

Research Article

Temporal variations in methane emissions from emergent aquatic macrophytes in two boreonemoral lakes

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Abstract. Methane (CH₄) emissions via emergent aquatic macrophytes can contribute substantially to the global CH₄ balance. We addressed temporal variability in CH₄ flux by using the static chamber approach to quantify fluxes from plots dominated by two species considered to differ in flux transport mechanisms (*Phragmites australis*, *Carex rostrata*). Temporal variability in daily mean emissions from early June to early October was substantial. The variable that best explained this variation was air temperature. Regular and consistent diel changes were absent and therefore less relevant to include when estimating or modelling CH₄ emissions. Methane emissions per m² from nearby plots were similar for *Phragmites australis* and *Carex rostrata* indicating that CH₄ production in the system influenced emissions more than the species identity. This study indicates that previously observed diel patterns and species-effects on emissions require further evaluation to support improved local and regional CH₄ flux assessments.

Keywords: Carex; CH₄ emission; emergent aquatic macrophytes; lakes; *Phragmites australis*; temporal variability.

Introduction

Methane (CH₄) impacts the global energy balance and climate, has substantially higher global warming potential than carbon dioxide (CO₂) per kg in a 100-year perspective, and accounts for some 20 % of radiative forcing (Myhre *et al.* 2013). Wetlands and inland waters are large contributors to overall CH₄ emission (Laanbroek 2010; Bastviken *et al.* 2011; Ciais *et al.* 2013; Kirschke *et al.* 2013), where anaerobic degradation of organic matter produces CH₄ through methanogenesis. Upon transport from the sediments, a large proportion of dissolved CH₄

can be oxidized in surface sediments or in the water (Bastviken *et al.* 2008; Conrad 2009; Laanbroek 2010; Duc *et al.* 2010). Therefore, the fluxes of dissolved CH₄ from surface waters are often smaller than flux pathways by which CH₄ can 'escape' oxidation, such as ebullition (flux by bubbles from sediments) and flux through rooted emergent aquatic macrophytes (Joabsson *et al.* 1999; Bastviken 2009). Such plants have well-developed aerenchyma in their stems and underground rhizomes that transport oxygen to roots (Laanbroek 2010). These gas transport systems can also transport CH₄ molecules

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from the root systems, bypassing the oxidation zone in the sediments and release methane directly into the atmosphere via stomata (Yavitt and Knapp 1998).

Emergent aquatic macrophytes in the arctic wet tundra play an important role in the exchange of CH₄ between the anaerobic environment and the atmosphere (Joabsson et al. 1999; Ding et al. 2005; Carmichael et al. 2014). It is widely suggested that emergent aquatic macrophytes are key sources of CH₄ emissions to the atmosphere through the aerenchyma (Ding et al. 2005; Bhullar et al. 2013). In addition, emergent aquatic macrophytes contribute carbon to the sediment during the growing season, by production of plant biomass and root leakage, further increasing CH₄ production in wetlands (Ding et al. 2005; Laanbroek 2010).

Molecular diffusion and convective flow are common processes that have been proposed to regulate transport of CH₄ in plant tissues (Kim et al. 1998), the latter depending on the pressure gradient in the plant (Tornberg et al. 1994). It has been suggested that observed differences between day and night in CH₄ fluxes may be due to molecular diffusion during night and convective flow during the day (Kim et al. 1998). The dominant type of gas transport can vary between species and previous work indicated a strong influence of convective gas flow in *Phragmites australis* (Armstrong and Armstrong 1991, 2014) whereas molecular diffusion through the aerenchyma has been suggested to dominate in *Carex rostrata* (Chanton and Whiting 1996). Therefore, it has been assumed that flux mediated by aquatic plants is highly species-dependent (Joabsson et al. 1999).

While plant species and gas transport mechanisms have been seen as important for wetland CH₄ emissions, fluxes can also be regulated by environmental variables, which can influence plant activity and ecosystem CH₄ production. For example, air temperature, wind, pressure, humidity and light are factors suggested to control the convective flow (Kim et al. 1998). Plant CH₄ exchange may also be affected by plant growth and amount of active aboveground biomass, for which net ecosystem exchange (NEE; net CO₂ exchange illustrating the overall balance between the photosynthesis and respiration) can be used as a proxy. The overall potential for CH₄ emissions is regulated by CH₄ production in sediments, which also depends on multiple environmental factors such as organic substrate supply and temperature (Yvon-Durocher et al. 2014).

Knowing the spatial and temporal variability in methane emission from emergent aquatic macrophytes is essential for assessing their importance, and for monitoring and modelling such emissions. Spatial variability in open water fluxes has recently been shown to depend on distance to inlets and on water depth (e.g. Natchimuthu

et al. 2016, 2017). For fluxes via emergent aquatic plants, spatial variability has frequently been associated with species distributions (see above), while considerable temporal variability has also been noted. Methane emissions from emergent aquatic macrophytes have been observed to vary over the growing season with a peak in the middle (Hyvönen et al. 1998; Juutinen et al. 2003; Kankaala et al. 2004; Duan et al. 2005; Kankaala et al. 2005). In lakes in both boreal (Kankaala et al. 2005) and arid environments (Duan et al. 2005), maximum CH₄ emission occurred in late June to mid-August, when temperature, growth and biomass peak. Other studies have also shown a positive relationship between CH₄ emissions and plant biomass during the growing season (Hirota et al. 2004; Kankaala et al. 2005), but this pattern is not always prominent (Kankaala et al. 2004).

There are also reports of considerable diel fluctuations in plant-mediated CH₄ emissions in the literature (Whiting and Chanton 1996; Käki et al. 2001; Juutinen et al. 2004; Ding et al. 2004; Duan et al. 2005). Previous studies have observed that emissions from *Typha latifolia* peak in the morning (Whiting and Chanton 1996), whereas *Peltandra virginica* (Whiting and Chanton 1996) and *Phragmites australis* (Brix et al. 1996) peak in the afternoon. Consequently, it has been suggested that diel fluctuations differ among species (Whiting and Chanton 1996). In addition, temperature, light, humidity and other environmental variables may influence diel patterns (Brix et al. 1996; Bergström et al. 2007; Duan et al. 2005; Hirota et al. 2004; Juutinen et al. 2004; Kankaala et al. 2005; Wang and Han 2005). The available studies on diel variations from specific stands of plants are limited as only a few diel cycles have been studied in each environment with relatively few measurements during the 24 h period (Hyvönen et al. 1998; Käki et al. 2001; Ding et al. 2004; Juutinen et al. 2004; Xing et al. 2004).

Altogether, there are a number of common assumptions about how species versus environmental factors influence fluxes, and about diel flux variability, that are based on limited data, and it is not clear to what extent the prevailing views are valid across ecosystems and over time. Hence, it is important to study diel variability for species with different evolutionary history as well as differences in morphological and anatomical characteristics over whole growth seasons to resolve the influence of environmental variables versus plant species differences and to allow the development of generally valid models.

