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Automated closed chamber measurements of methane fluxes from intact leaves and trunk of Japanese cypress

Kenshi Takahashi1,*, Yoshiko Kosugi2, Akito Kanazawa2 and Ayaka Sakabe2
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ABSTRACT
Continuous in situ measurements of methane (CH4) fluxes from intact leaves and trunk of Japanese cypress (Chamaecyparis obtusa Sieb. et Zucc) were conducted in a temperate forest from August 2009 to August 2010. An automated closed chamber system, which was used to evaluate CO2 exchange between the atmosphere and forest ecosystems, was coupled to a laser-based instrument to monitor CH4 concentrations. Temporal changes in CH4 concentrations from the foliage and trunk were measured at one-second intervals during chamber closure to determine CH4 fluxes between the leaf and trunk surfaces and the atmosphere. While recent studies have suggested that some plants emit CH4 under aerobic conditions, emission or uptake of CH4 in detectable amounts with our experimental system, by intact leaves or the trunk of C. obtusa, was not significantly observed throughout the measurement period.

Key words: methane; plant emission; aerobic conditions; cypress; chamber method.

1. Introduction
Recent experiments conducted by Keppler et al. (2006) suggested that CH4 emissions from terrestrial plants under aerobic conditions could be a significant source of atmospheric CH4, and that global emissions could range from 62 to 236 Tg CH4 yr⁻¹. Using different scaling approaches, subsequent studies have revised the upper limits of global aerobic CH4 emissions by vegetation to 1 to 213 Tg CH4 yr⁻¹ (Houweling et al., 2006; Kirschbaum et al., 2006; Parsons et al., 2006; Butenhoff and Khalil, 2007; Ferreti et al., 2007; Megonigal and Guenther, 2008; Bloom et al., 2010). However, since the mechanisms underlying CH4 emission are still largely unknown, any extrapolations to the global scale are highly speculative. The data collected to date for in situ CH4 exchange in plant communities under aerobic conditions has been conflicting, with some studies reporting significant CH4 emissions (Cao et al., 2008; McLeod et al., 2008; Vigano et al., 2008; Wang et al., 2008; Brüggemann et al., 2009; Bruhn et al., 2009; Qaderi and Reid, 2009), while others have reported no significant emissions (Dueck et al., 2007; Beerling et al., 2008; Kirschbaum and Walcroft, 2008; Megonigal and Guenther, 2008; Bowling et al., 2009; Nisbert et al., 2009). Similarly, no consensus currently exists on the effect of ultraviolet (UV) irradiation on CH4 emissions (McLeod et al., 2008; Vigano et al., 2008, 2009; Bowling et al., 2009; Bruhn et al., 2009; Qaderi and Reid, 2009). Thus, despite numerous studies having been conducted on the microscopic mechanisms associated with CH4 production and the impact that these have on the global CH4 budget, the issue of CH4 exchange in plants under aerobic conditions has not yet been satisfactorily resolved.

In Japan, artificial plantations of Japanese cypress (Chamaecyparis obtusa Sieb. Et Zucc) cover up to 10% of the total Japanese forest area (Japan Forestry Agency, 2005). Investigating whether C. obtusa emits CH4 significantly is thus important to develop an emission inventory of CH4 in Japan and to understand its impact on the atmospheric CH4 budget. Recently, laboratory incubations investigating CH4 emission from detached leaves of C. obtusa in a temperate forest have been made (Kamakura et al., in press), in which leaf smaples were collected at different heights within the canopy. Estimated CH4 fluxes were close to zero, which were several orders of magnitude smaller than, for example, those from some other C3 plants as reported by Keppler et al. (2006). In this study, we for the first time made an attempt to estimate CH4 fluxes from intact leaves and trunk, which are rather than detached tissues, of C. obtusa over the whole season using an automated, closed-chamber system coupled to a laser-based instrument that allowed in situ real-time detection of CH4.

2. Materials and methods
2.1 Study Site
Methane flux measurements were conducted in the Kiryu Experimental Watershed (KEW) in Shiga
Automated closed chamber measurements of methane fluxes from intact leaves and trunk of Japanese cypress

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Prefecture, central Japan, from August 2009 to August 2010. A detailed description of the site and a topographic map has been reported elsewhere (Ohkubo et al., 2007). The watershed has an area of 5.99 ha (1 ha = 10^4 m^2) and is mainly covered by Japanese cypress trees that were planted in 1959 (average height: 19 m, basal area: 43 m^2 ha^{-1}, total basal area 1853 stems ha^{-1} for 92%). The air temperature, which was measured with a platinum thermometer (HMP45C, Vaisala) 29 m above the ground, ranged from -4.4 to 33.7°C during the study period. Precipitation was measured using a tipping bucket rain gauge (RT-5, Ikeda Keiki). Short-wave downward radiation in the region of 305 - 2800 nm was measured using an albedo meter (CM14, Kipp & Zonen) 29 m above the ground.

