# Supplementary information

# Distinct patterns of distribution, community assembly and cross-domain co-occurrence of planktonic archaea in four major estuaries of China

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The supplementary information contains:

- 6 Figures
- 4 Tables

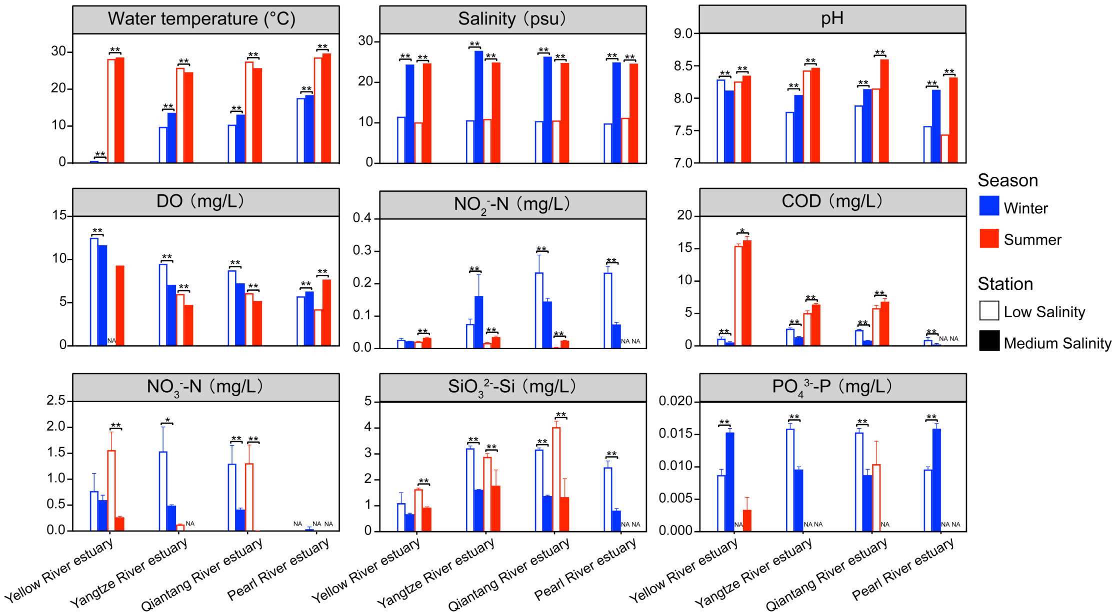

**Figure S1.** Dynamics of water environmental parameters across four estuaries during winter and summer cruises. Data present mean ± standard deviation (n = 5, except $NO_3^-$ in the medium-salinity station of Qiantang River estuary (summer cruise) and Pearl River estuary (winter cruise) and $PO_4^{3-}$ in the medium-salinity station of Yellow River estuary (summer cruise), for which n = 4). The significance of differences in environmental parameters between stations with low and medium salinity was tested using the Mann-Whitney *U* test ($^{*}P < 0.05$, $^{**}P < 0.01$) in each estuary during each seasonal cruise. DO, dissolved oxygen; $NO_2^-$, nitrite; COD, chemical oxygen demand; $NO_3^-$, nitrate; $SiO_3^{2-}$, silicate; $PO_4^{3-}$, phosphate. NA, not available.

