Solar radiation drives methane emissions from the shoots of Scots pine

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Summary

- Plants are recognized as sources of aerobically produced methane (CH₄), but the seasonality, environmental drivers and significance of CH₄ emissions from the canopies of evergreen boreal trees remain poorly understood.
- We measured the CH₄ fluxes from the shoots of Pinus sylvestris (Scots pine) and Picea abies (Norway spruce) saplings in a static, non-steady-state chamber setup to investigate if the shoots of boreal conifers are a source of CH₄ during spring.
- We found that the shoots of Scots pine emitted CH₄ and these emissions correlated with the photosynthetically active radiation. For Norway spruce, the evidence for CH₄ emissions from the shoots was inconclusive.
- Our study shows that the canopies of evergreen boreal trees are a potential source of CH₄ in the spring and that these emissions are driven by a temperature-by-light interaction effect of solar radiation either directly or indirectly through its effects on tree physiological processes.

Introduction

Vegetation has recently been recognized as an important component of the global cycle of methane (CH₄), a powerful greenhouse gas (Carmichael et al., 2014). Plants, both woody and herbaceous, can contribute to the CH₄ exchange of different ecosystems via various pathways: soil-derived CH₄ can be exported through stems (Pangala et al., 2013, 2015; Barba et al., 2019), CH₄ may be produced aerobically in foliage (Keppler et al., 2006; Brüggemann et al., 2009; Qaderi & Reid, 2009, 2011; Wang et al., 2009; Fraser et al., 2015; Martel & Qaderi, 2017, 2019), and it may be produced by microbial methanogenesis within core wood and potentially also other plant tissues (Yip et al., 2019; Puntikinen et al., 2021). These fluxes, however, have not been incorporated into global CH₄ budgets as fundamental questions about the mechanisms, drivers and quantities of plant CH₄ emissions remain unaddressed (Saunois et al., 2020).

Boreal forests cover c. 30% of the global forest area (Brantl et al., 2013), presenting an unignorable potential source of CH₄. Recent research shows that trees in temperate and boreal forests emit CH₄ in natural conditions (Covey et al., 2012; Machacova et al., 2016; Pirz & Mignonial, 2017; Covey & Mignonial, 2019) and can thus decrease the overall sink strength of upland forests, which is sustained by the oxidative consumption of CH₄ in the soil (Ito & Inatomi, 2012). Most of the research on CH₄ emissions from trees, however, has focused on emissions of soil-derived CH₄ from tree stems in tropical or temperate wetland forests (Terazawa et al., 2007; Pangala et al., 2013, 2015; Jeffrey et al., 2020). By contrast, relatively little is known about CH₄ emissions from the trees of the evergreen boreal forests, especially with respect to the potential aerobic in situ production and environmental drivers of CH₄ emission in the canopies. This lack of knowledge prevents us from constructing models for upscaling canopy emissions from regional scales to the global scale.

Emissions of CH₄ from forest canopies likely derive primarily from aerobic production in the foliage, but the biochemical origin and the source processes involved remain poorly understood. Laboratory studies with purified plant compounds and plant parts have demonstrated that compounds like pectin (Keppler et al., 2008; McLeod et al., 2008; Bruhn et al., 2009), lignin, cellulose (Vigano et al., 2008) and methionine (Althoff et al., 2014; Lenhart et al., 2015) can serve as precursors for CH₄ emissions in aerobic conditions, which are induced by various environmental drivers. Current evidence indicates that the unifying factor for aerobic CH₄ emissions from plants is the increased activity of reactive oxygen species (ROS) (McLeod et al., 2008; Messenger et al., 2009a,b), which is induced by environmental plant stressors such as ultraviolet (UV) radiation and heat (Bruhn et al., 2009; Qaderi & Reid, 2009; Fraser et al., 2015), and wounding of plant tissues (Wang et al., 2009). Despite the...
numerous studies focusing on potential precursors and drivers in laboratory settings, research on aerobic CH4 emission from intact plants in ambient outdoor conditions remains rare.

Fluxes of CH4 from the canopies of evergreen trees have only been reported in a handful of field studies. These results vary from small emissions early in the summer from Scots pine (Machacova et al., 2016) to both small emissions and apparent uptake by Norway spruce in the summer (Putkinen et al., 2021), and uptake in low light conditions late in the fall (Sundqvist et al., 2012). It remains unknown how the high seasonal variations typical to the boreal climate affect the canopy CH4 fluxes. Adaptation to the seasonal changes involves drastic alterations to the metabolic processes of evergreen trees which, in the spring in particular, affect their gas exchange and stress tolerance (Hari et al., 2017). For example, the emissions of volatile organic compounds (VOC) and methanol, a compound structurally similar to CH4, from vegetative buds and young shoots of Scots pine trees have been shown to peak in the spring and early in the summer (Aalto et al., 2014). Moreover, boreal conifers are exposed to photochemical stress under cold temperatures early in the spring, before their photosynthetic machinery has adapted to the increasing light levels (Otander et al., 1995; Vogg et al., 1998; Yang et al., 2020).

In this study, we aim to provide missing information about the canopy CH4 exchange of conifers in the spring. The overall objective of this study was to investigate whether the shoots of boreal conifer trees are a source of CH4 in the spring, and to examine whether solar radiation and ambient air temperature affect the CH4 exchange. We hypothesize that Scots pine and Norway spruce shoots act as net CH4 sources in the spring, that CH4 emissions increase under higher light and warmer temperature conditions within the ambient range, and that CH4 emissions increase over the spring with the increasing physiological activity of the trees.

