- 1 Supplementary Material: Lake mixing regime selects methane-
- 2 oxidation kinetics of the methanotroph assemblage

3 Dependency of the steady-state methane concentration in the epilimnion 4 on the half-saturation constant

5 We consider a well-mixed epilimnion that contains a microbial species B in μ M C 6 that grows on a single carbon substrate S in μ M C according to the Monod growth 7 kinetics. Furthermore, we assume that the substrate is released from the sediment 8 of the lake and that the concentration gradient from the sediment to the epilimnion 9 has reached a steady state. In this case, we have a constant volumetric flux F_S in μ M 10 C d⁻¹ into the epilimnion.

Without microbial growth, exchange with the atmosphere and substrateconsumption, the change of the substrate concentration in the epilimnion is simply

$$\frac{dS}{dt} = F_S \tag{1}$$

13 According to the Monod kinetics, the growth rate r in d⁻¹ of the microbial species is:

$$r = V_{max} \frac{S}{S + K_M} \tag{2}$$

14 where V_{max} is the maximum growth rate in d⁻¹ and K_M the half-saturation constant 15 in μ M.

16 Considering a mortality rate m in d⁻¹ and a substrate use efficiency y, the temporal 17 change of the bacterial species is:

$$\frac{dB}{dt} = (y \cdot r - m)B \tag{3}$$

18 Due to the growth of the bacterial species, the substrate concentration in the well-19 mixed epilimnion decreases with a rate of -rB and the complete system of 20 differential equations is:

$$\frac{dS}{dt} = F_S - rB$$

$$\frac{dB}{dt} = (y \cdot r - m)B$$
(4)

21 Steady-state consideration 1

A complete steady state is reached if both the concentration of the substrate as wellas the biomass of the microbial species remain constant over time:

$$\frac{dS}{dt} = 0 = F_S - rB$$

$$\frac{dB}{dt} = 0 = (y \cdot r - m)B$$
(5)

By rearranging the second equation, we get an equation for the equilibriumsubstrate concentration:

$$S^{eq} = \frac{K_M}{y \cdot V_{max}/m - 1} \tag{6}$$

According to equation 6, the equilibrium substrate concentration is indeed a function of the half-saturation constant K_M . However, the equilibrium concentration is not necessarily equal to the half-saturation constant. Depending on the ratio $y \cdot V_{max}/m$ the equilibrium concentration can be lower or higher than the halfsaturation constant.

The equilibrium concentration is smaller than the half-saturation constant for $y \cdot V_{max} > 2m$ and is higher than the half-saturation for $m < y \cdot V_{max} < 2m$. Because the maximum growth rate is likely higher than twice the mortality rate, the equilibrium concentration is smaller than the half-saturation constant.

35 Estimated equilibrium concentration in the epilimnion of Lake Rotsee: In the 36 epilimnion, we measured an average K_M of 1.8 μ M. Based on literature values, we 37 can assume a substrate use efficiency of 0.4 (Leak & Dalton, 1986) and a mortality of 38 0.022 day⁻¹ (Roslev & King, 1995). In the epilimnion, we measured an average 39 maximum methane oxidation rate of about 4 fmole h⁻¹ cell⁻¹. Assuming a cellular 40 carbon content of 0.42 pmole C cell⁻¹ (Oswald et al., 2015; Posch et al., 2001; 41 Romanova & Sazhin, 2010) this converts to a maximum methane oxidation rate of 42 0.2 day⁻¹.

43 With this, we would expect an equilibrium methane concentration of:

$$S^{eq} = \frac{1.8 \,\mu\text{M}}{0.4 \cdot 0.2 \,\text{day}^{-1}/0.022 \,\text{day}^{-1} - 1} = 0.7 \,\mu\text{M}$$
(7)

The highest measured maximum methane oxidation rate was 8.4 fmole h^{-1} cell⁻¹, which converts to a methane oxidation rate of 0.48 day⁻¹. This higher rate woul result in an equilibrium concentration of about 0.2 μ M.

These estimates should be treated with caution because all the values used for the calculation (in particular mortality and cellular carbon content) are fraught with considerable uncertainty. Nonetheless, it demonstrates that the expected methane concentration is lower than the half-saturation constant K_M .

51 Steady-state consideration 2

In an alternative approach we assume steady-state for the substrate concentrationbut not for the biomass:

$$\frac{dS}{dt} = 0 = F_S - rB$$

$$\frac{dB}{dt} = (y \cdot r - m)B$$
(8)

54 By rearranging the first equation, we get an equilibrium substrate concentration of:

$$S^{eq} = \frac{F_S \cdot K_M}{B \cdot V_{max} - F_S} \tag{9}$$

55 Estimated equilibrium concentration in the epilimnion of Lake Rotsee: In the epilimnion, we measured an average K_M of 1.8 μ M. With a cellular carbon content of 56 0.42 pmole C cell⁻¹ (Oswald et al., 2015; Posch et al., 2001; Romanova & Sazhin, 57 2010) and average cell numbers of $0.1 \times 10^5 - 2.5 \times 10^5$ cells mL⁻¹, we estimate average 58 biomass of 4 – 105 μ M. In the epilimnion we measured an average maximum 59 methane oxidation rate of about 4 fmole h⁻¹ cell⁻¹. Using the above cellular carbon 60 61 content, this converts to a maximum methane oxidation rate of 0.2 day⁻¹. Based on model work (Zimmermann et al., 2019), the median volumetric flux of methane into 62 63 the epilimnion is 5 μ M day⁻¹.

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65 Inserting these values into equation 9 results in an equilibrium methane 66 concentration of $0.6 - 1.5 \mu$ M, depending on the amount of biomass (lower 67 equilibrium concentrations with higher biomass). Again, the expected methane 68 concentration tends to be lower than the half-saturation constant K_M .

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