Bacterial community structure in a sympagic habitat expanding with global warming: brackish ice brine at 85–90 °N by Fernández-Gómez et al. (The ISME Journal 2018)
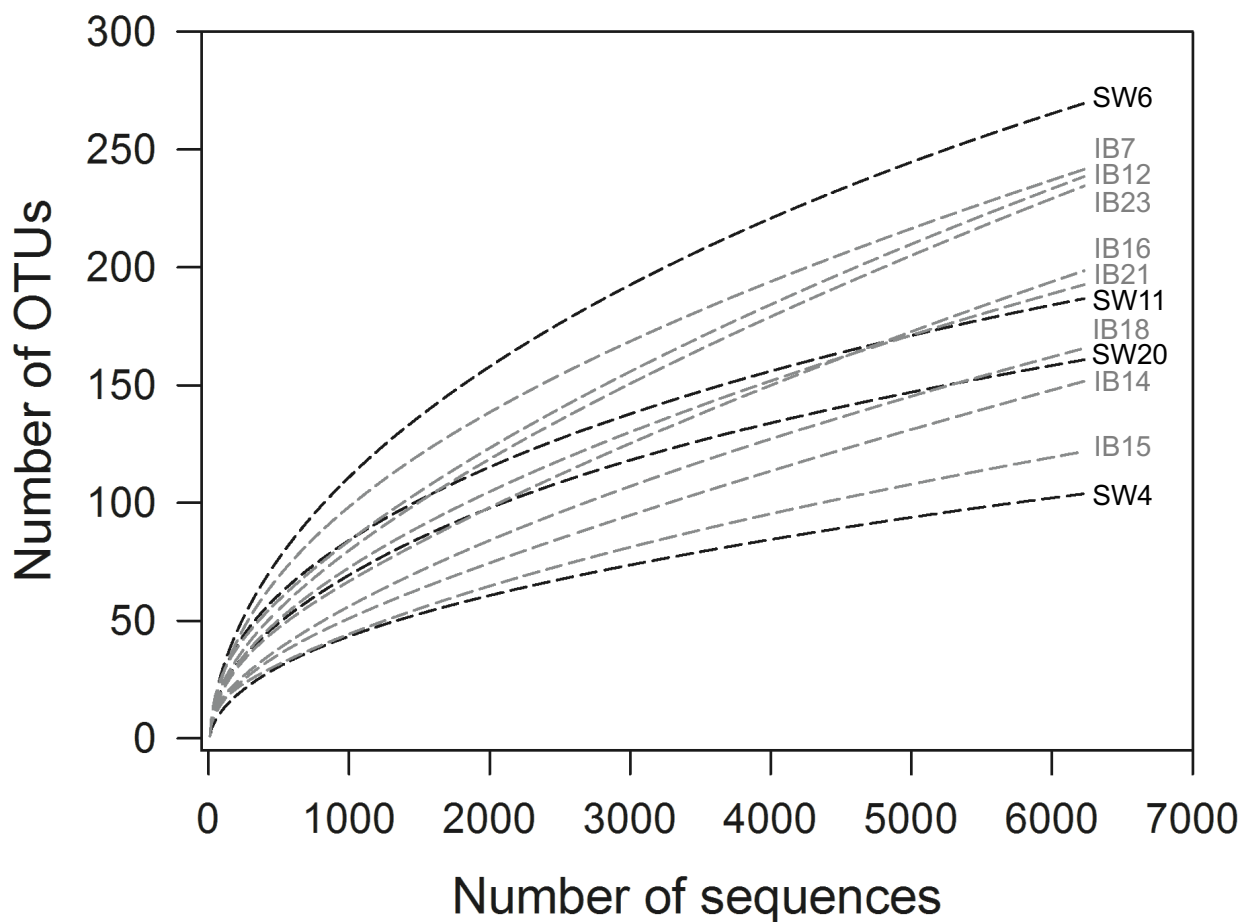
Supplementary Figures S1-S4

Figure S1. Rarefaction curves for the 12 samples from the Central Arctic Ocean. Grey curves represent the eight ice-brine samples (with prefix IB followed by sampling station number) and black curves represent the four immediate sub-ice seawater samples (with prefix SW followed by sampling station number).
