Manuscript ID: EGUSPHERE-2026-3709

Title: Proteobacteria Dominance and Neutral Assembly Processes of Bacterial Communities in the Chukchi Sea, Arctic

We greatly appreciate the reviewer for the thorough, rigorous and constructive comments, which substantially help us improve the scientific robustness, logical rigor and presentation quality of our manuscript. We have carefully addressed every comment, revised the main text, re-plotted figures, updated the supplementary table, and adjusted over-interpretative statements throughout the manuscript. Below is our detailed point-by-point response.

General-1: The classification into the three depth categories (S, M, and B) is not sufficiently clear or oceanographically justified … temperature and salinity should also be presented.

Response

We fully acknowledge this critical issue raised by the reviewer. In our initial version, S/M/B were loosely defined without explicit annotation that they were station-specific operational sampling layers rather than fixed hydrographic water masses, which caused ambiguity in ecological interpretation.

To fix this problem:

1. We have explicitly re-defined S (surface), M (middle), and B (bottom) as *relative sampling layers for each station* in the Methods section, and clearly distinguished them from geochemically defined water masses.
2. We supplemented the full in-situ hydrographic dataset including temperature, salinity, station coordinates, seafloor depth and bathymetric information into the revised Table S1. The complete station metadata table (latitude, longitude, bottom depth, geographic grouping) is provided in the revised supplementary material, as shown in the attached station list.
3. We revised the text and caption of Figure 1 to correct the description of station P2_08;
4. In the Discussion, we interpret bacterial community shifts together with real temperature-salinity profiles rather than only relying on the three simplified sampling groups.

All revisions and new environmental data make our grouping scheme transparent and reproducible for further oceanographic interpretation.

General-2: Given this limited sample set (8 stations with 3 depths each), I am not fully convinced that the stochastic process is identified as the dominant process. Unexplained variation may arise from unmeasured environmental variables, measurement error, or other sources of variation, and therefore cannot by itself be interpreted as evidence of stochasticity.

Response

We thank the reviewer for this valuable methodological reminder. We fully recognize the constraints brought by our sampling design (8 stations × 3 depths). We have substantially softened our conclusion and added explicit limitations in our manuscript:

1. We clearly state that the null-model outputs in this study are preliminary, hypothesis-generating results based on our limited sampling dataset, instead of a universal conclusion for the entire Chukchi Sea.
2. We add a paragraph in Discussion noting that unexplained community variation can originate from unmeasured environmental factors, sampling noise and analytical bias.
3. We modify our main conclusion: we only report that stochastic assembly processes show relatively higher contributions within our observed dataset, rather than claiming absolute

dominance across the whole region.

4. We keep the quantitative value in parentheses and emphasize that these estimated proportions carry large uncertainty with the current sample size.

General-3: The novelty of this work could be better presented in relation to previous work (Pan et al., 2025; Han et al., 2015).

Response

We appreciate this suggestion. We have enriched our introduction and discussion sections with careful comparison against the two cited classic regional studies and other published Chukchi Sea microbial papers. We restated the novelty of our work by highlighting the vertical community assembly mechanism (deterministic vs. stochastic processes) in this marginal Arctic sea, which complements previous surveys mainly focusing on horizontal biogeographic patterns and water-mass-associated microbial taxonomy. We explicitly contrast our findings with earlier results to better position our contribution in the regional research context.

General-4: Several statements in the Results and Discussion are not fully supported by the data presented and should be revised more carefully.

Response

We have gone through the whole manuscript sentence-by-sentence. All over-interpretive, speculative or data-unsupported claims have been removed, rephrased or moderated. We avoid causal inference where direct evidence is missing; all ecological inferences are strictly constrained to our 16S amplicon dataset and are cautiously discussed with proper citations of published literature. Line-specific revisions are provided below.

L77-81: Please clarify how these stations are classified as shelf and slope stations. Also, P2_08 appears close to the Northwind Ridge or the basin margin in Fig. 1, rather than clearly within the Canada Basin. Please provide station coordinates, bottom depths, bathymetric information, and the criteria used for the geographical classification. Overall, both the depth categories and the geographical classification of the stations require clarification.

Response

We thank the reviewer for this careful comment on our geographical and vertical-layer classifications. We acknowledge that the bathymetric criteria for station grouping were not explicitly described in the original manuscript, and we agree that station P2_08 is located near the Northwind Ridge at the margin of the Canada Basin, not in the central basin.

