. These differences could be explained by underlying differences in the physiological status or stress levels between the measurement saplings, but also underline the uncertainties remain in the measurements of extremely small fluxes.

Although high temperature levels induce CH₄ from plants and plant material independently from light under laboratory conditions, temperature is not likely an important independent driver of aerobic CH₄ in boreal forests where temperatures remain relatively cool even in the summer months. In study IV where no UV radiation or sunlight was provided, the CH₄ fluxes correlated with temperature during both light and dark hours, but the mean daily CH₄ fluxes showed small emissions only during an unexpected heat wave. Within the ambient temperature range, the increasing effect of heat to the overall CH₄ emissions from Scots pine shoots likely is negligible if the light conditions do not promote the production of CH₄, and this effect may further decrease after some days of exposure due to heat-acclimation (IV). Temperature does, however, enhance the CH₄-driving force of solar radiation (I; Fig. 5) already in relatively cool (> 10 °C) temperatures, thus, canopy-level CH₄ emissions from forests even in the boreal region may significantly increase during summer heat waves that occur together with high pressure.

Because light is the dominant driver of the shoot-level CH₄ emissions of Scots pines, it is also the main determinant of the diurnal and seasonal cycles of aerobic CH₄ emissions of

forest canopies (I, III, IV). PAR as a measure of solar radiation can, thus, be used to predict the upper limit of the light-driven CH₄ emissions in ambient environments (III). The diurnal patterns of shoot-level emissions of CH₄ observed in the greenhouse, supported by outdoor manual measurements (I, III) reveal almost immediate responses to light as an increase in the CH₄ flux. Similarly, the CH₄ emissions increased simultaneously with the heatwave in study IV, varying on a daily level along the temperature. These results show, that in the shoot-level CH₄ fluxes of forest canopies may also vary in shorter temporal scales, as the daily CH₄ flux depends also on variables such as cloudiness and daily temperature.

4.7 The emissions of methane from the shoots of Scots pine are independent from tree physiological functions

Drought did not significantly affect the CH₄ emissions of Scots pine shoots (IV), based on which, several conclusions can be made about the production process, physiological control, and the site of production of these CH₄ emissions. Firstly, the CH₄ emissions persisted despite the strong decline of the net uptake of CO₂ (Fig. 7), showing that there is no direct link between photosynthesis and the aerobic production process of CH₄. On process-level this means that metabolites of primary production are not immediate precursors of aerobically produced CH₄ in plants, supported by the numerous previous studies showing that plant structural compounds such as pectins and lignins release CH₄ in stress-induced, photochemical pathways (Kepler et al., 2008; McLeod et al., 2008; Viganò et al., 2008; Bruhn et al., 2009; Messenger et al., 2009a; Qaderi & Reid, 2009; Martel & Qaderi, 2017). Whereas this finding does not out rule the possibility of CH₄ release from also metabolites, such as methionine (Lenhart et al., 2015), it shows that CH₄ production occurs independently from photosynthesis. Correlation of CH₄ and CO₂ fluxes (I, Zhang et al., 2014) can therefore be explained by covariance of light and photosynthesis.

Interestingly, the CH₄ emissions were not increased during the drought, as could have been expected based on earlier findings on crops (Qaderi & Reid, 2009, 2011) and the conception that aerobic emissions of CH₄ from plants is induced by stress-related oxidative stress (Messenger et al., 2009b,a). It is possible that the drought-induced increase in the CH₄ production is masked by the overriding effect of the co-occurring heat wave and further compensated to the measured CH₄ flux on the leaf level by the drought-induced decrease of stomatal conductance. If this is the case, it implies that the stomata can partially but not fully restrict emission of CH₄ originating from the needle endosphere. More empirical evidence is, however, needed to describe the stomatal control of CH₄ emissions from conifer needles or that of CH₄ produced within the leaf tissues.

Finally, the microbial production of CH₄ in anaerobic microsites of the soil (Covey & Megonigal, 2019) or transport such soil-derived CH₄ up to the canopies via transpiration stream was inhibited by the drought, verifying that the measured CH₄ emissions were produced locally in the canopies. In addition to the process where CH₄ is photochemically released from plant compounds within the tissues of the foliage, CH₄ may be released from epicuticular waxes by photodegradation under high irradiation of UV-B (Bruhn et al., 2014). This process would naturally occur independently from stomatal conductance. Although the measurements of this study were done in conditions without high UV-B, this study does not distinguish whether the CH₄ emissions originated from the needle surfaces or from the endosphere.

4.8 Methane emissions from the canopies of Scots pine forests weaken the upland soil sink

While the results of this study show that the shoots of Scots pine emit CH₄ in favourable conditions, these emissions (medians 5.41 and 2.52 ng CH₄ g⁻¹ h⁻¹ of the garden and forest measurements, respectively) are only ~ 1 – 2 % of the emission factor (374 ng CH₄ g⁻¹ h⁻¹; Keppler et al., 2006) used in most of the global upscale estimates of aerobic CH₄ emissions from vegetation (Keppler et al., 2006; Kirschbaum et al., 2006; Parsons et al., 2006; Butenhoff & Khalil, 2007). Based on this study, the annual aerobic CH₄ production in boreal forests is similarly proportionally smaller than the initial estimates (Keppler et al., 2006), and hence, is only a minor factor in the global CH₄ budget. The discrepancies between the estimates likely result from the differences in the environmental conditions during the flux measurements, as Keppler's emission factor was based on measurements solely conducted in direct sunlight; the differences may further derive from possible variance in the emission capacities between plant species, and, thus, show the problematics of extrapolating results from individual studies and unilateral environmental conditions to global scale. Therefore, global estimates of the aerobic CH₄ emission should be reconciled from independent field studies, considering the regional differences in vegetation and climate.

