Supplementary material

A novel approach for high-frequency *in-situ* quantification of methane oxidation in peatlands

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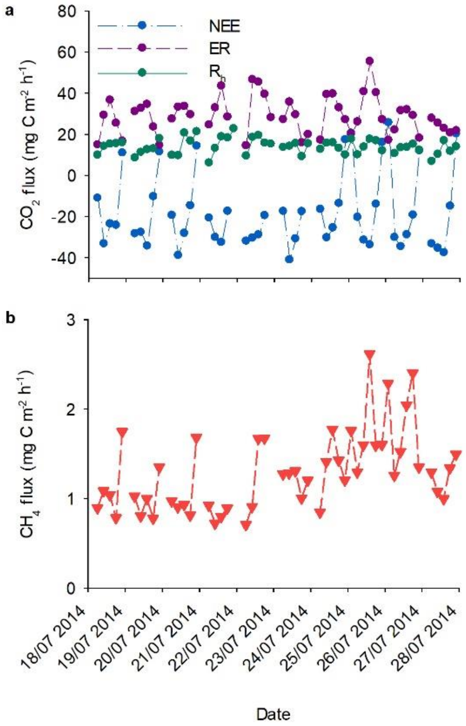

Figure S1 Four-hourly averages of a) net ecosystem exchange (NEE), ecosystem respiration (ER) and heterotrophic respiration (R_h) and b) CH_4 fluxes from undisturbed plots with transparent chambers in the Degerö mire during the measurement period from 18 to 27 July 2014. The NEE, ER and R_h all varied on a diurnal scale with highest CO_2 uptake by NEE and release by ER and R_h during mid-day. The net ecosystem exchange for the period fluctuated between -41 and 26 mg C m^{-2} h^{-1} (negative values representing net C uptake), and the average net ecosystem exchange of the period was -20.7 ± 18.4 mg C m^{-2} h^{-1} (uncertainties presented in this section are standard deviation). The ecosystem respiration fluctuated between 14 and 55 mg C m^{-2} h^{-1} during the same 10-day period, with an average of 30.3 ± 13.1 mg C m^{-2} h^{-1}. The heterotrophic respiration fluctuated between 6 and 23 mg C m^{-2} h^{-1} and averaged 13.9 ± 4.8 mg C m^{-2} h^{-1}. Thus, on average the heterotrophic respiration constituted almost 50 % of total ecosystem respiration. The CH_4 fluxes from the undisturbed plots fluctuated between 0.7 and 2.6 mg C m^{-2} h^{-1} and averaged 1.2 ± 0.7 mg C m^{-2} h^{-1}.