To test our hypotheses, we measured the CH4 exchange from the shoots of Scots pine and Norway spruce saplings early in the growing season in 2019 and 2020. Simultaneous with the CH4 flux measurements, we measured the environmental variables solar radiation and air temperature, and followed tree physiological activity during the breaking of winter dormancy.

Materials and Methods

Experimental design

The study was conducted in ambient conditions with potted 2–3-yr-old nursery saplings of Pinus sylvestris L. (Scots pine) and Picea abies (L.) Karst. (Norway spruce). The measurement site was located in Helsinki, Finland, in the courtyard of the Viikki Plant Growth Facility (lat. 60°13′40″N, long. 25°01′05″E). The measurements were made before and during the early growing season and repeated in two springs: 1 April–8 May in 2019 and 6 March–9 June in 2020. In 2019, we measured over a shorter period of time to confirm the CH4 emissions, and in 2020, we measured from late winter to early summer to cover the period from the spring recovery to the beginning of the annual growth. The balled and burlapped saplings were acquired in the autumn from commercial nurseries (Huutokoski Ltd (in 2019) and Harvila Ltd (in 2020)) and planted into 15–20 l plastic pots with a mixture of peat and humus, 6 months before the experiment. The potted saplings were planted, with their pots, in a sand bed outdoors between the glasshouses to overwinter. To avoid the formation of anaerobic conditions in the soil – in order to prevent potential anaerobic microbial methanogenesis occurring during the measurements – we used pots with drainage holes and irrigated the saplings only moderately; the saplings were kept in ambient outdoor conditions throughout the winter and during the experiment, except for the use of irrigation after longer dry periods, twice in spring 2019 and three times in spring 2020.

In both years, the gas exchange of four Scots pine and three Norway spruce shoots were repeatedly measured, each year with new saplings. The measurements were made nine times per week (three measurements each on 3 d) in 2019 and four times per week (two measurements each on 2 d) in 2020. An empty chamber was measured as a control at the beginning of each measurement round. Overall, there were 181 Scots pine, 53 Norway spruce and 55 empty chamber measurements made in 2019, and 205 Scots pine, 89 Norway spruce and 46 empty chamber measurements made in 2020. A brief summary of the CH4 fluxes from Norway spruce shoots measured in 2020 was presented previously by Putkinen et al. (2021); however, so far, no thorough analysis of this dataset has been reported. Our data from 2019 or from the Scots pine measurements of 2020 have not been presented before.

CH4 and CO2 flux measurements

To quantify the greenhouse gas exchange of the shoots and the background emissions potentially produced by chamber materials, CH4 and CO2 fluxes were measured using a manual chamber system, including transparent cylindrical shoot chambers (described in Machacova et al., 2016) and a portable greenhouse gas analyser (ABB Ultraportable Greenhouse Gas Analyzer; Los Gatos Research, San Jose, CA, USA). Each chamber (volume of 5.2 l) consisted of a permanently installed frame with a circular opaque bottom and top pieces made of polytetrafluoroethylene (PTFE) held together by four metal bars. The measured shoot and a fan to mix the headspace air were enclosed in the chamber; the chamber was then covered by a UV-transparent fluorinated ethylene propylene-foil (FEP) during each flux measurement. The opening for the stem in the bottom piece was sealed with adhesive putty (Blu Tack; Bostik SA, Colombes, France). The fluxes were measured in 7–10-min long chamber closures, during which the headspace air of the chamber was circulated in a closed loop between the analyser and the chamber. To maintain the shoots in ambient conditions, the FEP-foil was removed between each measurement.

Meteorological variables

Meteorological variables were continuously measured at the weather station of the Viikki Plant Growth Facility. The weather
station is positioned on the glasshouse roof, at a height of c. 7 m from the ground. The ambient air temperature (°C), global radiation (W m$^{-2}$), and photosynthetically active radiation (PAR; µmol m$^{-2}$ s$^{-1}$) were 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, Lincoln, NE, USA). The meteorological variables were averaged over 15 min in 2019 and 5 min in 2020. The total daily precipitation values for 2020 were obtained from the Kumpula measurement station of the Finnish Meteorological Institute (FMI), which is located c. 4 km from the measurement site.

**Spring recovery of the saplings**

To follow the spring recovery of the saplings in 2020, we monitored them for the seasonal increase in photosynthetic activity and changes in water status (Leinonen & Hänninen, 2002; Lin-tunen et al., 2018). The measurements were conducted on the same days as the gas exchange measurements, but in different individuals (four Scots pine and four Norway spruce saplings) to minimize any potential disturbance of the gas exchange measurements.

The photosynthetic potential of the saplings was defined by measuring the Chla fluorescence to analyse the maximum efficiency of photosystem II (PSII, $F_v/F_m$). The measured needles were first enclosed in lightproof clips for an hour to stop all photosynthetic reactions. Chlorophyll a fluorescence was then measured with the Field Fluorescence Monitoring System (FMS2+; Hansatech, King’s Lynn, UK), which applies a combination of low intensity light, to measure the minimum fluorescence ($F_0$), and brief saturating flashes (<1 s), to measure the maximum fluorescence ($F_m$), from which $F_v/F_m$ is calculated as follows:

$$F_v/F_m = (F_m - F_0)/F_m \quad \text{Eqn 1}$$

Low values of $F_v/F_m$ indicate that the light reactions are downregulated after the winter, whereas high values indicate that the photosynthetic apparatus is active (Linkosalo et al., 2014).