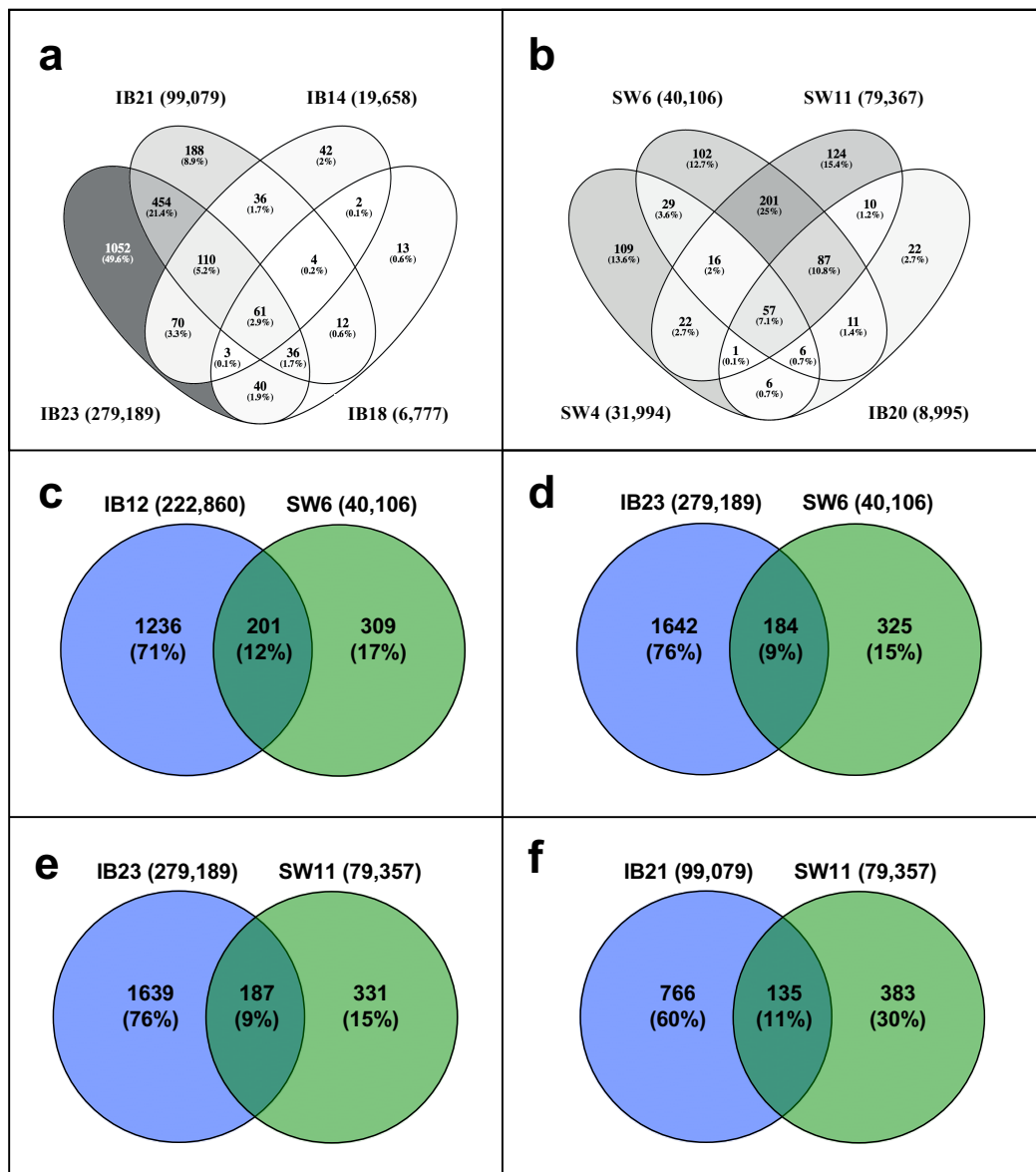
Figure S2. Series of Venn diagrams showing comparisons between samples to assess if OTU richness differs between ice brine (IB) and immediate sub-ice seawater (SW). (**a**) Comparison of four IB samples with different numbers of reads. (**b**) Comparison of four SW samples with different numbers of reads, (**c**) Comparison between samples IB12 and SW6 with different numbers of reads. (**d**) Comparison between samples IB23 and SW6 with different numbers of reads. (**e**) Comparison between samples IB2 and SW11 with different numbers of reads. (**f**) Comparison between samples IB21 and SW11with comparable numbers of reads. The number of reads is given in brackets after the sample number according to **Table S2**. Comparing four IB samples that yielded different numbers of sequences: IB18 (6777 reads), IB14 (19 658), IB21 (99 079) and IB23 (279 189) shows that the higher the number of sequences yielded, the more OTUs were recovered (Figure S2a). A similar comparison for the four SW samples: SW20 (8995), SW4 (31 994), SW6 (40 106) and SW11 (79 357) shows the same but the proportion of OTUs in the deepest sequenced sample is lower (IB 50%, SW 14%) (Figure S2b). This suggests that higher diversity is recovered in IB with an increasing number of reads than in SW. However, this is not convincing because the highest number of reads differs between the two habitats: 279 189 reads (IB23) are 3.5 times more than 79 357 reads (SW11). The next step is to assess the extent of recovery of OTUs from IB and SW samples and when increasing the number of reads in IB samples by 25% (from 222 860 to 279 189) the number of OTUs increases by 33% (from 1236 to 1642) but when increasing the number of reads in SW samples by 100% (from 40 106 to 79 357) the number of OTUs remains ~320 (Figure S2c-e). This result corroborates that of the rarefaction curves with most of the SW curves flattening earlier than the IB curves (Figure S1). A last comparison between samples with a similar number of reads, IB21 (99 079 reads, 766 OTUs) and SW11 (79 357 reads, 383 OTUs), again shows that IB was inhabited by more OTUs than SW relative to the number of reads (Figure S2f).
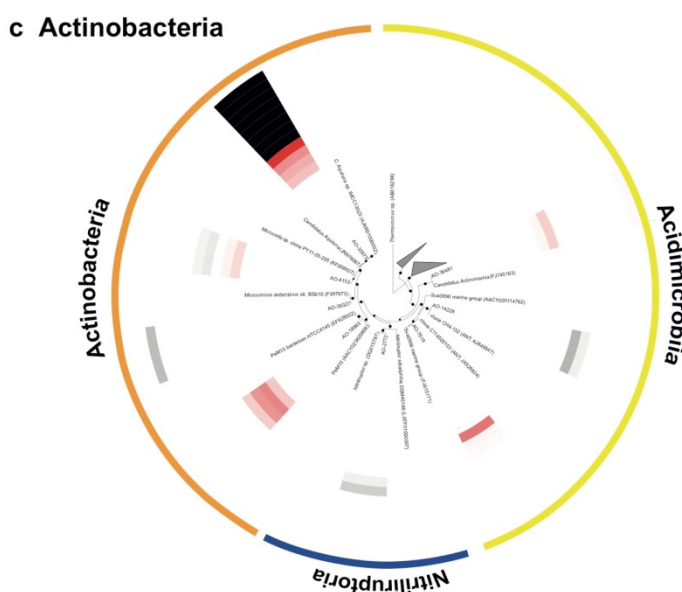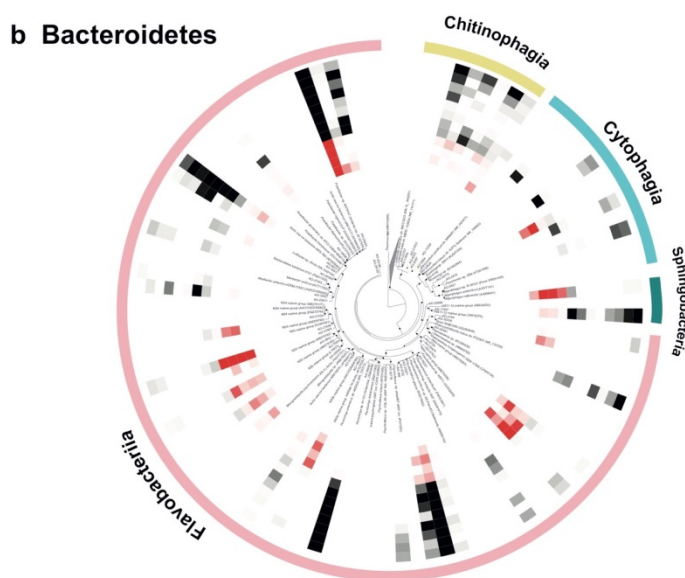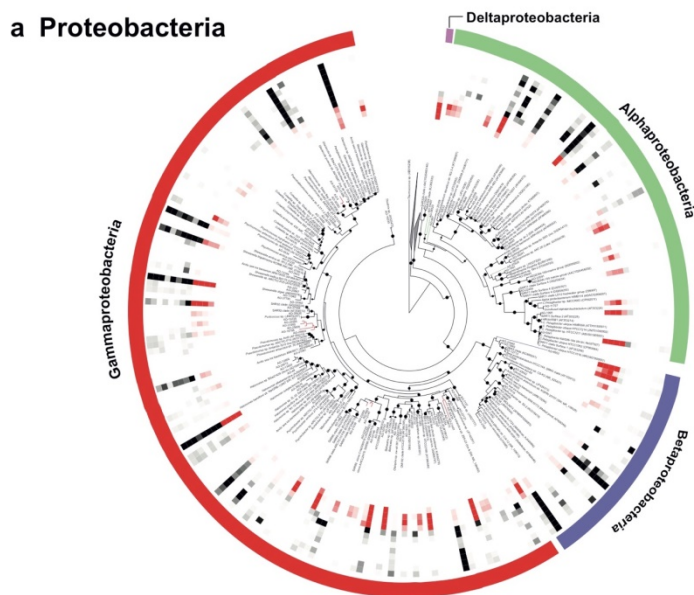
Figure S3. Phylogenetic trees based on OTUs with relative abundance ≥0.01% across all samples for each of the phyla Proteobacteria, Bacteroidetes and Actinobacteria. (a) Proteobacteria (109 OTUs). **(b)** Bacteroidetes (44 OTUs). **(c)** Actinobacteria (8 OTUs). The outer coloured ring denotes taxonomy (classes). The relative abundances of the OTUs per sampling station are indicated in shades of black for ice brine (IB) and in shades of red for immediate sub-ice SW with the sampling station order (from inside to outside the circle): SW4, SW6, SW11, SW20, IB7, IB12, IB14, IB15, IB16, IB18, IB21, IB23. Bootstrap values (ranging from 0.8 to 1.0) are indicated by dot size. Branches for expected new OTUs are marked with the colour of their class. Unknown clades are branch-coloured according to the colour of their phylum.
