

1 **Automated closed chamber measurements of methane fluxes from intact leaves** 2 **and trunk of Japanese cypress**

3 Kenshi Takahashi^{1*}, Yoshiko Kosugi², Akito Kanazawa² and Ayaka Sakabe²

4 ¹ Research Institute for Sustainable Humanosphere, Kyoto University, Gokasyo, Uji 611-0011, Japan

5 ² Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

6 * Corresponding author, E-mail: tkenshi@rish.kyoto-u.ac.jp

7 8 **ABSTRACT**

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

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

19 20 **1. Introduction**

21 Recent experiments conducted by Keppler et al. (2006) suggested that CH₄ emissions from terrestrial
22 plants under aerobic conditions could be a significant source of atmospheric CH₄, and that global emissions
23 could range from 62 to 236 Tg CH₄ yr⁻¹. Using different scaling approaches, subsequent studies have revised
24 the upper limits of global aerobic CH₄ emissions by vegetation to 1 to 213 Tg CH₄ yr⁻¹ (Houweling et al.,
25 2006; Kirschbaum et al., 2006; Parsons et al., 2006; Butenhoff and Khalil, 2007; Ferreti et al., 2007;
26 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 samples 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

53 Prefecture, central Japan, from August 2009 to August 2010. A detailed description of the site and a
54 topographic map has been reported elsewhere (Ohkubo et al., 2007). The watershed has an area of 5.99 ha (1
55 ha = 10^4 m²) and is mainly covered by Japanese cypress trees that were planted in 1959 (average height: 19 m,
56 basal area: 43 m² ha⁻¹, total basal area 1853 stems ha⁻¹ for 92%). The air temperature, which was measured
57 with a platinum thermometer (HMP45C, Vaisala) 29 m above the ground, ranged from - 4.4 to 33.7°C during
58 the study period. Precipitation was measured using a tipping bucket rain gauge (RT-5, Ikeda Keiki).
59 Short-wave downward radiation in the region of 305 - 2800 nm was measured using an albedo meter (CM14,
60 Kipp & Zonen) 29 m above the ground.

61

62 2.2 Experimental design

63 We used an automated closed-chamber system to investigate CH₄ exchange in intact leaves and
64 trunk of *C. obtusa* using a foliage chamber (L340 mm × W500 mm × H200 mm) and a trunk chamber (L300
65 mm × W300 mm × H300 mm), respectively. The same chamber system was used previously to measure
66 ecosystem respiration (Ohkubo et al., 2007). The foliage chamber was placed in the lower canopy at a height
67 of 17.2 m and enclosed a foliage surface area of 0.038 m². The diameter at breast height (DBH; 1.2 m above
68 the ground) and height of the sample tree were 0.18 m and 20.7 m, respectively. The trunk chamber was
69 placed at a height of 1.5 m and enclosed a trunk with a diameter of 180 mm. The DBH and height of the
70 sample tree were 0.18 m and 20.3 m, respectively. Each chamber was constructed of acrylic resin. Using a
71 quantum sensor (LP471PAR, DeltaOhm), the daily mean intensity of photosynthetically active wavelengths of
72 radiation inside the chamber was estimated to be 95% of that outside the chamber. The chambers remain open
73 most of the time and close for 120 seconds every 30 minutes. When the chamber is closed, a small ventilation
74 fan ensures that the density of the gas within the chamber is uniform. During closure, air is drawn
75 continuously from the target chamber through a polyfluoroacrylate tube (inner diameter: 4 mm) using a
76 diaphragm pump at a flow rate of approximately 1.8 L min⁻¹, and is returned to the chamber. To analyze the
77 CH₄ concentration of the sampled air, some of the main flow (0.7 L min⁻¹) is passed through a membrane
78 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). 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, $F_f^{CH_4}$ (nmol m⁻² s⁻¹), was calculated using the following equation (Ohkubo *et al.*, 2007):

$$F_f^{CH_4} = \frac{\Delta C}{\Delta t} \times \frac{V_f}{A_f} \quad (1)$$

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 (C (nmol m⁻³)) over time during chamber closure.

Methane flux from trunk, $F_t^{CH_4}$ (nmol m⁻² s⁻¹), was calculated using the following equation:

$$F_t^{CH_4} = \frac{\Delta C}{\Delta t} \times \frac{V_t}{V'_t} \times K_t \quad (2)$$

where V_t is the chamber volume excluding the enclosed trunk volume (m^3), V'_t the enclosed trunk volume ($6.03 \times 10^{-3} m^3$), and $\Delta C/\Delta t$ is as in Eq. (1). K_t ($m^3 m^{-2}$) 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 ($F_f^{CO_2}$) and trunks ($F_t^{CO_2}$) were estimated as described for Eqs. (1-2). Positive values for F_f and F_t indicate emission, whereas negative values does uptake.

