

# Methane exchange between tree stems and atmosphere in a boreal forest



*Photography: Laura Reinelt*

**Laura Reinelt**

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Supervisor: Elin Sundqvist, Department of Physical Geography and Ecosystems Science  
Lund University

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## Abstract

The role of vegetation in the global CH<sub>4</sub> budget and the processes involved in CH<sub>4</sub> emission by plants have been subject to debate in the past couple of years. This study contributes to this field of research by studying CH<sub>4</sub> fluxes between tree stems and the atmosphere on *pinus sylvestris* and *picea abies* in a boreal forest using chamber measurements. Additionally to CH<sub>4</sub> fluxes, CO<sub>2</sub> and H<sub>2</sub>O fluxes, sap flow and air temperature were measured in order to investigate which processes could be responsible for the CH<sub>4</sub> fluxes. Both positive and negative CH<sub>4</sub> fluxes between  $-1.92 \pm 0.15$  and  $2.99 \pm 0.29 \mu\text{g m}^{-2} \text{h}^{-1}$  could be detected. It can however not be determined for sure that they originated from the tree stems and not from air entering the chamber through untight spaces. No correlations between CH<sub>4</sub> fluxes and CO<sub>2</sub> or H<sub>2</sub>O fluxes or air temperature could be found. There was a significant positive correlation between CH<sub>4</sub> fluxes and sap flow in *picea abies*, but not in *pinus sylvestris*. Mediation of CH<sub>4</sub> solved in groundwater through the transpiration stream occurring simultaneously with uptake of CH<sub>4</sub> by methanotrophs were suggested as the processes that could most likely be responsible for the fluxes.

**Key words:** CH<sub>4</sub>, flux, tree stem, boreal forest

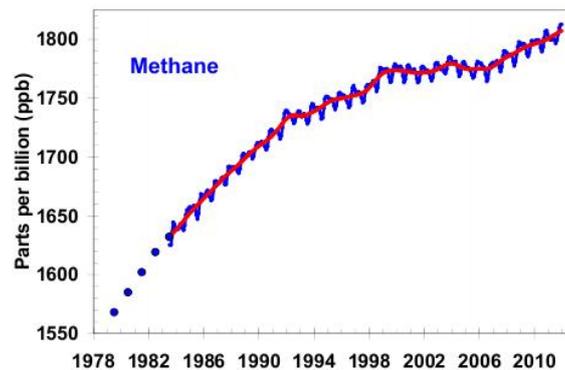
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## 1. Introduction

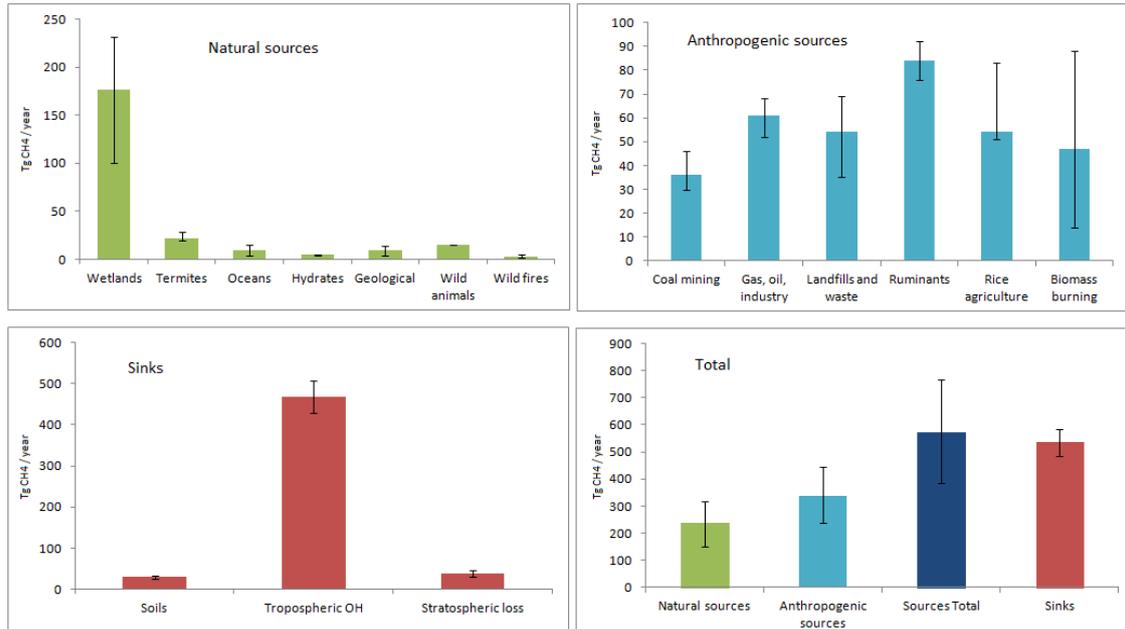
Methane (CH<sub>4</sub>) is an important greenhouse gas. According to the IPCC (2007) it has a global warming potential of 25 over a 100-year time horizon and an atmospheric lifetime of  $8.7 \pm 1.3$  years, so it is a much stronger greenhouse gas than CO<sub>2</sub> but it stays in the atmosphere shorter.

It is known from ice core measurements that atmospheric CH<sub>4</sub> concentration more than doubled since preindustrial times (Petit et al., 1999). In 2011, values for the annual mean atmospheric CH<sub>4</sub> concentration ranged between 1758 and 1874 ppb depending on the measurement station (Carbon Dioxide Information Analysis Center, 2013). Figure 1 shows the development of atmospheric CH<sub>4</sub> concentration during the past 24 years, which was not linear (NOAA Earth System Research Laboratory, 2012). The growth rate declined from 1983 until 1999 and then remained approximately constant until 2006. Since 2007 atmospheric CH<sub>4</sub> concentrations have been increasing again up to the present day. The mechanisms behind the variation in growth rate have not yet been completely understood, but a stabilization of fossil-fuel emissions (Aydin et al., 2011), a decrease in Northern Hemisphere microbial CH<sub>4</sub> sources (Kai et al., 2011) and an increased uptake by vegetation (Sundqvist et al., 2012) have been suggested to have caused the slowdown up to 2006. The recent increase has been attributed to high temperatures in the Arctic in 2007 and high precipitation in the Tropics in 2007 and 2008 (Dlugokencky et al., 2009).



**Figure 1:** Time series of atmospheric CH<sub>4</sub> concentration 1978-2012 (source: NOAA Earth System Research Laboratory, 2012)

Figure 2 shows the most important sinks and sources according to a review of relevant studies in the IPCC report from 2007. Anthropogenic sources such as ruminants, gas, oil, industry, rice agriculture, landfills and waste, biomass burning and coal mining are presently more important than natural sources. Wetlands are the overall largest source and much more important than other natural sources (termites, wild animals, oceans, geological sources, hydrates and wild fires). Studies indicate that wetlands might become even larger sources as a feedback mechanism to a global increase in temperature (Shindell et al., 2004). By far the largest sink of CH<sub>4</sub> is the oxidation by OH in the atmosphere. Other sinks are methanotrophs in the soil and stratospheric loss.



**Figure 2:** The most important CH<sub>4</sub> sources and sinks according to IPCC (2007). Bars represent mean values from eight studies during the period 1983-2001 that were reviewed in the IPCC Climate Change 2007 report; error bars represent the range of predictions from the different studies.

Recent studies indicate that the CH<sub>4</sub> budget presented in figure 2 might miss an important component as vegetation has been found to act as a source of CH<sub>4</sub>. The first report on this topic was published by Keppler et al. (2006) who found an emission of CH<sub>4</sub> from both detached plant parts and intact plants under aerobic conditions by a hitherto unknown process. This caused surprise and skepticism amongst the scientific community at first and some following studies (Kirschbaum and Walcroft, 2008, Dueck et al., 2007) did not show any aerobic CH<sub>4</sub> emission from vegetation. Other studies, however, could confirm Keppler et al.'s (2006) findings and today the existence of the phenomenon is widely accepted even though the mechanism and the magnitude are still debated (Bruhn et al., 2012). Surprisingly, Sundqvist et al. (2012) found that leaves of plants in Norunda forest, a boreal forest where also this study was carried out, acted as sinks of CH<sub>4</sub>.

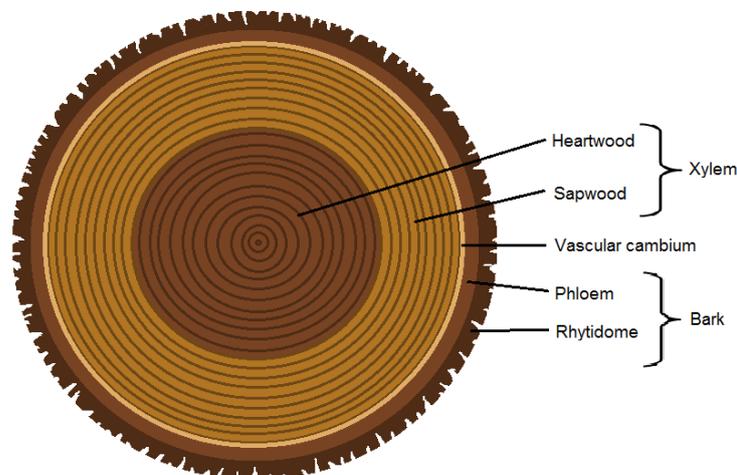
Apart from green plant parts, also tree stems have been found to act as sources of CH<sub>4</sub> in several studies (Gauci et al., 2010, Pangala et al., 2013, Terazawa et al., 2007, Zeikus and Ward, 1974, Mukhin and Voronin, 2011, Mukhin and Voronin, 2009, Rusch and Rennenberg, 1998), either by mediating CH<sub>4</sub> from the soil to the atmosphere or as a result of CH<sub>4</sub> production within the stem.