To assess the water status of the saplings, the needle water potential ($Ψ$) was measured at c. 09:00 h on the measurement days. A pair of needles was cut from each sapling, sealed into a plastic bag to avoid evaporation and immediately taken to the laboratory to be measured. A scalpel was used to apply a fresh cut to the needle sample before measuring the needle $Ψ$ using a pressure chamber (IS05D-EXP; PMS Instrument Co., Albany, OR, USA).

**Shoot sample processing and growth estimation**

To record the growth of the current-year ($Y_0$) shoot during the measurement campaign in 2020, the terminal buds developing into $Y_0$ shoots of the Scots pine saplings were photographed and their length was measured weekly. To obtain the biomasses of the foliage in the chambers, the shoots were collected once the campaign was over. The 1-yr-old ($Y_1$) and $Y_0$ needles were separated from the branches, oven-dried at 65°C for 72 h and weighed.

To calculate the gas fluxes per unit needle biomass, the $Y_0$ needle masses, which increased throughout the measurement campaigns, were estimated for each measuring day using the relative growth of the $Y_0$ shoots (i.e. shoot length on measurement day/shoot length at the end of the campaign). For this, we assumed that needle biomass increases proportionally with the elongation of the $Y_0$ shoot. For the Scots pine saplings of 2020, the relative growth was based on the measured shoot elongation growth. For the Norway spruce saplings of both years and the Scots pine saplings of 2019, the $Y_0$ relative growth was estimated using a model (Schiestl-Aalto et al., 2013) that describes the elongation growth of Scots pine shoots with respect to cumulative growing degree days above 5°C. To validate the suitability of the model for predicting the growth of our saplings, the relative growth, based on the measured $Y_0$ shoots in 2020, was compared to the relative growth obtained from the model in spring 2020.

**Gas flux calculations**

Each measurement closure was examined graphically, with gas concentrations plotted as a function of time. This was done to adjust the start and end times of the flux calculation in order to omit the values corresponding to instability in the measurement loop caused by mixing of the headspace gases at the beginning of the closure, leakage of the chambers, and inaccuracies in the recorded start and end times (Supporting Information Fig. S1). Closures with poor data quality were discarded (8.6% of all closures in 2019 and 20.8% in 2020).

During the measurements, we noticed that the greenhouse gas analyser’s automatic correction for H$_2$O spectral interferences did not work accurately: increases in H$_2$O concentrations led to apparent decreases in the measured CH$_4$ concentrations. To correct for this source of error in the CH$_4$ measurements, we conducted a laboratory experiment to empirically determine a correction factor $f$ for the H$_2$O interference: A 0.8 l empty chamber equipped with an injection septum was flushed with dry air and then connected in a closed loop with the analyser. Next, c. 1 ml of water was injected to the chamber. As the measurement loop air became saturated with water vapour, the humidity in the analyser’s measurement chamber increased rapidly, while the (actual) dry CH$_4$ mixing ratios remained unchanged; $f$ was determined as the regression slope between the measured dry CH$_4$ mixing ratio and the mixing ratio of H$_2$O. The experiment was replicated six times, resulting in a value $f$ ($\approx 9.122 \times 10^{-7}$ ppm CH$_4$ ppm$^{-1}$ H$_2$O, 95% confidence interval 0.452 $\times 10^{-7}$ ppm CH$_4$ ppm$^{-1}$ H$_2$O), which was used to correct the measured CH$_4$ concentration values:

$$[\text{CH}_4]_{	ext{dry,corr}}(t) = [\text{CH}_4]_{	ext{dry,raw}}(t) - [\text{H}_2\text{O}](t) \cdot f \quad \text{Eqn 2}$$

The flux rates of CH$_4$ from shoots and the empty chamber were calculated using the least squares method, defining the best linear fit for the concentration change over the closure time (dC/dt). The hourly flux rates from the shoots were then calculated.
using \( \frac{dC}{dt} \) and normalized to the dry weight of the needles of each chamber-enclosed shoot:

\[
F = \frac{dC}{dt} \cdot \frac{M \cdot V}{m_{\text{needles}}} \cdot \frac{p}{R \cdot T} \cdot 3600
\]  
Eqn 3

\( F \), CH4 flux (ng g\(^{-1}\) (needle dry weight) h\(^{-1}\)); \( M \), molar mass of CH4 (1.604 \times 10\(^{10}\) ng mol\(^{-1}\)); \( m_{\text{needles}} \), needle mass (g dry weight of \( Y_0 + Y_1 \) needles taking into account to the growth over the campaign); \( p \), pressure (assumed 101 325 Pa); \( R \), gas constant (8.31446 J mol\(^{-1}\) K\(^{-1}\)); \( T \) (K), sample temperature as it enters the analyser; \( V \), shoot chamber volume including the loop volume (5.2 \times 10\(^{-3}\) m\(^3\)).