We have made the following revisions:

1. Bathymetry-based geographical classification criteria are explicitly stated in Section 2.1:

- Shelf stations: seafloor depth < 200 m;
- Slope stations: seafloor depth ranging 200–2000 m;
- Basin-margin station (P2_08): seafloor depth > 2000 m, located adjacent to the Northwind Ridge at the Canada Basin margin. The text has been revised to remove the misleading “within the Canada Basin” statement.

2. Full station metadata including sample ID, latitude, longitude, seafloor bottom depth, and geographical category are compiled in Table S1(a). All in-situ hydrographic, nutrient and chlorophyll-a data are provided in Table S1(b). The table notes explicitly distinguish station-relative vertical layers (S/M/B) from fixed absolute-depth bins, and highlight that mid-water and near-bottom samples are closely spaced at station R13.

3. The caption and text annotation of Figure 1 have been updated to reflect the revised description

for station P2_08 (the bathymetric basemap was kept unchanged, while all written descriptions of station type were corrected).

These changes make both geographical and vertical-water-column grouping fully transparent and reproducible.

L82: S, M, and B are sampling depth categories, not water masses.

Response

We accept this correction. Throughout the revised manuscript, we consistently refer to S, M and B as operational sampling depth layers / sampling categories. We have removed any wording that could mislead readers into treating them as predefined hydrographic water masses. We also add this clarification in the Methods section.

L96: The primer pair used is expected to amplify both bacterial and archaeal 16S rRNA genes. Please explain why archaeal sequences were not included in the analysis.

Response

We appreciate the reviewer's careful comment.

The 515F–926R primer pair is known to amplify both bacterial and archaeal 16S rRNA gene amplicons. We provide the explanation for excluding archaeal sequences from the present analysis as follows:

First, the primary research objective of this manuscript focuses on exploring the distribution pattern and environmental drivers of bacterial communities. Second, preliminary taxonomic classification revealed that archaeal sequences recovered from the 515F–926R dataset were of very low abundance across all samples, which cannot support robust statistical analysis of archaeal assemblages.

Notably, we have independently performed amplicon sequencing for archaea using archaea-specific primers (524F10extF/Arch958RmodR) within the same project to achieve sufficient coverage of archaeal communities. Comprehensive analyses of archaeal communities based on this dedicated dataset will be presented in a separate follow-up article.

In bioinformatic processing, we implemented a domain-level taxonomic filter to retain only OTUs assigned to d__Bacteria, and archaeal sequences were removed before all downstream ecological analyses. We have supplemented this rationale clearly in the Methods section of the revised manuscript.

L108:Please provide more detail on how turnover and nestedness were calculated and explain their ecological meaning.

Response

We thank the reviewer for this valuable suggestion. We have supplemented detailed computational procedures and ecological interpretations of turnover and nestedness components of β-diversity in the revised manuscript.

Briefly, following the framework of Baselga (2010), the total Sørensen dissimilarity (*βsor*) was partitioned into two additive fractions: species turnover (*βsim*) and nestedness-resultant dissimilarity (*βsne*). Calculations were conducted on an OTU presence–absence matrix (0 = absence, 1 = presence) using the beta.pair function in the betapart R package (v1.6). Ecologically, (*βsim*) represents community dissimilarity generated by species turnover, i.e., reciprocal replacement of species between sites without marked changes in species richness. In contrast, (*βsne*) describes dissimilarity originating from nestedness, where differences in community composition are driven by uneven species richness: species-poor communities form subsets of

richer communities via selective species loss or colonization. We calculated the relative contributions of turnover and nestedness as (*βsim/βsor*) and (*βsne/βsor*), and visualized the proportions of similarity, turnover and nestedness across all sample pairs using a ternary plot.

We have supplemented this rationale clearly in the Methods section of the revised manuscript.

L122:I cannot clearly identify strong taxonomic stratification in Fig. 2 because the M and B communities appear relatively similar and the major taxonomic groups remain broadly consistent.

Response

We thank the reviewer for this careful observation. We fully agree that the middle (M) and bottom (B) bacterial communities share similar dominant taxonomic composition, while the surface (S) community exhibits pronounced divergence from both M and B layers.

We have revised the wording in the manuscript to avoid overstating the stratification pattern and described the vertical variation more accurately. The revised text clarifies that the surface community is distinctly differentiated, whereas the middle and bottom assemblages show relatively similar taxonomic composition.

