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

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7  
8 **ABSTRACT**

9 Continuous *in situ* measurements of methane (CH<sub>4</sub>) 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 CO<sub>2</sub> exchange between the atmosphere and  
12 forest ecosystems, was coupled to a laser-based instrument to monitor CH<sub>4</sub> concentrations. Temporal changes  
13 in CH<sub>4</sub> concentrations from the foliage and trunk were measured at one-second intervals during chamber  
14 closure to determine CH<sub>4</sub> fluxes between the leaf and trunk surfaces and the atmosphere. While recent studies  
15 have suggested that some plants emit CH<sub>4</sub> under aerobic conditions, emission or uptake of CH<sub>4</sub> 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 CH<sub>4</sub> emissions from terrestrial  
22 plants under aerobic conditions could be a significant source of atmospheric CH<sub>4</sub>, and that global emissions  
23 could range from 62 to 236 Tg CH<sub>4</sub> yr<sup>-1</sup>. Using different scaling approaches, subsequent studies have revised  
24 the upper limits of global aerobic CH<sub>4</sub> emissions by vegetation to 1 to 213 Tg CH<sub>4</sub> yr<sup>-1</sup> (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 CH<sub>4</sub>

27 emission are still largely unknown, any extrapolations to the global scale are highly speculative. The data  
28 collected to date for *in situ* CH<sub>4</sub> exchange in plant communities under aerobic conditions has been conflicting,  
29 with some studies reporting significant CH<sub>4</sub> emissions (Cao et al., 2008; McLeod et al., 2008; Vigano et al.,  
30 2008; Wang et al., 2008; Brüggemann et al., 2009; Bruhn et al., 2009; Qaderi and Reid, 2009), while others  
31 have reported no significant emissions (Dueck et al., 2007; Beerling et al., 2008; Kirschbaum and Walcroft,  
32 2008; Megonigal and Guenther, 2008; Bowling et al., 2009; Nisbert et al., 2009). Similarly, no consensus  
33 currently exists on the effect of ultraviolet (UV) irradiation on CH<sub>4</sub> emissions (McLeod et al., 2008; Vigano et  
34 al., 2008, 2009; Bowling et al., 2009; Bruhn et al., 2009; Qaderi and Reid, 2009). Thus, despite numerous  
35 studies having been conducted on the microscopic mechanisms associated with CH<sub>4</sub> production and the  
36 impact that these have on the global CH<sub>4</sub> budget, the issue of CH<sub>4</sub> exchange in plants under aerobic conditions  
37 has not yet been satisfactorily resolved.

38 In Japan, artificial plantations of Japanese cypress (*Chamaecyparis obtusa* Sieb. Et Zucc) cover up  
39 to 10% of the total Japanese forest area (Japan Forestry Agency, 2005). Investigating whether *C. obtusa* emits  
40 CH<sub>4</sub> significantly is thus important to develop an emission inventory of CH<sub>4</sub> in Japan and to understand its  
41 impact on the atmospheric CH<sub>4</sub> budget. Recently, laboratory incubations investigating CH<sub>4</sub> emission from  
42 detached leaves of *C. obtusa* in a temperate forest have been made (Kamakura et al., in press), in which leaf  
43 samples were collected at different heights within the canopy. Estimated CH<sub>4</sub> fluxes were close to zero, which  
44 were several orders of magnitude smaller than, for example, those from some other C<sub>3</sub> plants as reported by  
45 Keppler et al.(2006). In this study, we for the first time made an attempt to estimate CH<sub>4</sub> fluxes from intact  
46 leaves and trunk, which are rather than detached tissues, of *C. obtusa* over the whole season using an  
47 automated, closed-chamber system coupled to a laser-based instrument that allowed *in situ* real-time detection  
48 of CH<sub>4</sub>.

49

## 50 **2. Materials and methods**

### 51 *2.1 Study Site*

52 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 =  $10^4$  m<sup>2</sup>) and is mainly covered by Japanese cypress trees that were planted in 1959 (average height: 19 m,  
56 basal area: 43 m<sup>2</sup> ha<sup>-1</sup>, total basal area 1853 stems ha<sup>-1</sup> 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 CH<sub>4</sub> 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 m<sup>2</sup>. 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<sup>-1</sup>, and is returned to the chamber. To analyze the  
77 CH<sub>4</sub> concentration of the sampled air, some of the main flow (0.7 L min<sup>-1</sup>) is passed through a membrane  
78 dryer (PD-50T-48, Perma Pure Inc.) to remove moisture before being diverted to a CO<sub>2</sub>/H<sub>2</sub>O analyzer (LI-840,

79 LiCor) and a CH<sub>4</sub> analyzer (FMA-100, Los Gatos Research). After analysis, the gas (0.7 L min<sup>-1</sup>) was returned  
80 to the main flow before returning to the chamber.