The CO2 flux was estimated using an exponential function fitted to the concentration over time \( t \):

\[
C(t) = C_{\text{lim}} + (C_0 - C_{\text{lim}}) \cdot e^{-k \cdot t}
\]  
Eqn 4

\( C_0 \), initial concentration; \( C_{\text{lim}} \), the asymptote; \( e \), Euler’s number (2.71828); \( k \), rate constant. The initial \( \frac{dC}{dt} \) was calculated as the initial slope of the following function:

\[
\frac{dC}{dt} = -k \cdot (C_0 - C_{\text{lim}})
\]  
Eqn 5

The CO2 flux was calculated in a similar manner to the CH4 flux (Eqn 3), but in terms of mg g\(^{-1}\) (needle dry weight) h\(^{-1}\) and using the molar mass of CO2 (4.401 \times 10\(^{-4}\) ng mol\(^{-1}\)).

To correct for the background CH4 emitted by the chamber materials (Table S1), the CH4 fluxes of the empty chambers were first tested for their sensitivity to environmental variables by performing linear analyses for CH4 fluxes as functions of global radiation and air temperature. For 2020, when such sensitivities were not detected and the mean of the empty chamber CH4 did not significantly change over the campaign, the mean empty chamber CH4 flux was subtracted from the shoot chamber CH4 fluxes. For 2019, when the linear model revealed a significant correlation between the empty chamber CH4 flux and global radiation (as well as PAR), this model was used to predict the subtracted chamber background flux for each shoot chamber closure based on the corresponding mean global radiation.

Finally, the method detection limit (MDL) for a single chamber closure was defined as three times the SD of the apparent CH4 flux measured for the empty chambers. Separate detection limits were calculated for 2019 (123.3 ng CH4 h\(^{-1}\)) and 2020 (120.9 ng CH4 h\(^{-1}\)). When scaled to the average shoot dry weight for each species and each year, the MDL ranged from 5.12 to 27.2 ng CH4 g\(^{-1}\) dry weight (DW) h\(^{-1}\) (Table 1). These detection limits decrease with \( \sqrt{n} \) for repeated measurements, resulting in detection limits between 0.73 and 6.48 ng CH4 g\(^{-1}\) DW h\(^{-1}\) for the average number of measurements conducted over a 2-wk period.

### Statistical analysis

To assess the trends in CH4 fluxes over the two springs, the data were divided into chronological subgroups, each consisting of measurements from a 2-wk period. The differences in the mean CH4 fluxes between these subgroups were tested with a linear mixed-effects analysis using the individual tree as a random intercept, and multiple comparisons of means. Further, the differences between the CH4 emission of each shoot chamber and the empty chamber background emission were tested with an independent \( t \)-test.

To test if the CH4 fluxes were influenced by environmental variables or the uptake rate of CO2 in each tree species, we performed a linear mixed-effects analysis, where the model was used to express the correlation of CH4 fluxes as a function of CO2 fluxes, PAR, global radiation, and ambient air temperature. Further, the relationship between PAR and global radiation was tested with a linear model. As with the analysis of the subgroups, the nonindependence of the data points of the repeated measurements was solved by adding a random effect for individual trees. In addition, the covariance of PAR and the temperature was analysed with a linear regression model.

To distinguish between PAR and air temperature as the potential drivers of CH4 emissions, the data were divided into groups of low and high PAR values (PAR ≤ 500 and ≥ 500 mol m\(^{-2}\) s\(^{-1}\), respectively), after which the groups were analysed separately. To test for an interaction effect of air temperature and light on CH4 emissions, a factor variable

| Measure | Unit | Year | Tree species |
|---------|------|------|--------------|
| Detection limit per chamber\(^a\) | ng CH4 h\(^{-1}\) | 120.9 | 123.3 | – |
| Detection limit per measurement\(^b\) | ng CH4 g\(^{-1}\) DW h\(^{-1}\) | 5.12 | 27.2 | Scots pine |
| Average shoot DW | g | 23.6 | 4.54 | Norway spruce |
| Median of measurements (n) per 2-wk period | g | 17 | 16 | Norway spruce |
| Range of measurements (n) per 2-wk period | g | 49–63 | 24–39 | Scots pine |
| Detection limit per 2-wk period\(^c\) | ng CH4 g\(^{-1}\) DW h\(^{-1}\) | 0.73 | 5.23 | Scots pine |
| Detection limit for the amount of CH4 released into the enclosure chamber during a single closure (not scaled to shoot size), defined as three times the SD of the emissions measured in the empty chamber. | ng CH4 h\(^{-1}\) | 120.9 | 123.3 | – |

\(^a\)Typical detection limit for CH4 emissions from a spruce or pine shoot, defined as the detection limit described in (\(^a\)) divided by the average shoot dry weight for each year and species.

\(^b\)Typical detection limit for CH4 emissions from a spruce or pine shoot, defined as the detection limit described in (\(^a\)) divided by the square root of the number of replicate measurements (median of the number of measurements per 2-wk period).
was created which grouped the data into four bins based on temperature (every five degrees from 0°C to 20°C), after which Helmert contrasts were applied to the variable to enable testing between the subsequent levels. A linear mixed-effects analysis was then performed using PAR, the temperature bins and the interaction between PAR and the bin variable as independent variables, and individual trees as random intercepts.

All data analysis was conducted using the R v.4.0.5 software environment (R Core Team, 2017), with the additional packages NLME v.3.1-152 (Pinheiro et al., 2021), multcomp v.1.4-17 (Hothorn et al., 2008) and lme4 v.1.1-27.1 (Bates et al., 2015).