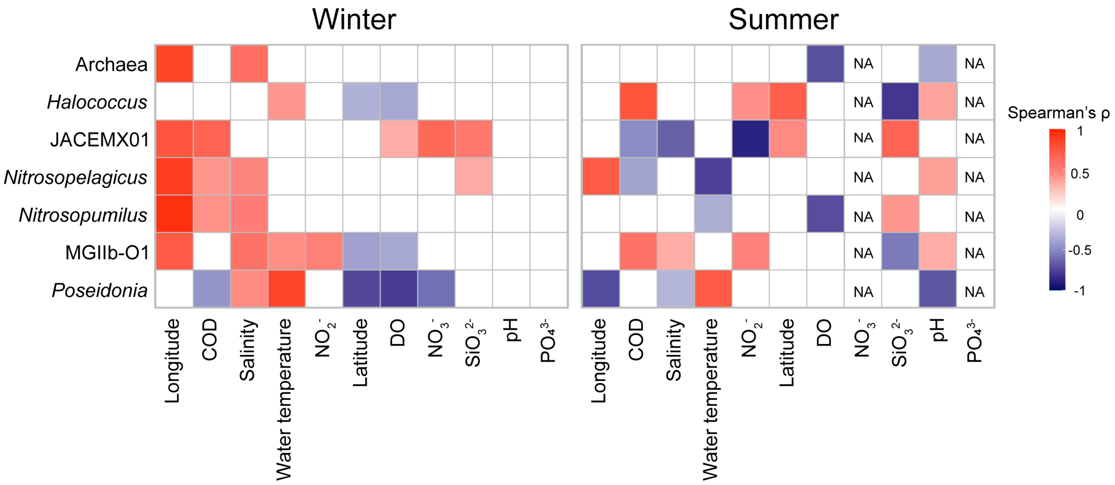

**Figure S2.** Heatmap showing Spearman's correlations of environmental and geographic factors with relative abundances of total archaea and major archaeal genera in prokaryotic communities during the winter or summer cruise. Colored cells indicate significance ($P < 0.05$). Cells with NA (not available) indicate that no calculations are performed when the coverage of environmental data less than 75% during the winter or summer cruise. COD, chemical oxygen demand; DO, dissolved oxygen.

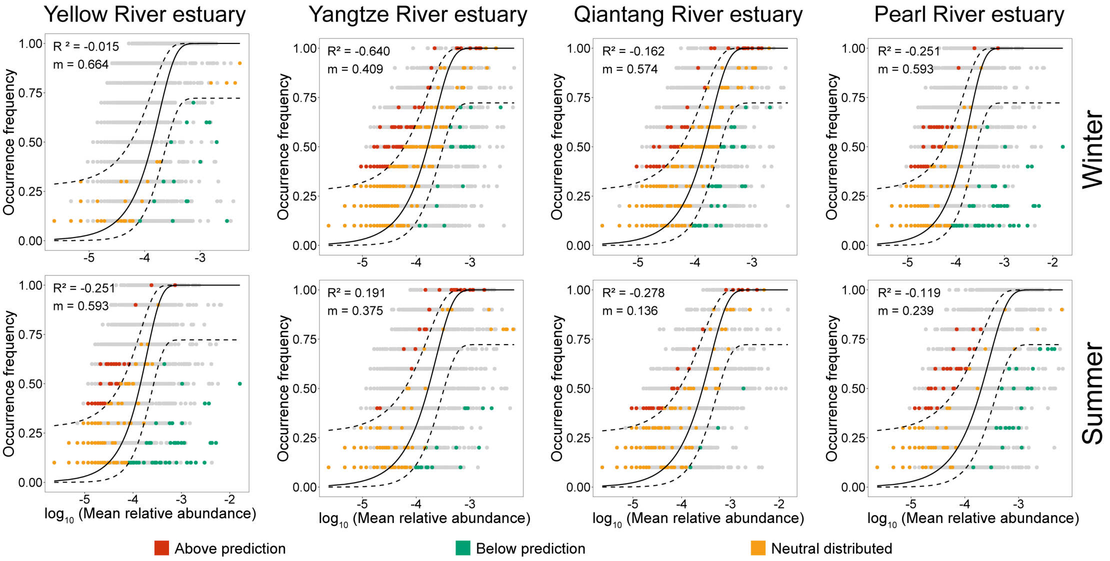

**Figure S3.** Fit of the neutral models for prokaryotic communities in each estuary during the winter or summer cruise with the metacommunity cross all four estuaries as the source. Archaeal ZOTUs that occurred more frequently than the model prediction are shown in red, while those occurred less frequently than predicted are shown in green. Dashed lines represent 95% confidence intervals around the model prediction and the archaeal ZOTUs fall within the confidence intervals are considered as neutrally distributed and shown in orange. Bacterial ZOTUs are shown in grey. $R^2$ values present the goodness of model fitting, ranging from 0 (no fit) to 1 (perfect fit).