81 The CH<sub>4</sub> analyzer used in this study employed cavity enhanced absorption spectroscopy with a diode  
82 laser of around 1.6 μm; the instrument permits interference-free real-time monitoring of CH<sub>4</sub> concentration at  
83 atmospheric levels (Hendriks *et al.*, 2008; Smeets *et al.*, 2009). Before deploying the CH<sub>4</sub> analyzer in the field,  
84 the instrumental performance was tested using the Allan variance method (e.g., Eugster and Plüss, 2010). The  
85 mixing ratio of CH<sub>4</sub> in a dry compressed air cylinder (Masuda Medical Instruments) was measured over  
86 several hours with 1-Hz resolution, and resultant time series of the data providing 2496 ± 0.9 ppb (mean ±  
87 SD) were subjected to the Allan variance analysis. This assessment suggested that the Allan deviation of this  
88 analyzer was 0.7 ppb with 1-s integration time (i.e., ±0.03% against a background concentration of 2496 ppb),  
89 which is similar to that for the same kind of this instrument as reported recently (Eugster and Plüss, 2010).  
90 The overall precision of our measurement system was estimated using standard gas (1.773 ppm in synthetic  
91 air, Kyoto Teisan). The minimum measurable CH<sub>4</sub> flux, estimated from the overall precision of our system at  
92 atmospheric levels of CH<sub>4</sub>, were ±0.77 and ±0.11 nmol m<sup>-2</sup> s<sup>-1</sup> for foliage and trunk chambers, respectively.

93 The zero offset of the CH<sub>4</sub> analyzer was calibrated against pure nitrogen gas every day. The span of  
94 the CH<sub>4</sub> analyzer was calibrated against standard gas every week. No serious drifts in zero and span readings  
95 were observed throughout the measurement period (<1.0%). For the CO<sub>2</sub>/H<sub>2</sub>O analyzer, the zero offset was  
96 checked with pure nitrogen gas every day. The CO<sub>2</sub> span was calibrated using a standard gas cylinder and  
97 water vapor using a dew-point generator (LiCor, LI-610).

98 Methane flux from foliage,  $F_f^{CH_4}$  (nmol m<sup>-2</sup> s<sup>-1</sup>), was calculated using the following equation  
99 (Ohkubo *et al.*, 2007):

$$100 \quad F_f^{CH_4} = \frac{\Delta C}{\Delta t} \times \frac{V_f}{A_f} \quad (1)$$

101 where  $V_f$  (m<sup>3</sup>) is the chamber volume,  $A_f$  (m<sup>2</sup>) the enclosed foliage surface area (0.038 m<sup>2</sup>), and  $\Delta C/\Delta t$  the rate  
102 of change in CH<sub>4</sub> concentration inside the chamber ( $C$  (nmol m<sup>-3</sup>)) over time during chamber closure.

103 Methane flux from trunk,  $F_t^{CH_4}$  (nmol m<sup>-2</sup> s<sup>-1</sup>), was calculated using the following equation:

104 
$$F_t^{CH_4} = \frac{\Delta C}{\Delta t} \times \frac{V_t}{V'_t} \times K_t \quad (2)$$

105 where  $V_t$  is the chamber volume excluding the enclosed trunk volume ( $m^3$ ),  $V'_t$  the enclosed trunk volume  
106 ( $6.03 \times 10^{-3} m^3$ ), 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  
107 average trunk respiration per unit of ground area at the site. This equation is based on the assumption that the  
108 efflux per trunk volume is uniform. In addition,  $CO_2$  fluxes from foliage ( $F_f^{CO_2}$ ) and trunks ( $F_t^{CO_2}$ ) were  
109 estimated as described for Eqs. (1-2). Positive values for  $F_f$  and  $F_t$  indicate emission, whereas negative values  
110 does uptake.