Results

Meteorological conditions

In 2019, the start of spring, defined as the time when the average 24-h temperature permanently exceeds 0°C, occurred on 13 March, and the thermal growing season started on 26 April (FMI). In spring 2020 (Fig. 1) the measurements followed a snowless and relatively mild winter. The spring started on the 1 March and the thermal growing season on 18 April in Helsinki. Photosynthetically active radiation and global radiation were collinear in both years (Photosynthetically active radiation and global radiation were almost identical in all three time groups throughout the campaign, from mid-April to mid-May (4.62 ± 0.46, 4.89 ± 0.67 and 4.93 ± 0.36 ng CH4 g−1 DW h−1, respectively). The mixed-effects linear model and multiple comparison of means indicated a statistically significant difference in the CH4 fluxes in early to mid-April (Group 3, 16.1 ± 2.70 ng CH4 g−1 DW h−1), whereas in 2019 the fluxes remained almost identical in all three time groups throughout the campaign, from mid-April to mid-May (4.62 ± 0.46, 4.89 ± 0.67 and 4.93 ± 0.36 ng CH4 g−1 DW h−1, respectively). The mixed-effects linear model and multiple comparison of means indicated a statistically significant difference in the CH4 fluxes in early to mid-March 2020 (Group 1) in comparison to late-April 2020 (Group 3, P = 0.001) and mid-to-late May 2020 (Group 6, P = 0.046). In 2019, no change in emission rates was observed during the campaign. As for the Norway spruce, no distinct changes in CH4 fluxes were confirmed in either year (Fig. 3a), although the measurements of late April to mid-May 2020 suggest net CH4 emissions. The observed CH4 fluxes were lowest (0.90 ± 1.45 ng CH4 g−1 in 2019 and −4.34 ± 3.52 ng CH4 g−1 in 2020) in mid-to late April (Group 4).

Shoot growth and Chla fluorescence

In the 2020 campaign when we followed the spring recovery of the trees, the first visible signs of the start of the new annual growth were noticed on 21 April. Based on the Fv/Fm values, the spring recovery of the photosystem started simultaneously with the visible shoot growth and was complete at the end of May (Fig. 1b, c. 0.8 being a typical summer value for Scots pine and Norway spruce in Finland, Linkosalo et al., 2014). As for the water status of the saplings, the needle water potential increased after the winter dehydration, reaching a nearly saturated state in early April (Fig. 1b). From early April to late April, the water potential decreased down to −1.2 MPa possibly due to the fact that there was an increased transpiration demand but the cold soil was still limiting the water uptake of the saplings. From late April to early June, water potential started to increase again.

Shoot methane fluxes

We observed CH4 emissions from the shoots of both Scots pine and Norway spruce in spring 2019 and spring 2020, of which the emissions of Scots pine could be verified statistically; CH4 emissions measured from chambers containing Scots pine shoots were significantly higher than fluxes measured from the empty chambers for all but one shoot chamber (Fig. 2a). The range of variation differed between the years and species: the flux rates of Scots pine ranged from 1.59 to 7.46 ng CH4 g−1 DW h−1 in 2019 and from −0.09 to 15.16 ng CH4 g−1 DW h−1 in 2020 (inter-quartile range; IQR). The flux rates of Norway spruce ranged from −2.21 to 5.21 ng CH4 g−1 DW h−1 in 2019 and from −6.60 to 9.05 ng CH4 g−1 DW h−1 in 2020 (IQR). The CH4 flux of the empty chambers was 27.38 ± 5.44 ng−1 h−1 (mean ± SE) in 2019 and 11.76 ± 6.06 in 2020. The empty chamber fluxes are presented relative to the shoot chamber fluxes (Fig. 2) before correcting for the background CH4 emitted by the chamber materials or scaling to shoot dry weight.

Methane fluxes over the spring

Over spring 2020, the CH4 emissions from Scots pine increased between March and mid-April, after which the increase levelled off (Fig. 3a). When the fluxes were examined in groups based on pooled measurements from 2-wk time intervals, the mean flux was lowest in early to mid-March (Group 1, 1.75 ± 1.37 ng CH4 g−1 DW h−1, mean ± SE). In 2020, the highest mean positive fluxes were measured in early to mid-April (Group 3, 16.1 ± 2.70 ng CH4 g−1 DW h−1), whereas in 2019 the fluxes remained almost identical in all three time groups throughout the campaign, from mid-April to mid-May (4.62 ± 0.46, 4.89 ± 0.67 and 4.93 ± 0.36 ng CH4 g−1 DW h−1, respectively). The mixed-effects linear model and multiple comparison of means indicated a statistically significant difference in the CH4 fluxes in early to mid-March 2020 (Group 1) in comparison to late-April 2020 (Group 3, P = 0.001) and mid-to-late May 2020 (Group 6, P = 0.046). In 2019, no change in emission rates was observed during the campaign. As for the Norway spruce, no distinct changes in CH4 fluxes were confirmed in either year (Fig. 3b), although the measurements of late April to mid-May 2020 suggest net CH4 emissions. The observed CH4 fluxes were lowest (0.90 ± 1.45 ng CH4 g−1 in 2019 and −4.34 ± 3.52 ng CH4 g−1 in 2020) in mid-to late April (Group 4).

Methane and photosynthetically active radiation, solar radiation and air temperature

The CH4 fluxes of Scots pine correlated positively with global radiation and PAR (Fig. 4a) in both 2019 and 2020 (P < 0.001). The relationship between PAR and CH4 fluxes was also examined between each of the time groups, but no statistically significant trend in this relationship was observed over the spring in either year.