Scots pine canopies of boreal forests are likely a CH₄ source of only minor importance on a global scale, but these emissions may notably decrease the sink strength of the boreal upland forest soil. Although the CH₄ emissions measured on the shoot level of Scots pine are small (0.56 – 1.2 μg CH₄ m⁻² h⁻¹, assuming specific leaf area of 45 cm² g⁻¹ DW, Xiao et al., 2006) in comparison to the CH₄ uptake of the soil (-119 μg CH₄ m⁻² h⁻¹; Vainio et al., 2021), the contribution of canopy emissions to the ecosystem scale CH₄ exchange is emphasised due to the proportionally large leaf area of the foliage (LAI 4.5 in a Scots pine stand in the Hyytiälä research forest; Kolari & Aalto, 2022). Estimation of the ecosystem scale aerobic emission based on these numbers show that the canopy emissions of CH₄ may decrease the soil sink strength by 2.1 – 4.6 %. Given the relatively conservative emission factors chosen here as well as the high spatiotemporal variance in the CH₄ fluxes of the boreal forest floor (Vainio et al., 2021), this percentage may yet be an underestimation of the contribution of canopy emissions to the CH₄ flux of boreal forests. Therefore, it is important to gain more data of shoot-level CH₄ fluxes from field measurements, to further refine the estimates of the source strength of aerobic CH₄ emissions of tree canopies.

4.9 Prospects for future research

By showing that shoot-level CH₄ emissions occur independently from photosynthesis, this study rules out intermediates of the primary metabolism as the dominant biochemical source of aerobic CH₄ release. For future research, one approach to improving the global upscale models of the aerobic CH₄ emission could be to confirm their exact precursors in living plants and by applying species distribution models, quantify the potential of global plant biomass to release abiotic CH₄. Identification of the precursors of CH₄ could be attempted by e.g. utilizing pulse-chase labelling method (Epron et al. 2012), where the stable carbon isotope ¹³C is followed through plant pools, from the photosynthesised ¹³CO₂ label into the compounds of the plant and finally into the emission of ¹³CH₄. One weakness of this approach is, however, the difficulty of detecting the change in the isotopic composition of CH₄ in emissions barely above the detection limits of the current GHG analysers.

This study also provides insight into the contribution of canopy CH₄ fluxes to the forest CH₄ exchange in boreal forests by describing the shoot-level CH₄ emissions of one of the most common conifer species in the boreal region of the Eurasian continent, and by showing how these emissions respond to light and increase of air temperature. The next step in quantifying the global aerobic CH₄ emission is to conduct more field-scale measurements to further define whether the responses of the shoot emissions to these environmental drivers vary between different species, climates and seasons. Such temporal and spatial broadening of the research scope will provide empirical data to support and increase the accuracy of global upscale models, but it also requires significant measurement efforts from the global research community. Automatization and the establishment of continuous canopy CH₄ flux measurements to existing field stations, such as the forest SMEAR stations, will facilitate the gathering of data and provide an ecosystem-scale context for the shoot-level measurements.

5 CONCLUSIONS

This study investigated three aspects that determine how tree canopies contribute to the CH₄ cycle of boreal forests: (1) the flux rates and environmental drivers of the CH₄ exchange by the shoots on Scots pine, (2) the tree physiological determinants and their implications to the source process of aerobic CH₄ emissions, and (3) the significance of the aerobic emission from Scots pine canopies in boreal upland forests. Following conclusions can be made based on the findings:

The potential for aerobic CH₄ production process is ubiquitously present in the shoots of Scots pine trees, but these emissions are only ~1 – 2 % of the initial estimates of aerobic emissions from plants (Keppler et al., 2006). Thus, the aerobic emissions from the canopies of boreal forests are only a minor global source of CH₄ when compared to other natural sources of CH₄, such as the tropical wetlands.

The CH₄ emissions from Scots pine shoots are released from plant compounds in an abiotic, photochemical process that is not linked to photosynthesis-related primary metabolism but is similar to the process of aerobic CH₄ release that has been shown before from structural and other non-labile plant compounds. Aerobic CH₄ production in the shoots of Scots pine originates from a dominantly light-driven process at rates that are determined not only by light intensity but also the spectral composition of light. In ambient conditions, solar radiation is the main driver for these emissions which are further enhanced by increase of temperature.

The CH₄ emissions of forest canopies are likely to show considerable temporal and spatial variation because the positive net CH₄ flux at the shoot level requires both the conditions under which the production of CH₄ is induced, and production rates high enough to overpower the possible parallel, consuming processes in CH₄ the canopies. On ecosystem level, the canopy emissions may decrease the sink strength of the upland soils by a conservative estimate of ~ 5%, when taking into account the spatiotemporal variation of both the canopy emissions (this study) and the upland soil uptake (Vainio et al., 2021). This proportion of canopy level CH₄ emission to the boreal upland forest CH₄ flux becomes higher during the warm and sunny periods in the summer.

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