111 Figure 1 shows a typical trace of the raw time series obtained for  $CH_4$  and  $CO_2$  concentrations in the  
112 foliage, trunk, and three different soil chambers during their closures on 4 September, 2009 at 14:00 - 14:15  
113 local time (LT); a program-controlled valve was used to switch between the gas sampling lines connected to  
114 the different five chambers at 3-minute intervals. While analysis of the soil data is beyond the scope of this  
115 paper, temporal changes in  $CH_4$  and  $CO_2$  concentrations in all of the chambers obtained 75 sec after valve  
116 actuation were subjected to linear regression analysis to determine  $\Delta C/\Delta t$  in Eqs. (1-2); all calculations  
117 considered the time lag associated with the movement of sampled air to the analyzers and the gas flush times  
118 of the optical cavity in the analyzers.

119 A potential problem associated with determining  $\Delta C/\Delta t$  was the presence of artifacts related to the  
120 apparent dilution of  $CH_4$  due to the increase in water molecules derived from the transpiring leaves in the  
121 foliage chamber. In this study, a membrane dryer was used to remove water vapor from the air samples prior  
122 to  $CH_4$  analysis. However, the drying efficiency of such dryers has been reported to be dependent upon  
123 operational conditions (Leckrone and Haynes, 1997). We therefore measured the residual water vapor exiting  
124 the membrane dryer using the LI-840 analyzer (Fig. 1), and the data were used to compensate for the observed  
125 temporal changes in  $CH_4$  concentrations. The mixing ratio of residual water vapor was less than 4.8 permille  
126 and the rate of increase during chamber closure was less than  $0.01$  permille  $s^{-1}$  over the entire observation  
127 period.

128

129 **3. Results and discussion**

130 *3.1 Foliage*

131 Figure 2 shows an example of temporal variations in CH<sub>4</sub> fluxes between the foliage and trunk of *C.*  
132 *obtusa* and the atmosphere, as well as CO<sub>2</sub> fluxes and changes in air temperature and solar radiation during 1 -  
133 8 September, 2009. The quoted uncertainty in the individual CH<sub>4</sub> flux values was equivalent to two standard  
134 deviations from the least-squares regression and the uncertainty in the measurement of CH<sub>4</sub> concentration.  
135 The CO<sub>2</sub> fluxes from foliage followed a diel cycle in response to photosynthetic uptake and respiratory release,  
136 but such a diel cycle was not clearly observed in the CH<sub>4</sub> fluxes. The CH<sub>4</sub> fluxes were very low and around or  
137 below the minimum detection limit of our instrumentation. Very low fluxes are not contradictory to the results  
138 reported by Kamakura et al. (in press), although our experimental design is different from their study. The  
139 CH<sub>4</sub> flux distributions in this study were not significantly different from zero (p-value > 0.01, two-tailed t-test).  
140 In order to investigate the seasonal characteristics of the CH<sub>4</sub> flux distributions, the obtained data were  
141 summed over three-month intervals as shown in Figure 3. Results indicate that seasonal variation in the flux  
142 distributions was unclear (p > 0.01) and intact leaves of *C. obtusa* did, in our experimental system, neither  
143 emit nor absorb detectable amounts of CH<sub>4</sub> for all seasons (within  $\pm 1.0 \text{ nmol m}^{-2} \text{ s}^{-1}$ ), with the differences  
144 from zero statistically indistinguishable (p > 0.01). No clear diel or seasonal changes in  $F_f^{\text{CH}_4}$  were observed  
145 with our experimental system, suggesting that changes in air-temperature and solar radiation (at least  
146 wavelengths longer than about 380 nm, see following paragraph) do not control the emission of CH<sub>4</sub> from  
147 leaves of *C. obtusa* at rates detectable in our system.