There have been no measurements of CH<sub>4</sub> exchange between tree stems and atmosphere in a boreal forest before, so the aim of this project was to study CH<sub>4</sub> exchange between tree stems and atmosphere on pine (*Pinus sylvestris*) and spruce (*picea abies*) in a boreal forest. More specifically, the evolution of CH<sub>4</sub> fluxes during the course of a day and potential relations to CO<sub>2</sub> and H<sub>2</sub>O fluxes, sap flow and temperature were studied.

## 2. Background

### 2.1 Tree stem physiology

A tree stem supports the tree, stores water, carbohydrates and minerals, conducts water and minerals from the roots to upper parts of the tree and transports and stores gases, hormones and nutrients. Tree stems typically consist of **xylem** and **bark** (see figure 3). The xylem is the inner column of wood arranged in annual rings. In mature trees only the outer, younger part of the xylem, called **sapwood**, is composed of living cells and conducts **sap**, a liquid that consists of mostly water with substances such as minerals and nutrients solved in it. If the sapwood is damaged, **resin**, a semifluid liquid consisting mostly of different kinds of acids, is conducted to the wound to prevent insect attacks and fungus infections. As the xylem ages, its cells die off and form **heartwood**, which still supports the tree but does not take part in the tree's metabolism anymore. Often it is darker in color than the sapwood. In a thin tissue between xylem and phloem, the **vascular cambium**, new cells are formed for both xylem and bark. The bark serves as a protection for the soft and vulnerable vascular cambium and the sapwood against physical injuries and infections and it conducts sugars and amino acids. The living inner part of the bark is called **phloem** and also dies off with age to form the **rhytidome** which can have various shapes depending on the tree species. (Kozłowski and Pallardy, 1997)



**Figure 3:** Schematic structure of a tree stem.

**Transpiration**, the loss of water from plants through evaporation, occurs mainly through the stomata in the leaves but also from other plant parts. The magnitude of transpiration from stems depends on the permeability of the wood to gases which can be highly variable depending on the species (Sorz and Hietz, 2006), sap flow and temperature. (Kozłowski and Pallardy, 1997)

**Respiration** is the oxidation of nutrition and release of CO<sub>2</sub> performed by cells in order to access energy. All living cells respire for maintenance. In growing tissues, such as the

vascular cambium, additionally growing respiration occurs. Consequently, stem respiration is highest in the vascular cambium. Within the xylem, respiration decreases inwards. Oxygen can be supplied to the wood inside the stem through radial diffusion of air into the stem or through transpiration of sap containing oxygen. Some species adapted to inundated conditions have structures to supply their roots with oxygen from the atmosphere such as aerenchyma (tissues with large intercellular spaces within the stem) and small lenticels within the vascular cambium. (Kozłowski and Pallardy, 1997)

## **2.2 Processes possibly involved in CH<sub>4</sub> fluxes from tree stems**

There are a number of processes mentioned in the literature that can be responsible for CH<sub>4</sub> emission from tree stems. Methanogenic archaea produce CH<sub>4</sub> under anaerobic conditions by taking part in the complex process of anaerobic decomposition. In simple terms, anaerobic bacteria first degrade organic carbon compounds to acetate, H<sub>2</sub>, CO<sub>2</sub> and formate which are then transformed into CO<sub>2</sub> and CH<sub>4</sub> by methanogenic archaea (Stams and Plugge, 2010). Anaerobic conditions in tree stems leading to CH<sub>4</sub> emission have been suggested to be caused by a beginning, not yet visible heart rot fungus infection inside the tree stem (Covey et al., 2012) and by wet heartwood (Zeikus and Ward, 1974). It is likely that CH<sub>4</sub> emissions tend to be higher at breast height than at the bottom of the stem if CH<sub>4</sub> is produced within the stem (Covey et al., 2012) as oxygen concentration decreases with stem height (Eklund, 2000).

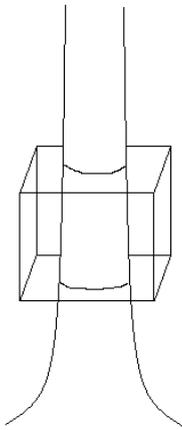
CH<sub>4</sub> that was produced in the soil by anaerobic decomposition can be mediated into the atmosphere by tree stems. (Terazawa et al., 2007, Gauci et al., 2010, Pangala et al., 2013, Rusch and Rennenberg, 1998) This does not imply that the soil around the tree necessarily releases CH<sub>4</sub>. The CH<sub>4</sub> content can vary within the soil profile and especially the groundwater can contain concentrations of CH<sub>4</sub> much higher than the concentration in water equilibrated with the atmosphere. (Terazawa et al., 2007) CH<sub>4</sub> from deep soil layers with a net CH<sub>4</sub> production can bypass lower soil layers with a net CH<sub>4</sub> uptake through the tree stem. The CH<sub>4</sub> can either be solved in water and leave the tree stem with the transpiration stream or it can be transported from the soil in gaseous form through the same pathways that supply the roots with oxygen (see 2.1) in some tree species adapted to inundated soil. It is likely that CH<sub>4</sub> emissions tend to be higher at the bottom of the stem than at breast height if CH<sub>4</sub> is mediated from the soil. (Terazawa et al., 2007)

Nonmicrobial CH<sub>4</sub> production, which can even occur under aerobic conditions, is nowadays well-documented for green plant parts (Bruhn et al., 2012), but has not been shown unambiguously in woody plant tissue. However, purified lignin and cellulose, which are parts of woody tissues, have been shown to emit CH<sub>4</sub> under aerobic conditions (Vigano et al., 2008), so it is possible that this process could occur in woody tissues. The process has been shown to be stimulated by injury (Wang et al., 2009, Wang et al., 2011), UV light (Vigano et al., 2008), temperature (Vigano et al., 2008) and anoxia (Wang et al., 2011). Some studies, as summarized in review article by Bruhn et al. (2012), indicate that the process could at least partly be based on the production of reactive oxygen species (ROS) under stress conditions.

Only one process is known that could be responsible for CH<sub>4</sub> uptake from tree stems. Methanotrophic bacteria consume CH<sub>4</sub> under aerobic conditions and can often be found at anaerobic-aerobic interfaces. Almost all of them are obligate methanotrophs. Methanotrophic bacteria have been found in various plant tissues, including bark (Iguchi et al., 2012).

### 2.3 Methods used in other studies to measure CH<sub>4</sub> fluxes on tree stems

In most studies with direct measurements of CH<sub>4</sub> fluxes on tree stems, transparent stem chambers were used that enclosed a part of the tree stem (Pangala et al., 2013, Gauci et al., 2010, Rusch and Rennenberg, 1998) (see figure 4). Terazawa et al. used a steel chamber that was placed on the tree stem. In all studies, gas samples were extracted from the chambers in intervals of 10 to 60 min and the CH<sub>4</sub> contents of the gas samples were measured to extrapolate CH<sub>4</sub> fluxes. Pangala et al., Gauci et al. and Terazawa et al. measured in situ gas fluxes in wetland trees while Rusch and Renneberg took entire trees to the lab and exposed the bare roots to CH<sub>4</sub> in order to study CH<sub>4</sub> mediation in the gaseous form.



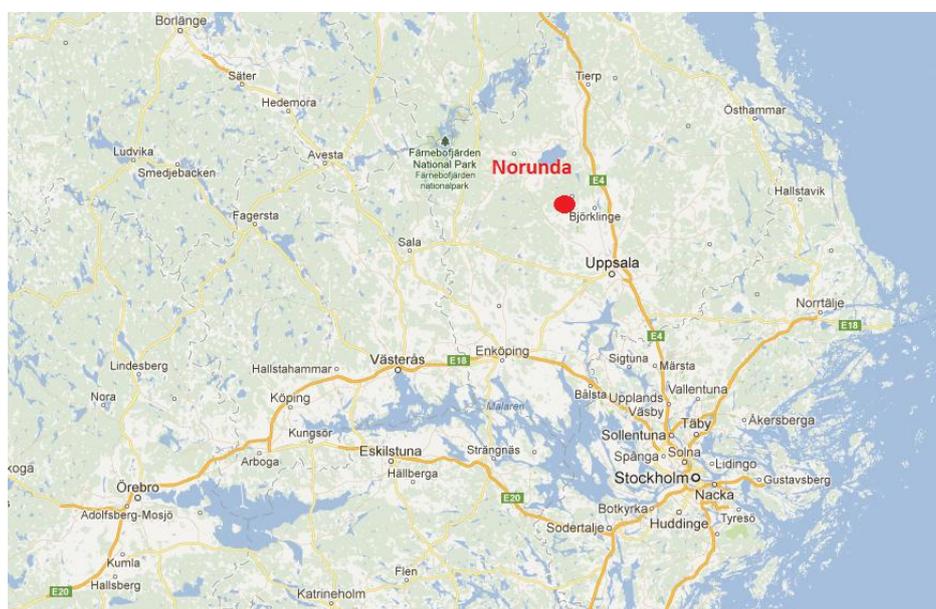
**Figure 4:** Schematic drawing of a stem chamber of the kind used by Pangala et al. (2013), Gauci et al. (2010) and Rusch and Rennenberg (1998).

In other studies, core samples were taken from tree stems and analyzed for CH<sub>4</sub> content (Zeikus and Ward, 1974, Covey et al., 2012). Covey et al. extrapolated CH<sub>4</sub> stem fluxes from trunk content.

Mukhin and Voronin (2011) analyzed CH<sub>4</sub> fluxes from samples of tree stems in the laboratory that had been dried at room temperature for 24 hours. They also measured fluxes after hydration with distilled water on the same samples. Other samples were dried at 105°C previous to the measurements.