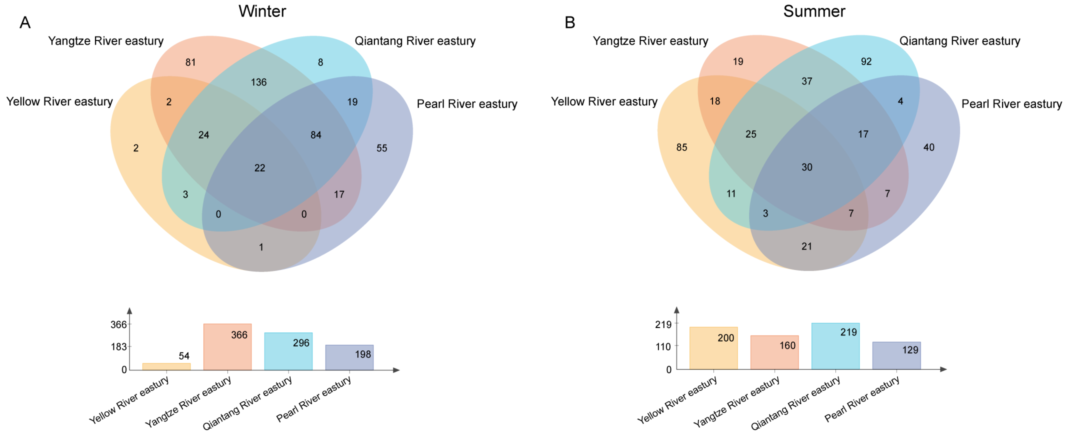

**Figure S4.** Venn diagrams showing the numbers of archaeal ZOTUs being unique and shared in four estuaries during winter (A) and summer cruises (B). The bar plots below the Venn diagrams present the number of archaeal ZOTUs in each estuary.

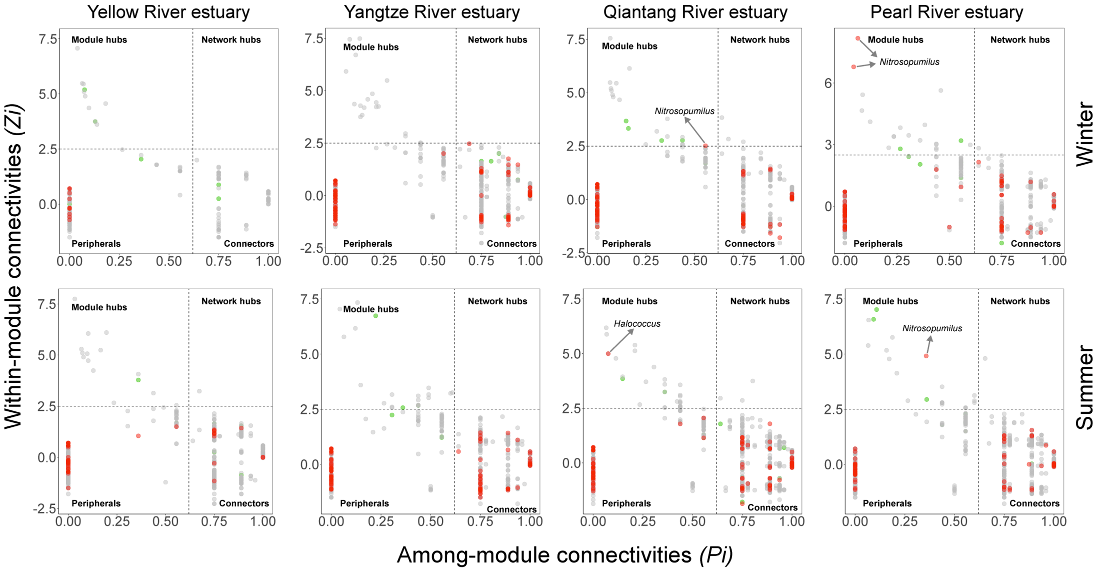

**Figure S5.** Classification of the nodes in the microbial co-occurrence networks. Four categories can be defined for each node: (1) peripheral nodes ($Zi \leq 2.5$, $Pi \leq 0.62$), (2) connectors ($Zi \leq 2.5$, $Pi > 0.62$), (3) module hubs ($Zi > 2.5$, $Pi \leq 0.62$), and (4) network hubs ($Zi > 2.5$, $Pi > 0.62$). Archaeal, bacterial, and phytoplanktonic ZOTUs are shown in red, grey, and green, respectively.