148 Interestingly, various authors have reported that CH<sub>4</sub> emissions from the structural component pectin,  
149 as well as from fresh and dried leaf material, occur in response to UV irradiation (McLeod et al., 2008; Viganò  
150 et al., 2008, 2009, Bruhn et al., 2009; Qaderi and Reid, 2009). On the other hand, Bowling et al. (2009)  
151 reported that no evidence was found for a significant foliar CH<sub>4</sub> source in the vegetation canopy under high  
152 UV irradiance conditions. Unfortunately, in this study, we were unable to examine whether such UV-induced,  
153 non-enzymatic processes contribute to CH<sub>4</sub> emissions because the chambers were constructed of acrylic,  
154 which filters out most of the solar UV (the cut-off wavelength of 380 nm was estimated using a UV-VIS

155 spectrometer (U-2001, Hitachi)). Using chambers constructed of UV-transparent glass would enable us to  
156 address this issue. On the other hand, we have also been investigating CH<sub>4</sub> exchange on a canopy scale in  
157 KEW using a technique employing relaxed eddy accumulation; the obtained results have revealed the  
158 existence of seasonal variations in CH<sub>4</sub> flux with emission (from the forest to the atmosphere) maxima  
159 occurring in September to October and uptake (from the atmosphere to the forest) maxima occurring in May  
160 to June (Sakabe et al., 2011). Since surface UV intensity on Honshu Island in Japan peaks near the summer  
161 solstice and troughs near the winter solstice, even if UV light-induced processes promoted the emission of  
162 CH<sub>4</sub> from intact *C. obtusa* leaves, such emissions would not markedly affect CH<sub>4</sub> flux on a canopy scale.

163

### 164 3.2 Trunk

165 The results obtained for trunk were similar to those for foliage, i.e. as shown in Figs 2 and 3, no  
166 significant emission or uptake of CH<sub>4</sub> was recorded and no diel or seasonal changes were evident over the  
167 course of the study (within  $\pm 0.14 \text{ nmol m}^{-2} \text{ s}^{-1}$ ), with the differences from zero statistically indistinguishable ( $p$   
168  $> 0.01$ ). The CH<sub>4</sub> fluxes were very low and around or below the minimum detection limit of our  
169 instrumentation. Recent incubation studies by Wang et al. (2008, 2009) have shown that CH<sub>4</sub> emissions from  
170 detached xerophyte stems under aerobic conditions are both complex and vary depending on the species. Thus,  
171 much still remains to be done with regard to clarifying CH<sub>4</sub> exchange between tree trunks and the atmosphere  
172 under aerobic conditions.

173

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178

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259 **Figure captions**

260 **Figure 1** Typical time series for CH<sub>4</sub> and CO<sub>2</sub> 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. CH<sub>4</sub> concentrations were recorded at one-second intervals, while CO<sub>2</sub> and H<sub>2</sub>O  
265 concentrations at 10-second intervals. Analysis of soil data is beyond the scope of this paper.

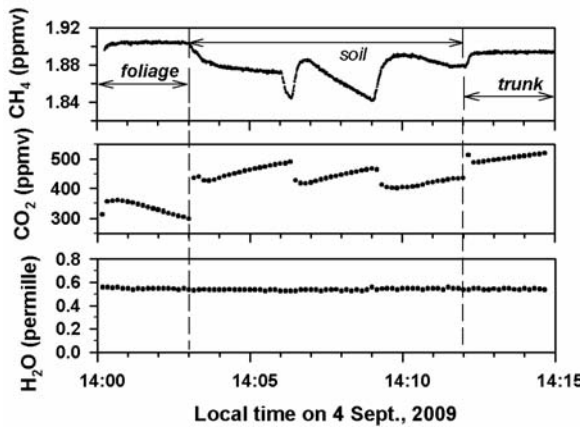
266

267 **Figure 2** Temporal variations in CH<sub>4</sub> and CO<sub>2</sub> 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

