

Title: Niche partitioning of methane-oxidizing bacteria along the
oxygen-methane counter gradient of stratified lakes

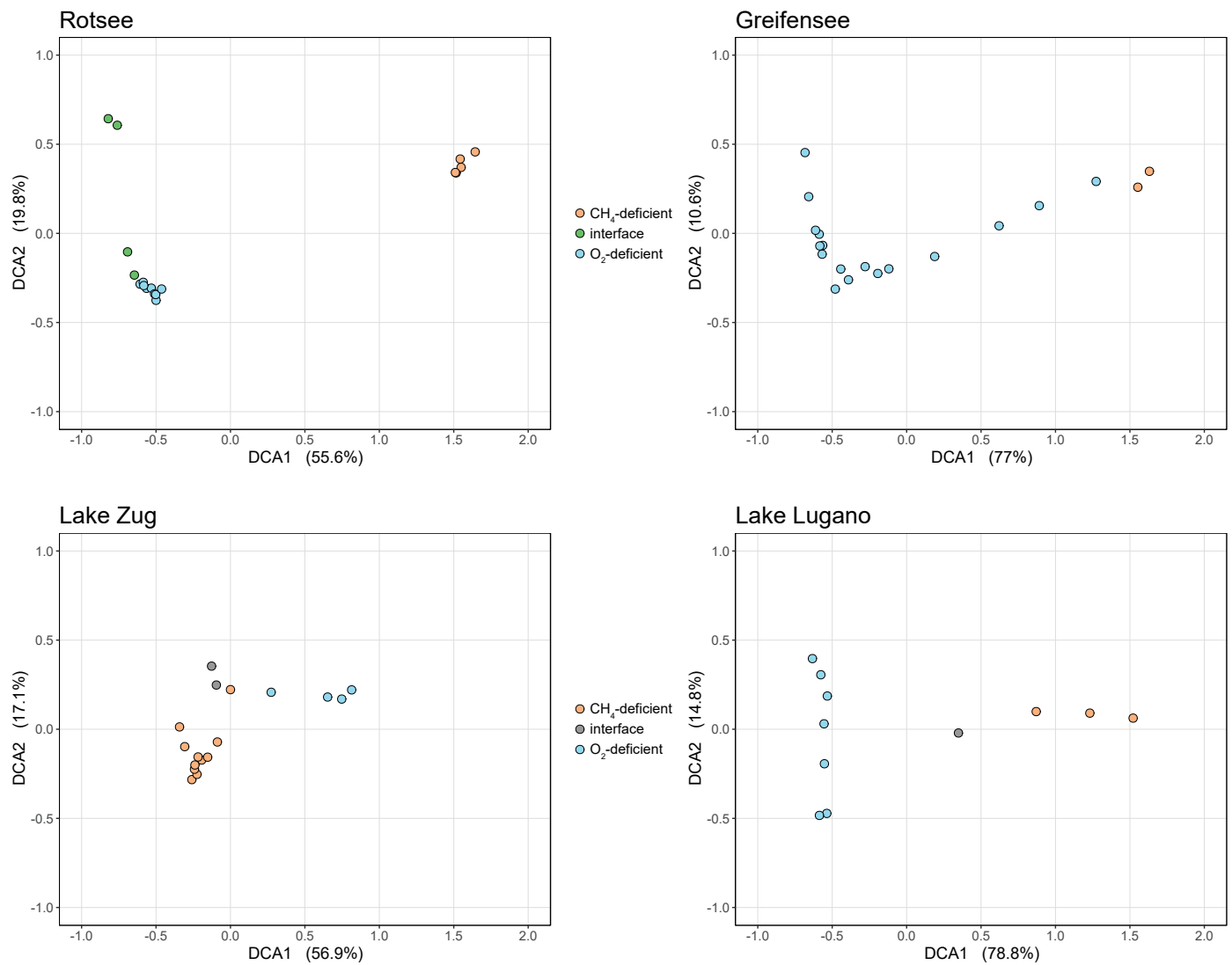
Running title: Methanotrophs in the oxygen-methane counter gradient

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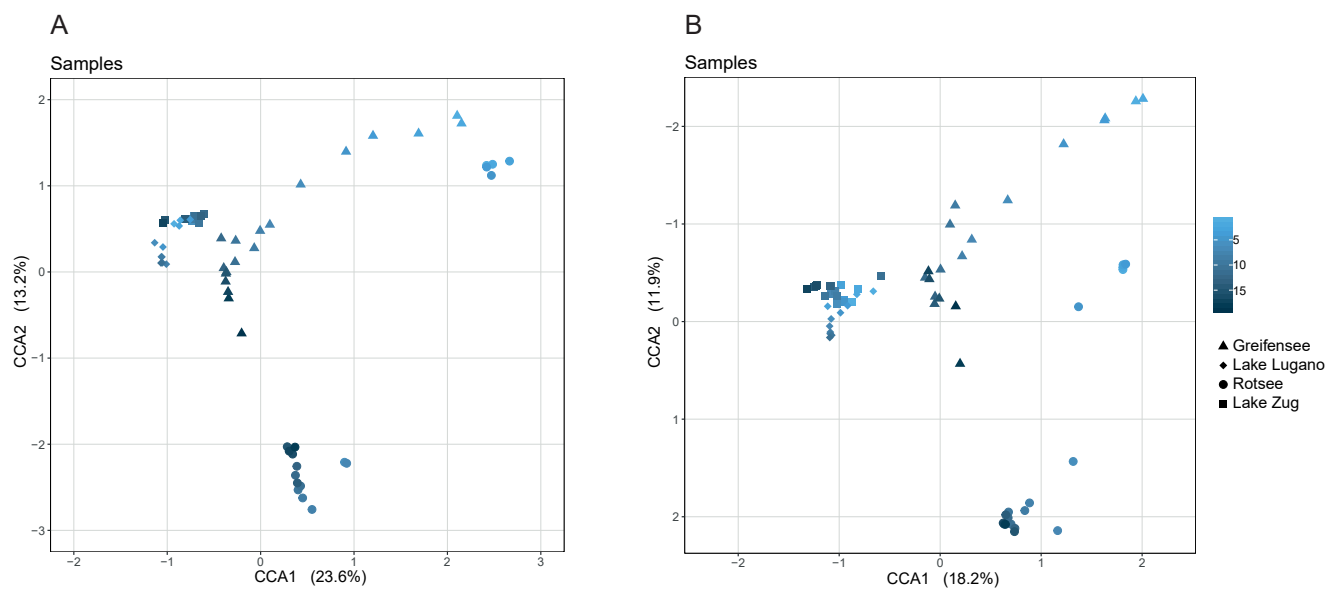