Figure 1 shows a typical trace of the raw time series obtained for CH_4 and CO_2 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 CH_4 and CO_2 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 CH_4 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 CH_4 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 CH_4 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 $0.01 \text{ permille s}^{-1}$ over the entire observation period.

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 $\pm 1.0 \text{ nmol m}^{-2} \text{ s}^{-1}$), with the differences from zero statistically indistinguishable (p > 0.01). No clear diel or seasonal changes in $F_{\text{f}}^{\text{CH}_4}$ 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; Viganò 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

spectrometer (U-2001, Hitachi)). Using chambers constructed of UV-transparent glass would enable us to address this issue. On the other hand, we have also been investigating CH₄ exchange on a canopy scale in KEW using a technique employing relaxed eddy accumulation; the obtained results have revealed the existence of seasonal variations in CH₄ flux with emission (from the forest to the atmosphere) maxima occurring in September to October and uptake (from the atmosphere to the forest) maxima occurring in May to June (Sakabe et al., 2011). Since surface UV intensity on Honshu Island in Japan peaks near the summer solstice and troughs near the winter solstice, even if UV light-induced processes promoted the emission of CH₄ from intact *C. obtusa* leaves, such emissions would not markedly affect CH₄ flux on a canopy scale.

163

164 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 $\pm 0.14 \text{ nmol m}^{-2} \text{ s}^{-1}$), 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.

173

174 Acknowledgments

The authors thank to Drs. Masayuki Ito (Kyoto Univ.) and Mai Kamakura (Nara Womens' Univ.) for fruitful discussions. This study was supported in part by a Grant-in-Aid from the MEXT. The authors are grateful to the anonymous referees for their constructive comments.

178

179 References

180 Beerling, D.J., Gardiner, T., Leggett, G., McLeod, A., Quick, W.P., 2008. Missing methane emissions from

181 leaves of terrestrial plants. *Glob. Change Biol.* 14, 1821-1826.
 182 Bloom, A.A., Lee-Taylor, J., Madronich, S., Messenger, D.J., Palmer, P.I., Reay, D.S., McLeod, A.R., 2010.
 183 Global methane emission estimates from ultraviolet irradiation of terrestrial plant foliage. *New Phytol.*
 184 187, 417-425.
 185 Bowling, D.R., Miller, J.B., Rhodes, M.E., Burns, S.P., Monson, R.K., Baer, D., 2009. Soil, plant, and
 186 transport influences on methane in a subalpine forest under high ultraviolet irradiance. *Biogeosciences*
 187 6, 1311-1324.
 188 Brüggemann, N., Meier, R., Steigner, D., Zimmer, I., Louis, S., Schnitzler, J.P., 2009. Nonmicrobial aerobic
 189 methane emission from poplar shoot cultures under low-light conditions. *New Phytol.* 182, 912-918.
 190 Bruhn, D., Mikkelsen, T.N., Obro, J., Willats, W.G.T., Ambus, P., 2009. Effects of temperature, ultraviolet
 191 radiation and pectin methyl esterase on aerobic methane release from plant material. *Plant Biol.* 11,
 192 43-48.
 193 Butenhoff, C.L., Khalil, M.A.K., 2007. Global methane emissions from terrestrial plants. *Environ. Sci. Tech.*
 194 41, 4032-4037.
 195 Cao, G.M., Xu, X.L., Long, R.J., Wang, Q.L., Wang, C.T., Du, Y.G., Zhao, X.Q., 2008. Methane emissions by
 196 alpine plant communities in the Qinghai-Tibet Plateau. *Biol. Lett.* 4, 681-684.
 197 Dueck, T.A., de Visser, R., Poorter, H., Persijn, S., Gorissen, A., de Visser, W., Schapendonk, A., Verhagen, J.,
 198 Snel, J., Harren, F.J.M., Ngai, A.K.Y., Verstappen, F., Bouwmeester, H., Voesenek, L., van der Werf, A.,
 199 2007. No evidence for substantial aerobic methane emission by terrestrial plants: a C-13-labelling
 200 approach. *New Phytol.* 175, 29-35.
 201 Eugster, W., Plüss, P., 2010. A fault-tolerant eddy covariance system for measuring CH₄ fluxes. *Agric. Forest*
 202 *Meteorol.* 150, 841-851.
 203 Ferretti, D.F., Miller, J.B., White, J.W.C., Lassey, K.R., Lowe, D.C., Etheridge, D.M., 2007. Stable isotopes
 204 provide revised global limits of aerobic methane emissions from plants. *Atmos. Chem. Phys.* 7,
 205 237-241.
 206 Hendriks, D.M.D., Dolman, A.J., van der Molen, M.K., van Huissteden, J., 2008. A compact and stable eddy