2.2 Experimental design

We used an automated closed-chamber system to investigate CH4 exchange in intact leaves and trunk of C. obtusa using a foliage chamber (L340 mm × W500 mm × H200 mm) and a trunk chamber (L300 mm × W300 mm × H300 mm), respectively. The same chamber system was used previously to measure ecosystem respiration (Ohkubo et al., 2007). The foliage chamber was placed in the lower canopy at a height of 17.2 m and enclosed a foliage surface area of 0.038 m^2. The diameter at breast height (DBH; 1.2 m above the ground) and height of the sample tree were 0.18 m and 20.7 m, respectively. The trunk chamber was placed at a height of 1.5 m and enclosed a trunk with a diameter of 180 mm. The DBH and height of the sample tree were 0.18 m and 20.3 m, respectively. Each chamber was constructed of acrylic resin. Using a quantum sensor (LP471PAR, DeltaOhm), the daily mean intensity of photosynthetically active wavelengths of radiation inside the chamber was estimated to be 95% of that outside the chamber. The chambers remain open most of the time and close for 120 seconds every 30 minutes. When the chamber is closed, a small ventilation fan ensures that the density of the gas within the chamber is uniform. During closure, air is drawn continuously from the target chamber through a polyfluoroacrylate tube (inner diameter: 4 mm) using a diaphragm pump at a flow rate of approximately 1.8 L min^{-1}, and is returned to the chamber. To analyze the CH4 concentration of the sampled air, some of the main flow (0.7 L min^{-1}) is passed through a membrane dryer (PD-50T-48, Perma Pure Inc.) to remove moisture before being diverted to a CO2/H2O analyzer (LI-840, LiCor) and a CH4 analyzer (FMA-100, Los Gatos Research). After analysis, the gas (0.7 L min^{-1}) was returned to the main flow before returning to the chamber.

The CH4 analyzer used in this study employed cavity enhanced absorption spectroscopy with a diode laser of around 1.6 μm; the instrument permits interference-free real-time monitoring of CH4 concentration at atmospheric levels (Hendriks et al., 2008; Smeets et al., 2009). Before deploying the CH4 analyzer in the field, the instrumental performance was tested using the Allan variance method (e.g., Eugster and Plüss, 2010). The mixing ratio of CH4 in a dry compressed air cylinder (Masuda Medical Instruments) was measured over several hours with 1-Hz resolution, and resultant time series of the data providing 2496 ± 0.9 ppb (mean ± SD) were subjected to the Allan variance analysis. This assessment suggested that the Allan deviation of this analyzer was 0.7 ppb with 1-s integration time (i.e., ±0.03% against a background concentration of 2496 ppb), which is similar to that for the same kind of this instrument as reported recently (Eugster and Plüss, 2010).

Methane flux from foliage, \( F_f^{\text{CH}_4} \) (nmol m^{-2} s^{-1}), was calculated using the following equation ([Ohkubo et al., 2007]):

\[
F_f^{\text{CH}_4} = \frac{\Delta C}{\Delta t} \times \frac{V_f}{A_f}
\]

where \( V_f \) (m^3) is the chamber volume, \( A_f \) (m^2) the enclosed foliage surface area (0.038 m^2), and \( \Delta C/\Delta t \) the rate of change in CH4 concentration inside the chamber (nmol m^{-3}) over time during chamber closure.

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The overall precision of our measurement system was estimated using standard gas (1.773 ppm in synthetic
air, Kyoto Teisan). The minimum measurable CH₄ flux, estimated from the overall precision of our system at
atmospheric levels of CH₄, were ±0.77 and ±0.11 nmol m⁻² s⁻¹ for foliage and trunk chambers, respectively.

The zero offset of the CH₄ analyzer was calibrated against pure nitrogen gas every day. The span of
the CH₄ analyzer was calibrated against standard gas every week. No serious drifts in zero and span readings
were observed throughout the measurement period (<1.0%). For the CO₂/H₂O analyzer, the zero offset was
checked with pure nitrogen gas every day. The CO₂ span was calibrated using a standard gas cylinder and
water vapor using a dew-point generator (LiCor, LI-610).