### 3. Methods

The study site, Norunda forest, is located in central Sweden, about 30 km north of Uppsala (lat. 60°05'N. lon. 17°29'E) and is part of the southern part of the boreal forest zone (see figure 5). The mature boreal forest is dominated by Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) with only a small fraction (15%) of deciduous trees. The part of the forest where the measurements were conducted was about 100 years old and had been thinned in the winter of 2008-9. The soil is sandy, has a thin organic layer and is very compact below 70 cm where almost no roots can be found. The depth to the groundwater table is highly variable. Sundqvist (oral communication) measured a net CH<sub>4</sub> uptake of ca. 8 μmol m<sup>-2</sup> h<sup>-1</sup> by the soil in non-inundated areas and net emission close to an inundated spot.



**Figure 5:** Map showing the location of Norunda forest. Map created with Google maps

During the study period in Norunda (22<sup>nd</sup> to 26<sup>th</sup> of April 2013) the area was more wet than usual during this time of the year and parts of the forest ground were inundated. This was due to the weather conditions during the preceding months: the soil had been very moist after heavy precipitation in the beginning of the winter. Then the temperatures had dropped below zero and the winter had additionally brought large amounts of snow.

CH<sub>4</sub>, H<sub>2</sub>O and CO<sub>2</sub> fluxes on tree stems were measured using a chamber built from a tin can (see figure 6) with a cylindrical shape, a radius of 5cm and thus an area of 0.0079m<sup>2</sup> that was covered by the chamber on the tree. The chamber had a volume of 1000 cm<sup>3</sup>. It was connected to a Los Gatos Research laser gas analyzer (LGR) by two 15 m long gas lines with an inner radius of 2 mm and thus a volume of 377cm<sup>3</sup>. The total volume in which the gas circulated was consequently 0.001377 m<sup>3</sup>. The LGR registered gas concentrations in ppm every second and corrected them for water vapor automatically.

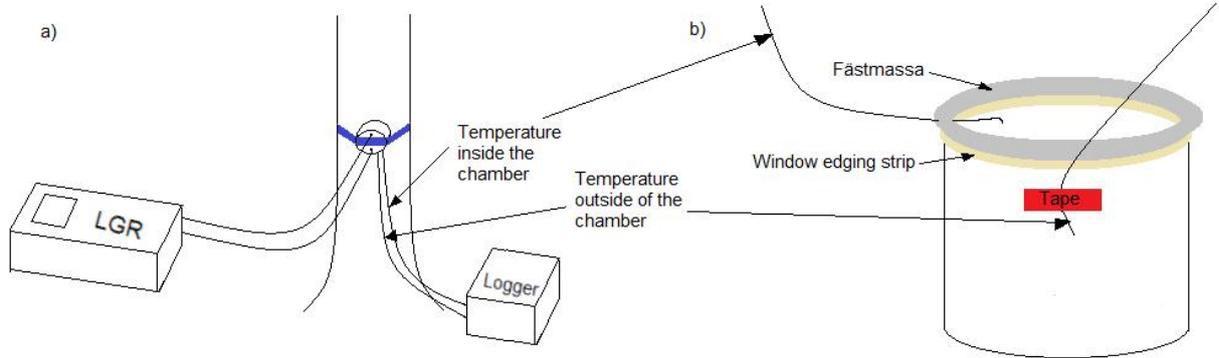
On the 25<sup>th</sup> of April 2013 seven flux measurements of 15 min each (10 min for the second measurement on spruce) with a ventilation period of at least 10 min in between each measurement were taken at breast height on a pine with a circumference of 1.25 m at breast height. On the 26<sup>th</sup> of April 2013 the same procedure was repeated for a spruce with a circumference of 63 cm at breast height. Both trees were located in a non-inundated area.

After testing several ways of attaching the chamber to the stem the following method was chosen: First, a part of the bark was removed at the spot chosen for the measurement. On pine, a large part of the bark was removed as the rhytidome consisted of loose layers and had deep vertical furrows, so that it would not have been possible to seal the chamber gas tightly without removing the rhytidome and a part of the phloem. However it was made sure that the vascular cambium and the xylem were not damaged in any place, to avoid that an increased accumulation of resin in this part of the stem would not bias the measurements. Very deep furrows were filled with Fästmassa 312 (Dana Lim A/S) before installing the chamber. On spruce, only a few irregularities were removed from the phloem. Then, window edging strip was adhered to the edge of the chamber, followed by a layer of Fästmassa that was attached upon the window edging strip and the chamber was pressed against the tree stem and firmly attached to the tree with a belt. The window edging strip served to enlarge the area of the edge of the chamber so that the pressure applied on the chamber would press the Fästmassa against the tree and not only the edges of the chamber into the Fästmassa. Both Fästmassa and window edging strip did not show any CH<sub>4</sub>, CO<sub>2</sub> or H<sub>2</sub>O fluxes when tested over 15 min.

Before starting the measurements on each tree it was tested if the chamber sat gas-tightly by blowing onto the area where the chamber sat on the stem. It was not possible to attach the chamber completely gas-tightly on any of the trees, but untight spaces were reduced as much as possible. Due to the larger stem diameter this was more successful on the pine than on the spruce. In between the measurements the chamber was ventilated for 10 min by removing the cable from the outflow opening of the chamber and suspending it next to the chamber.

To make sure that the measurements were not biased by a large temperature difference of the stem at the measuring spot compared to the rest of the stem air temperature was measured both inside the chamber by a sensor cable laid through the Fästmassa and outside of the chamber at a shaded spot directly next to the chamber. Temperature values were saved every 10 s into a Campbell Scientific data logger of the model CR1000. When necessary the chamber was shaded with sheets of white paper to avoid heating it by direct sun irradiation.

Previous to the measurement the chamber was placed on the soil, where as expected an uptake of CH<sub>4</sub> could be observed and on standing water where a release of CH<sub>4</sub> could be observed to test the functioning of the setup and the instrument.



**Figure 6:** Setup: a) placement of the chamber on the stem and connection with LGR and logger, b) preparation of the chamber with temperature sensors and tightening material.

The two trees chosen were part of another project within which their sap flow density was being measured every 30 min using the Granier system (Lagergren, 2001). The Granier system consisted of two copper-constantan thermocouples inside steel needles that had been inserted into the sapwood. One of them was heated with a constant power, so that an increase in sap flow would dissipate heat away from the sensor and decrease the measured temperature. Sap flow density could be then calculated from the temperature difference between the heated and the unheated sensor.

The data was evaluated using MATLAB. The measured gas concentrations were converted from ppm to  $\mu\text{mol m}^{-2}$  using the following equation:

$$c_{\mu\text{mol}/\text{m}^2} = \frac{c_{\text{ppm}} * p_{\text{mean}}}{R * T_{\text{chamber}} (\text{K})} \quad \text{with } p_{\text{mean}} = 101325 \text{ Pa} \quad (\text{eq. 1})$$

To determine gas fluxes, a linear regression was performed on minute 2-6 for  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and on minute 2-14 (2-9 in the case of the second measurement on spruce) for  $\text{CH}_4$ . The slope of the linear regression was then converted into flux by using the following equation with molar masses of  $16 \text{ g mol}^{-1}$  ( $\text{CH}_4$ ),  $18 \text{ g mol}^{-1}$  ( $\text{CO}_2$ ) and  $44 \text{ g mol}^{-1}$  ( $\text{H}_2\text{O}$ ):

$$\text{flux}_{\mu\text{g}/(\text{m}^2\text{s})} = \text{slope}_{\mu\text{mol}/(\text{m}^2\text{s})} * \frac{\text{area}_{\text{chamber opening}}}{\text{volume}_{\text{chamber} + \text{gas lines}}} * \text{molar mass} \quad (\text{eq. 2})$$

$\text{CH}_4$  fluxes were plotted against the corresponding  $\text{CO}_2$  and  $\text{H}_2\text{O}$  fluxes and a linear regression was performed to investigate whether there was a correlation between  $\text{CH}_4$  and other gas fluxes. Fluxes that were not significant ( $p$ -value > 0.05) were not included in further analyses.

Sap flow and temperatures during the measurement days were plotted using MATLAB. Also,  $\text{CH}_4$  fluxes were plotted against an average of sap flow during the corresponding measuring period. The same was done for temperature. A linear regression was

performed to investigate whether there was a correlation between CH<sub>4</sub> fluxes and sap flow and/or temperature.

The LGR had a precision of 0.25 ppb for a measurement period of 100 s. With the pessimistic estimation that thus a change of 0.5 ppb within 15 minutes could be detected and using equations 1 and 2 the minimum detectable flux at 10°C was 0.015 µg m<sup>-2</sup> h<sup>-1</sup>.

## 4. Results

### 4.1 Tree stem CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>O fluxes

Table 1 shows gas fluxes and  $R^2$ -values for each of the measurement periods. Plots of the corresponding gas concentration development in µmol m<sup>-3</sup> can be found in the appendix (figure I-VI). Most  $p$ -values were < 0.05.

**Table 1:** Gas fluxes obtained from linear regression for each of the measurement periods with standard errors and  $R^2$ -values. Values marked in grey have  $p$ -values > 0.05.