In the present study, both diel and seasonal variability in CH₄ emissions from areas dominated by the emergent aquatic macrophytes *Phragmites australis* (Cav.) Steud. and *Carex rostrata* Stokes in two hemiboreal lakes in the southwest of Sweden were measured. The aims were:

- i. Evaluate diel and seasonal patterns during the same time periods in plant-mediated CH₄ emissions from emergent aquatic macrophytes species reported to have different dominating flux transport mechanisms (convective or diffusive);
- ii. To evaluate links between CH₄ fluxes and environmental variables that are potentially related to temporal patterns in plant mediated CH₄ emissions, such as air temperature, light, air pressure, humidity, NEE of CO₂, wind, species and biomass.

Methods

Study site

The field work was carried out from June to October 2014 in two lakes located in the Skogaryd Research Catchment (www.fieldsites.se (13 July 2017); near Vänersborg, Sweden): Lake Erssjön and Lake Följesjön in the drainage area of Bäveån in Västra Götaland County. Both lakes are located in the hemiboreal zone in the southwest of Sweden. Erssjön (N 58° 22.2786', E 12° 9.7175') is a small open-water lake (6.2 ha) with a mean depth of 1.7 m and a maximum depth of 4.5 m. The vegetation along the lake margin is dominated by *Phragmites australis*, and different species of *Carex*, including *C. rostrata* in the shallower parts, with smaller contributions of *Equisetum fluviatile* and *Typha latifolia*, while *Nymphaea alba* is present at deeper water down to ~2 m depth. The surrounding area of Erssjön consists of coniferous forest with a small proportion of deciduous trees, as well as agricultural land adjacent to the northern shore of the lake. Följesjön (N 58° 22.5250', E 12° 9.2237') on the other hand, is smaller (3.8 ha), shallower (mean and max depth of 0.5 and 1.5 m, respectively) and almost the entire surface of the lake is dominated by emergent and floating-leaved wetland species. Only small patches of open water remain and even if named as a lake ('sjön' = 'the lake' in Swedish), large parts of Följesjön share most characteristics with a plant-covered fen-type wetland. In the middle part of Följesjön, vegetation is dominated by *Phragmites australis*, *Carex* spp. with smaller contributions of *Typha latifolia* while *Sphagnum* spp. and *Myrica gale* are abundant along the shore. The vegetation surrounding Följesjön is similar to the vegetation around Erssjön, except that there is no agricultural land adjacent to Följesjön. The lakes are hydrologically connected, as one of the main streams providing water to Följesjön comes from Erssjön. The distance between the lakes is ~1 km.

The concentration of P, N and Fe was higher in Följesjön than in Erssjön, whereas mean dissolved O₂ concentration was lower in Följesjön than in Erssjön

Table 1 Lake characteristics in Lake Erssjön and Lake Följesjön. Wind, temperature and air pressure records from May to October 2014.

Lake	Erssjön		Följesjön	
Area (ha)	6.2		3.8	
Mean depth (m)	1.7		0.5	
	Mean	SD	Mean	SD
tot-P (µg L ⁻¹)	33.73	10.56	50.77	13.73
tot-N (mg L ⁻¹)	1.03	0.16	1.43	0.25
tot-Fe (µg L ⁻¹)	1378	671	1592	500
tot-Mn (µg L ⁻¹)	135	97	108	47
O ₂ (mg L ⁻¹)	7.37	1.39	4.72	1.70
Wind (m s ⁻¹)	1.68	1.01	1.24	0.35
Air temperature (°C)	14.3	5.99	15.18	5.89
Incoming rad (short wave) (W m ⁻²)	250	151	246	123
Air pressure (atm)	0.99	0.00	0.99	0.00

(Table 1). Weather variables did not differ considerably between the two lakes.

Sampling sites and duration

Five sampling sites (three in Erssjön and two in Följesjön) were chosen considering both accessibility and the abundance of the target plant species (Fig. 1). Within each lake, the sites were all located close to each other to minimize the difference between the sites in other aspects than the dominating plant species. The water depth in each sampling site was ~10–20 cm. Each site was dominated by one species; *P. australis* or *C. rostrata*. The sampling sites were located in the northern part of Erssjön and in the central part of Följesjön. Initially there were four sampling sites, three of them possible to reach from boardwalks and the fourth site directly reachable from the shore of Erssjön. Due to the high water level in Erssjön in the end of July, the original shore sampling site could not be reached without disturbing the sediment (mixing the sediment can affect the release of CH₄). This sampling site was therefore moved permanently to a similar nearby area (the fifth site), easily reached from a boardwalk, for the remaining four measurement occasions (Fig. 1).

Measurements in Erssjön were conducted once each hour during 24 h on the two first measurement occasions (9–10 June and 29–30 June 2014) and once every second hour during 24 h on five later occasions (20–21 July, 8–9 August, 29–30 August, 21–22 September and 12–13 October). Samplings in Följesjön took place each

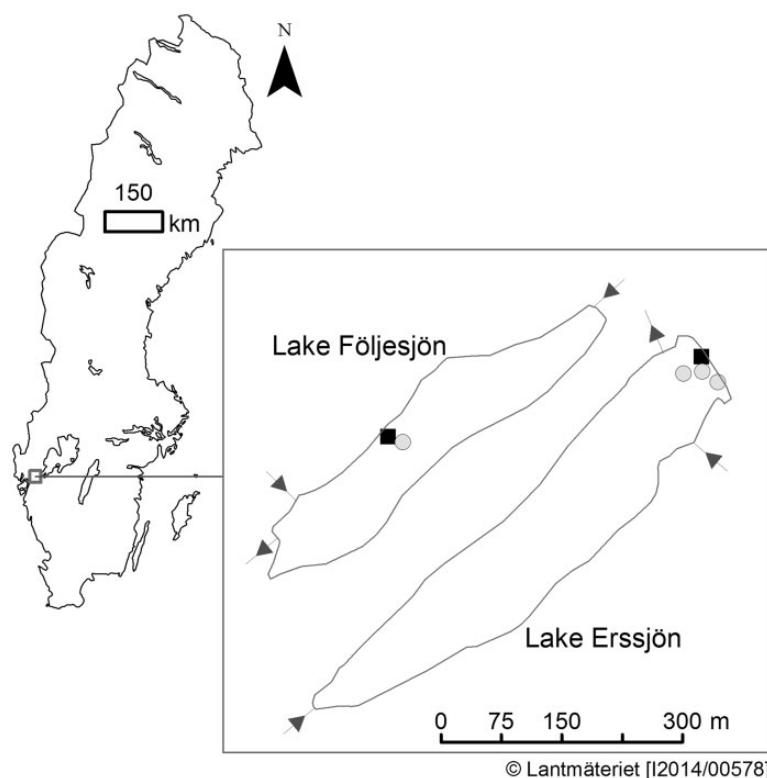


Figure 1. Map of the study area and the sampling sites. Squares represent *Carex rostrata*; circles, *Phragmites australis* (in Erssjön, where the sampling point was moved, the initial one is the left of the two circles).

hour during 24 h on 1–2 July and every second hour during 24 h on five later measurement occasions (21–22 July, 9–10 August, 30–31 August, 23–24 September and 13–14 October).

