## **1** Supplementary Information

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## 3 High peatland methane emissions following permafrost thaw: enhanced acetoclastic 4 methanogenesis during early successional stages Liam Heffernan<sup>1,2\*</sup>, Maria A, Cavaco<sup>3\*</sup>, Maya P. Bhatia<sup>3</sup>, Cristian Estop-Aragonés<sup>4</sup>, Klaus-5 Holger Knorr<sup>4</sup>, David Olefeldt<sup>1</sup> 6 7 <sup>1</sup> Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2H1, 8 Canada.<sup>2</sup> Evolutionary Biology Centre, Department of Ecology and Genetics/Limnology, 9 Uppsala University, Norbyvägen 18D, 752 36, Uppsala, Sweden.<sup>3</sup> Department of Earth and 10 11 Atmospheric Sciences, University of Alberta, Edmonton, AB T6G 2H1, Canada.<sup>4</sup> Institute of 12 Landscape Ecology, Ecohydrology and Biogeochemistry Group, University of Münster, 13 Münster, Germany 14 \*Corresponding and co-first authors: Liam Heffernan (liam.heffernan@ebc.uu.se) and Maria

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Figure S1. (a) Averaged seasonal (May – September, individual months in lighter shades) depth profile of soil temperature in the young (green) and mature (yellow) bog. Green and yellow horizontal lines represent thaw depth, or transition between peat that accumulated before and after permafrost thaw, in the young (YB; ~30 cm) and mature bog (MB; ~70 cm) respectively. (b) Positive apparent fractionation factor ( $\alpha_c$ ) response to soil temperature for data pooled for shallow peat that accumulated post-thaw and deep peat that accumulated prethaw for the young and mature bog.

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Figure S2. Archaeal community composition throughout all stages of peat or pore water sampled per depth. Highest taxonomic (phylum) level down to lowest taxonomic level (genus) is shown on the y axis for all archaeal organisms. Phylum is shown in the largest font, with ensuing classes shown in bold. Lowest taxonomic assignment is presented down to the genus, shown in italics. Depth at which samples were obtained are shown on the x axis, with each panel demonstrating the relative abundance of archaeal members at each stage of peat or pore water. The black

- 45 colour denotes putatively acetoclastic methanogens, while purple denotes putatively hydrogenotrophic methanogens and blue represents non-
- 46 methanogenic taxa.



Figure S3. Seasonal surface (a) CH<sub>4</sub> emissions , (b) CO<sub>2</sub> emissions as ecosystem respiration, (c) ratio between CO<sub>2</sub> emissions (as ecosystem respiration) and CH<sub>4</sub>, for the young bog and mature bog. (a) and (b) CH<sub>4</sub> and CO<sub>2</sub> land-atmosphere fluxes were measured once a month from May – September 2018. (c) Ratio of CH<sub>4</sub>:CO<sub>2</sub> emissions demonstrates relative importance of CH<sub>4</sub> for overall C emissions and is calculated as CH<sub>4</sub>/(CH<sub>4</sub> + CO<sub>2</sub>). Both CH<sub>4</sub> and CO<sub>2</sub> fluxes were standardized to per g C to calculate this ratio.



Figure S4. Keeling plots to determine the  $\delta^{13}$ C-CH<sub>4</sub> signature of CH<sub>4</sub> fluxes from the (a) young bog and (b) mature bog collected in September and October 2016. The intercept from Keeling plots is used to determine the  $\delta^{13}$ C-CH<sub>4</sub> signature of CH<sub>4</sub> fluxes. Each collar was measured twice (A and B) in September and October 2016. (a) September A: intercept = -66.13,  $R^2 = 0.36$ , September B: intercept = -64.94,  $R^2 = 0.43$ , October A: intercept = -66.43,  $R^2 = 0.98$ , October B: intercept = -68.42,  $R^2 = 0.94$ . (b) September A: intercept = -73.59,  $R^2 = 0.48$ , September B: intercept = -77.12,  $R^2 = 0.15$ , October A: intercept = -86.57,  $R^2 = 0.88$ , October B: intercept = -76.46,  $R^2 = 0.32$ .