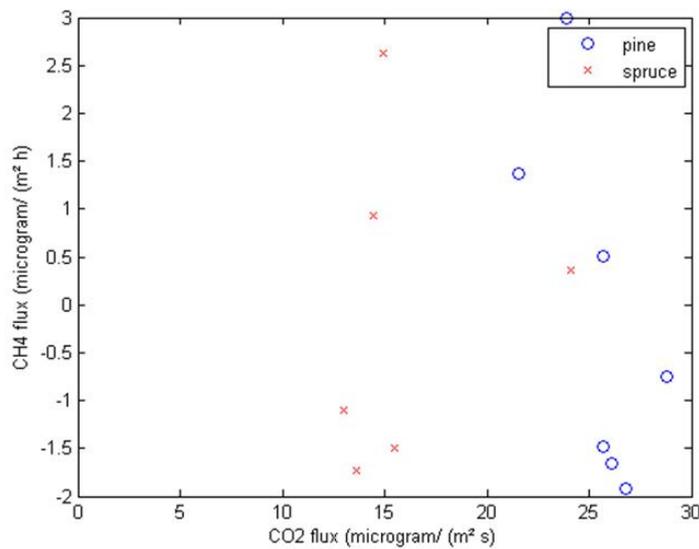
Measurement	CH <sub>4</sub> flux (µg m <sup>-2</sup> h <sup>-1</sup> )	Std error	$R^2$	CO <sub>2</sub> flux (µg m <sup>-2</sup> s <sup>-1</sup> )	Std error	$R^2$	H <sub>2</sub> O flux (µg m <sup>-2</sup> s <sup>-1</sup> )	Std error	$R^2$
Pine 25/04/2013. 10:40-10:55	-1.48	0.14	0.13	25.70	0.22	0.98	220.85	11.21	0.58
Pine 25/04/2013. 11:05-11:15	2.99	0.29	0.19	23.90	0.11	0.99	189.05	11.63	0.48
Pine 25/04/2013. 13:20-13:35	-1.92	0.15	0.18	26.81	0.17	0.99	196.33	11.12	0.52
Pine 25/04/2013. 13:45-14:00	-1.65	0.16	0.13	26.09	0.13	0.99	243.86	9.74	0.69
Pine 25/04/2013. 14:10-14:25	0.51	0.15	0.01	25.69	0.13	0.99	198.12	9.60	0.60
Pine 25/04/2013. 14:35-14:50	1.37	0.17	0.08	21.54	0.13	0.99	86.03	14.20	0.11
Pine 25/04/2013. 15:00-15:15	-0.75	0.15	0.03	28.80	1.52	0.56	647.37	43.49	0.44
Spruce 26/04/2013. 09:30-09:45	-1.49	0.15	0.11	15.46	0.17	0.97	36.87	11.18	0.04
Spruce 26/04/2013. 09:55-10:10	2.62	0.16	0.27	14.92	0.11	0.98	64.65	11.41	0.10
Spruce 26/04/2013. 10:20-10:35	0.36	0.15	0.00	24.12	0.13	0.97	5.35	10.09	0.00
Spruce 26/04/2013. 10:45-11:00	-1.10	0.15	0.06	13.03	0.14	0.97	1.38	11.08	0.00
Spruce 26/04/2013. 11:10-11:25	-1.73	0.14	0.16	13.65	0.13	0.98	-44.91	10.38	0.06
Spruce 26/04/2013. 12:35-12:50	-1.74	0.15	0.15	1.91	2.90	0.00	220.85	10.98	0.00
Spruce 26/04/2013. 13:00-13:15	0.93	0.14	0.05	14.47	0.97	0.44	189.04	16.34	0.16

For pine, CH<sub>4</sub> fluxes ranged between  $-1.92 \pm 0.15$  and  $2.99 \pm 0.29$  µg m<sup>-2</sup> h<sup>-1</sup> and  $R^2$ -values ranged between 0.01 and 0.19. Fluxes on spruce ranged between  $-1.73 \pm 0.14$  and  $2.62 \pm 0.16$  µg m<sup>-2</sup> h<sup>-1</sup>,  $R^2$ -values between 0.00 and 0.27.

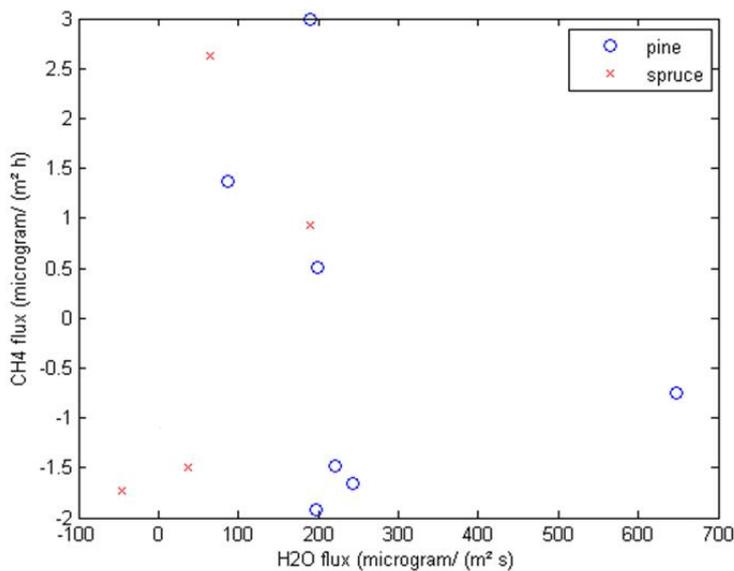
Most of the CO<sub>2</sub> fluxes exhibited  $R^2$ -values  $\geq 0.97$ . They ranged between  $21.54 \pm 0.13$  and  $28.80 \pm 1.52 \mu\text{g m}^{-2} \text{s}^{-1}$  for pine and between  $13.03 \pm 0.14$  and  $24.12 \pm 0.13 \mu\text{g m}^{-2} \text{s}^{-1}$  for spruce. Three measurements had much lower  $R^2$ -values between 0 and 0.56 and one of them had a  $p$ -value  $> 0.05$  (see table 1).

H<sub>2</sub>O fluxes on pine ranged between  $86.03 \pm 14.20$  and  $647.37 \pm 43.49 \mu\text{g m}^{-2} \text{s}^{-1}$  with  $R^2$ -values between 0.11 and 0.69. For spruce the fluxes ranged between  $36.87 \pm 11.18$  and  $189.04 \pm 16.34 \mu\text{g m}^{-2} \text{s}^{-1}$  with  $R^2$ -values between 0.00 and 0.16. Three of them had  $p$ -values  $> 0.05$ .

Figure 7 and 8 show CH<sub>4</sub> fluxes plotted against the corresponding CO<sub>2</sub> and H<sub>2</sub>O fluxes respectively. The linear regressions gave  $p$ -values  $> 0.05$  for all of the datasets.



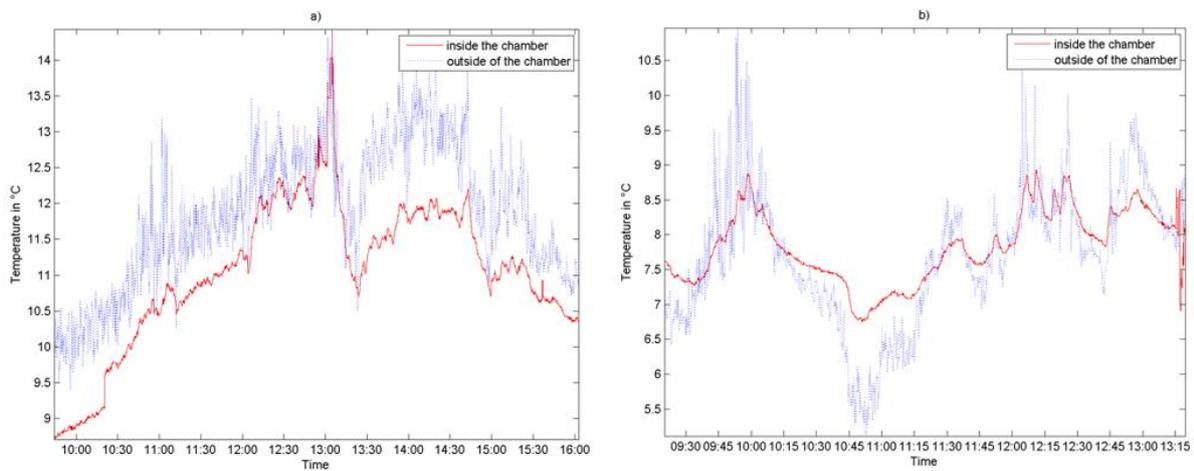
**Figure 7:** CH<sub>4</sub> fluxes plotted against corresponding CO<sub>2</sub> fluxes.



**Figure 8:** CH<sub>4</sub> fluxes plotted against corresponding H<sub>2</sub>O fluxes

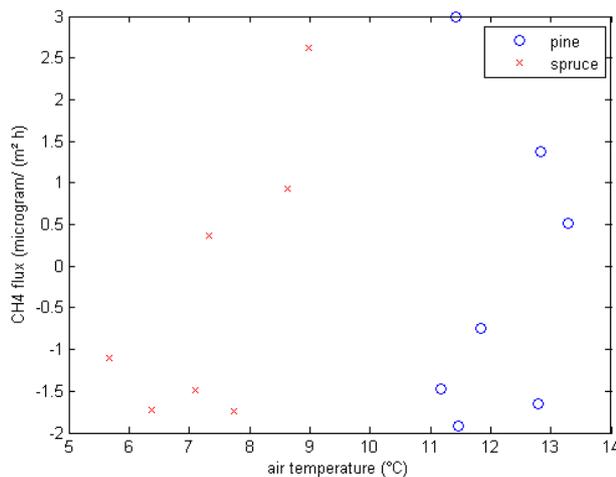
## 4.2 Temperature

Figure 9 shows the temperatures measured inside and outside of the chamber during the measurement periods. A peak in temperature around 13:00 that was caused by touching the sensor in the course or adjustments on the setup was removed from the data. For the measurement on pine the temperature outside of the chamber ranged between 9 and 14 °C. The temperature inside the chamber was slightly (not more than 2 °C) lower and ranged between 9 and 13 °C. For the measurement on spruce the temperature outside of the chamber ranged between 5 and 11 °C. The temperature inside the chamber followed the variation outside of the chamber but with lower maximum and higher minimum values. It ranged between 7 and 9 °C.