Sampling method and measurement duration

A closed-chamber method was used to measure CH_4 flux from the areas with emergent aquatic macrophytes. This means that we measured the flux through macrophytes combined with flux through water (the latter being approximately one order of magnitude smaller than the former in the present study). The chamber (d 43 cm, w 43 cm, h 137.5 cm: volume 197.4 L) was made of plastic pipes, built in a rectangular block shape (Fig. 2) and covered with transparent airtight plastic. The plastic material was made of four layers: 40 µm nylon, 6.5 µm methylene-vinyl-alcohol, 40 µm nylon and 100 µm polyethylene (Otto Nielsen Emballage, 2800 Lyngby, Denmark). The chamber was then put over a stand of emergent aquatic macrophytes in order to collect the gas, floating on Styrofoam rods along the lower edges of the plastic pipe frame and with the plastic entering 5 cm into the water ensuring a good seal. The chamber was equipped with two computer CPU fans, run with 12 V batteries for air circulation and a thermometer to measure

the temperature inside the chamber. Gas was sampled from the inside of the chamber through two transparent PVC tubes (outer diameter 5 mm and inner diameter 3 mm) being ~5 m long. One end was attached inside the chamber and the other end to an LGR-UGGA (Los Gatos Research Ultraportable Greenhouse Gas Analyser), one tube for air entering the LGR-UGGA and the other for outgoing air back to the chamber creating a closed loop between the chamber and the LGR-UGGA. Concentrations of CH_4 and CO_2 were logged with the LGR-UGGA every 20 s during 5 min on each plant site. Between measurements, the chamber was removed from the site and vented until the gas levels decreased to the same level as the surrounding air.

Environmental variables

Air temperature ($^{\circ}\text{C}$), relative humidity (%) and air pressure (mb) were measured outside the chamber, with a pocket weather station (Anemometer Silva ADC) during each CH_4 flux measurement. Wind speed (m s^{-1}) was obtained from a weather station near each lake (measured wind speed at 30 min intervals at 4.7 m height in Erssjön and at 2.3 m height in Följesjön (data provided by Eric Sahlée and Anna Rutgersson, Department of Earth Sciences, Uppsala University and Leif Klemetsson,

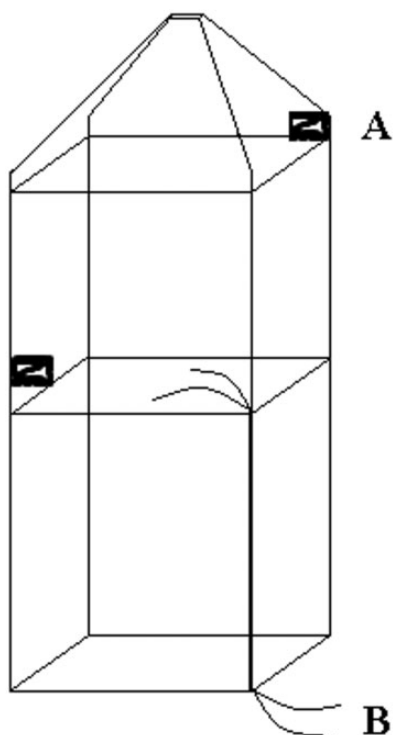


Figure 2. Model of the chamber used for measuring plant-mediated CH_4 emissions. A is a fan run with a 12 V battery (placed at 100 as well as 60 cm height), B is two transparent PVC tubes transporting the air between the chamber and the LGR (Los Gatos Research (LGR) Ultraportable Greenhouse Gas Analyser, UGGA). The chamber is covered with airtight plastic.

Department of Earth Sciences, University of Gothenburg). Missing values for wind speed were replaced by values from another nearby weather station located on a mire 500 m east of Erssjön (measured at 2.25 m; provided by University of Gothenburg). Light (incoming solar radiation) was measured next to the chamber, with a PAR sensor (HOBO Photosynthetic Active Light Smart Sensor) connected to a HOBO® H21-002 Micro Station Data Logger, which logged incoming light at 10 s intervals. The O_2 concentration in the water was measured every 15 min at a depth of 0.5 m below the water surface with a HOBO oxygen logger probe (HOBO U26 Dissolved Oxygen Logger). Samples for total phosphorous P, total N, and total Fe and total Mn were collected in 0.5 L polyethylene bottles at 0.5 m depth (one sample per location collected every other week as a part of the regular monitoring program in the area). Total N and P were analysed spectrophotometrically after chemical reduction to ammonia (N) or oxidation to phosphate (P), respectively, and addition of reagent compounds, according to analytical standard ISO 15681-1:2003 and SS-EN ISO 11905-1:1997, respectively. Fe and Mn were analysed using a Nexion 300D ICP-MS (Perkin Elmer).

Biomass estimation

The aboveground biomass in each chamber was estimated after every sampling day, in total seven times per site. For *P. australis*, plant height was recorded and the number of shoots counted. Biomass in each chamber was estimated from regression equations that were developed by measuring the height of plants and counting shoots in nearby plots, followed by harvesting shoots at the water surface to determine dry weight biomass. For *Carex rostrata*, the shoot density and average height was measured in the chambers on each measurement day. Then, after the measurements, a nearby area (i.e. outside the chambers) with similar shoot density and average height were harvested for biomass determination. The harvested shoots for both species were dried for 48 h at 60°C and plant biomass dry weight (g DW m^{-2}) was determined.

Calculations

The plant CH_4 flux was calculated from the linear change in the chambers gas concentration over time according to Bäckstrand et al. (2008). The method was modified to fit a larger chamber and a different type of gas measurement. The first minute of each 5 min measurement period was omitted, due to heterogeneous mixing-effects before the gas in the tubing and the LGR measurement cell reflected average chamber gas content. Multiple slopes of the gas concentration versus time were calculated for 2-min periods, each period offset by 20 s. In total seven such 2-min slopes were calculated for each 5-min period. From the seven slopes, the one with the highest r^2 -value was chosen for flux estimation.

The following equation was used to calculate the regression slope for each 2-min period, in parts per million per day,

$$\frac{\text{ppm}}{\text{day}} = \frac{\sum(t_i - \bar{t})(\text{ppm}_i - \overline{\text{ppm}})}{\sum(t_i - \bar{t})^2} \quad (1)$$

where ppm_i and t_i are the starting concentration (in ppm) and starting time for each 2-min period and $\overline{\text{ppm}}$ and $\bar{t}$ are the mean of ppm and time for each 2-min period.