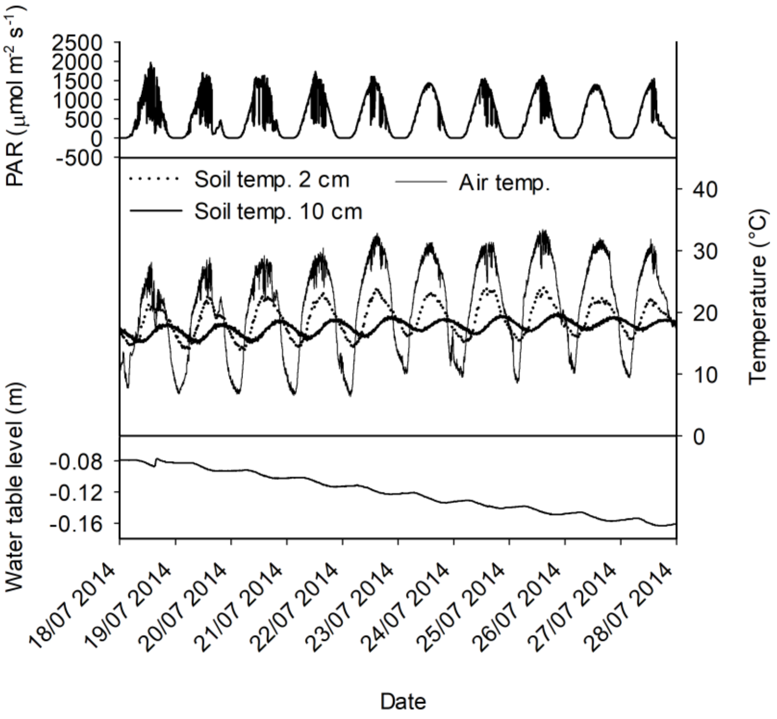

Figure S2 20 second averages of photosynthetically active radiation (PAR), and air temperature in 10 cm height, and 5 minute averages of soil temperature at 2 and 10 cm depth, and water table level at the experimental site in the Degerö mire during the measurement period from 18 to 27 July, 2014.

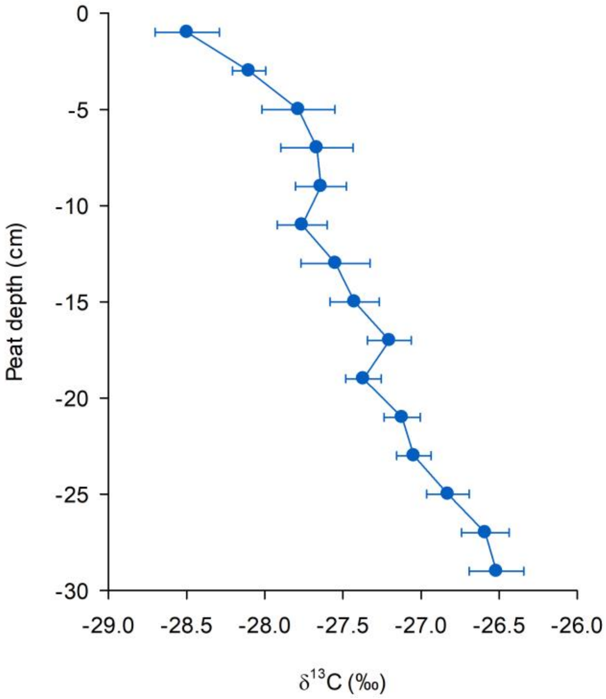

Figure S3 Carbon isotopic signature (δ^{13}C) of peat in the Degerö mire measured on 2-cm peat core sections (n = 120). Error bars show standard error.

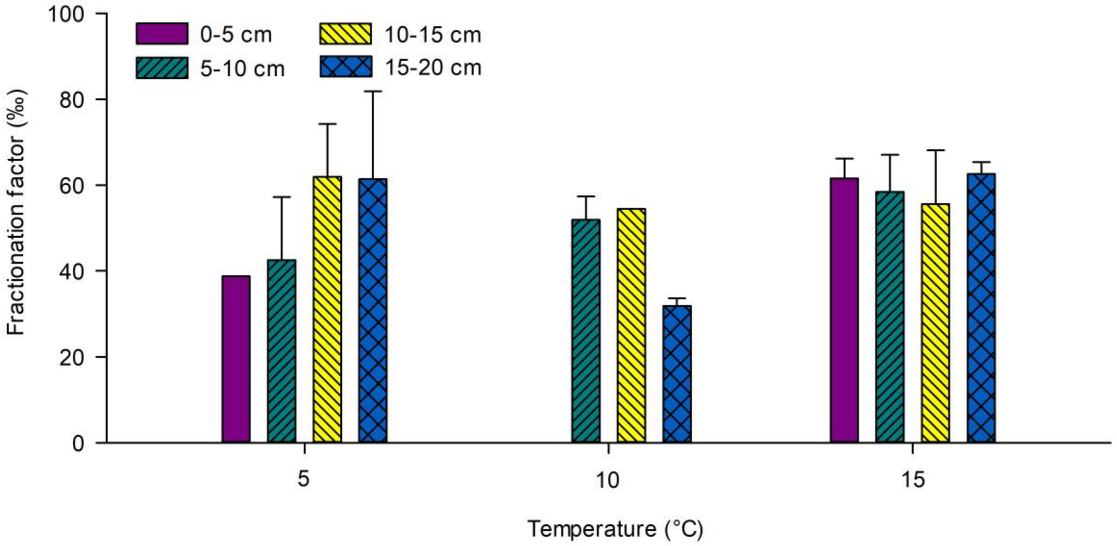

Figure S4 Mean fractionation factors measured at 5, 10 and 15 °C in peat cores of depths 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm from the Degerö mire. Error bars show standard error.