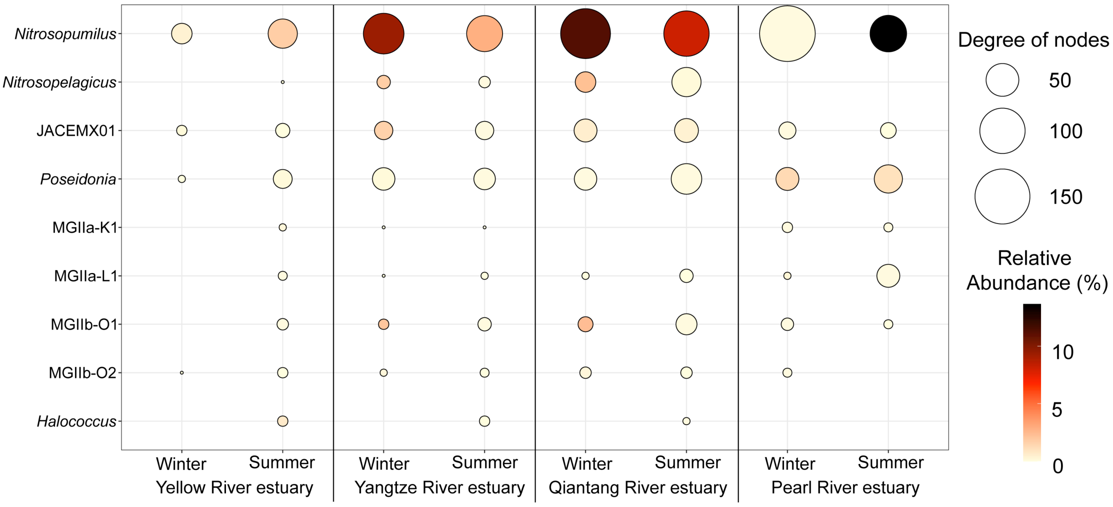

**Figure S6.** Bubble plot summarizing degree of nodes of cross-domain associations of major archaeal genera with their relative abundances in prokaryotic communities in each estuary during the winter and summer cruises. The size of bubbles indicates degree of nodes. The color of bubbles indicates the relative abundances of archaeal genera in prokaryotic communities.

**Table S1.** Three-way repeated measures analysis of variance (ANOVA) testing the influence of *Estuary*, *Season*, *Station*, and their interactions on relative abundances of total archaea and dominant archaeal taxa in prokaryotic communities.