The CH_4 flux (F) for the selected 2-min period was calculated according to

$$F = \frac{\frac{\text{ppm}}{\text{day}} \cdot \frac{P_{\text{tot}}}{10^5} \cdot \frac{V}{RT}}{A} \cdot 1000 \quad (2)$$

where ppm/day is the selected regression slope (Equation 1) from CH_4 concentration measurements, P_{tot} is the measured air pressure (atm), V is chamber volume

(L), R is the universal gas constant ($0.082056 \text{ L atm K}^{-1} \text{ mol}^{-1}$), T is temperature (K) and A is the chamber's base area (m^2). The value 1000 is used for unit conversion from $\text{mol m}^{-2} \text{ d}^{-1}$ to $\text{mmol m}^{-2} \text{ d}^{-1}$. CH_4 emissions from the emergent aquatic macrophytes were also calculated per g DW biomass in each measurement site ($\text{mmol g DW}^{-1} \text{ d}^{-1}$). When calculating net flux in this manner, all three types of CH_4 release from the area covered by the chamber are included, i.e. emission via plants, diffusion across the water surface and minor events of ebullition (larger events were excluded, see below).

Ebullition

Ebullition releases can be suspected when there is a rapid sudden increase in CH_4 concentration (also resulting in a reduced R^2 over the whole 2-min period). Large ebullition events are therefore easily detected in the regression plot of CH_4 against time. Such an ebullition event occurred one time only, during the last measurement in Följesjön, and this value was excluded. Ebullition events being too small to detect in this way can be considered insignificant compared with the plant-mediated fluxes.

Diffusive CH_4 flux

CH_4 concentration in the water was measured twice in each lake, once in July and once in October, to estimate the diffusive CH_4 flux. Water samples were taken with a 10 mL plastic syringe and ~5 mL water was collected to rinse the syringe prior to sampling. During this rinsing, care was taken to also remove air bubbles from the syringe. When the rinsing water had been discarded, 5 mL water were sampled 3 cm below the surface, and, after visually confirming absence of bubbles, the sample was injected into a pre-capped vial (20 mL) filled with 100 μL H_3PO_4 and N_2 at atmospheric pressure. CH_4 concentration in the water was analysed in the laboratory by a gas chromatograph (Agilent 6890 with Haysep N column and flame ionization detector calibrated with certified gas standards).

The diffusive CH_4 flux can be expressed with the equation:

$$F = k \times (C_w - C_{eq}) \quad (3)$$

where F is diffusive CH_4 flux in $\text{mmol m}^{-2} \text{ d}^{-1}$, k is the gas transfer velocity (m d^{-1}), C_w is the measured CH_4 concentration in the water (mmol m^{-3}), and C_{eq} is the CH_4 concentration in the water if it was in equilibrium with the air concentration (estimated from air concentrations and Henry's Law). The k -value was derived from independent flux and concentration measurements on the nearby open water (see, e.g. Bastviken et al. 2010

and Natchimuthu et al. 2016 for method details). This can be considered to yield overestimated diffusive flux as previous work has indicated that k -values are much lower in stands of macrophytes where the water surface is wind-sheltered than for open water (Kosten et al. 2016; Attermeyer et al. 2016).

Statistical analyses

Methane emissions were analysed in three ways, with focus (i) on the possible existence of diel patterns; (ii) simple relationships between mean diel CH_4 flux per m^2 and different environmental variables; and (iii) on modelling all data using all explanatory variables.

To evaluate whether there were consistent diel patterns in CH_4 emissions among the 25 daily time series (one incomplete series excluded), we used intra-class correlation (Rusak et al. 1999). We first divided the day into 10 time classes, calculating an average per class (there were 1–4 values per time class). These calculations were conducted with SPSS 24, using the two-way mixed model, with absolute agreement.

The relationships between mean diel CH_4 flux and temperature, wind, light and net CO_2 exchange, respectively, were evaluated using linear regression. Relationships for which the slopes had P -values < 0.05 were considered significant.

In approach (iii), all 5 min flux measurements ($N = 288$) were analysed using a generalized linear model (GLZ, McCullagh and Nelder 1989) with a log-link function and Gamma distribution using the software R (R Core Team 2013). Explanatory variables were lake, plant species, air temperature, temperature inside the chamber, light, air pressure, air humidity, wind speed, plant biomass, CO_2 flux, date and time, where date and time were treated as separate variables in the model. A quadratic term of time and date was included, as we expected a nonlinear response of CH_4 flux during the day and over the growing season (based on previous studies, a peak in plant CH_4 emission was expected during the day and highest emissions were expected in the summer during the peak of the growing season; Van der Nat et al. 1998; Kankaala et al. 2005). An AIC-based model averaging and selection approach was used (Symonds and Moussalli 2011).

In model selection and averaging, candidate models are the set of all possible models that are nested within the full model and the AIC values (Akaike Information Criterion; Akaike 1974) for candidate models are used as a measure of model fit. The difference in AIC between models is used to rank models, to determine the importance of explanatory variables and to estimate regression coefficients. Model selection was implemented using the MuMIn-package (Barton 2016), and continuous

explanatory variables were standardized to have mean = 0 and SD = 0.5 prior to analysis. Correlations between explanatory variables were calculated to assess the degree of collinearity. Humidity and temperature inside the chamber were excluded from the model due to their high correlation with air temperature ($r > 0.7$). The fitted full model was tested for collinearity (Zuur et al. 2010) with the vif function (car-package; Fox and Weisberg 2011); detecting only minimal collinearity ($\text{vif} < 3$). Furthermore, residuals from the fitted model did not show heteroscedasticity.

Linear regression is sensitive to un-equal sampling intensity along the explanatory gradients. The greater number of measurements in the beginning of the growing season might bias the results and thus lead to erroneous conclusions. Therefore, every second value, from a total of 24 measurements in 9–10 June, 29–30 June and 1–2 July were omitted, in order to have a consistent dataset with twelve measurements per day. Autocorrelation between consecutive measurement during a day was detected with the ar-function (in the stats-package in R), for days with 24 measurements. However, there was less autocorrelation when every second value was deleted, so no further process for handling autocorrelation in the model was needed.