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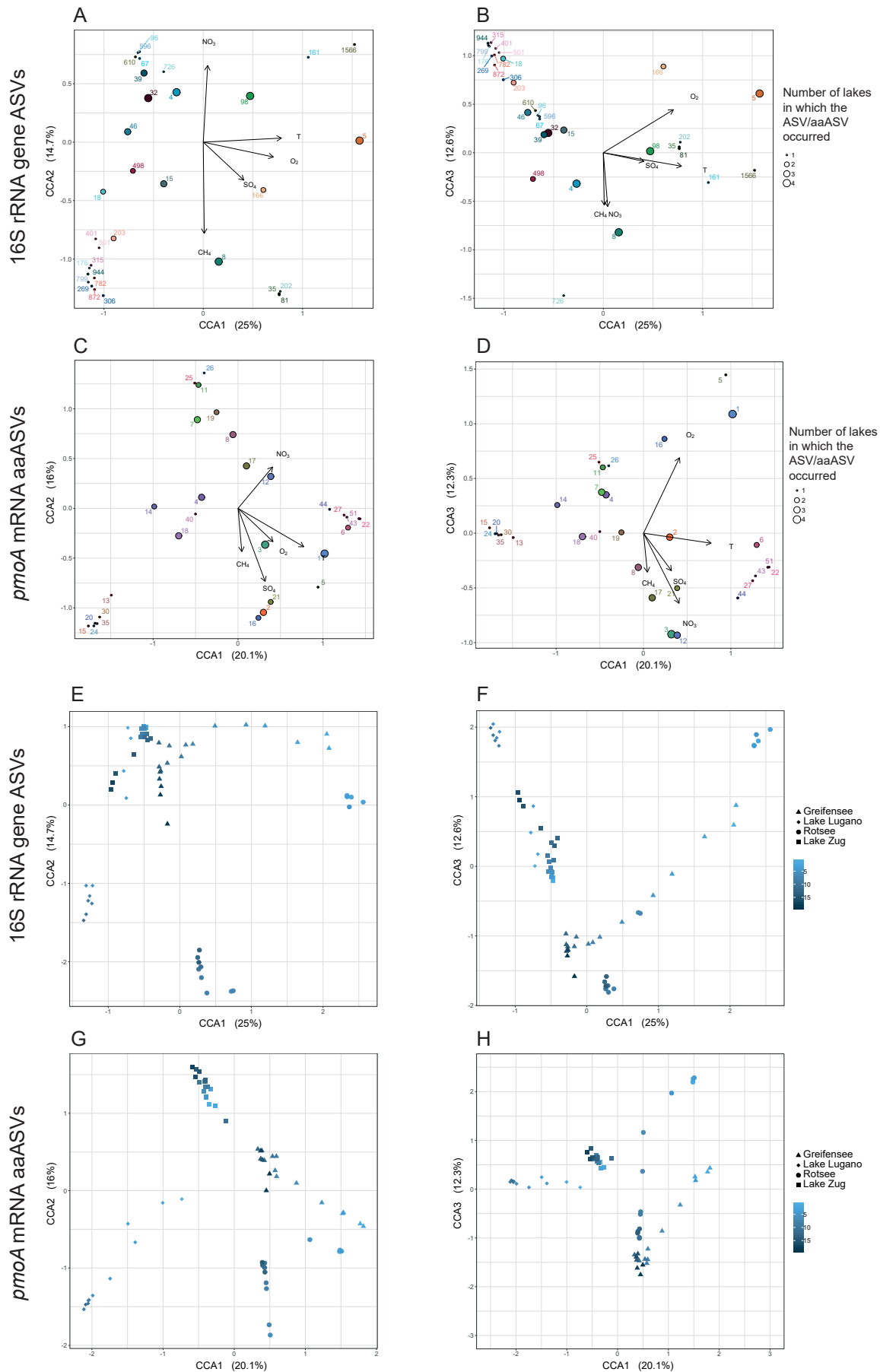
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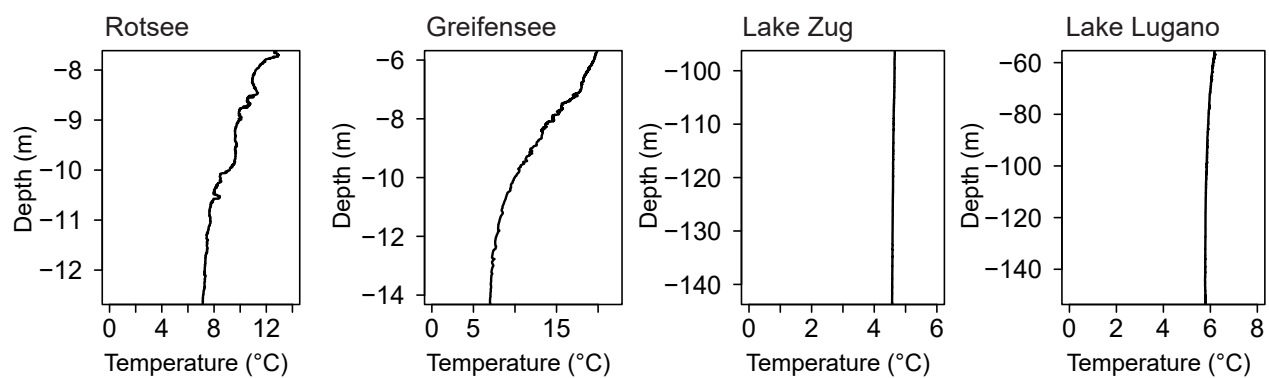
Supplementary Figure S1 Detrended correspondence analysis (DCA) calculated based on a Bray-Curtis dissimilarity matrix using relative abundances of MOB 16S rRNA gene ASVs compared to all MOB ASVs (sample sum=1). Samples are shown separately for each lake and were colored according to their corresponding niche (see Figure 1 and 2), which are based on oxygen and methane availability. Please note that in Greifensee only methane-deficient and oxygen-deficient were captured.