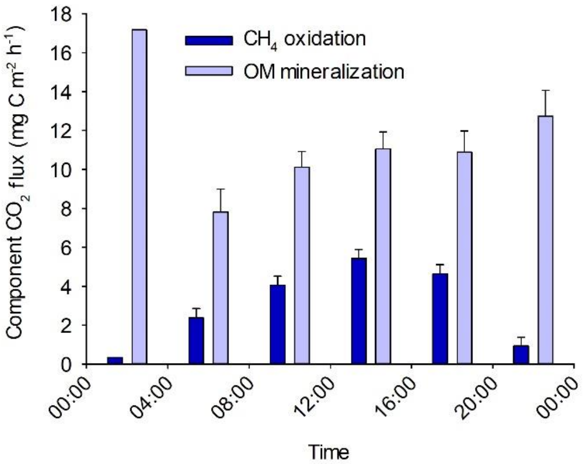

Figure S5 Four-hour means of heterotrophic CO_2 fluxes from vegetation-free plots (all photosynthetic biomass removed) in the Degerö mire, derived from CH_4 oxidation and organic matter (OM) mineralization. Error bars show standard error.

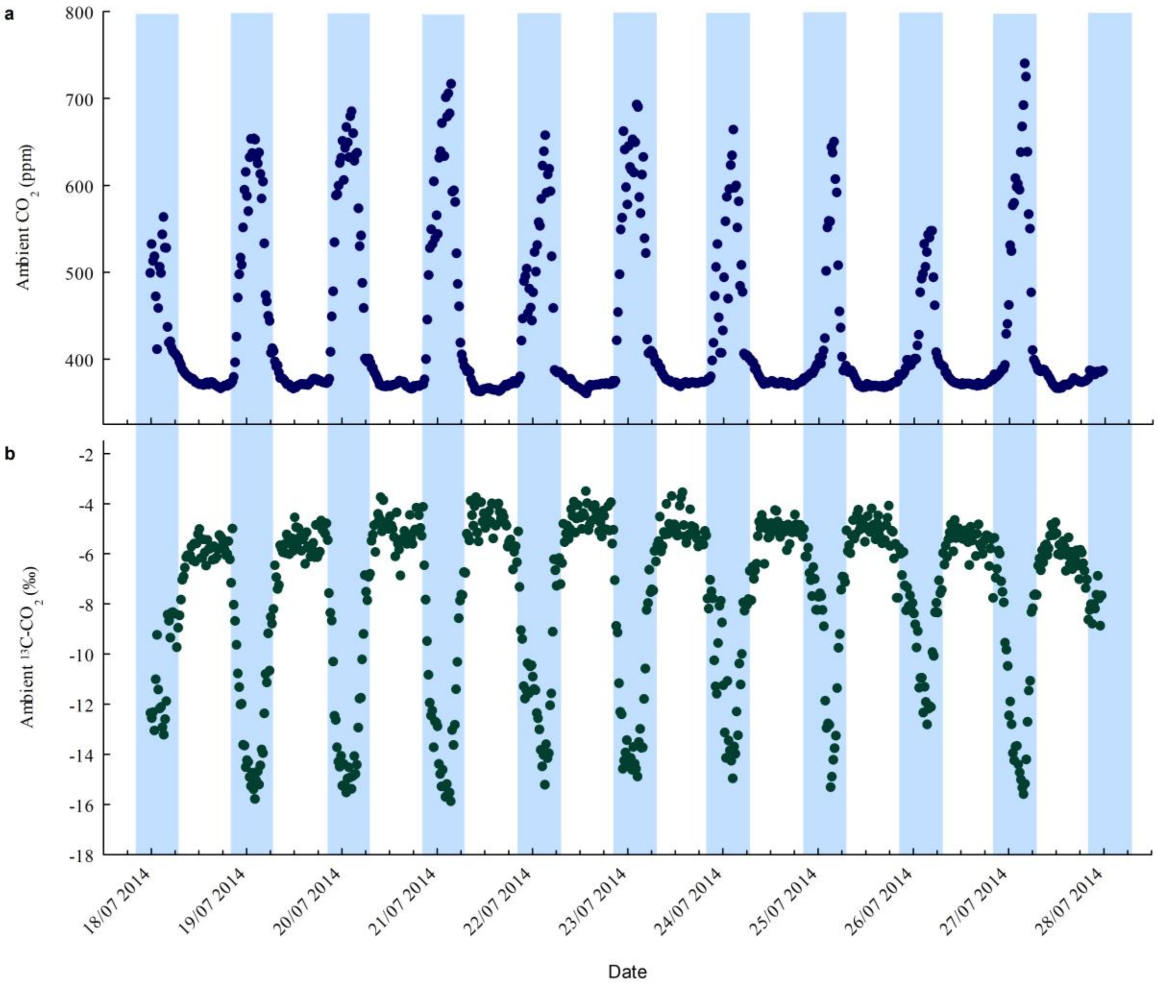

Figure S6 a) Ambient air CO_2 concentration 10 cm above the mire surface and **b)** the isotopic signature of CO_2 at the Degerö mire during the measurement period 18-27 July 2014. Light blue areas indicate nighttime (20:00-07:00). During nighttime there was a stratification of the atmosphere above the soil causing build-up of higher CO_2 concentrations with lower isotopic signature. This could have potentially influenced the diffusion of ^{12}C-CO_2 relative to ^{13}C-CO_2 out of the soil (due to a lowered concentration gradient) leading to overestimation of the flux isotopic signature (signatures would appear to be more enriched). In addition, lower fluxes during night made it more difficult to establish good keeling plots, and thus more nighttime keeling intercepts failed to pass the quality criteria and were excluded from further analysis.

Sensitivity analysis

When using the mixing model, we recognize that the fractionation factor is a crucial variable influencing the magnitude of our results and that this factor could potentially vary over a growing season [1]. We therefore made a sensitivity analysis (Figure S7, Figure S8 and Table S1) in order to assess the effect of using the most extreme fractionation factors from literature (3 [2] and 60 ‰ [3]), as well as the minimum and maximum measured carbon isotopic ($\delta^{13}C$) signatures of organic matter (OM) (-29.7 and -25.9 ‰) and pore water methane (CH_4) (-73.5 and -58.9 ‰). The lowest estimate for the relative contribution of CH_4 oxidation to heterotrophic respiration (R_h) across the measurement period was 16.0 % and was given by the combination of the lowest $\delta^{13}C$ signatures of OM and pore water CH_4 combined with the highest fractionation factor. The highest estimate (56.6 %) was given by the combination of the highest $\delta^{13}C$ signatures of peat and CH_4 and the lowest fractionation factor. The 5th and 95th percentiles were 17.0 and 53.7 % respectively. This analysis therefore shows that our used estimate of 20.1 % of R_h is rather conservative, lying in the lower range as seen on Figure S7 and S8 (black lines on panel e) on both figures).