The CH4 fluxes of Scots pine also positively correlated with ambient air temperature in both 2019 (R² = 0.19, P < 0.001) and 2020 (R² = 0.06, P = 0.009). To some extent, this correlation likely resulted from the co-variance between air temperature and PAR (R² = 0.04, P < 0.01 in 2019 and R² = 0.23, P < 0.001 in 2020) as the regression residuals of fluxes with temperature were still correlated to PAR but not vice versa. In 2019, CH4 fluxes were only correlated with air temperature under high PAR conditions (≥ 500 µmol m−2 s−1, R² = 0.16, P < 0.001) (Fig. 5a). Furthermore, the slopes of the mixed-effects linear model fits for CH4 and PAR correlation increased with air temperature between all of the temperature...
Using Helmert contrasts, all of the levels were significant (Fig. 5b).

For Norway spruce, the CH$_4$ fluxes did not correlate with global radiation, PAR (Fig. 4b) or air temperature in either year.

Methane emissions and photosynthesis

The CH$_4$ emissions from the Scots pine correlated positively with CO$_2$ uptake in both 2019 and 2020 ($R^2 = 0.38$ and 0.08...
Fig. 2 The apparent CH₄ emission/uptake (i.e. not scaled to needle dry weight; ng h⁻¹) in the empty measurement chambers (dark grey) with respect to chambers containing shoots (light grey) of Scots pine (a) or Norway spruce (b) in 2019 and 2020, before correction for the chamber background flux. The horizontal lines in the boxes represent median values, the boxed areas represent the inter-quartile range, the whiskers show the minimum and maximum values, and the black dots are outlying values. The grey horizontal dashed line indicates the zero-line for the apparent CH₄ emission/uptake. Statistically significant differences between each shoot chamber and the empty chamber of the corresponding year are indicated by an asterisk (*, P < 0.05).

Fig. 3 The variability of CH₄ fluxes (ng g⁻¹ dry weight h⁻¹) of Scots pine (a) and Norway spruce (b) within temporal subgroups in spring 2020 (grey) and spring 2019 (white). The horizontal lines in the boxes indicate the median values, the boxed areas represent the inter-quartile range, and the whiskers show the minimum and maximum values. The compact letter display (CLD) indicates the statistically significant differences between the temporal subgroups of Scots pine in 2020. The grey horizontal dashed line indicates the zero-line for CH₄ fluxes.
Fig. 4 Mixed-effects linear model fits showing the overall relationship of the CH$_4$ fluxes (ng g$^{-1}$ dry weight h$^{-1}$) of Scots pine and Norway spruce with photosynthetically active radiation (PAR, µmol m$^{-2}$ s$^{-1}$) and global radiation (W m$^{-2}$) in 2019 (black points and solid lines) and 2020 (white points, black dashed line). The statistics for the linear fits are for the fluxes as a function of PAR. For Scots pine (a) they are $P < 0.001$ for both years, $y = 0.004x + 0.686$ and $R^2 = 0.45$ for 2019 and $y = 0.007x - 0.160$ and $R^2 = 0.09$ for 2020. For Norway spruce (b) the model showed no statistical significance in either year. The grey horizontal dashed line indicates the zero-line for CH$_4$ fluxes.

Fig. 5 The interaction effect of photosynthetically active radiation (PAR) and temperature to CH$_4$ emissions. (a) Linear model fits showing the relationship between ambient air temperature (°C) and CH$_4$ emissions (ng g$^{-1}$ dry weight h$^{-1}$) for Scots pine in 2019, in groups of low (dashed line, $y = 0.063x + 0.847$, $P = 0.19$, $R^2 = 0.032$) and high (solid line, $y = 0.412x + 2.46$, $P < 0.001$, $R^2 = 0.19$) PAR (µmol m$^{-2}$ s$^{-1}$). (b) The slopes (±SE) of the mixed-effects linear model fits for CH$_4$ and PAR correlation, divided into four temperature bins. The grey horizontal lines indicate the zero-line for CH$_4$ emissions and slopes.
The linear fits are ranging from $$-C_0$$ to $$+C_1$$, respectively, $$P < 0.0001$$ (Fig. 6). For Norway spruce, no correlation between CH$_4$ fluxes and CO$_2$ was detected in either year.

**Discussion**

The shoots of Scots pine are a source of CH$_4$

In this study, we show that the shoots of Scots pine are a source of CH$_4$ emissions. Our greenhouse gas measurements, conducted from the shoots of young saplings in a garden setup, showed CH$_4$ emissions c. 20–30 times higher in sunlight (PAR $\geq 500$ µmol m$^{-2}$ s$^{-1}$) and of the same order of magnitude in cloudy conditions (PAR $\leq 500$ µmol m$^{-2}$ s$^{-1}$) as those observed from the shoots of mature Scots pine trees measured during overcast weather (Machacova et al., 2016) (0.225 ng CH$_4$ g$^{-1}$ DW h$^{-1}$, assuming a specific leaf area of 45 cm$^2$ g$^{-1}$ DW (Xiao et al., 2006)). Our low-PAR CH$_4$ emissions were also similar in magnitude to those measured from poplar cultures under low-light conditions (Brüggemann et al., 2009). We show CH$_4$ emissions c. 2–3 times smaller than the lowest CH$_4$ fluxes reported by Keppler et al. (2006), and of the same order of magnitude as those measured from plant biomass in laboratory experiments by Martel & Qaderi (2017).