Results

Plant mediated and diffusive CH_4 emissions

Our flux estimates ranged from 1 to 87 $\text{mmol m}^{-2} \text{d}^{-1}$ and 1 to 73 $\text{mmol m}^{-2} \text{d}^{-1}$ for *P. australis* and *C. rostrata*,

respectively (Table 2; equivalent to 0.7–58 and 0.7–49 $\text{mg CH}_4 \text{ m}^{-2} \text{h}^{-1}$). When calculating CH_4 emissions from the emergent aquatic macrophytes per m^2 , the diffusive flux of CH_4 and ebullition were included in the total emission. The measured diffusive emissions ranged from 0.11 to 2.46 $\text{mmol m}^{-2} \text{d}^{-1}$, which represented 1–22 % of the total mean emission from the emergent aquatic macrophytes on the day that measurements were made (Table 2). In an extensive, previous 2-year study of the two lakes, median diffusive fluxes ranged from 0.1 to 1.0 $\text{mmol m}^{-2} \text{d}^{-1}$ (Natchimuthu et al. 2016) confirming that diffusive flux, where plants were present, was small relative to plant-mediated fluxes. Further, these numbers are most likely overestimates of the diffusive flux at the measurement plots because gas exchange rates are lower among plants than for open water (see Methods section).

Variability between lakes, plant species and measurement days

The 24-h mean CH_4 emissions per m^2 differed between lakes with higher fluxes in Följesjön (Fig. 3). There were no differences in these CH_4 emissions (per m^2) between *P. australis* and *C. rostrata* within the same lake (Fig. 3). The highest 24-h mean fluxes occurred in the summer (June–August) and the lowest fluxes were recorded during September and October in both lakes (Table 2 and Fig. 3). There was a 5-fold difference between measurement days with highest and lowest flux for both plants species. The variability between measurement days were

Table 2 Maximum, minimum and mean of CH_4 emission ($\text{mmol m}^{-2} \text{d}^{-1}$) for *P. australis* and *C. rostrata* in Lake Erssjön and Lake Följesjön, throughout the study period in 2014 ($n = 7$).

CH_4 emission ($\text{mmol m}^{-2} \text{d}^{-1}$)			Measurement occasion						
			9–10 June	29 June to 2 July	20–22 July	8–10 Aug.	29–31 Aug.	21–24 Sep.	12–14 Oct.
Erssjön	<i>P. australis</i>	Maximum	86.7	15.4	37.6	38.0	26.8	11.7	5.46
		Minimum	6.60	2.39	1.87	6.07	6.78	0.94	1.60
		Mean	21.6	4.78	11.1	15.4	12.7	4.11	3.42
	<i>C. rostrata</i>	Maximum	20.7	10.8	–	17.8	14.5	4.21	5.10
		Minimum	7.20	5.20	–	9.09	8.2	2.01	1.05
		Mean	14.7	6.75	–	13.0	10.0	2.88	2.14
	<i>P. australis</i>	Maximum	–	44.1	55.4	77.6	40.1	59.2	35.40
		Minimum	–	2.69	19.3	11.7	8.57	1.98	5.74
		Mean	–	16.9	32.6	49.9	19.8	15.6	13.7
Följesjön	<i>C. rostrata</i>	Maximum	–	31.5	73.0	54.1	29.1	28.4	34.2
		Minimum	–	9.90	24.6	20.6	8.29	4.12	4.05
		Mean	–	18.6	37.0	32.5	16.0	10.6	15.1

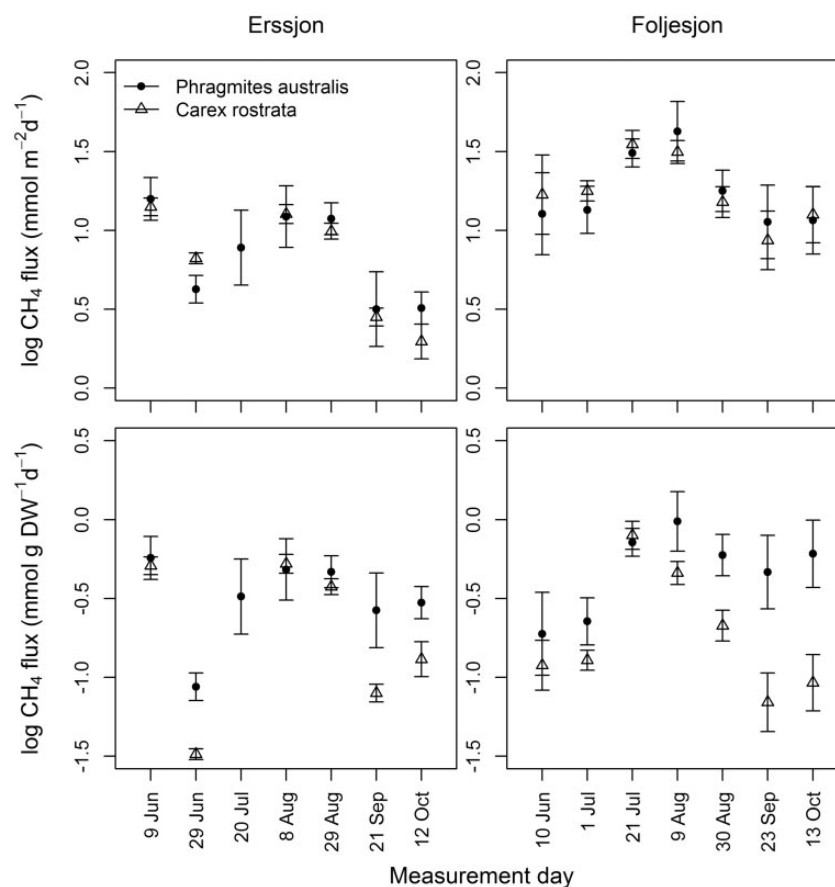


Figure 3. Fluxes of CH₄ (diel average with CI_{95%}, $n = 7$) from sites dominated by *Phragmites australis* and *Carex rostrata* in Erssjön and Följesjön during June to October 2014. Top graphs show fluxes calculated per m² and bottom graphs fluxes calculated per g DW.

consistent for both *P. australis* and *C. rostrata*, i.e. low and high fluxes occurred during the same days for both species.

When expressing 24-h mean fluxes per g DW (instead of per m²) the temporal pattern among measurement days remains, but there were differences between plots with different plants, with lower fluxes per g DW from *Carex rostrata* stands some of the days (Fig. 3).

Diel variation in CH₄ emission

There was variability in CH₄ emissions within days (Fig. 4). However, recurrent peaks in CH₄ emissions at the same time on the different measurement days (i.e. a consistent diel pattern) were not found for the two species (intra-class correlation coefficient based on both species: 0.055, $P = 0.316$; coefficients for *P. australis* and *C. rostrata* were 0.014 and 0.017, respectively). Variation during the days was higher for *P. australis* than for *C. rostrata* in Erssjön, where *P. australis* had both higher and lower emissions registered each measurement day compared to *C. rostrata* (Table 2).

Mean daily CH₄ flux and single environmental variables

There was a significant relationship between mean daily CH₄ emissions per m² and mean diel temperature (Fig. 5). There were no significant relationships between diel means of wind speed, light levels or net CO₂ exchange and CH₄ emissions (Fig. 5).