**Figure 9:** Temperatures inside and outside of the chamber during the measurement a) on pine the 25/04/2013 b) on spruce the 26/04/2013.

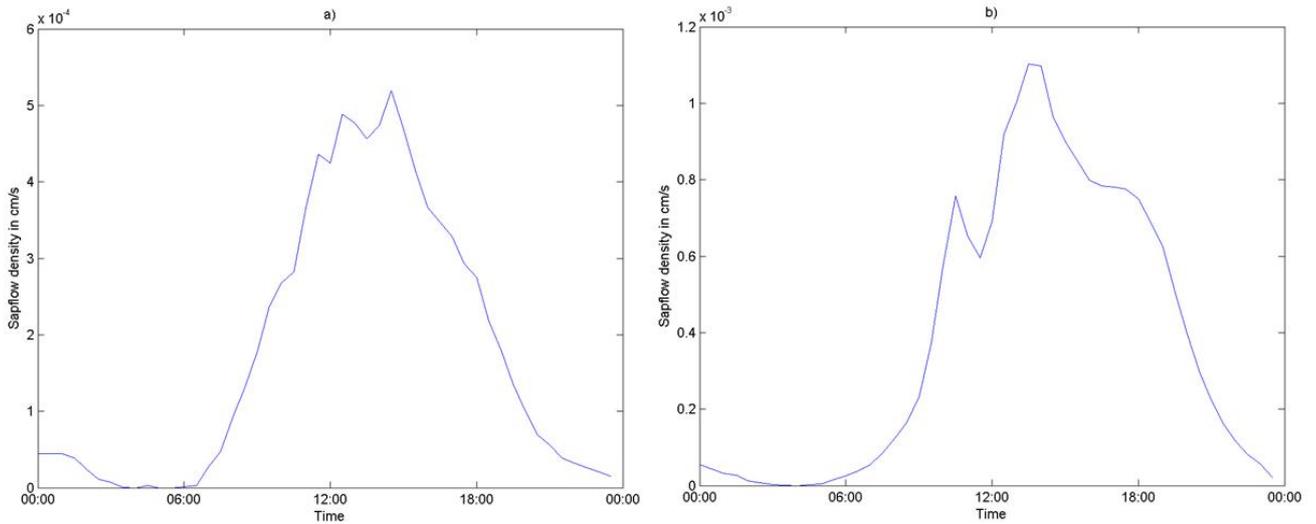
In figure 10 CH<sub>4</sub> fluxes were plotted against the average temperature during the corresponding measurement period. None of the *p*-values of the linear regressions performed on the data for pine and the data for spruce shown in figure 8 was < 0.05.



**Figure 10:** CH<sub>4</sub> fluxes plotted against the average temperature during the corresponding measurement.

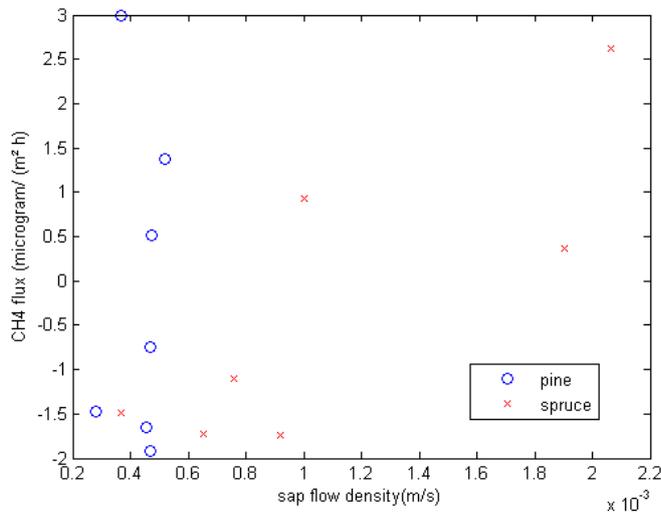
### 4.3 Sap flow

Figure 11 shows sap flow densities during the measurement period on each tree. In both trees, sap flow density was zero or very low during the night and started increasing at about 06:00. In pine a maximum flow density of  $5.2 \cdot 10^{-4} \text{ m s}^{-1}$  was reached at 14:30 and in spruce a maximum flow density of  $1.6 \cdot 10^{-3} \text{ m s}^{-1}$  was reached at 12:30. After this maximum sap flow density decreased in both trees until it was almost zero at 00:00.



**Figure 11:** Sap flow a) on pine the 25/04/2013 b) on spruce the 26/04/2013.

In figure 12,  $\text{CH}_4$  fluxes were plotted against the average sap flow density during the corresponding measurement period. For spruce the  $p$ -value of the linear regression was 0.02, for pine it was  $> 0.05$ .



**Figure 12:**  $\text{CH}_4$  fluxes plotted against the average sap flow density during the corresponding measurement

## 5. Discussion

### 5.1 Results

Both positive and negative CH<sub>4</sub> fluxes could be detected in the chamber. The changes in CH<sub>4</sub> concentration over the measured time periods did however not exceed the magnitudes of changes in CH<sub>4</sub> concentration in the atmosphere outside of the chamber. It is thus not possible to definitely determine to which extent the measured changes in concentrations were due to fluxes from the tree stems or caused by diffusion of air from the atmosphere into the chamber through spaces that could not be tightened. If the changes in CH<sub>4</sub> concentration were due to tree stem fluxes, the results indicate that both net emission and net uptake of CH<sub>4</sub> occurred from the tree stems at different time periods. The plot of CH<sub>4</sub> concentrations between 14:10 and 14:25 on pine in figure I f even seems to have captured a moment when the stem switched from being a sink to being a source of CH<sub>4</sub>.

The noise exhibited by the data is too large to have originated from imprecision of the LGR. Instead, its most probable origin is air diffusing into the chamber from the atmosphere through spaces that could not completely be tightened. The soil in Norunda acted as sink of different magnitude in some and as source in other places, so small-scale turbulences in the forest most likely caused the CH<sub>4</sub> content of the atmosphere around the chamber to vary within small spacial and temporal scales. Both wind and turbulences from the outside and the circulation of gas within chamber and LGR can increase the diffusion between atmosphere and chamber.

13 out of 14 CO<sub>2</sub> fluxes were significant and positive as expected due to the occurrence of stem respiration. The measurements with low  $R^2$ -values exhibited peaks (see figure II g and III f, g) that are probably caused by people passing by close to the chamber and breathing. As there were more research projects going on in Norunda at the same time this could not always be avoided. Significant stem transpiration (emission of H<sub>2</sub>O) could be detected in all of the measurements on pine but in only 4 out of 7 of the measurements on spruce. No relationship could be found between CH<sub>4</sub> flux and either of the other gas fluxes.

The temperature differences between air in the chamber and the atmosphere can be explained by the reduced air circulation within the chamber compared to the atmosphere. As they did not exceed 2°C it is improbable that heating or cooling effects caused by the chamber biased the measurements. The average of the annual maximum temperatures in Uppsala between 1991 and 2010 was 29.87 °C (Uppsala Universitet), which is ca. 15-25 °C warmer than the temperatures prevailing during the study periods. Dunfield et al. (1993) found optimum temperatures for methanotrophs in temperate and subarctic peat soils at 20-25 °C and for methanogenics at 25-30 °C so it would be interesting to study fluxes during warmer temperatures. No relationship could be found between CH<sub>4</sub> fluxes and air temperature in the atmosphere. As temperatures inside the tree stem and in the soil are not the same as air temperatures and react to changes in air temperature with a delay CH<sub>4</sub> emissions could still have been related to soil and stem temperatures even though no correlation with air temperature could be found.

A positive linear relationship between CH<sub>4</sub> fluxes and sap flow density was found for spruce, but not for pine. As sap flow densities overall were higher for spruce than for pine it is possible that this relationship is only present if sap flow density is sufficiently high. The maximum sap flow density in 2012 was  $2.0 \cdot 10^{-3} \text{ m s}^{-1}$  for the pine and  $2.2 \cdot 10^{-3} \text{ m s}^{-1}$  for the spruce. This is  $1.5 \cdot 10^{-3} \text{ m s}^{-1}$  higher than the maximum on the measurement day for pine and  $0.6 \cdot 10^{-3} \text{ m s}^{-1}$  higher than the maximum on the measurement day for spruce, so it would also be interesting to study gas fluxes at higher sap flow densities.

If the fluxes originated from the stems and not from untight spaces between chamber and stem, speculations about possible processes involved in CH<sub>4</sub> fluxes can be made based on the evaluation of the collected data. It seems like methane production and/or mediation and consumption occurred in the trees simultaneously and that their proportion varied over time. As methanotrophic bacteria tend to be close to places where CH<sub>4</sub> is produced this would not be surprising. There is no cause for the assumption that CH<sub>4</sub> consumption by methanotrophic bacteria varied much over such small temporal scales, so it is likely that the emission of CH<sub>4</sub> varied over time instead. The correlation between CH<sub>4</sub> emission and sap flow density in spruce suggests that mediation of CH<sub>4</sub> solved in water from the soil could be the process responsible for CH<sub>4</sub> emission. Ebullition of CH<sub>4</sub> mediated from the soil, as also observed by Pangala et al. (2013), could be responsible for small-timescale variations in CH<sub>4</sub> emissions. The fact that the soil emitted CH<sub>4</sub> in wetter areas of the forest indicates that the ground water contains CH<sub>4</sub> which could be mediated through tree stems. It speaks against this process that no correlation between CH<sub>4</sub> fluxes and H<sub>2</sub>O fluxes could be found, as CH<sub>4</sub> emissions would be expected to occur simultaneously with H<sub>2</sub>O emissions if CH<sub>4</sub> is transported via the transpiration stream. Fast temporal variations in CH<sub>4</sub> production that occurs within the stem on the other hand would be surprising. It is however possible that several CH<sub>4</sub> emission processes occurred simultaneously.