| | | Total Archaea | | *Halococcus* | | JACEMX01 | | *Nitrosopelagicus* | | *Nitrosopumilus* | | MGIIb-O1 | | *Poseidonia* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Estuary** | **Factor** | F | $P$ | F | $P$ | F | $P$ | F | $P$ | F | $P$ | F | $P$ | F | $P$ |
| All | *Estuary* | 25.77 | **< 0.001** | 1.392 | 0.253 | 22.71 | **< 0.001** | 39.09 | **< 0.001** | 21.64 | **< 0.001** | 19.20 | **< 0.001** | 63.06 | **< 0.001** |
| | *Season* | 2.653 | 0.108 | 1.553 | 0.217 | 33.23 | **< 0.001** | 90.93 | **< 0.001** | 7.015 | **0.010** | 56.95 | **< 0.001** | 2.432 | 0.124 |
| | *Station* | 0.000 | 0.994 | 0.763 | 0.386 | 33.99 | **< 0.001** | 36.02 | **< 0.001** | 7.251 | **0.009** | 53.17 | **< 0.001** | 6.434 | **0.014** |
| | *Estuary* × *Season* | 38.19 | **< 0.001** | 1.402 | 0.250 | 20.12 | **< 0.001** | 31.34 | **< 0.001** | 34.64 | **< 0.001** | 18.97 | **< 0.001** | 3.613 | **0.018** |
| | *Estuary* × *Station* | 24.79 | **< 0.001** | 0.870 | 0.461 | 17.15 | **< 0.001** | 28.72 | **< 0.001** | 32.17 | **< 0.001** | 17.25 | **< 0.001** | 3.916 | **0.012** |
| | *Season* × *Station* | 98.46 | **< 0.001** | 0.774 | 0.382 | 10.73 | **0.002** | 36.60 | **< 0.001** | 86.59 | **< 0.001** | 51.46 | **< 0.001** | 61.68 | **< 0.001** |
| | *Estuary* × *Season* × *Station* | 19.41 | **< 0.001** | 0.866 | 0.463 | 20.55 | **< 0.001** | 33.87 | **< 0.001** | 19.42 | **< 0.001** | 16.65 | **< 0.001** | 46.73 | **< 0.001** |
| Yellow River estuary | *Season* | 12.12 | **0.003** | 1.439 | 0.248 | 9.654 | **0.007** | 8.203 | **0.011** | 0.000 | 1.000 | 24.25 | **< 0.001** | 18.68 | **0.001** |
| | *Station* | 5.473 | **0.033** | 0.842 | 0.372 | 2.980 | 0.104 | 0.068 | 0.798 | 0.000 | 1.000 | 11.80 | **0.003** | 0.202 | 0.659 |
| | *Season* × *Station* | 5.682 | **0.030** | 0.842 | 0.372 | 1.676 | 0.214 | 0.068 | 0.798 | 2.000 | 0.176 | 14.62 | **0.001** | 0.145 | 0.709 |
| Yangtze River estuary | *Season* | 36.00 | **< 0.001** | 7.714 | **0.013** | 30.85 | **< 0.001** | 56.15 | **< 0.001** | 23.56 | **< 0.001** | 25.91 | **< 0.001** | 5.166 | **0.037** |
| | *Station* | 7.288 | **0.016** | 0.857 | 0.368 | 26.60 | **< 0.001** | 0.807 | 0.382 | 14.57 | **0.002** | 26.50 | **< 0.001** | 6.333 | **0.023** |
| | *Season* × *Station* | 1.524 | 0.235 | 0.095 | 0.762 | 23.26 | **< 0.001** | 0.093 | 0.764 | 2.557 | 0.129 | 25.47 | **< 0.001** | 17.14 | **0.001** |
| Qiantang River estuary | *Season* | 10.61 | **0.005** | 2.068 | 0.170 | 2.032 | 0.173 | 41.86 | **< 0.001** | 0.444 | 0.515 | 30.81 | **< 0.001** | 1.793 | 0.199 |
| | *Station* | 10.27 | **0.006** | 2.068 | 0.170 | 6.522 | **0.021** | 43.68 | **< 0.001** | 0.927 | 0.350 | 26.00 | **< 0.001** | 2.069 | 0.170 |
| | *Season* × *Station* | 42.53 | **< 0.001** | 2.068 | 0.170 | 3.434 | 0.082 | 49.78 | **< 0.001** | 21.04 | **< 0.001** | 25.26 | **< 0.001** | 24.11 | **< 0.001** |
| Pearl River estuary | *Season* | 73.16 | **< 0.001** | 3.600 | 0.076 | 5.115 | **0.038** | - | - | 105.5 | **< 0.001** | 8.219 | **0.011** | 3.089 | 0.098 |
| | *Station* | 68.86 | **< 0.001** | 0.400 | 0.536 | 0.442 | 0.515 | - | - | 103.4 | **< 0.001** | 8.219 | **0.011** | 4.606 | **0.048** |
| | *Season* × *Station* | 114.5 | **< 0.001** | 0.400 | 0.536 | 1.434 | 0.249 | - | - | 104.6 | **< 0.001** | 7.253 | **0.016** | 51.72 | **< 0.001** |

Data in bold indicate significant influence ($P < 0.05$).