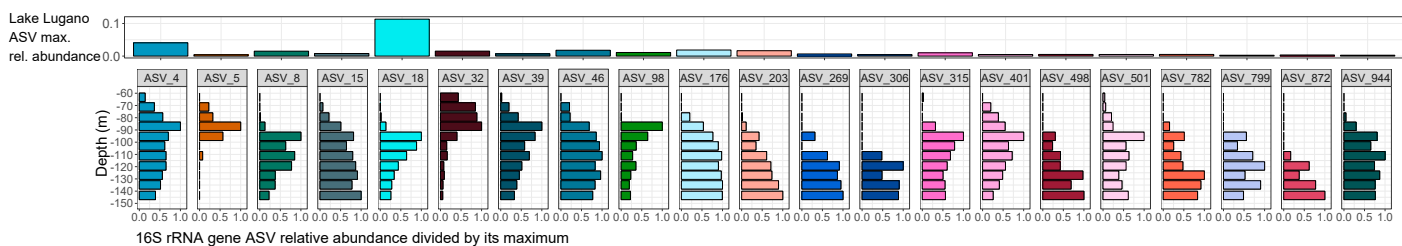
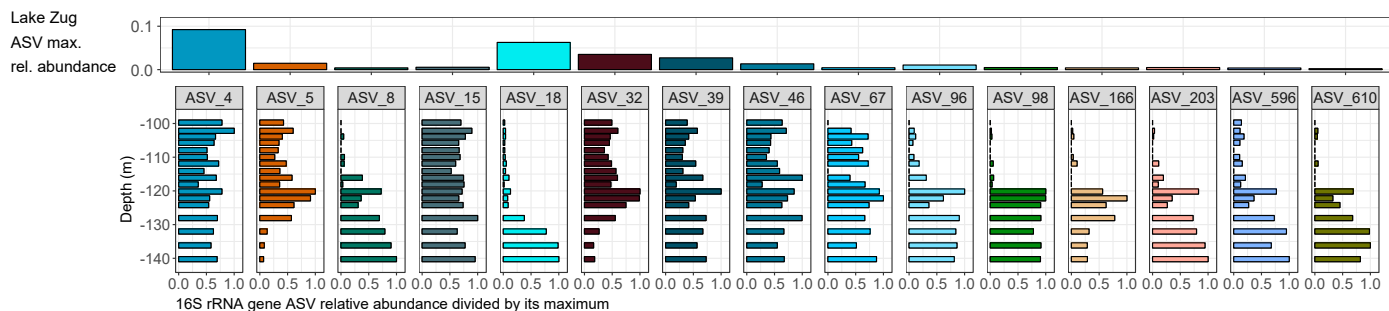
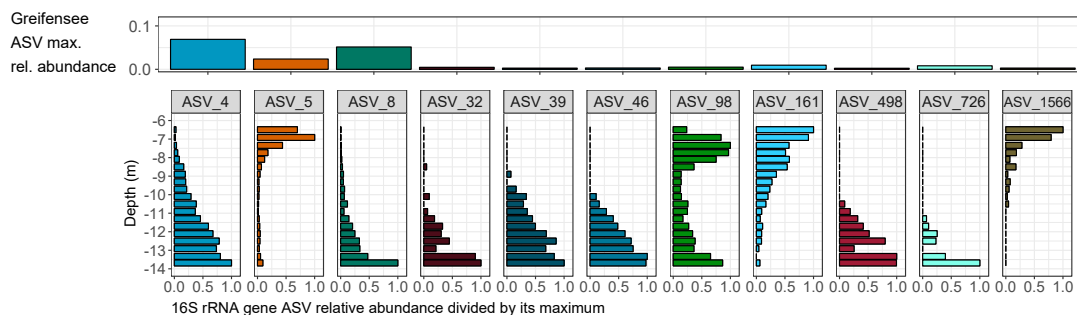
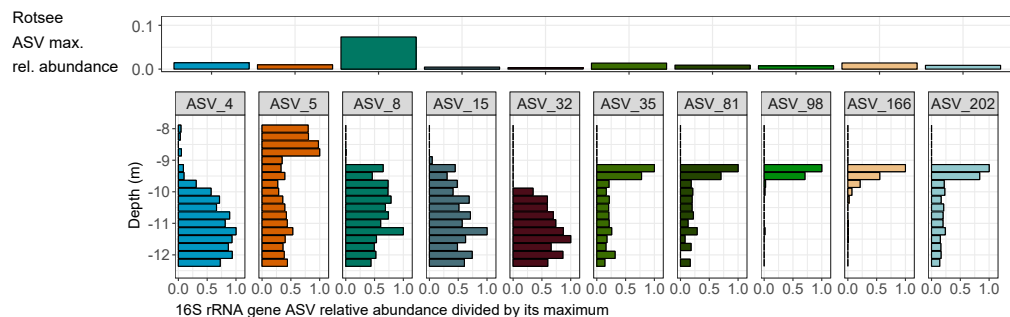
Supplementary Figure S2 Canonical correspondence analysis (CCA) calculated based on a Chi-square dissimilarity matrix using relative abundances compared to all MOB sequences in a sample (sample sum=1) and standardized physicochemical parameters (T=Temperature). **A)** samples scores of 16S rRNA gene ASVs **B)** samples scores of *pmoA* mRNA aaASVs. The color gradient corresponds to ranked depths of each lake, from the shallowest (light blue) to the deepest (dark blue) depth.



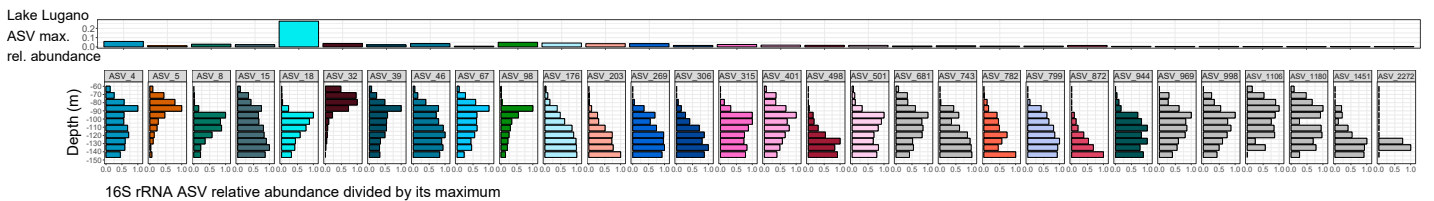