Methane flux from foliage, \( F_{CH4}^{fol} \) (nmol m⁻² s⁻¹), was calculated using the following equation
(Ohkubo et al., 2007):

\[
F_{CH4}^{fol} = \frac{\Delta C}{\Delta t} \times \frac{V_f}{A_f} \tag{1}
\]

where \( V_f \) (m³) is the chamber volume, \( A_f \) (m²) the enclosed foliage surface area (0.038 m²), and \( \Delta C/\Delta t \) the rate
of change in CH₄ concentration inside the chamber (nmol m⁻³) over time during chamber closure.

Methane flux from trunk, \( F_{CH4}^{trunk} \) (nmol m⁻² s⁻¹), was calculated using the following equation:
3. Results and discussion

3.1 Foliage

Figure 2 shows an example of temporal variations in CH4 fluxes between the foliage and trunk of *C. obtusa* and the atmosphere, as well as CO2 fluxes and changes in air temperature and solar radiation during 1 - 8 September, 2009. The quoted uncertainty in the individual CH4 flux values was equivalent to two standard deviations from the least-squares regression and the uncertainty in the measurement of CH4 concentration.

The CO2 fluxes from foliage followed a diel cycle in response to photosynthetic uptake and respiratory release, but such a diel cycle was not clearly observed in the CH4 fluxes. The CH4 fluxes were very low and around or below the minimum detection limit of our instrumentation. Very low fluxes are not contradictory to the results reported by Kamakura et al. (in press), although our experimental design is different from their study. The CH4 flux distributions in this study were not significantly different from zero (p-value > 0.01, two-tailed t-test).

In order to investigate the seasonal characteristics of the CH4 flux distributions, the obtained data were summed over three-month intervals as shown in Figure 3. Results indicate that seasonal variation in the flux distributions was unclear (p > 0.01) and intact leaves of *C. obtusa* did, in our experimental system, neither emit nor absorb detectable amounts of CH4 for all seasons (within ±1.0 nmol m⁻² s⁻¹), with the differences from zero statistically indistinguishable (p > 0.01). No clear diel or seasonal changes in \( F_{\text{CH4}} \) were observed with our experimental system, suggesting that changes in air-temperature and solar radiation (at least wavelengths longer than about 380 nm, see following paragraph) do not control the emission of CH4 from leaves of *C. obtusa* at rates detectable in our system.

Interestingly, various authors have reported that CH4 emissions from the structural component pectin, as well as from fresh and dried leaf material, occur in response to UV irradiation (McLeod et al., 2008; Vigano et al., 2008, 2009; Bruhn et al., 2009; Qaderi and Reid, 2009). On the other hand, Bowling et al. (2009) reported that no evidence was found for a significant foliar CH4 source in the vegetation canopy under high UV irradiance conditions. Unfortunately, in this study, we were unable to examine whether such UV-induced, non-enzymatic processes contribute to CH4 emissions because the chambers were constructed of acrylic, which filters out most of the solar UV (the cut-off wavelength of 380 nm was estimated using a UV-VIS

\[
F_{\text{CH4}} = \frac{\Delta C}{\Delta t} = \frac{V_f}{V_t} \times K_t
\]

where \( V_t \) is the chamber volume excluding the enclosed trunk volume (m³), \( V_f \) the enclosed trunk volume (6.03×10⁻³ m³), and \( \Delta C/\Delta t \) is as in Eq. (1). \( K_t \) (m³ m⁻²) is the coefficient converting the trunk chamber data into average trunk respiration per unit of ground area at the site. This equation is based on the assumption that the efflux per trunk volume is uniform. In addition, CO2 fluxes from foliage (\( F_{\text{CO2}} \)) and trunks (\( F_{\text{CO2}} \)) were estimated as described for Eqs. (1-2). Positive values for \( F_t \) and \( F_f \) indicate emission, whereas negative values do not.

Figure 1 shows a typical trace of the raw time series obtained for CH4 and CO2 concentrations in the foliage, trunk, and three different soil chambers during their closures on 4 September, 2009 at 14:00 - 14:15 local time (LT); a program-controlled valve was used to switch between the gas sampling lines connected to the different five chambers at 3-minute intervals. While analysis of the soil data is beyond the scope of this paper, temporal changes in CH4 and CO2 concentrations in all of the chambers obtained 75 sec after valve actuation were subjected to linear regression analysis to determine \( \Delta C/\Delta t \) in Eqs. (1-2); all calculations considered the time lag associated with the movement of sampled air to the analyzers and the gas flush times of the optical cavity in the analyzers.