Our results, together with those of Machacova et al. (2016), indicate that boreal conifer shoots have the potential for aerobic formation of CH$_4$, a phenomenon first introduced by Keppler et al. (2006) and later confirmed by a number of studies (Brüggemann et al., 2009; Bruhn et al., 2012; Martel & Qaderi, 2017; Covey & Megonigal, 2019). The fluxes detected in our study (ranging from −28 to 37 ng g$^{-1}$ DW h$^{-1}$ for both species and years) were comparable to those reported by Dueck et al. (2007) (−10 to 42 ng g$^{-1}$ DW h$^{-1}$), but our better detection limit (Table 1) allowed us to confidently detect fluxes of this magnitude, especially for Scots pine in 2019. Therefore, the lack of confirmation of CH$_4$ emission reported in some previous studies might have been due to their detection limits being too high.

We acknowledge that the process of CH$_4$ production cannot be directly assumed to be aerobic purely because the emissions are measured from shoots (Covey & Megonigal, 2019). Our experimental setup, however, limits the CH$_4$ production in potential sources other than needles to a minimum – although boreal trees, including Scots pines, have been shown to emit soil-derived, anaerobically produced CH$_4$ from their stems, substantial transport of CH$_4$ from the soil is mainly associated with locations where the water table is relatively high (Machacova et al., 2014). Moreover, in well-drained upland sites, shoot CH$_4$ emissions exceeded stem emissions 41-fold (Machacova et al., 2016). It should therefore be noted that our measured saplings were planted into well-drained pots on a sand bed and only moderately irrigated. Although the presence of anaerobic microsites in the pots cannot be fully ruled out as possible sites of microbial methanogenesis, CH$_4$ formation at such sites would be negligible relative to the quantities of CH$_4$ we measured from the shoots. Therefore, it is most likely that the CH$_4$ was produced in the needles.

Our results for the Norway spruce do not show evidence of CH$_4$ emissions. Other authors have reported net uptake of CH$_4$ by shoots of Norway spruce (Sundqvist et al., 2012), indicating that there may be parallel, possibly microbial, processes contributing to the overall canopy-level CH$_4$ fluxes of conifers. We observed apparent net CH$_4$ uptake during individual measurements in 2019 and 2020 (Figs 1–3). These observations may indicate net CH$_4$ uptake by microbial methanotrophs, which have been detected in the phyllosphere of Norway spruce (Putinen et al., 2021). The concurrent uptake of CH$_4$ with emissions would explain the lower CH$_4$ fluxes observed in Norway spruce shoots. The observed apparent CH$_4$ uptake rates, however, generally remained within the measurement uncertainty; hence, our data provides no further evidence for or against CH$_4$ uptake.

**Methane emissions are controlled by solar radiation and further enhanced by temperature**

The CH$_4$ emissions correlated with PAR and global radiation in Scots pine (Fig. 4), making our study one of the first to demonstrate solar radiation as a driver of aerobic CH$_4$ emissions from the shoots of conifers under ambient conditions. The only field study that has addressed this question before showed no correlation with solar radiation in Japanese cypress (Kamakura et al., 2012), although sunshine exposure had already been shown to increase CH$_4$ emissions from living plants in the laboratory by Keppler et al. (2006).

Previously, positive correlations between CH$_4$ emissions and radiation (and air temperature) have been shown in laboratory conditions with dried and fresh bulk plant biomass, structural components like pectin and lignin, and surface waxes (McLeod et al., 2008; Vigano et al., 2008; Bruhn et al., 2014). In these studies, UV-light, especially in the UVB region, was identified as a key driver for CH$_4$. These experiments were, however, conducted mostly at UV radiation levels (up to 7 W m$^{-2}$ UVB) that
were much higher than typical UVB radiation in Finland during springtime (daily maximum \( c. 1–4 \text{ W m}^{-2} \) UVB in March–June). The CH\(_4\) emissions in our study may also depend on radiation in other spectral regions, for example UVA and blue light, which has been recently shown to induce aerobic CH\(_4\) production in canola (Martel & Qaderi, 2019). Besides the apparent direct effects of solar radiation, our measurements also show a correlation between CH\(_4\) emission and CO\(_2\) uptake; therefore, it is possible that the source process of the CH\(_4\) emissions is, to at least some extent, associated with other light-driven tree physiological processes, such as the interaction of the methionine cycle and C\(_4\) metabolism, which is closely linked to photosynthesis.

In our 2019 campaign, we found a strong correlation between CH\(_4\) emissions and air temperature for Scots pine shoots in direct sunlight, whereas in cloudy conditions there was no correlation (Fig. 5a). Notably, although sunshine generally increases the air temperature close to the ground as well as in the closed measurement chambers early in the spring in Finland, in April–May of 2019 PAR only explained \( c. 4\%\) of the changes in ambient air temperature. Temperature-driven CH\(_4\) release from plants and plant compounds has been reported in multiple studies (Keppler et al., 2006; Vigano et al., 2008; Bruhn et al., 2009); however, these experiments were conducted in laboratory conditions and mostly outside the ambient temperature range (with temperatures as high as 80°C). Our results show that even on warmer days the CH\(_4\) emissions remained low if the sky was overcast, whereas in warmer and sunny conditions the effect of solar radiation on CH\(_4\) emissions was enhanced (Fig. 5b), pointing towards a temperature-by-light interaction effect. Thus, although air temperature is not an independent driver of shoot CH\(_4\) emissions in other spectral regions, for example UVA and blue light, which has been recently shown to induce aerobic CH\(_4\) production in canola (Martel & Qaderi, 2019). Besides the apparent direct effects of solar radiation, our measurements also show a correlation between CH\(_4\) emission and CO\(_2\) uptake; therefore, it is possible that the source process of the CH\(_4\) emissions is, to at least some extent, associated with other light-driven tree physiological processes, such as the interaction of the methionine cycle and C\(_4\) metabolism, which is closely linked to photosynthesis.