The key vertical stratification signals mainly lie in the striking decline of typical surface groups (Cyanobacteria, Bacteroidia) from the surface to deeper layers, as well as shifts in the relative proportions of other clades (e.g., Marinimicrobia_SAR406_clade, SAR324_cladeMarine_group_B) between the surface and deeper waters, despite the similarity between M and B.

L129:It is unclear what is meant by “dramatic shifts” in Alphaproteobacteria. Based on Fig. 2, the relative abundance change does not appear substantially greater than that of Cyanobacteria. In addition, because Alphaproteobacteria is a broad taxonomic class, this statement should either be quantified or supported by analyses at a lower taxonomic level.

Response

We thank the reviewer for this critical comment. We agree that the term "dramatic shifts" was subjective and not adequately supported by the class-level data in Fig. 2, especially given that Cyanobacteria exhibited comparable or even larger changes in relative abundance across depths. We also acknowledge that Alphaproteobacteria is a broad taxonomic class, and meaningful interpretation of its depth-related dynamics would require higher-resolution analysis (e.g., at the order or family level), which is beyond the scope of the current study.

To avoid overinterpretation, we have removed the relevant statement regarding Alphaproteobacteria from the revised manuscript (L129). The text now simply states that the cumulative proportion of minor bacterial classes was markedly higher in mid and bottom waters, without making unsupported claims about any specific taxon. We believe this revision ensures a more rigorous and data-consistent presentation of our results.

We are grateful to the reviewer for helping us improve the clarity and objectivity of our manuscript.

L151:The statement that “M and B partially overlapped” may reflect the strong overlap and heterogeneity in the underlying depth categories.

Response

We thank the reviewer for this insightful comment. We agree that partial overlap between M and B samples indicates relatively high compositional similarity alongside within-group heterogeneity for mid and bottom water communities. We have refined the manuscript wording to clarify this observation, avoiding overstatement of separation.

L159:A broader calculated niche breadth does not by itself demonstrate expanded resource use.

Response

We thank the reviewer for this important methodological caveat. We fully agree that a broader calculated niche breadth (Bcom) does not directly demonstrate expanded resource use, as it is an indirect measure of habitat occupancy along environmental gradients rather than a direct assay of substrate or resource utilization.

To address this, we have revised the relevant sentence in L159 to avoid overinterpretation. The revised text now states that the significantly broader niche width in mid and bottom waters suggests wider environmental tolerance or occupancy of a broader range of environmental conditions, rather than claiming expanded resource use.

We believe this revision makes our interpretation more rigorous and data-consistent. We are grateful to the reviewer for prompting this important refinement.

L185:“L” is not shown in Fig. 5a

Response

Thank you for pointing out this inconsistency. The abbreviation “L: Light” was mistakenly retained in the figure caption, while the Light variable was not included in the final db-RDA analysis. We have removed “L: Light” from the list of abbreviations in Figure 5 caption in the revised manuscript.

L187-188: The network analysis is based on only 8 samples per depth category. This limitation needs to be explained more explicitly.

Response

Thank you for raising this critical point. Each co-occurrence network was generated with eight samples for each depth group, and this limited sample size may produce spurious correlations in Spearman-based network inference. We have added a caveat in the revised text to explicitly acknowledge this constraint and remind readers to interpret the network outputs cautiously.

L191: The M layer network appears to contain more highly connected nodes than the S and B networks. However, this comparison is difficult to interpret because the depth categories are broad and environmentally heterogeneous. In addition, Fig. 6a does not indicate which panels correspond to S, M, and B.

Response

Thank you for this valuable comment. We fully agree that broad depth grouping and underlying environmental heterogeneity complicate direct cross-network comparisons of node connectivity. We have added text in the manuscript to acknowledge this interpretive caveat. Meanwhile, we have revised Figure 6a by adding explicit panel labels (S, M, B) below each subplot to clearly denote Surface, Middle, and Bottom layers in the revised figure.

L216: The authors should briefly explain the ecological meanings of dispersal limitation and drift. More importantly, I am not convinced that the relative contributions of these processes can be robustly estimated from the present dataset and sampling design.

Response

Thank you for this valuable and critical comment. We fully agree that brief definitions for key assembly-process terms are necessary for readability, and that relative-contribution values derived from the null model are sensitive to sampling design, spatial coverage and sample size.