A potential problem associated with determining \( \Delta C/\Delta t \) was the presence of artifacts related to the apparent dilution of CH4 due to the increase in water molecules derived from the transpiring leaves in the foliage chamber. In this study, a membrane dryer was used to remove water vapor from the air samples prior to CH4 analysis. However, the drying efficiency of such dryers has been reported to be dependent upon operational conditions (Leckrone and Haynes, 1997). We therefore measured the residual water vapor exiting the membrane dryer using the LI-840 analyzer (Fig. 1), and the data were used to compensate for the observed temporal changes in CH4 concentrations. The mixing ratio of residual water vapor was less than 4.8 permille and the rate of increase during chamber closure was less than 0.01 permille s⁻¹ over the entire observation period.
\[ F_{i}^{\text{CH}_4} = \frac{\Delta C}{\Delta t} = \frac{V_{i}' - V_{i}'}{V_{i}'} \times K_{i} \]  

where \( V_{i} \) is the chamber volume excluding the enclosed trunk volume (m\(^3\)), \( V_{i}' \) the enclosed trunk volume (6.03 \times 10^{-3}\) m\(^3\)), and \( \Delta C/\Delta t \) is as in Eq. (1). \( K_{i} \) (m\(^3\)/m\(^2\)) is the coefficient converting the trunk chamber data into average trunk respiration per unit of ground area at the site. This equation is based on the assumption that the efflux per trunk volume is uniform. In addition, CO\(_2\) fluxes from foliage (\( F_{i}^{\text{CO}_2} \)) and trunks (\( F_{i}'^{\text{CO}_2} \)) were estimated as described for Eqs. (1-2). Positive values for \( F_{i} \) and \( F_{i}' \) indicate emission, whereas negative values does uptake.

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3.2 Trunk

The results obtained for trunk were similar to those for foliage, i.e. as shown in Figs 2 and 3, no significant emission or uptake of CH4 was recorded and no diel or seasonal changes were evident over the course of the study (within ±0.14 nmol m-2 s-1), with the differences from zero statistically indistinguishable (p > 0.01). The CH4 fluxes were very low and around or below the minimum detection limit of our instrumentation. Recent incubation studies by Wang et al. (2008, 2009) have shown that CH4 emissions from detached xerophyte stems under aerobic conditions are both complex and vary depending on the species. Thus, much still remains to be done with regard to clarifying CH4 exchange between tree trunks and the atmosphere under aerobic conditions.

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References


Figure captions

**Figure 1** Typical time series for CH$_4$ and CO$_2$ concentrations in foliage, trunks and three different soil chambers on 4 September 2009, from 14:00 to 14:15 LT. Concentration of residual water vapor exiting the membrane dryer was also recorded (see text). At intervals of three minutes, a program-controlled valve switched between the lines connecting the different measurement chambers. The target chamber closed 30 sec after valve actuation. CH$_4$ concentrations were recorded at one-second intervals, while CO$_2$ and H$_2$O concentrations at 10-second intervals. Analysis of soil data is beyond the scope of this paper.

**Figure 2** Temporal variations in CH$_4$ and CO$_2$ fluxes between leaves or trunk and the atmosphere, and air temperature and solar radiation from September 1 to 8, 2009. Positive flux values indicate emission from leaves/trunk to the atmosphere, whereas negative values uptake. No precipitation was observed during this period. Arrows in the margin indicates the minimum measurable fluxes (see text).

**Figure 3** Box-and-whisker plots depicting seasonal CH$_4$ fluxes from leaves ($F_{l}^{CH_4}$) and trunk ($F_{t}^{CH_4}$). Data were divided into autumn (1 Sept., 2009 to 30 Nov., 2009; $n = 4145$ for foliage, $n = 4053$ for trunk), winter (1 Dec., 2009 to 28 Feb., 2010; $n = 1248$ for foliage, $n = 4102$ for trunk), spring (1 Mar., 2010 to 31 May, 2010; $n = 4175$ for foliage, $n = 4367$ for trunk), and summer (1 June, 2010 to 31 Aug., 2010; $n = 1375$ for foliage, $n = 2153$ for trunk). Boxes enclose all values between the 25th and 75th percentiles, whereas whiskers encompass all values within the 5th to 95th percentile range. Solid horizontal bars in the boxes indicate median values and horizontal broken bars indicate the mean values. Mean values for the foliage data are 0.007, -0.016, -0.003, and 0.008 nmol m$^{-2}$s$^{-1}$ for autumn, winter, spring and summer, respectively. Mean values of the trunk data are 0.003, -0.003, -0.002, and 0.003 nmol m$^{-2}$s$^{-1}$ for autumn, winter, spring and summer, respectively. Dotted lines indicate the minimum measurable fluxes (see text).
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