In conclusion, our study provides evidence that conifers emit CH\(_4\) from their shoots, suggesting that the evergreen boreal forest regions are exposed to photo-oxidative stress during early spring when the increasing levels of light energy cannot yet be utilized as photosynthesis is inhibited due to low temperature (Karpinski et al., 1993; Ottander et al., 1995; Vogg et al., 1998). Moreover, previous studies show that the production of ROS, which is induced by a number of plant stressors, may initiate the formation of CH\(_4\) within the plant (Messener et al., 2009b; Wang et al., 2009; Bruhn et al., 2012; Liu et al., 2015). Although our results do not rule out the seasonal increase in solar radiation as an explanation for the increasing trend in CH\(_4\) fluxes between March and April, the timing of the highest measured CH\(_4\) emissions of Scots pine in early to mid-April raises the question of whether the increased susceptibility of the shoots to light before the full upregulation of the photosynthetic machinery promotes the formation of CH\(_4\), possibly via ROS-related pathways (Messener et al., 2009a).

After the gradual increase in early March to mid-April (Fig. 2a), CH\(_4\) emissions did not continue to increase with further advancing of the spring. This was despite increases in solar radiation and in the ratio of newly grown \( Y_0 \) to year-old \( Y_1 \) shoot biomass in the measurement chambers. Thus, we found no evidence for a difference in CH\(_4\) emissions from \( Y_0 \) and \( Y_1 \) shoots, whereas such differences were found in methanol emissions from Scots pine shoots (Aalto et al., 2014), indicating buds and growing shoots act as a strong source of methanol but not CH\(_4\).

Uncertainties in the measurements

Our results show variability in the observed CH\(_4\) fluxes between the two measurement years for both Scots pine and Norway spruce, with notably larger variance in the observed fluxes of 2020 (Fig. 2). To some extent, these differences arise from technical limitations in the measurements of leaf-level CH\(_4\) fluxes that are very small relative to the atmospheric background concentration (Kohl et al., 2021). The variation in the observed CH\(_4\) emission ranges of Scots pine shoots between the years can be furthermore explained by differences in the growth patterns of the saplings that resulted in a lower needle biomass : chamber volume ratio in 2020; the measured biomass values for the Scots pine shoots were significantly smaller in 2020 than in 2019 (Table 1). As a result, the CH\(_4\) concentration changes during the chamber closure were smaller and, consequently, the measurement uncertainty was larger due to a higher proportion of the fluxes being close to the detection limit. Similarly, the lower needle biomass : chamber volume ratio could also explain why the measurements provided generally inconclusive evidence for CH\(_4\) emissions from the shoots of the Norway spruces. Finally, linear models with PAR, temperature and the uptake rate of CO\(_2\) all predicted very similar if not identical CH\(_4\) emission rates for the Scots pine in both years (Figs 3, 6), validating our findings about the relationship of these factors to the CH\(_4\) emissions.

Conclusions

In conclusion, our study provides evidence that conifers emit CH\(_4\) from their shoots, suggesting that the evergreen boreal forest
canopies may decrease the CH₄ sink strength of boreal upland forests. We identified solar radiation as a driver for shoot CH₄ emissions through a temperature-by-light interaction effect. Measurements conducted under more controlled conditions will help to further clarify the individual effects of light and temperature on CH₄ production in the needles. We also demonstrated that in the springtime, the environmental conditions in the boreal regions, potentially together with the physiological state of the trees, favour CH₄ formation in the shoots of evergreen conifers. Although our work increases our knowledge of canopy level CH₄ fluxes from conifers by providing the first results of springtime CH₄ emissions, long-term measurements are needed to characterize the seasonality of these fluxes in order to evaluate their significance for the CH₄ exchange at ecosystem level.

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Author contributions

The study was conceptualized by M Pihlatie, SAMT, LK and MK. The measurements were conducted by SAMT, M Patama, AZ, AL, RL and LK. The data analysis was conducted by SAMT, MK and LK. M Pihlatie, AL, MK and LK provided supervision and guidance to graduate students. SAMT wrote the first draft of the manuscript, all other co-authors contributed to the final version.

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Data availability

The original data are available at 10.5281/zenodo.6367101. Additional data related to this article may be requested from the corresponding author (SAMT).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Example plots showing the adjustment of the start and end times of measurement closures, based on a graphical assessment of CH$_4$ and CO$_2$ mixing ratios as a function of time.

**Table S1** Table presenting the chamber background CH$_4$ fluxes defined from empty chamber measurements, and shoot CH$_4$ fluxes defined as shoot chamber fluxes before scaling to dry weight and after subtracting the chamber background flux.

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