In other studies of in situ CH<sub>4</sub> fluxes on tree stems, emissions in the range of 4.37-838  $\mu\text{g m}^{-2} \text{ h}^{-1}$  (Gauci et al., 2010), 17.0-185  $\mu\text{g m}^{-2} \text{ h}^{-1}$  (Pangala et al., 2013) and 50-200  $\mu\text{g m}^{-2} \text{ h}^{-1}$  (Terazawa et al., 2007) were measured. The lowest fluxes measured by Gauci et al. are close to the fluxes measured in this study, the others are one to two orders of magnitude higher. This is not surprising as all of the other studies were conducted on wetland trees. in warmer climate zones and on angiosperms which have been reported to be more permeable to radial gas diffusion than conifers such as pine and spruce (Sorz and Hietz, 2006).

## 5.2 Suggestions for further studies

The most important task for the setup of further studies is to quantify and to reduce the amount of air that enters the chamber during the measurements. Blank measurements on an object with a structure similar to a tree stem close to the trees that will be studied can be used to quantify errors from untight spaces. To reduce errors, plastic foil can be wrapped around tree and chamber as a wind protection. If the tree has a diameter which is too small to attach the chamber tightly, a part of the chamber could be cut off so that it

fits the curvature of the tree. In the other studies of in situ CH<sub>4</sub> fluxes (see background) no air was circulated continuously through the chamber. Instead, samples of air were taken in certain intervals. This technique has the advantage that diffusion between chamber and atmosphere is reduced, but the disadvantage that short-term variations in fluxes as they seem to be present in Norunda cannot be observed. If the aim of a study is to relate CH<sub>4</sub> fluxes to other variables and to study the processes involved, measuring continuously consequently is the better method. If the study aims at determining net fluxes over a longer period to evaluate the role of trees in the global CH<sub>4</sub> budget taking air samples out of the chamber is a more suitable technique.

To measure fluxes with a higher accuracy, either the measuring periods can be extended or a chamber with a smaller ratio between volume and area of the opening can be used. If the tree diameter is not too large and the bark is relatively smooth, a stem chamber such as used by for example Gauci et al. (2010) can be a good solution. When larger changes in gas concentration are being measured it may be necessary to also correct the concentration values for the change CO<sub>2</sub>.

Covey et al. argued that CH<sub>4</sub> fluxes could be extrapolated from concentrations of CH<sub>4</sub> measured in core samples by pointing at a study that found linear relations between xylem CO<sub>2</sub> content and CO<sub>2</sub> stem fluxes (Steppe et al., 2007). Especially on trees with uneven bark this technique is much simpler than measuring fluxes. It misses however the effect of methanotrophs on CH<sub>4</sub> on its way from the heart of the stem into the atmosphere. As more respiration occurs in the outer layers of tree stems than inside methanotrophs are likely to be most numerous within the outermost parts of the tree stem where they find well-aerated conditions. Consequently, it is not generally possible to extrapolate net CH<sub>4</sub> fluxes from core concentrations. Still, studying core concentrations can be a very good way to obtain information about processes occurring inside the tree.

Not only can measurement techniques be made more precise for future research, there are also many options to study the condition under which CH<sub>4</sub> is emitted or taken up by tree stems and the processes involved. It would for example be interesting to measure at different heights of the same tree stem to obtain information about whether emitted CH<sub>4</sub> was produced in the tree stem or in the soil. Measurements could also be taken at trees growing at lowland-sites with higher soil moisture content and be compared to measurements at drier upland-sites. Even though Norunda forest is dominated by pine and spruce, measurements on the few angiosperms could be interesting as their stems are more permeable to gases. First, however, an evaluation about the influence of daily temperature and sap flow variations on CH<sub>4</sub> fluxes would be necessary.

Additionally, measurements of CH<sub>4</sub> fluxes from core samples that have been dried before such as performed by Mukhin and Voronin (2011) can give information about a potential CH<sub>4</sub> production inside the wood and exclude the soil as a CH<sub>4</sub> source. It would also be interesting to measure with a transparent chamber to find out whether (UV-) radiation influences CH<sub>4</sub> fluxes. Additional analysis of trees and environment can be helpful to determine the processes behind CH<sub>4</sub> fluxes, for example groundwater CH<sub>4</sub> content at different depths and traces of fungus infection or wet heartwood in core samples.

## 6. Conclusion

The results of this study indicate that both emission and uptake of CH<sub>4</sub> might be occurring from tree stems in boreal forests. As the measured fluxes were very small (between  $-1.92 \pm 0.15$  and  $2.99 \pm 0.29 \mu\text{g m}^{-2} \text{h}^{-1}$ ) it can however not be determined for sure that they originated from the tree stems and not from air entering the chamber through untight spaces. If the fluxes did originate from the tree stems they were probably caused by mediation of CH<sub>4</sub> solved in groundwater through the transpiration stream occurring simultaneously with uptake of CH<sub>4</sub> by methanotrophs. It can be stated that no emissions in an order of magnitude as large as found from wetland tree stems in other studies were found.

To better understand the processes behind potential CH<sub>4</sub> fluxes between tree stems and atmosphere in boreal forests and to be able to estimate their impact on the global CH<sub>4</sub> budget further studies are needed. They should include measurements during the warm summer months when methanogenic activity is likely to be higher and be carried out with improved methods.