covariance set-up for methane measurements using off-axis integrated cavity output spectroscopy. Atmos. Chem. Phys. 8, 431-443.

Houweling, S., Röckmann, T., Aben, I., Keppler, F., Krol, M., Meirink, J.F., Dlugokencky, E.J., Frankenberg, C., 2006. Atmospheric constraints on global emissions of methane from plants. Geophys. Res. Lett. 33.L15821, doi:10.1029/2006GL026162.

Japan Forestry Agency, 2005. Annual report on trends of forest and forestry. In: The Ministry of Agriculture, Forestry and Fisheries of Japan (Ed.).

Kamakura, M., Kosugi, Y., Nakagawa, R., Itoh, M., 2011. Methane flux of leaves in a tropical rainforest and a temperate conifer forest. J. Agric. Meteorol., in press.

Keppler, F., Hamilton, J.T.G., Brass, M., Röckmann, T., 2006. Methane emissions from terrestrial plants under aerobic conditions. Nature 439, 187-191.

Kirschbaum, M.U.F., Bruhn, D., Etheridge, D.M., Evans, J.R., Farquhar, G.D., Gifford, R.M., Paul, K.I., Winters, A.J., 2006. A comment on the quantitative significance of aerobic methane release by plants. Funct. Plant Biol. 33, 521-530.

Kirschbaum, M.U.F., Walcroft, A., 2008. No detectable aerobic methane efflux from plant material, nor from adsorption/desorption processes. Biogeosciences 5, 1551-1558.

Leckrone, K.J., Hayes, J.M., 1997. Efficiency and temperature dependence of water removal by membrane dryers. Anal. Chem. 69, 911-918.

McLeod, A.R., Fry, S.C., Loake, G.J., Messenger, D.J., Reay, D.S., Smith, K.A., Yun, B.W., 2008. Ultraviolet radiation drives methane emissions from terrestrial plant pectins. New Phytol. 180, 124-132.

Megonigal, J.P., Guenther, A.B., 2008. Methane emissions from upland forest soils and vegetation. Tree Physiol. 28, 491-498.

Nisbet, R.E.R., Fisher, R., Nimmo, R.H., Bendall, D.S., Crill, P.M., Gallego-Sala, A.V., Hornibrook, E.R.C., Lopez-Juez, E., Lowry, D., Nisbet, P.B.R., Shuckburgh, E.F., Sriskantharajah, S., Howe, C.J., Nisbet, E.G., 2009. Emission of methane from plants. Proceedings of the Royal Society B-Biological Sciences 276, 1347-1354.

233 Ohkubo, S., Kosugi, Y., Takanashi, S., Mitani, T., Tani, M., 2007. Comparison of the eddy covariance and
 234 automated closed chamber methods for evaluating nocturnal CO₂ exchange in a Japanese cypress
 235 forest. *Agricul. Forest Meteorol.* 142, 50-65.
 236 Ohte, N., Tokuchi, N., Suzuki, M., 1997. An in situ lysimeter experiment on soil moisture influence on
 237 inorganic nitrogen discharge from forest soil. *J. Hydrol.* 195, 78-98.
 238 Qaderi, M.M., Reid, D.M., 2009. Methane emissions from six crop species exposed to three components of
 239 global climate change: temperature, ultraviolet-B radiation and water stress. *Physiol. Plant.* 137,
 240 139-147.
 241 Parsons, A.J., Newton, P.C.D., Clark, H., Kelliher, F.M., 2006. Scaling methane emissions from vegetation.
 242 *Trends in Ecology and Evolution* 21, 423-424.
 243 Sakabe, A., Hamotani, K., Kosugi, Y., Ueyama, M., Takahashi, K., Kanazawa, A., Itoh, M., 2011.
 244 Measurement of methane flux over an evergreen coniferous forest canopy using a relaxed eddy
 245 accumulation system with tunable diode laser spectroscopy detection. *Theor. Appl. Climatol.* (in press)
 246 Smeets, C., Holzinger, R., Vigano, I., Goldstein, A.H., Röckmann, T., 2009. Eddy covariance methane
 247 measurements at a Ponderosa pine plantation in California. *Atmos. Chem. Phys.* 9, 8365-8375.
 248 Vigano, I., van Weelden, H., Holzinger, R., Keppler, F., McLeod, A., Röckmann, T., 2008. Effect of UV
 249 radiation and temperature on the emission of methane from plant biomass and structural components.
 250 *Biogeosciences* 5, 937-947.
 251 Vigano, I., Röckmann, T., Holzinger, R., van Dijk, A., Keppler, F., Greule, M., Brand, W.A., Geilmann, H.,
 252 van Weelden, H., 2009. The stable isotope signature of methane emitted from plant material under UV
 253 irradiation. *Atmos. Environ.* 43, 5637-5646.
 254 Wang, Z.P., Han, X.G., Wang, G.G., Song, Y., Gulledge, J., 2008. Aerobic methane emission from plants in
 255 the Inner Mongolia steppe. *Environ. Sci. Tech.* 42, 62-68.
 256 Wang, Z.P., Gulledge, J., Zheng, J.Q., Liu, W., Li, L.H., Han, X.G., 2009. Physical injury stimulates aerobic
 257 methane emissions from terrestrial plants. *Biogeosciences* 6, 615-621.
 258