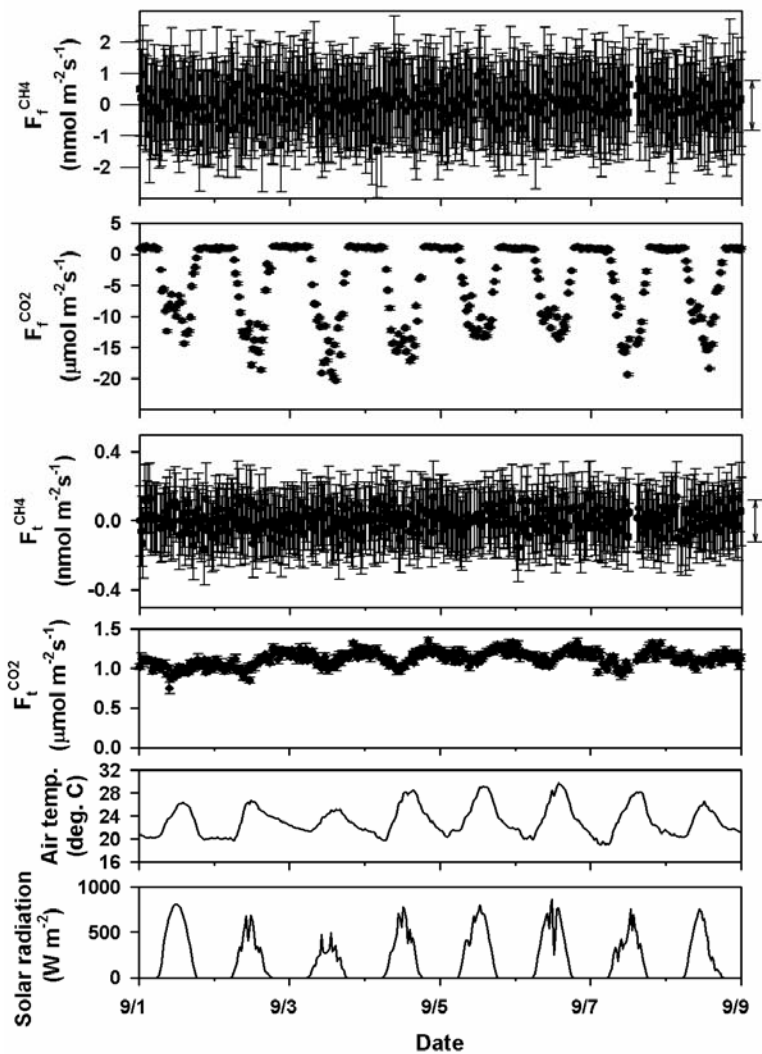
272 **Figure 3** Box-and-whisker plots depicting seasonal CH<sub>4</sub> fluxes from leaves ( $F_f^{CH_4}$ ) and trunk ( $F_t^{CH_4}$ ). Data  
273 were divided into autumn (1 Sept., 2009 to 30 Nov., 2009;  $n = 4145$  for foliage,  $n = 4053$  for trunk), winter (1  
274 Dec., 2009 to 28 Feb., 2010;  $n = 1248$  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 25<sup>th</sup> and 75<sup>th</sup> percentiles, whereas whiskers encompass  
277 all values within the 5<sup>th</sup> to 95<sup>th</sup> 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<sup>-2</sup>s<sup>-1</sup> 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<sup>-2</sup>s<sup>-1</sup> for autumn, winter, spring and summer, respectively. Dotted lines  
281 indicate the minimum measureable fluxes (see text).

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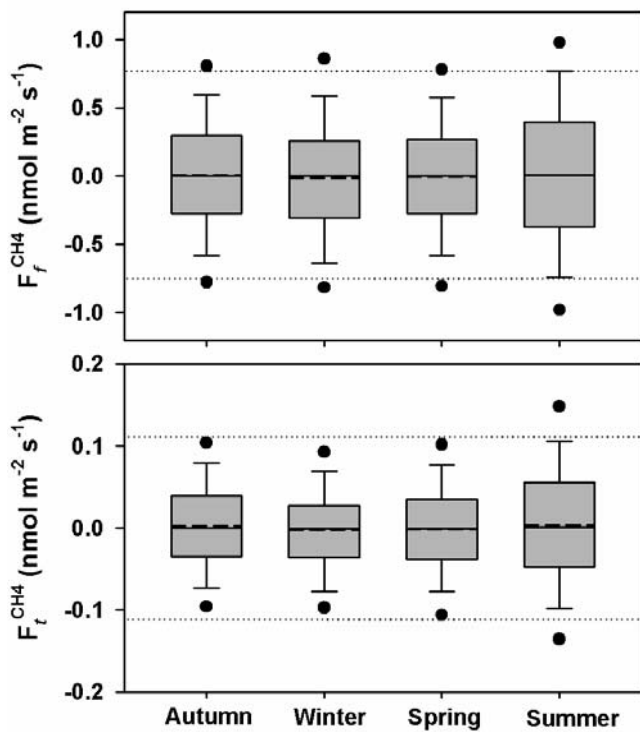
284 Figure 1 Takahashi et al.



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286 **Figure 2** Takahashi et al.

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290 Figure 3 Takahashi et al.

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