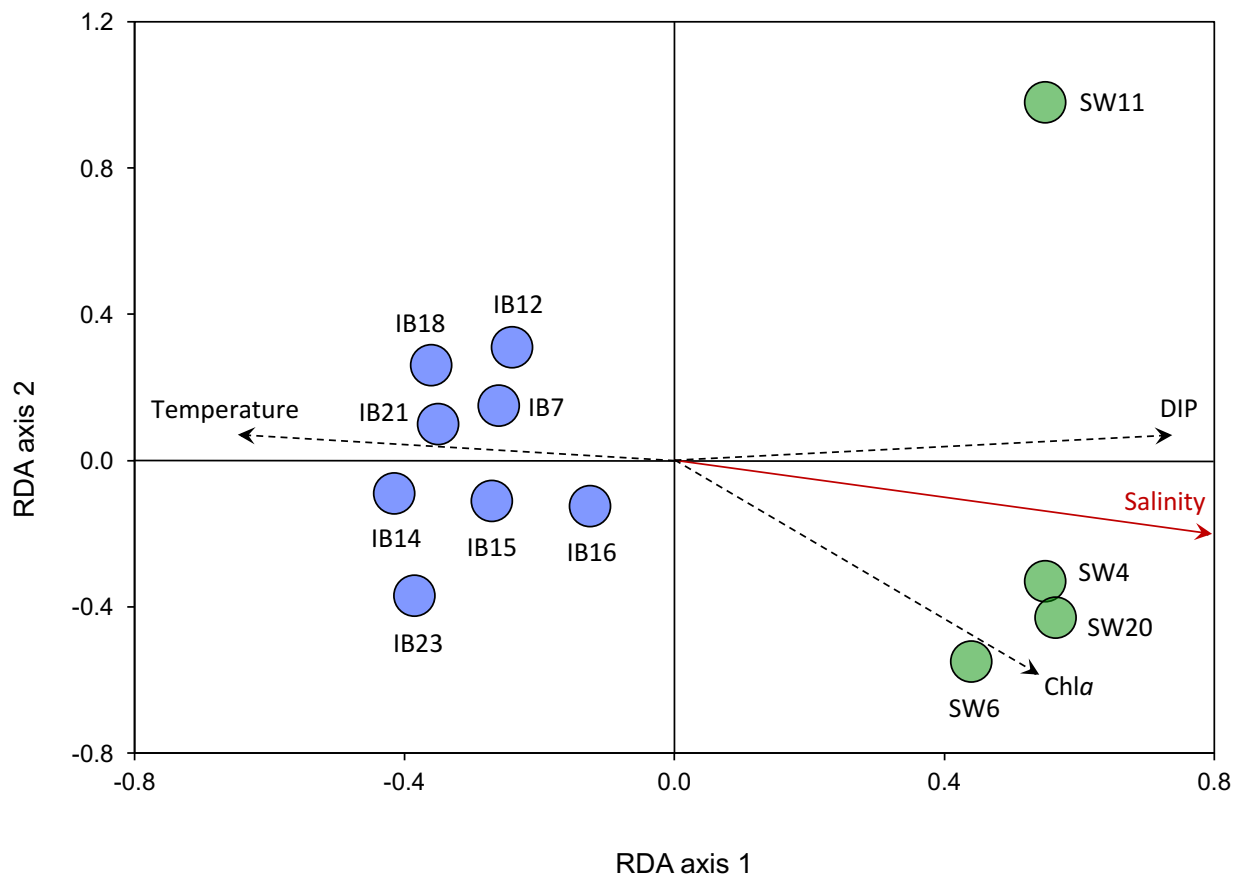
Figure S4. Redundancy Analysis (RDA) illustrating the influence of environmental factors in shaping bacterial community composition. The analysis is based on rarefied data of OTU abundance and ten abiotic and biotic variables (salinity, temperature, DIN, DIP, DSi, POC, PON, POP, bacterial density and Chl*a* concentration; **Table S1**). The RDA displayed a significant association (F = 9.18, p = 0.002) between community composition and the ten variables. Salinity was the most important and only statistically significant variable (red arrow) by its own, explaining 37.5% of the variance in community composition. Three additional variables each explained a smaller part of the variance (DIP: 12.5%, Chl*a*: 11.3%, temperature: 3.6%) but they were not statistically significant on their own (dashed arrows). Sampling stations are indicated by centroids (spheres), blue for ice brine (IB) and green for immediate sub-ice seawater (SW), and the four variables that together influenced community composition most are indicated by arrows. The arrow length corresponds to the variance explained by the variable and the arrow direction indicates an increasing rate of change of the variable. The perpendicular distance between the sampling station centroids and the axes in the plot reflects their correlations with the respective significant variables; the smaller the distance the stronger the correlation.