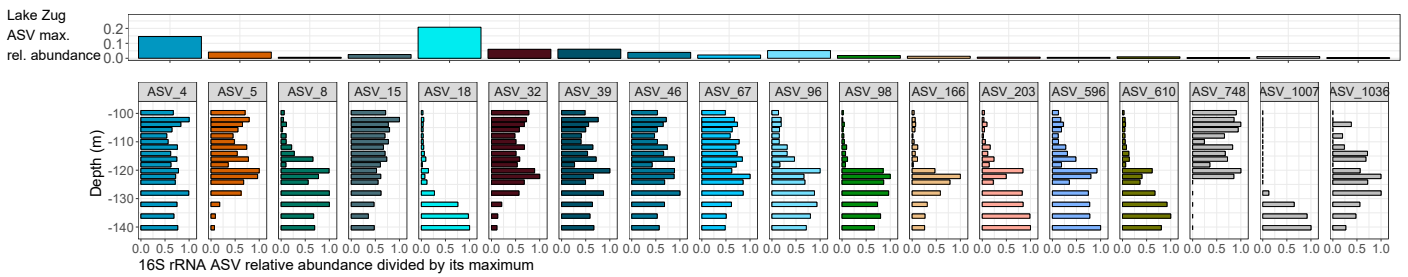
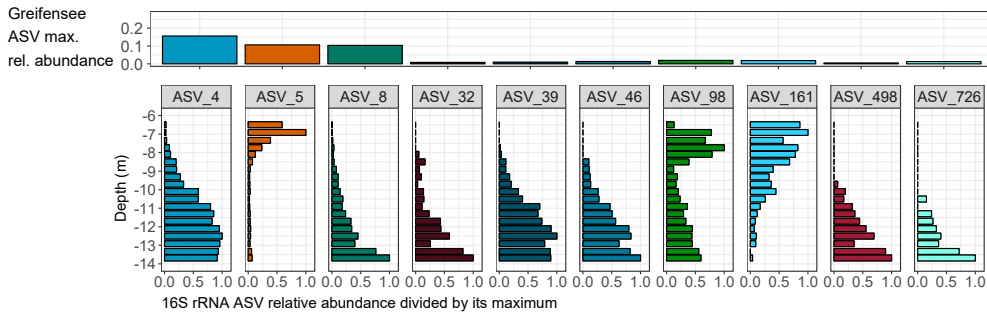
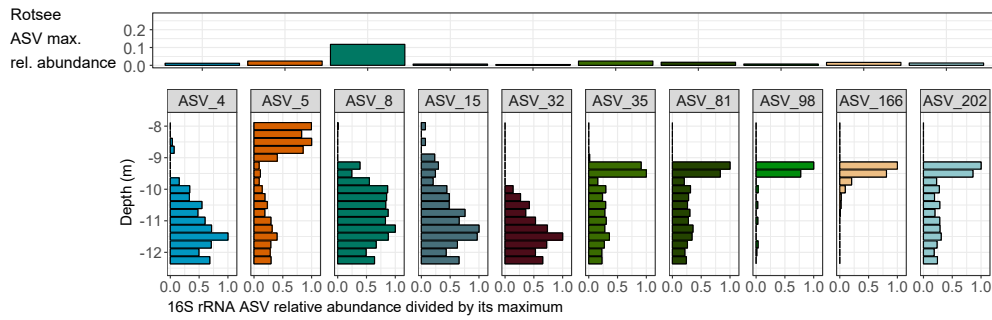
Supplementary Figure S3 Canonical correspondence analysis (CCA) calculated based on a Chi-square dissimilarity matrix using relative abundances compared to all MOB sequences in a sample (sample sum=1) and selected physicochemical variables (T=temperature). The first three axes are shown. Taxa scores of 16S rRNA gene ASVs are shown in A and B, corresponding sample scores in E and F. Taxa scores of *pmoA* mRNA aaASVs are shown in C and D and corresponding sample scores in G and H. A-D colors are taxon specific and the dot