259 **Figure captions**

260 **Figure 1** Typical time series for CH₄ and CO₂ concentrations in foliage, trunks and three different soil
261 chambers on 4 September 2009, from 14:00 to 14:15 LT. Concentration of residual water vapor exiting the
262 membrane dryer was also recorded (see text). At intervals of three minutes, a program-controlled valve
263 switched between the lines connecting the different measurement chambers. The target chamber closed 30 sec
264 after valve actuation. CH₄ concentrations were recorded at one-second intervals, while CO₂ and H₂O
265 concentrations at 10-second intervals. Analysis of soil data is beyond the scope of this paper.

266

267 **Figure 2** Temporal variations in CH₄ and CO₂ fluxes between leaves or trunk and the atmosphere, and air
268 temperature and solar radiation from September 1 to 8, 2009. Positive flux values indicate emission from
269 leaves/trunk to the atmosphere, whereas negative values uptake. No precipitation was observed during this
270 period. Arrows in the margin indicates the minimum measureable fluxes (see text).

271

272 **Figure 3** Box-and-whisker plots depicting seasonal CH₄ fluxes from leaves ($F_f^{CH_4}$) and trunk ($F_t^{CH_4}$). Data
273 were divided into autumn (1 Sept., 2009 to 30 Nov., 2009; $n = 4145$ for foliage, $n = 4053$ for trunk), winter (1
274 Dec., 2009 to 28 Feb., 2010; $n = 1248$ for foliage, $n = 4102$ for trunk), spring (1. Mar., 2010 to 31 May, 2010;
275 $n = 4175$ for foliage, $n = 4367$ for trunk), and summer (1 June, 2010 to 31 Aug., 2010; $n = 1375$ for foliage, n
276 $= 2153$ for trunk). Boxes enclose all values between the 25th and 75th percentiles, whereas whiskers encompass
277 all values within the 5th to 95th percentile range. Solid horizontal bars in the boxes indicate median values and
278 horizontal broken bars indicate the mean values. Mean values for the foliage data are 0.007, -0.016, -0.003,
279 and 0.008 nmol m⁻²s⁻¹ for autumn, winter, spring and summer, respectively. Mean values of the trunk data are
280 0.003, -0.003, -0.002, and 0.003 nmol m⁻²s⁻¹ for autumn, winter, spring and summer, respectively. Dotted lines
281 indicate the minimum measureable fluxes (see text).

282

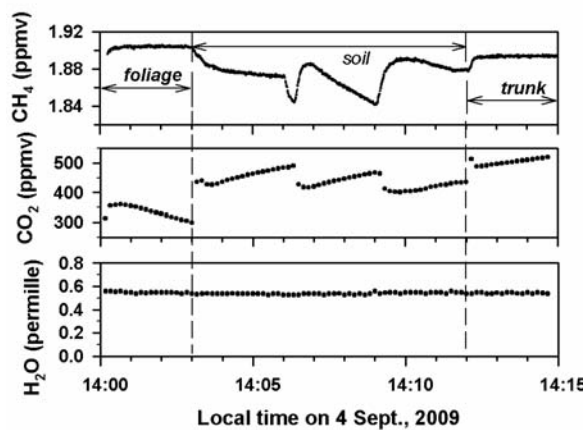


Figure 1 Takahashi et al.