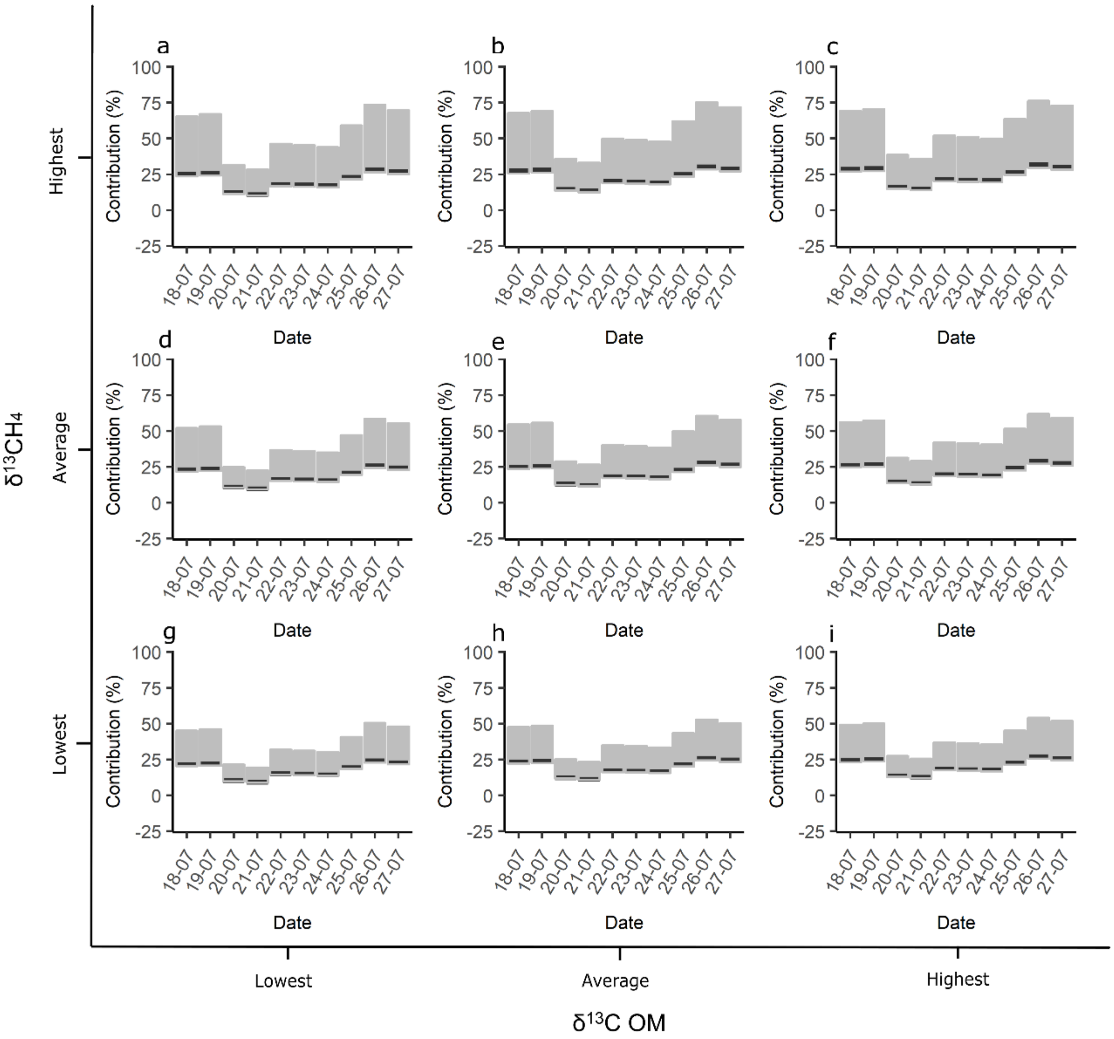

Figure S7 Daily averages of contribution of CH_4 oxidation to R_h CO_2 fluxes for the period 18 to 27 July 2014 based on mixing models using minimum, average, and maximum measured $\delta^{13}C$ signatures of OM ($\delta^{13}C$ OM) and pore water CH_4 ($\delta^{13}CH_4$). The black lines indicates when the measured fractionation factor is used, and the grey areas indicate the range given by using the extreme values from literature (3‰, upper range and 60‰, lower range).