Accordingly, we have added explicit ecological explanations for dispersal limitation and drift directly within the results text, and contextualized both processes under the neutral community assembly framework. Meanwhile, we have de-emphasized the exact 90 % value in the main clause:

the general pattern that stochastic processes constitute the overwhelming majority of contributions is stated in the main sentence, while the numerical value (>90 %) is moved into parenthesis as supplementary information alongside Figure 7a. We further added a clear caveat that these null-model-derived quantitative estimates should be treated as preliminary, hypothesis-generating observations instead of definitive conclusions, to avoid over-interpretation of modelled percentages. Although the quantitative outputs carry uncertainties, the overall observed pattern is consistent with previously reported paradigms of stochastic dominance for Arctic microbial communities.

L234-237: I am not sure what evidence from the present dataset supports the statement that Alphaproteobacteria are associated with primary production or chlorophyll a. According to Table S1, only two samples showed relatively elevated chlorophyll a, while most values were low. It is therefore unclear whether the present dataset supports this association. Similarly, I cannot see how the increase in Gammaproteobacteria was linked specifically to organic matter input from sea-ice melting. Was sea-ice melt or melt-derived organic matter directly measured?

Response

We sincerely appreciate the reviewer's critical and constructive comments, which helped us avoid over-interpretation of our dataset.

For the relationship between Alphaproteobacteria and photic-zone processes: We fully agree that our in-situ chlorophyll a data is limited, with only a small subset of samples showing high chlorophyll-a concentrations, precluding a robust direct statistical correlation between Alphaproteobacteria abundance and measured Chl-a values within our dataset.

Accordingly, we have revised our manuscript text. Instead of directly linking Alphaproteobacteria to measured chlorophyll-a/ primary production values from our own data, we now base our inference on the co-distribution pattern: both Alphaproteobacteria and Cyanobacteria peaked in the surface layer and declined towards deeper waters. Cyanobacteria are typical photic-zone microbes. This co-occurrence pattern suggests a potential ecological linkage of Alphaproteobacteria to photic-zone biogeochemical cycling, supported by previous Arctic microbial studies (Galand et al., 2010; Han et al., 2014).

Regarding Gammaproteobacteria: We acknowledge that we did not directly quantify sea-ice melt fractions or sea-ice-melt-derived organic matter in this work. The original manuscript incorrectly attributed the depth-increasing pattern of Gammaproteobacteria specifically to sea-ice-melt organic matter, which represented an over-interpretation. We have completely removed the "sea-ice melting organic matter" statement from the revised text. In the revised version, we describe that Gammaproteobacteria gradually increased with depth and levelled-off in mid-and-bottom waters, which reflects their physiological adaptability to deep-water physicochemical environments and accumulated sedimentary organic matter.

L238-244: Neither the present dataset nor the cited references demonstrate that members of the SAR406 clade are barophilic. The suggested role in refractory DOM degradation and carbon sequestration should also be presented more cautiously because no functional measurements were made.

Response

We thank the reviewer for this important criticism. We fully agree that our 16S rRNA amplicon dataset cannot provide direct phenotypic evidence for barophily of the SAR406 clade. Therefore, we have removed the "barophilic" description in the revised text.

Furthermore, since our study lacks functional measurements, metagenomic data and experimental evidence, we have entirely deleted speculative statements regarding refractory dissolved organic matter degradation and carbon sequestration. We now only describe the vertical distribution pattern and deep-habitat preference of the SAR406 clade. The revised text is marked within the manuscript.

L250-252:What data support the statement that M and B layer communities are associated specifically with nutrient cycling and that the observed pattern demonstrates habitat filtering?

Response

We thank the reviewer for this important comment. Our 16S rRNA amplicon dataset lacks direct functional measurements of nutrient-cycling processes. Therefore, we have removed statements linking mid- and bottom-layer taxa to biogeochemical cycling of nutrients.

Moreover, community compositional differences across water layers alone cannot definitively demonstrate habitat filtering, since other assembly processes such as ecological drift and dispersal limitation can produce similar vertical patterns. We have deleted the strong claim "essentially reflects the habitat filtering effect". In the revised text, we only note that the observed vertical community pattern is consistent with vertical environmental gradients under sea-ice melting conditions. All modifications are marked in the manuscript.

L253-254：The higher alpha diversity in deeper samples does not by itself demonstrate that deeper water is a microbial "diversity reservoir." The term may be appropriate in some contexts, but this interpretation is not established by the present dataset.

Response

We agree with the reviewer's criticism. Higher alpha-diversity values in deeper samples alone cannot confirm that deeper water represents a microbial "diversity reservoir". We have fully removed this interpretive statement from the revised text.