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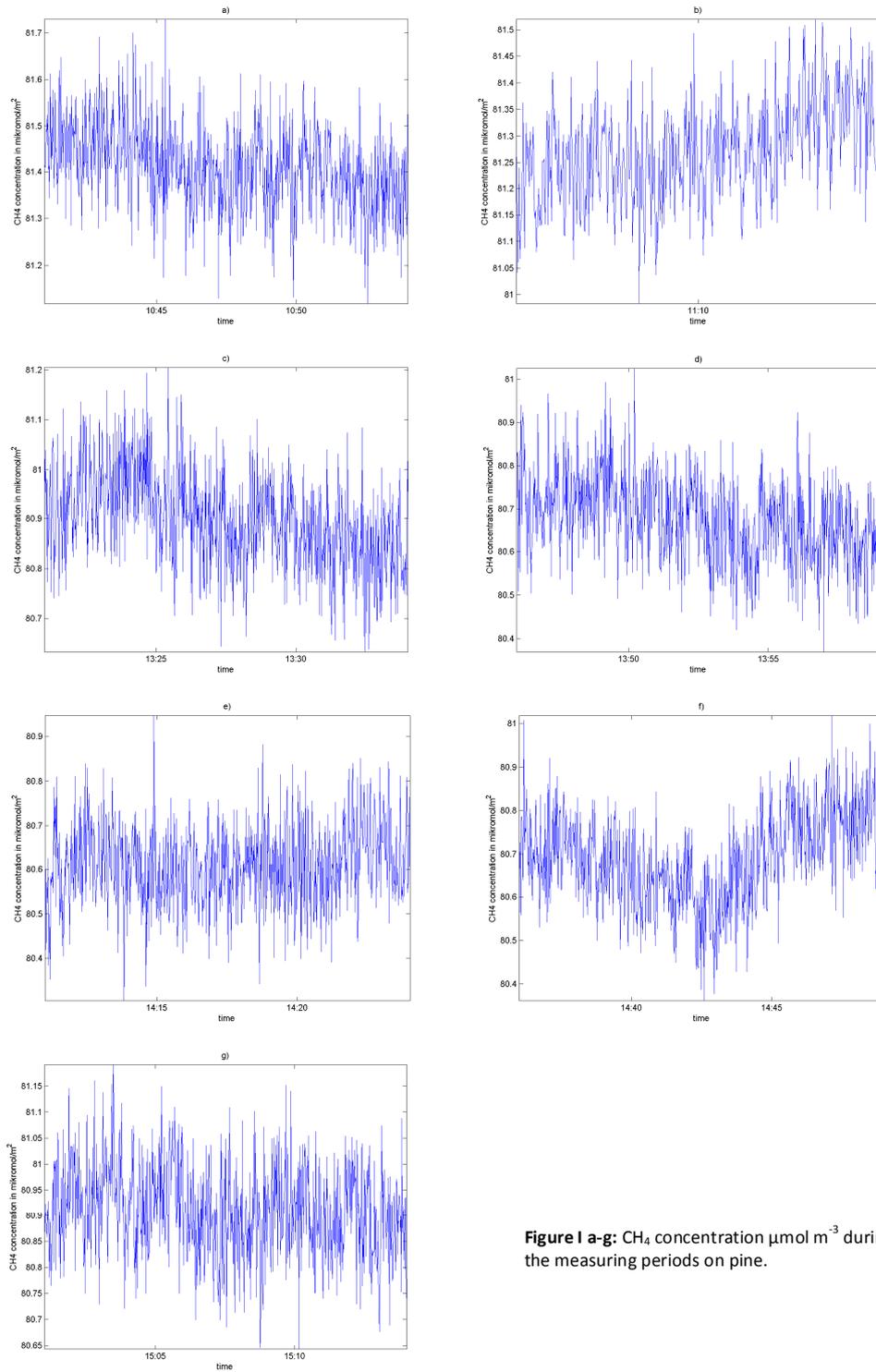
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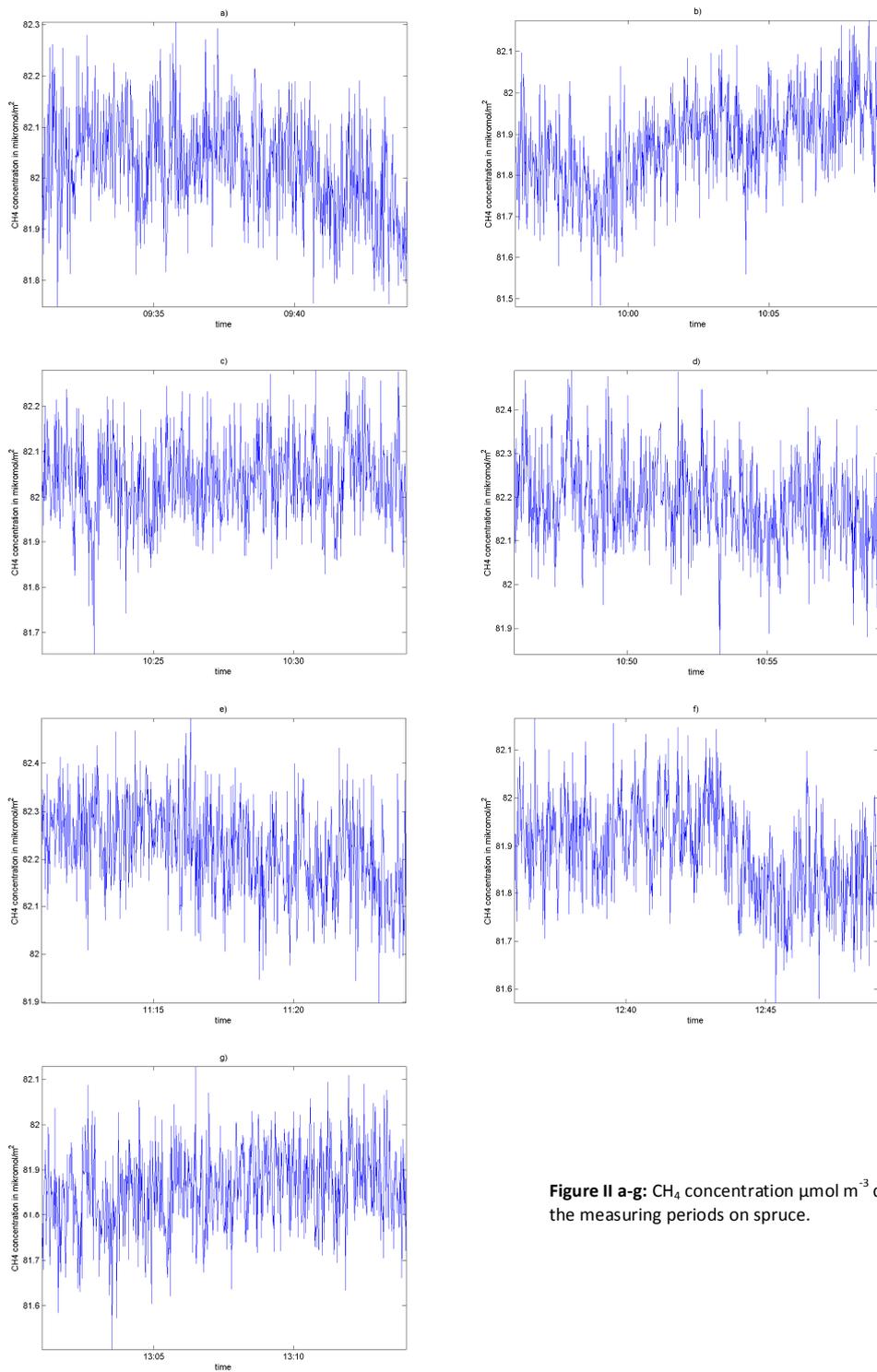
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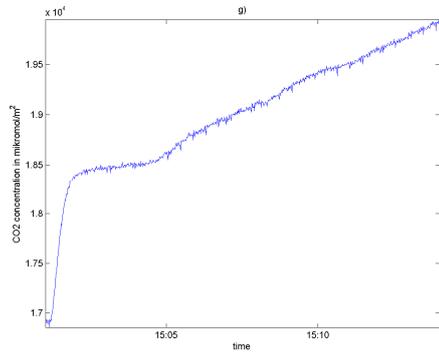
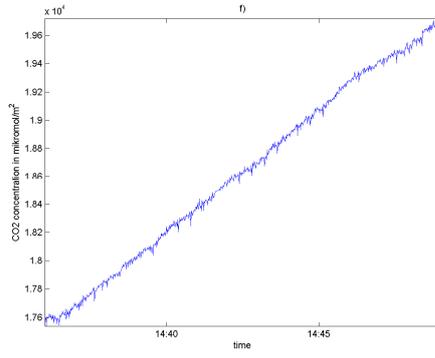
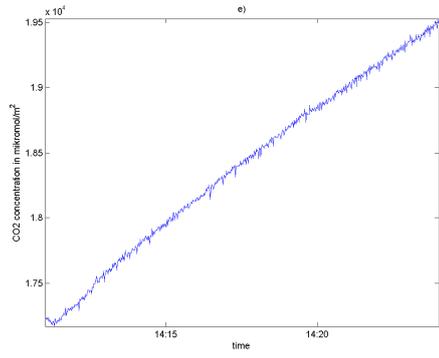
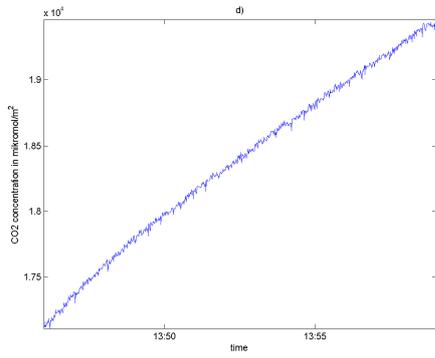
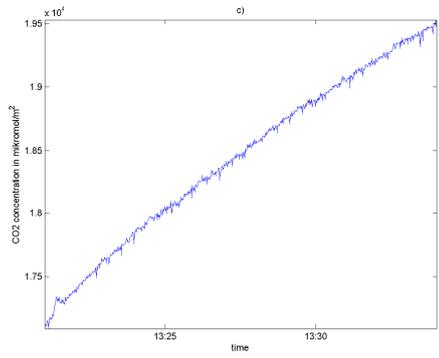
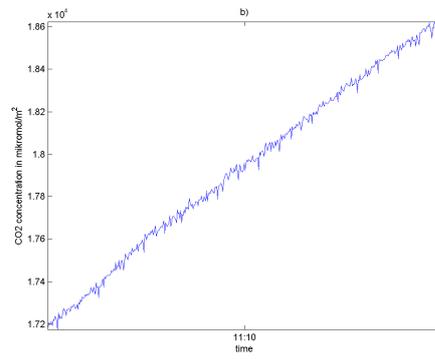
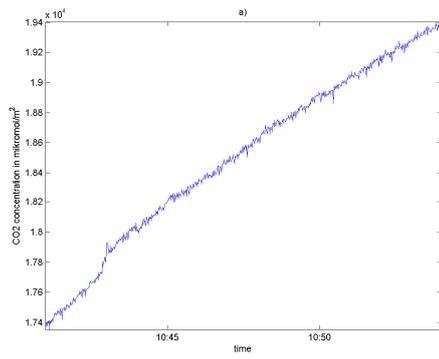
## 8. Appendix



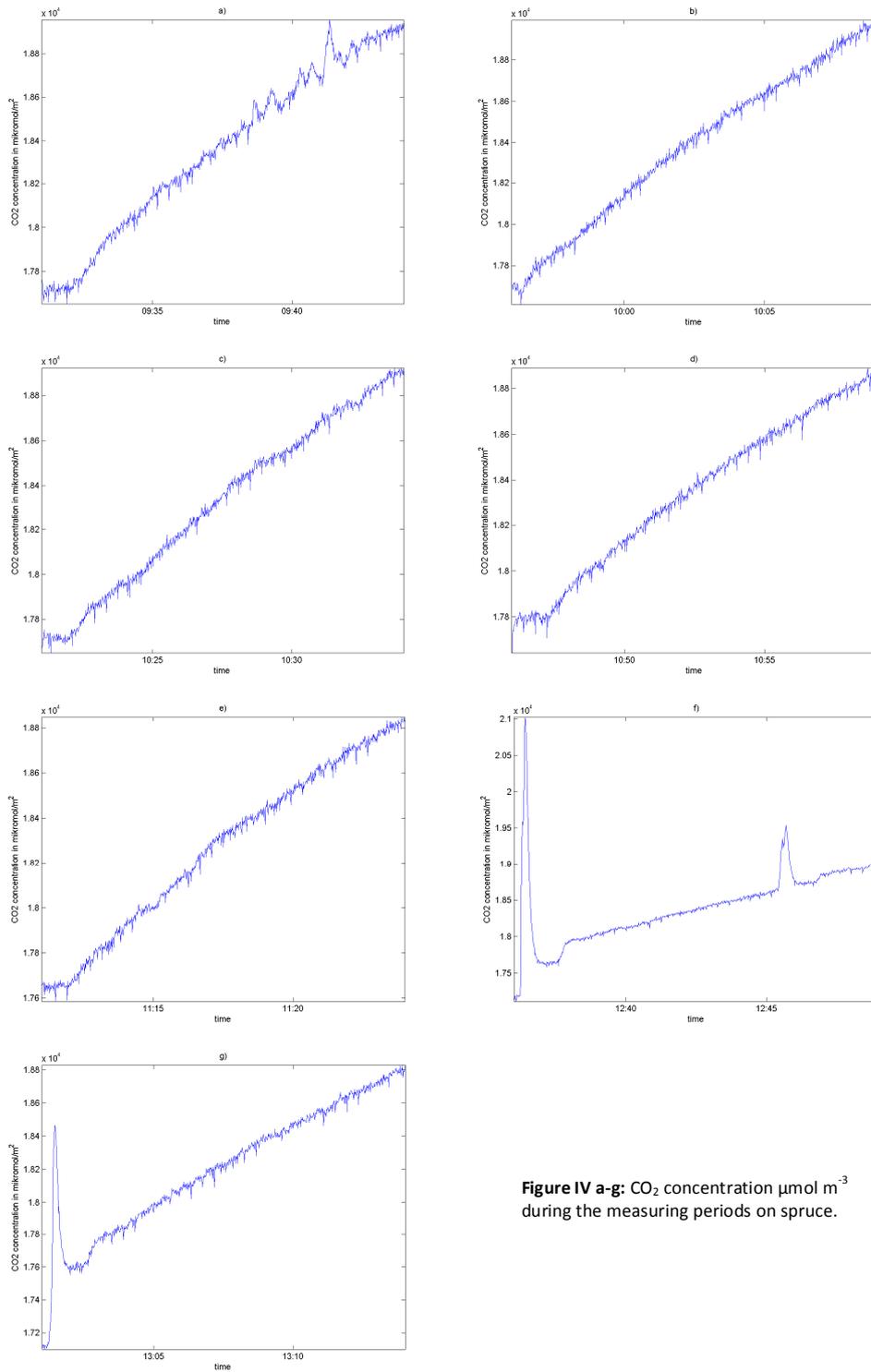
**Figure I a-g:** CH<sub>4</sub> concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on pine.