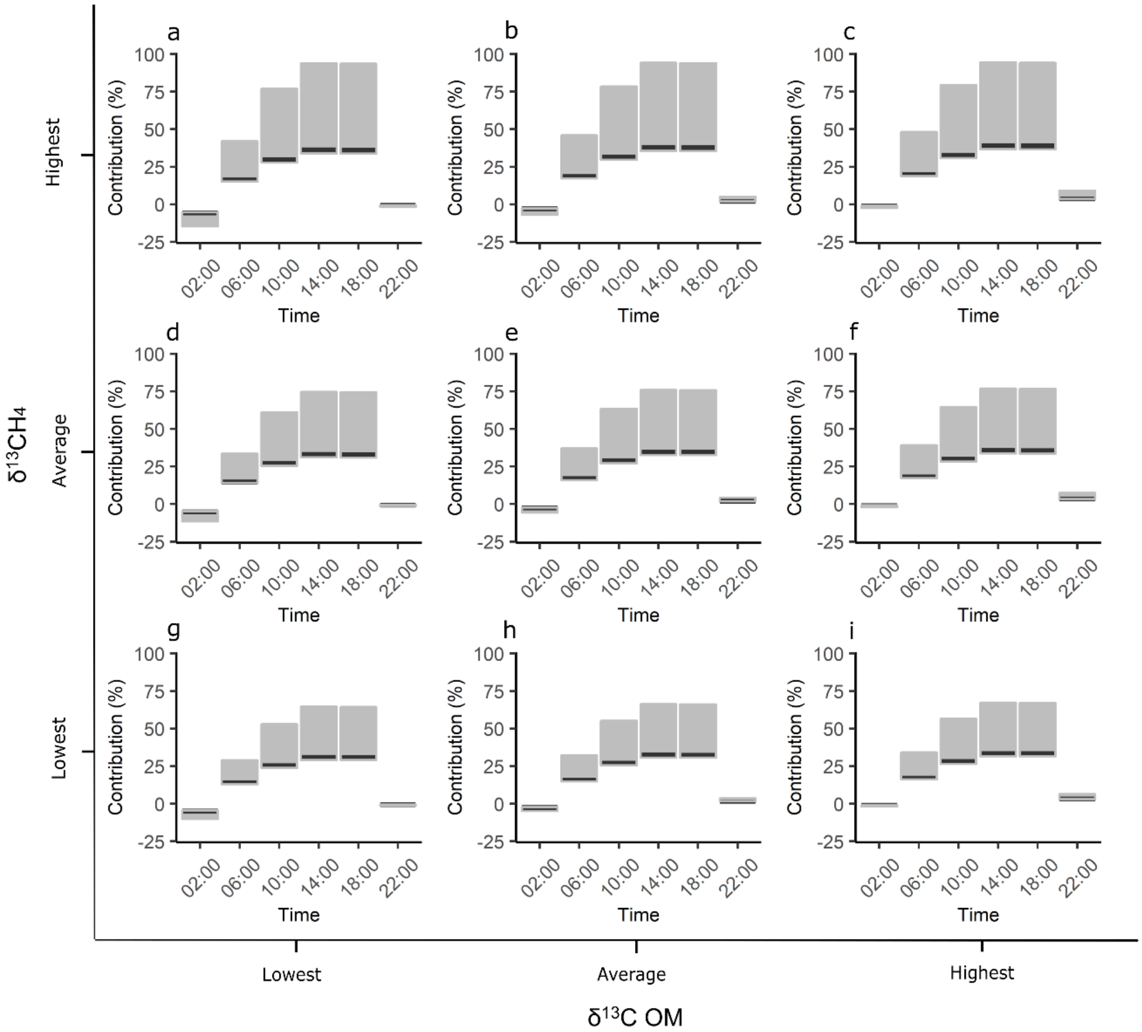

Figure S8 4-hourly averages of the contribution of CH_4 oxidation to total R_h CO_2 fluxes for the period 18 to 27 July 2014 based on mixing models using minimum, average, and maximum measured $\delta^{13}C$ signatures of OM ($\delta^{13}C$ OM) and pore water CH_4 ($\delta^{13}CH_4$). The black lines indicate when the measured fractionation factor is used, and the grey areas indicate the range given by using the extreme values from literature (3 ‰, upper range and 60 ‰, lower range).