We now only state that the increase of alpha-diversity with depth is consistent with earlier findings in polar marine environments (Kraemer et al., 2024). We have also softened the wording for β-diversity by replacing "may be attributed to" with "may be associated with" to avoid over-confident causal inference. Changes are marked in the revised manuscript.

L485：The DOI link is incorrect

Response

We thank the reviewer for catching this error. The original reference contained an incorrect publication year and DOI for Stegen et al. We have corrected both the publication year (2012→2013) and the DOI to (https://doi.org/10.1038/ismej.2013.93) in the reference list.

Table S1：Units are missing for all environmental variables. The reported numerical precision is also inconsistent.

Response

Thank you for this important comment.

We have added complete units for each environmental variable within the table header. Numerical precision has been standardized across the whole supplementary table.

We have also revised the table caption and footnote following journal formatting requirements. The updated Table S1(b) is included in the revised supplementary material.

Once again, we sincerely thank the reviewer for the careful evaluation and all the valuable suggestions, which greatly improve the quality and scientific rigor of our manuscript.

(a)

STATION_NAME	Latitude	Longitude	Bottom_depth_m	Geographical_category
R11_S	73.99468	-168.50477	162	Shelf
R11_M	73.99468	-168.50477	162	Shelf
R11_B	73.99468	-168.50477	162	Shelf
R12_S	74.73367	-168.4864	170	Shelf
R12_M	74.73367	-168.4864	170	Shelf
R12_B	74.73367	-168.4864	170	Shelf
R13_S	75.50528	-168.6236	164	Slope
R13_M	75.50528	-168.6236	164	Slope
R13_B	75.50528	-168.6236	164	Slope
R16_S	77.76623	-168.42512	515	Slope
R16_M	77.76623	-168.42512	515	Slope
R16_B	77.76623	-168.42512	515	Slope
P1_07_S	75.03767	-164.19582	575	Shelf
P1_07_M	75.03767	-164.19582	575	Shelf
P1_07_B	75.03767	-164.19582	575	Shelf
P2_04_S	76.87042	-165.79413	1076	Slope
P2_04_M	76.87042	-165.79413	1076	Slope
P2_04_B	76.87042	-165.79413	1076	Slope
P2_05_S	76.6204	-163.73822	544	Slope
P2_05_M	76.6204	-163.73822	544	Slope
P2_05_B	76.6204	-163.73822	544	Slope
P2_08_S	75.66578	-156.5149	1175	Basin-margin
P2_08_M	75.66578	-156.5149	1175	Basin-margin
P2_08_B	75.66578	-156.5149	1175	Basin-margin

TableS1. Physical and chemical properties in 8 Stations.

(b)