size shows the number lakes in which the the ASV/aaASV occurred. E-H the color gradient corresponds to ranked depths of each lake form the shallowest (light blue) to the deepest (dark blue) depth.



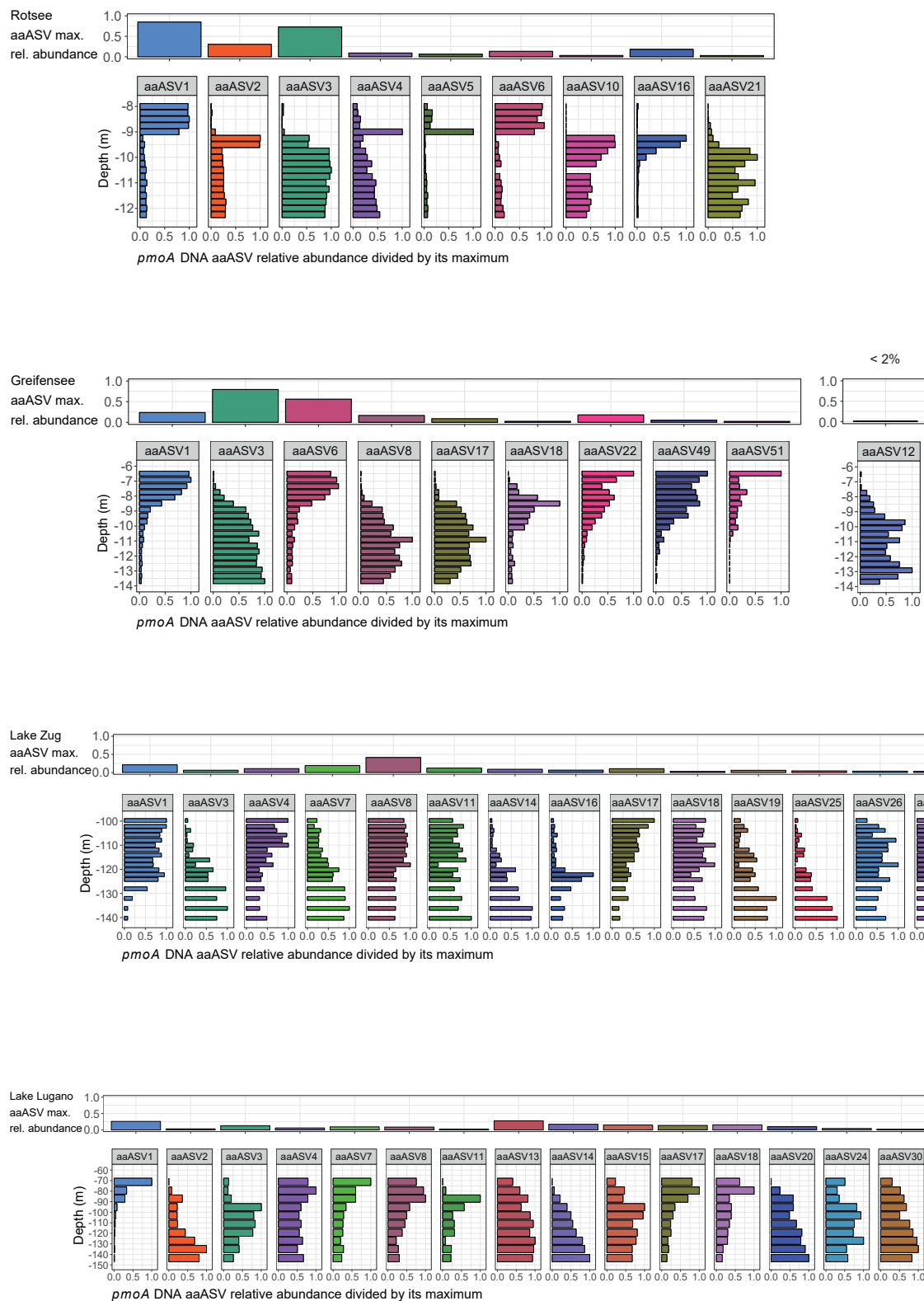
Supplementary Figure S4 Vertical profiles of temperature in Rotsee, Greifensee, Lake Zug and Lake Lugano across the oxygen-methane counter gradient.



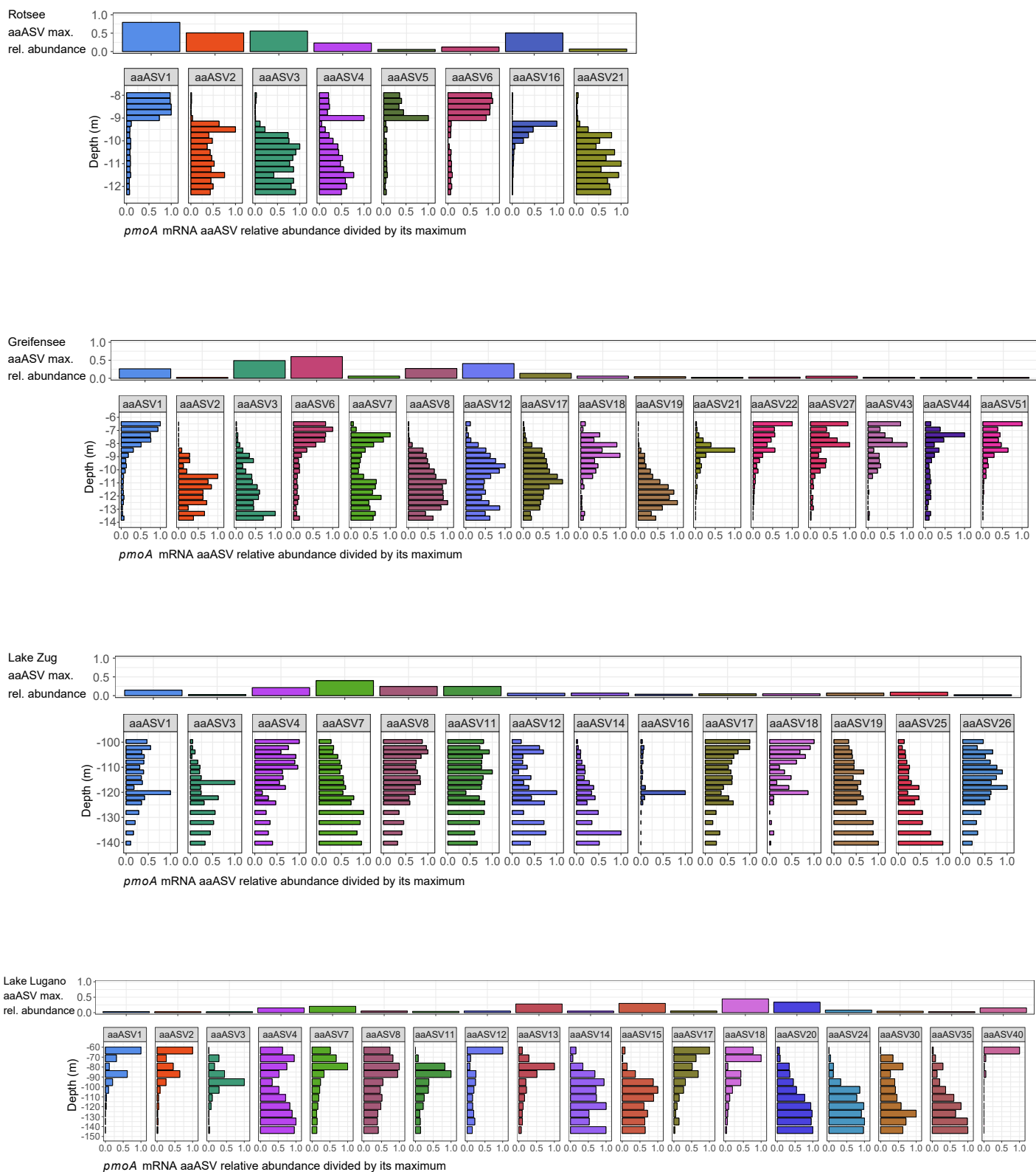
Supplementary Figure S5 Vertical distribution patterns of MOB 16S rRNA gene ASVs in Rotsee, Greifensee, Lake Zug and Lake Lugano along the oxygen-methane counter gradient. On top the maximum relative abundance compared to all bacterial 