Modelling CH₄ flux

The results from the regressions of daily means on CH₄ emission per m² were confirmed by the AIC-based GLZ model selection and averaging procedure using $N = 288$ data points. The analysis found that models that contained lakes and air temperature were better at explaining methane flux (Table 3). These variables were included in the all best fitting candidate models (i.e. relative importance = 1). The model average parameter estimate indicated that methane flux increased with temperature and was higher in Följesjön than in Erssjön (Table 3). An increase in light and wind had a (significant) negative effect on CH₄ emissions, while plant species,

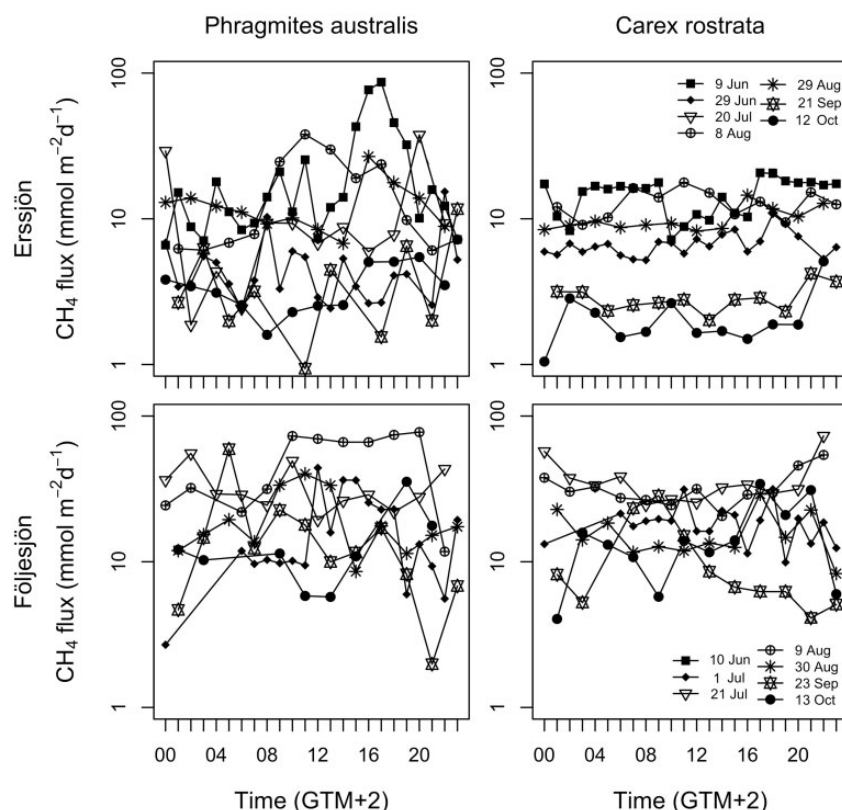


Figure 4. Diel variation in CH_4 fluxes from sites dominated by *Phragmites australis* and *Carex rostrata* in Erssjön and Följesjön, for each measurement occasion during the growing season (June to October, $n = 7$). Measured in Swedish summertime (GMT + 2).

biomass dry weight, NEE of CO_2 , and time of day were much less important (Table 3).

Discussion

No clear diel flux patterns

While our flux values correspond with previously recorded ranges for CH_4 flux via *P. australis* and various *Carex* species (mean fluxes reported to 0–80 and 0–33 $\text{mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$; examples of ours and previously reported fluxes provided in Table 4), our results challenge common views on diel variability. We found that peaks in plant-mediated CH_4 emissions occurred during all hours of the day but were not very strong, nor did they dominate the total daily flux (Fig. 4). Furthermore, the diel patterns for *P. australis* did not coincide with patterns for *C. rostrata*.

Previous studies of diel variability often found clear flux peaks and it has been suggested that predominating convective flux mechanisms actively regulated by the plants lead to a stronger diel cycle than when diffuse and more passive flux through the plant dominates (Whiting and Chanton 1996; Juutinen et al. 2004; Duan et al. 2005). The timing of the observed flux peaks has

been shown to vary between species (Brix et al. 1996; Whiting and Chanton 1996). However, it is sometimes unclear how stable the diel patterns were over time when patterns from only a single day are reported (e.g. Whiting and Chanton 1996; Van der Nat et al. 1998; Ding et al. 2004). The present study reports diel measurements during seven measurement days, and clearly shows that the diel patterns vary considerable among them.

Gas exchange by plants is thought to be controlled by stomatal opening allowing gas diffusion into or out from the aerenchyma, in some species facilitated by pressure-driven convection. The convection is controlled by internal/external differences in relative humidity (RH) or temperature, or by wind (reviewed by Sorrell and Brix 2013 and Armstrong and Armstrong 2014). Aquatic plants do not need to save water and can keep stomata open during night (Käki et al. 2001). Accordingly, diffusion-driven gas exchange is often found to lack clear diel patterns for emergent aquatic plants. The absence of strong and consistent diel patterns was therefore expected for *C. rostrata*, but was surprising for *P. australis*, which commonly is found to have gas exchange that is positively related to insolation (Table 4). Light has been suggested to influence convection-driven diel variability

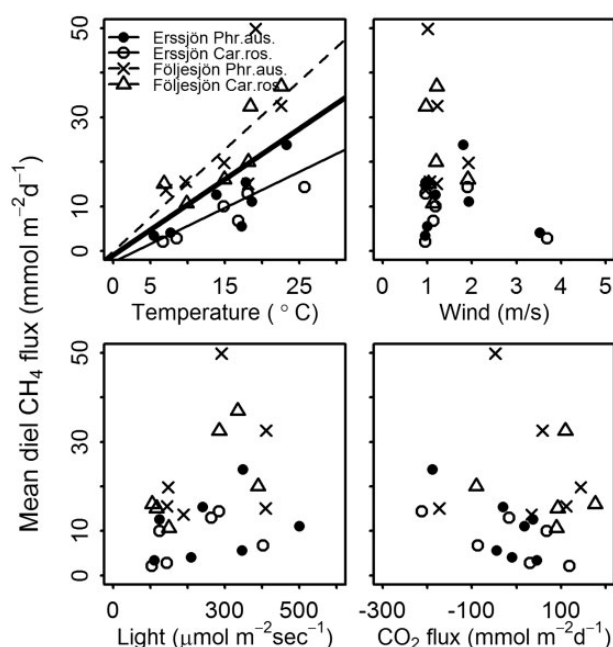


Figure 5. Mean diel CH_4 emissions in relation to air temperature (t_{air}), wind, light and CO_2 flux (i.e. net ecosystem CO_2 exchange) for measurement plots with *Phragmites australis* and *Carex rostrata* in Lake Ersjön and Lake Följesjön, respectively. Lines show significant linear regressions. The CH_4 flux (F_{CH_4} ; $\text{mmol m}^{-2} \text{d}^{-1}$) were similar among species (see text) and the dashed and thin solid lines represent the regressions for both species combined in Följesjön ($F_{\text{CH}_4} = 1.529 \cdot t_{\text{air}} - 0.068$; $P = 0.009$; adj. $R^2 = 0.46$) and Ersjön ($F_{\text{CH}_4} = 0.811 \cdot t_{\text{air}} - 2.487$; $P < 0.001$; adj. $R^2 = 0.63$), respectively. The thick solid line denotes the overall regression for all data ($F_{\text{CH}_4} = 1.136 \cdot t_{\text{air}} - 0.984$; $P = 0.003$; adj. $R^2 = 0.30$). No significant relationships were found for wind, light or CO_2 flux.