STATION_NAME	$NH4^+$ ($\mu g\ L^{-1}$)	SiO_2-Si ($\mu g\ L^{-1}$)	DO ($\mu mol\ L^{-1}$)	$PO4^{3-}$ ($\mu g\ L^{-1}$)	NO^{2-} ($\mu g\ L^{-1}$)	D (m)	chla ($mg\ m^{-3}$)	T(°C)	Salinity	Latitude	Longitude
R11_S	0.44	25.0	917.45	134.0	5.31	0	0.02	-1.34	30.13	73.99468	-168.50477
R12_S	0.80	144.0	821.18	19.7	0.43	0	0.00	-1.3	29.83	74.73367	-168.4864
R13_S	8.11	175.0	869.22	134.0	0.00	0	0.34	-1.32	29.96	75.50528	-168.6236
R16_S	0.00	142.0	962.91	21.2	0.00	0	3.61	-1.29	29.35	77.76623	-168.42512
P1_07_S	4.87	144.0	813.59	27.3	0.00	0	0.02	-1.21	28.98	75.03767	-164.19582
P2_04_S	2.55	148.0	840.77	22.3	0.00	0	0.00	-1.26	28.50	76.87042	-165.79413
P2_05_S	0.64	154.0	805.45	28.6	0.00	0	0.01	-1.12	28.50	76.6204	-163.73822
P2_08_S	8.20	124.0	791.74	74.9	0.00	0	0.00	-0.63	27.18	75.66578	-156.5149
R11_M	1.61	1334.0	529.82	43.0	0.72	100	0.02	-1.54	32.77	73.99468	-168.50477
R12_M	0.00	1005.0	612.40	60.7	0.78	100	0.00	-1.65	32.71	74.73367	-168.4864
R13_M	0.00	787.0	529.18	43.0	0.31	150	0.01	-0.57	34.14	75.50528	-168.6236
R16_M	0.00	250.0	601.17	26.9	0.00	200	0.01	-0.58	34.47	77.76623	-168.42512
P1_07_M	0.00	725.0	528.46	43.3	0.40	200	0.00	-0.66	34.15	75.03767	-164.19582
P2_04_M	0.00	154.0	605.57	29.4	0.00	500	0.00	0.88	34.86	76.87042	-165.79413
P2_05_M	0.00	389.0	537.86	40.2	0.00	200	0.00	-0.68	34.27	76.6204	-163.73822
P2_08_M	0.00	244.0	601.98	28.0	0.00	500	0.00	0.84	34.86	75.66578	-156.5149
R11_B	1.34	1593.2	514.67	41.4	1.13	162	0.00	-0.12	34.49	73.99468	-168.50477
R12_B	0.94	750.0	511.04	43.3	1.74	170	0.00	-0.43	34.26	74.73367	-168.4864
R13_B	0.00	736.0	525.46	41.4	0.86	164	0.01	-0.42	34.29	75.50528	-168.6236
R16_B	0.00	184.0	605.11	28.3	0.00	515	0.00	0.84	34.87	77.76623	-168.42512
P1_07_B	0.00	219.0	609.23	26.5	0.63	575	0.00	0.81	34.87	75.03767	-164.19582
P2_04_B	0.00	214.0	604.36	29.9	0.00	1076	0.00	0.52	34.88	76.87042	-165.79413
P2_05_B	0.00	124.0	604.16	27.7	0.00	544	0.00	0.78	34.88	76.6204	-163.73822
P2_08_B	1.85	256.0	617.89	45.4	0.00	1175	0.00	-0.06	34.90	75.66578	-156.5149

NH_4^+: ammonium nitrogen; SiO_2-Si: silicate; DO: dissolved oxygen; PO_4^{3-}: phosphate; NO_2^-: nitrite; D: water depth; chla: chlorophyll-*a*;T:temperature

TableS1. Physical and chemical properties in 8 Stations.

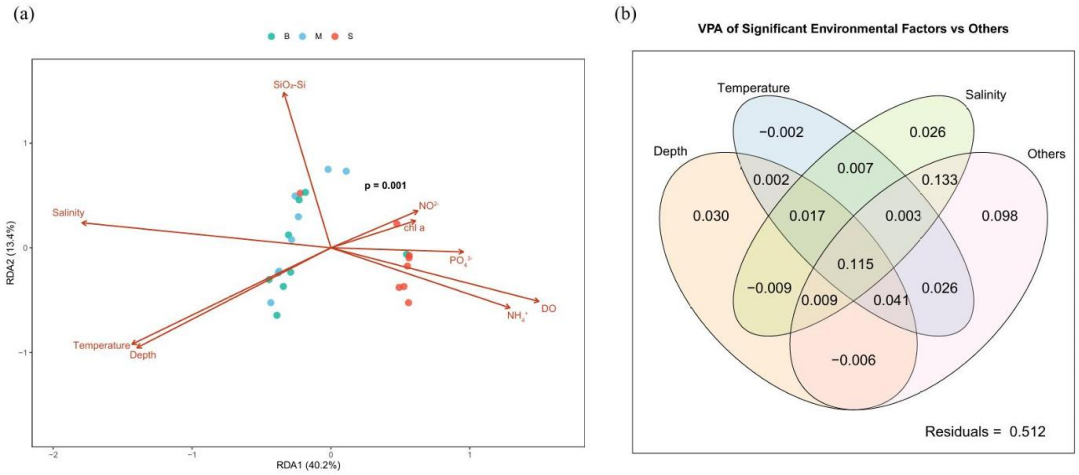

Figure 5: Links between water environmental variables and bacterial community composition based on db-RDA and VPA. (a) db-RDA ordination (Bray-Curtis distance) of bacterial communities. colored points denote different sample groups. (b) VPA Venn diagram showing adjusted R^2 of community variation explained by depth, SiO_2, DO and other environmental factors. Values < −0.01 are omitted. Abbreviations: NO_2^-, nitrite; PO_4^{3-}, phosphate; SiO_2-Si, silicate; NH_4^+, ammonium nitrogen; DO, dissolved oxygen;chl a, chlorophyll a.