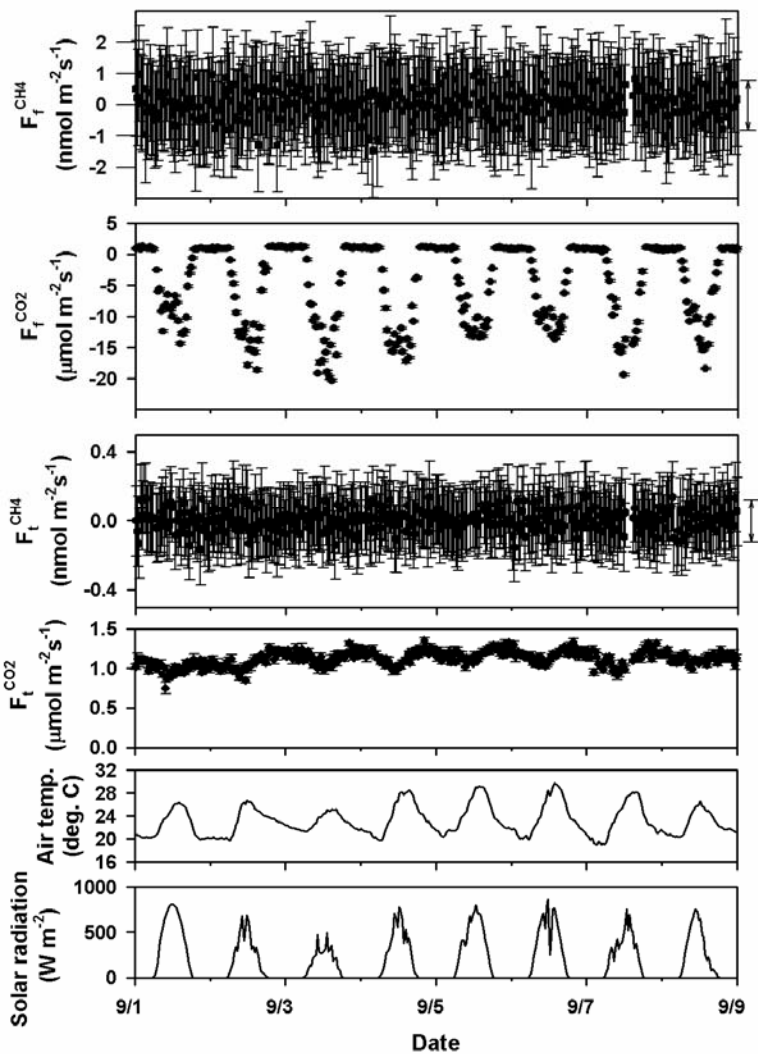


Figure 2 Takahashi et al.

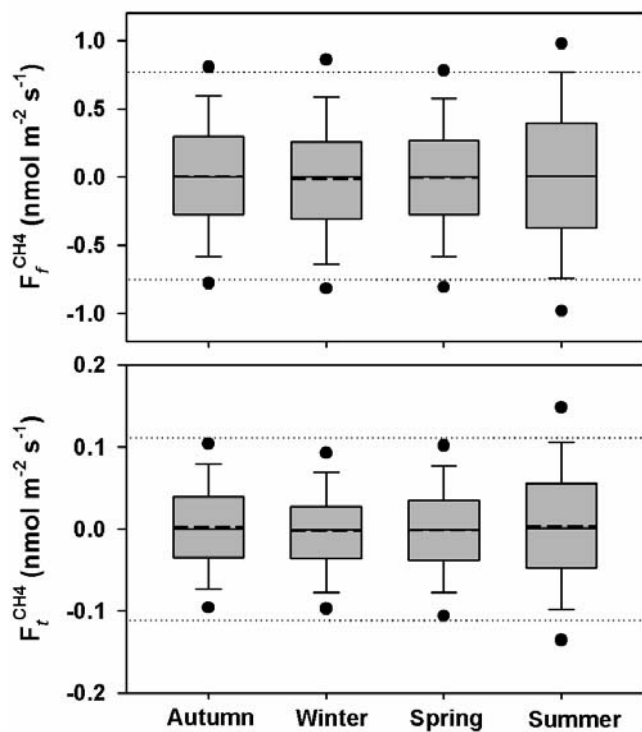


Figure 3 Takahashi et al.