in fluxes, presumably by affecting RH and temperature gradients. The detailed AIC-based GLZ model selection and averaging showed that light and wind were of intermediate importance and had a weak negative correlation with CH_4 flux (Table 3). The GLZ includes all time points and combines both diel and seasonal data so the wind and light correlations may not primarily be associated with diel variability. However, it is possible that the long days and short (if any) dark periods during summertime at northern latitudes may make the diel gas exchange patterns less predictable than at more southern latitudes having more pronounced day-night differences in light. For example, diel patterns related to the light cycle were observed from measurements in August and September only (when nights get darker) at a lakeshore site in Finland, while patterns were unclear during May to July (brighter nights) (Käki et al. 2001). Altogether, our result indicated that CH_4 fluxes from *P. australis* may not always show distinct diel patterns, and that further work addressing diel variability at different locations and under different conditions is needed.

What controls plant-mediated CH_4 emissions?

Methane emissions from both species were on average three times higher in Följesjön than in Ersjön (Table 1). This is in line with the study by Natchimuthu et al. (2016) that concludes that the amount of released CH_4 from the open water areas in Följesjön exceeded open water fluxes from Ersjön. Possible explanations include that Följesjön was very shallow, had thick organic-rich sediments, and a higher standing crop of emergent vegetation leading to a high production of organic matter substrates for methanogenesis per unit area. This is a speculation based on the overall abundance of macrophytes and bulk organic matter, as measuring the in-situ production of the specific original plant material fuelling microbial communities including methanogens in sediments is difficult and beyond the scope of this study. However, variables associated with high primary production or high levels of organic matter are known to stimulate CH_4 production and fluxes (e.g. Segers 1998; Bastviken et al. 2004; Bridgham et al. 2013), giving some support for this potential explanation of the higher overall fluxes from Följesjön.

The temporal variability of CH_4 emissions within each system was related to temperature (Table 3 and Fig. 5). Although we used air temperature, the temperature of the sediment was probably more important (Johansson et al. 2004; Duan et al. 2005), which provides an additional reason for the lake differences; Följesjön was the shallowest lake, hence likely to have a higher sediment temperature during the growing season. The sediment in a shallower lake with a smaller water volume is also likely to respond faster to changes in air temperatures. Several studies have demonstrated a relationship between CH_4 emissions and sediment temperature, in accordance with the high temperature-sensitivity of methanogenesis (Segers 1998; Wang and Han 2005; Duc et al. 2010; Yvon-Durocher et al. 2014; Turetsky et al. 2014). In contrast, oxidation of CH_4 seems unaffected by temperature (most often limited by substrate supply; Nykänen et al. 1998; Duc et al. 2010).

It should be noted that temperature can have a direct influence on process rates, as discussed above, but also is correlated with seasonal fluctuations and production of organic substrates for methanogenesis (Laanbroek 2010; Carmichael et al. 2014). Several studies reported maximum biomass during the growing season as the key explanatory factor for CH_4 emissions (Hirota et al. 2004; Juutinen et al. 2004; Kankaala & Bergström 2004). However, in the present study and in that by Kankaala et al. (2004), biomass could not explain the temporal variability in CH_4 emissions per m^2 (Table 3), suggesting that direct temperature effects or organic matter from

Table 3 The effect of categorical and continuous variables on emergent aquatic macrophytes CH₄ emissions per m². Data from the full generalized linear model (GLZ; estimate) and from a model selection and averaging of nested candidate GLZ-models with standardized continuous variables (model average estimate, standard error (SE), z-value, 95 % confidence interval (CI) and relative importance for variables). Values marked with bold text are statistically significant.

Methane flux (mmol m ⁻² d ⁻¹)	Estimate	Standardized values					
		MAE*	SE	z-value	CI interval		RI*
					Lower	Upper	
Intercept	2.502e + 07	2.637	0.069	37.962	2.501	2.773	
Lake Erssjön (compared with Lake Följesjön)	−9.995e − 01	−0.991	0.097	10.202	−1.181	−0.800	1.0
Air temperature (C°)	5.826e − 02	0.896	0.172	5.182	0.557	1.235	1.0
Light (μmol m ⁻² s ⁻¹)	−2.929e − 04	−0.242	0.111	2.178	−0.459	−0.024	0.9
Wind (m/s)	−8.890e − 02	−0.218	0.095	2.291	−0.404	−0.031	0.93
Date 1 (Measurement day)	1.894e − 05	−0.279	0.135	2.057	−0.545	−0.013	0.9
Date 2 (Measurement day)**	−6.740e − 15	−0.342	0.187	1.821	−0.710	0.026	0.9
<i>P. australis</i> (compared to <i>C. rostrata</i>)	1.488e − 01	0.172	0.090	1.913	−0.004	0.348	0.77
Air pressure (atm)	6.357e − 01	0.028	0.102	0.270	−0.172	0.227	0.27
Biomass (g DW)	−1.011e − 03	−0.145	0.115	1.259	−0.370	0.081	0.50
NEE (net ecosystem exchange of CO ₂)	9.841e − 05	0.092	0.085	1.077	−0.075	0.259	0.48
Time 1 (Time of the day)	−3.516e − 02	−0.013	0.089	0.148	−0.189	0.163	0.12
Time 2 (Time of the day)**	1.234e − 11	0.115	0.238	0.481	−0.353	0.582	0.12

*MAE (Model Average Estimate) and RI (Relative Importance).

**Quadratic term.

other sources (e.g. the catchment) could also influence fluxes.

Wind speed and light were shown to weakly affect CH₄ emissions from *P. australis* and *C. rostrata* negatively in both lakes (Table 3). In contrast, many previous studies showed that light had a positive effect on the CH₄ emission from *P. australis* in relation to diel patterns (discussed above; Table 4). According to Duan et al. (2005) light can affect CH₄ emissions also on a larger scale (e.g. seasonal patterns) during the period when the emergent aquatic macrophytes transport gas with convective flow (light-dependent plant activity suggested to regulate gas exchange). In the study by Juutinen et al. (2004), light could explain 39–73 % of the variation in plant-mediated CH₄ emissions during the middle of the growing season. However, light often co-varies with temperature, other weather variables, and primary production or net CO₂ exchange, and it is often unclear if effects from light, temperature and other variables can be separated. Our AIC-based GLZ model selection and averaging procedure is one way to approach this challenge and it clearly showed that temperature was more strongly related to the flux than light and wind, with no clear link between CH₄ flux and net CO₂ exchange.