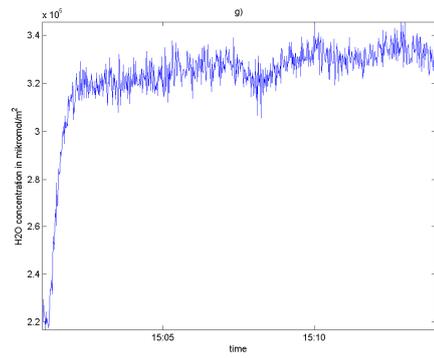
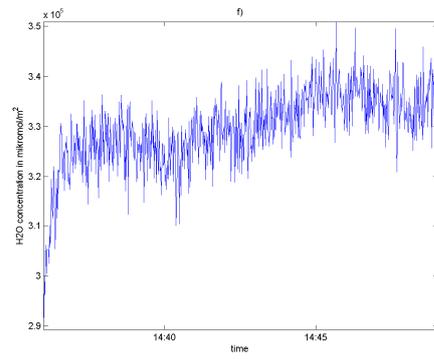
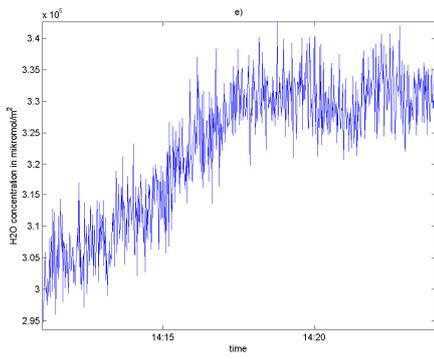
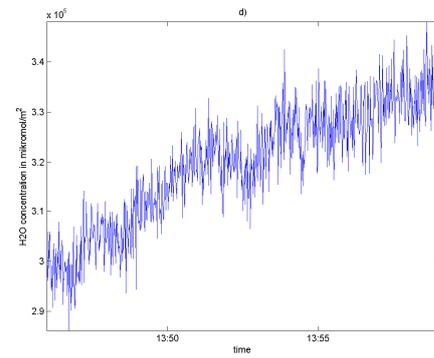
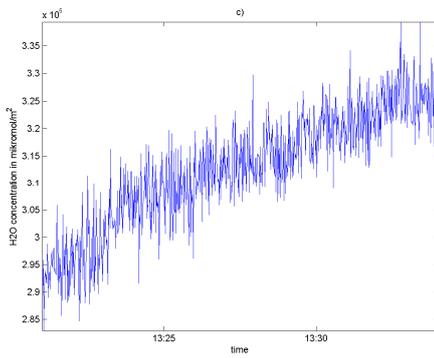
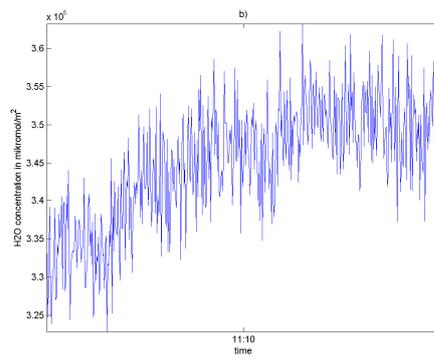
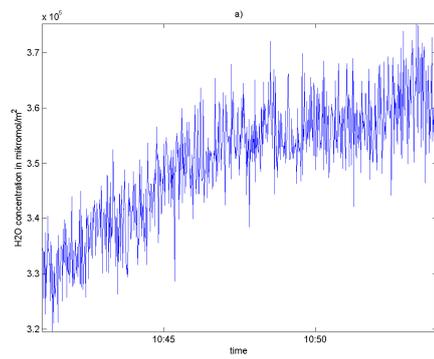
**Figure II a-g:** CH<sub>4</sub> concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on spruce.



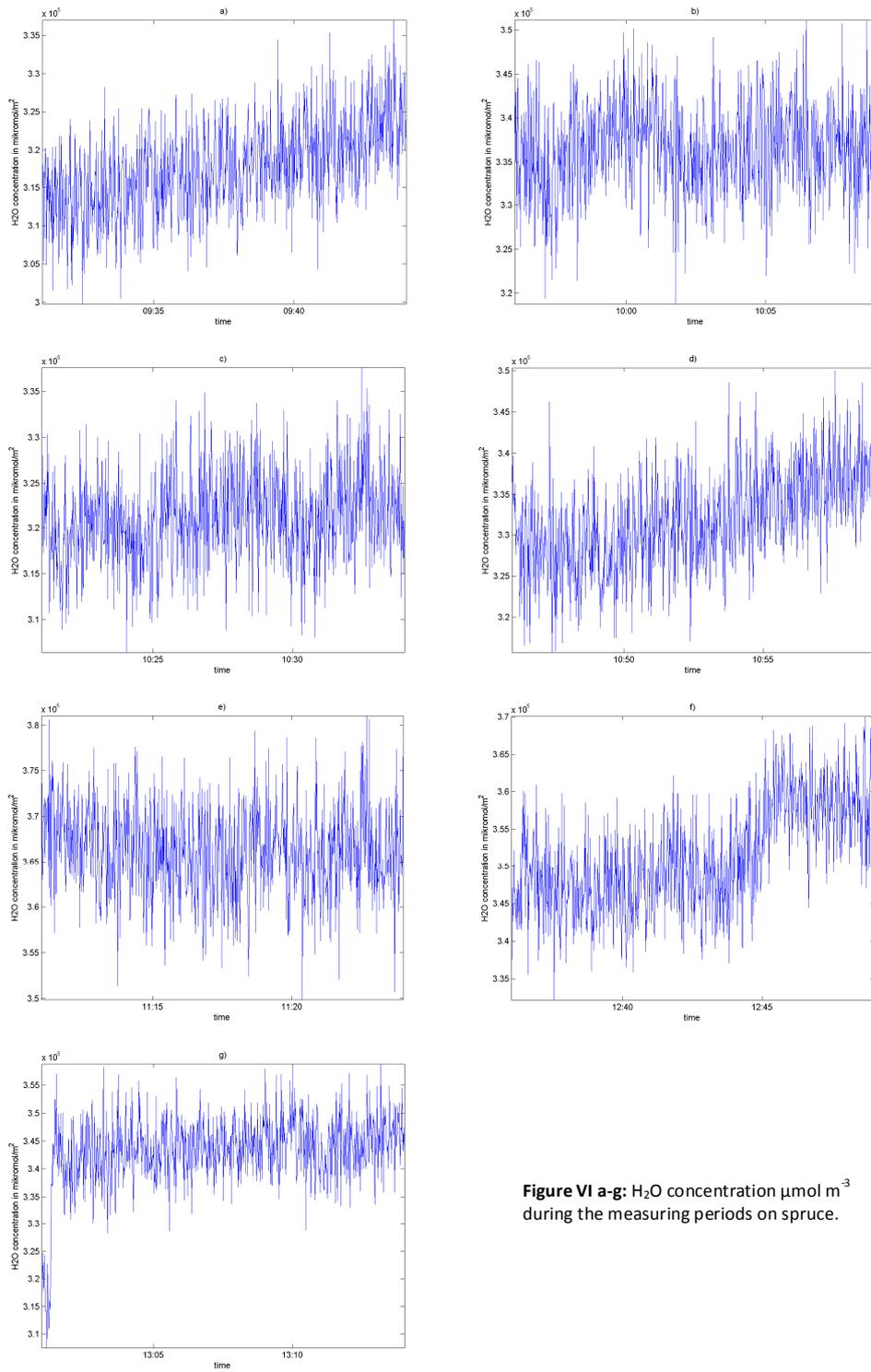
**Figure III a-g:** CO<sub>2</sub> concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on pine.



**Figure IV a-g:** CO<sub>2</sub> concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on spruce.



**Figure V a-g:** H<sub>2</sub>O concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on pine.



**Figure VI a-g:** H<sub>2</sub>O concentration  $\mu\text{mol m}^{-3}$  during the measuring periods on spruce.