16S rRNA gene sequences for each ASV is shown. Colors are ASV specific. ASVs are sorted according to their numbering.



Supplementary Figure S6 Vertical distribution patterns of MOB 16S rRNA ASVs in Rotsee, Greifensee, Lake Zug and Lake Lugano along the oxygen-methane counter gradient. On top the maximum relative abundance compared to all bacterial 16S rRNA sequences for each ASV is shown. Colors are ASV specific. ASVs in grey do not have a corresponding 16S rRNA gene ASV with applied filtering thresholds. ASVs are sorted according to their numbering.



Supplementary Figure S7 Vertical distribution patterns of *pmoA* DNA aaASVs in Rotsee, Greifensee, Lake Zug and Lake Lugano along the oxygen-methane counter gradient. On top the maximum relative abundance compared to all *pmoA* DNA sequences for each aaASV is shown. Colors are aaASV specific. Note that *pmoA* DNA from the shallowest depth (63 m) of Lake Lugano could not be amplified. aaASVs are sorted according to their numbering.



Supplementary Figure S8 Vertical distribution patterns of *pmoA* mRNA aaASVs in Rotsee, Greifensee, Lake Zug and Lake Lugano along the oxygen-methane counter gradient. On top the maximum relative abundance compared to all *pmoA* mRNA sequences for each aaASV is shown. Colors are aaASV specific. aaASVs are sorted according to their numbering.