Did plant species identity influence emissions?

Plant species are expected to differ in the amount of CH₄ they can emit due to, e.g. the extent of their root system, the amount of biomass they have, and differences in flux modes (diffusive or convective). Plant-mediated CH₄ emissions are usually reported per m², a unit useful for scaling up estimates of emission. On the other hand, CH₄ emissions per plant biomass unit may be more appropriate if focusing on emission mechanisms and plant architecture. In the present study we therefore reported both. We expected that the convective flux mode (*P. australis*) should cause higher emissions than the passive one (*C. rostrata*). However, sites with *P. australis* and *C. rostrata* always showed similar fluxes per m² in the same systems on nearby sites (Fig. 3). Further, if comparing results from the lakes in Fig. 3, it is clear that the same flux rates could be sustained by widely different plant densities (maximum biomass values for *P. australis* were 25 and 60 g m⁻² and for *C. rostrata* 204 and 139 g m⁻² for Erssjön and Följesjön, respectively). The differences found in the fluxes per g DW plant biomass therefore largely reflected differences in standing crop biomass of the two species, and did not provide any clear information about fluxes per se or about flux regulation. Hence, most of the

Table 4 Examples of studies addressing diel variability in CH₄ flux from areas with *Phragmites australis* or *Carex* species.

Type of environment and location	Dominating plants	Time period	Mean flux range (mg CH ₄ m ⁻² d ⁻¹)		Diel flux pattern	No of diel cycles reported	Method used for flux measurements	Source
			Low	High				
Lakeshore sites, central Finland	<i>P. australis</i>	July to October	0	32	24h mean = 0.58 to 0.91 times the mean for daytime flux. Most shallow sites showed less clear diel variability	many (unclear)	Automatic flux chamber; ca 5 measurements per diel cycle	Juutinen et al. (2004)
Constructed wetland, The Netherlands	<i>P. australis</i>	Unclear	20	80	Flux positively related with PAR. Up to 2-fold difference between day and night.	1 (unclear)	Chambers connected to analyzer. 15-50 min enclosure.	Van der Nat et al. (1998)
Eutrophic lake shore, southern Finland	<i>P. australis</i>	May to September	1	18	Highest flux at noon in August and September. Irregular patterns other times.	7	manual sampling over 9 min every 6th h.	Käki et al. (2001)
Temperate fen, Germany	<i>P. australis</i>	9 days, August	3.5	11	increasing mean fluxes from 06-14 (2-fold increase)	1-6 (unclear)	Transparent chambers; 40 min incubation; syringe sampling. Time period of 06-14 studied.	Günther et al. (2014)
Two fens in Belarus; focus on shallow water plots	<i>P. australis</i>	2 days, June, August	2	20	Fluxes in both transparent and dark chambers increased with PAR. >2-fold higher flux during daytime.	2	Chamber; discrete samples taken over 8-12 min	Minke et al. (2014)
March, Nebraska, USA	<i>P. australis</i>	65 days, July–September	8	25	Fluxes highest midmorning-noon. 2-fold higher flux during daytime.	4; 2-day periods	Eddy covariance	Kim et al. (1998)
Hemiboreal lake-wetland, southwest Sweden	<i>P. australis</i>	June to October	11	33	No consistent diel pattern	6 x 2	Chambers connected to analyzer. 5 min enclosure; fluxes measured every 2h.	This study
Hemiboreal lake shore, southwest Sweden	<i>P. australis</i>	June to October	3	58	No consistent diel pattern	7 x 2	Chambers connected to analyzer. 5 min enclosure; fluxes measured every 2h.	This study
Lakeshore sites, central Finland	<i>Carex</i> spp.	August	0	32	No strong diel pattern	12	Automatic flux chamber; ca 5 measurements per diel cycle	Juutinen et al. (2004)
Temperate fen, Germany	<i>C. acutiformis</i>	9 days, August	8	11	No clear diel patterns	1-6 (unclear)	Transparent chambers; 40 min incubation; syringe sampling; time period of 06-14 studied.	Günther et al. (2014)
Eutrophic marsh, China	<i>C. lasiocarpa</i>	August	20	33	Increasing flux from 03 to 09 (up to 1.5-fold increase)	3	Flux chambers sampled manually; 30 min enclosure time; 3 h enclosure frequency	Ding et al. (2004)
Hemiboreal lake-wetland, southwest Sweden	<i>C. rostrata</i>	June to October	3	16	No consistent diel pattern	6	Chambers connected to analyzer. 5 min enclosure; fluxes measured every 2 h.	This study
Hemiboreal lake shore, southwest Sweden	<i>C. rostrata</i>	June to October	3	14	No consistent diel pattern	6	Chambers connected to analyzer. 5 min enclosure; fluxes measured every 2 h.	This study

variability in plant-mediated flux per m² appeared related to temperature and between-lake differences in environmental conditions as discussed above, while plant species/flux mode had, at most, a minor influence. We suggest additional studies in multiple systems to investigate under what conditions plant species communities affect fluxes, while trying to separate effects of plant species and other variables regulating CH₄ production on the specific site.

Conclusions

In summary, and in contrast to many previous studies on CH₄ emissions via aquatic macrophytes, we found the following:

1. Diel variability in CH₄ fluxes from *P. australis* and *C. rostrata*, representing plants with convective and diffusive flux modes, respectively, were irregular in magnitude and timing and unpredictable based on data from seven days distributed from June to October.
2. The 24-h mean fluxes per m² on nearby sites were similar between species and highly temperature-dependent. Differences between lakes were consistent with factors influencing system CH₄ fluxes such as macrophyte standing biomass and water depth. No clear influence of other studied potential predictors such as light, wind, pressure, and NEE of CO₂ were found.
3. All available data from this study indicate similar total CH₄ flux per m² from plots with *P. australis* and *C. rostrata*. Hence, fluxes were not controlled by the dominating plant species but more likely by the overall CH₄ production in the systems.

Given these results, the presence and importance of diel variability in plant fluxes, as well as the importance of macrophyte species composition under various conditions needs to be re-evaluated and assessed systematically over time across a range of environments and species.

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Contributions by the Authors

D.B. and P.M. conceived the study; all authors contributed to the design; L.T. collected the data; L.T. and L.W. analysed the data; all authors contributed to evaluation and interpretation of the results; L.T. drafted the text (as part of a MSc thesis) with all authors contributing substantially to the current version.

Conflict of Interest Statement

None declared.

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