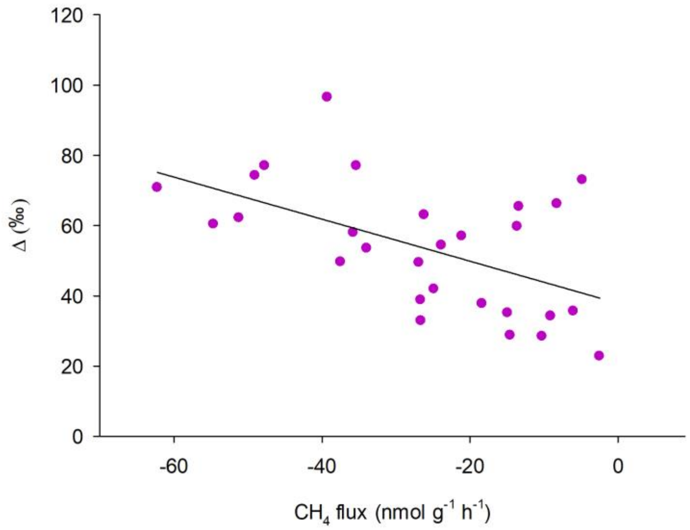

Figure S9 Permil fractionation factors (Δ) from the incubation experiment plotted against the corresponding CH_4 fluxes. The line shows linear regression which had R^2=0.29 and $p < 0.0001$.

Table S1 Averages of contribution from CH_4 oxidation to heterotrophic respiration at Degerö Stormyr for the measurement period 18 to 27 July 2014 as influenced by the isotopic signatures of peat C ($\delta^{13}C$ OM) and pore water CH_4 ($\delta^{13}CH_4$) and the fractionation factor used in the two-source mixing model. The ranges in $\delta^{13}C$ OM and $\delta^{13}CH_4$ represent minimum, average and maximum measured values. The range in fractionation factors represent minimum and maximum values from literature as well as the average measured value for the mire.

Contribution from CH_4 oxidation to heterotrophic respiration (%)				
$\delta^{13}CH_4$	**Fractionation factor**	**$\delta^{13}C$ OM**		
		Low (-29.7 ‰)	Average (-27.4 ‰)	High (-25.9 ‰)
Low (-73.5 ‰)	Low (3 ‰)	35.4	38.4	40.3
	Measured (54.0 ‰)	16.9	18.9	20.1
	High (60 ‰)	16.0	17.8	18.9
Average (-67.2 ‰)	Low 3 ‰)	40.9	44.1	46.0
	Measured (54.0 ‰)	18.1	**20.1**	21.4
	High (60 ‰)	17.0	18.9	20.1
High (-58.9 ‰)	Low (3 ‰)	51.5	54.7	56.6
	Measured (54.0 ‰)	19.9	22.1	23.4
	High (60 ‰)	18.6	20.6	21.9

References:

1. Zhang, G.; Yu, H.; Fan, X.; Ma, J.; Xu, H. Carbon isotope fractionation reveals distinct process of CH_4 emission from different compartments of paddy ecosystem. *Sci. Rep.* **2016**, *6*, 27065, doi:10.1038/srep27065.
2. Happell, J.D.; Chanton, J.P.; Showers, W.S. The Influence of Methane Oxidation on the Stable Isotopic Composition of Methane Emitted from Florida Swamp Forests. *Geochim. Cosmochim. Acta* **1994**, *58*, 4377-4388, doi: 10.1016/0016-7037(94)90341-7.
3. Cadieux, S.B.; White, J.R.; Sauer, P.E.; Peng, Y.B.; Goldman, A.E.; Pratt, L.M. Large fractionations of C and H isotopes related to methane oxidation in Arctic lakes. *Geochim. Cosmochim. Acta* **2016**, *187*, 141-155, doi:10.1016/j.gca.2016.05.004.