**Table S2.** Mantel tests demonstrating Spearman's correlations of the compositional variation of archaeal communities (based on Bray-Curtis dissimilarity) with variation in bacterial community composition (based on Bray-Curtis dissimilarity), environmental variables (based on Euclidean distance), and geographic distance (with 999 permutations) during the winter or summer cruise. Partial-Mantel tests demonstrating correlations between the compositional variation of archaeal communities and key significantly correlative factors (with Mantel $\rho > 0.4$) after controlled by each other. Three WYE_L samples, three WYE_M samples, and five WP_L samples of archaeal communities were excluded due to low read counts. Communities and environmental profiles with missing data were removed to generate a fully overlapping data set for each test.

| Winter | | | | | | | | Summer | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mantel | | | Controlled by | Partial-Mantel | | | Variable | Mantel | | | Controlled by | Partial-Mantel | | |
| | ρ | ***P*** | n | | ρ | ***P*** | n | | ρ | ***P*** | n | | ρ | ***P*** | n |
| Bacteria | 0.880 | **0.001** | 29 | Geo. | 0.731 | **0.001** | 29 | Geo. | 0.700 | **0.001** | 40 | Bacteria | 0.653 | **0.001** | 40 |
| Bacteria | - | - | - | Temp. | 0.637 | **0.001** | 29 | Geo. | - | - | - | $NO_2^-$ | 0.782 | **0.001** | 30 |
| Bacteria | - | - | - | DO | 0.706 | **0.001** | 29 | Bacteria | 0.597 | **0.001** | 40 | Geo. | 0.526 | **0.001** | 40 |
| Bacteria | - | - | - | COD | 0.840 | **0.001** | 29 | Bacteria | - | - | - | $NO_2^-$ | 0.799 | **0.001** | 30 |
| Bacteria | - | - | - | $SiO_3^{2-}$ | 0.845 | **0.001** | 29 | $NO_2^-$ | 0.499 | **0.001** | 30 | Geo. | 0.611 | **0.001** | 30 |
| Bacteria | - | - | - | $NO_3^-$ | 0.839 | **0.001** | 28 | $NO_2^-$ | - | - | - | Bacteria | -0.046 | 0.754 | 30 |
| Geo. | 0.840 | **0.001** | 29 | Bacteria | 0.628 | **0.001** | 29 | $SiO_3^{2-}$ | 0.305 | 0.002 | 30 | - | - | - | - |
| Geo. | - | - | - | Temp. | 0.525 | **0.001** | 29 | Salinity | 0.267 | **0.001** | 40 | - | - | - | - |
| Geo. | - | - | - | DO | 0.715 | **0.001** | 29 | $NO_3^-$ | 0.135 | 0.018 | 24 | - | - | - | - |
| Geo. | - | - | - | COD | 0.809 | **0.001** | 29 | pH | 0.084 | 0.095 | 40 | - | - | - | - |
| Geo. | - | - | - | $SiO_3^{2-}$ | 0.811 | **0.001** | 29 | Temp. | 0.070 | 0.067 | 40 | - | - | - | - |
| Geo. | - | - | - | $NO_3^-$ | 0.823 | **0.001** | 28 | DO | -0.002 | 0.529 | 35 | - | - | - | - |
| Temp. | 0.820 | **0.001** | 29 | Bacteria | 0.374 | **0.001** | 29 | COD | -0.025 | 0.672 | 30 | - | - | - | - |
| Temp. | - | - | - | Geo. | 0.439 | **0.001** | 29 | $PO_4^{3-}$ | -0.362 | 0.968 | 9 | - | - | - | - |
| Temp. | - | - | - | DO | 0.499 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| Temp. | - | - | - | COD | 0.786 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| Temp. | - | - | - | $SiO_3^{2-}$ | 0.768 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| Temp. | - | - | - | $NO_3^-$ | 0.817 | **0.001** | 28 | - | - | - | - | - | - | - | - |
| DO | 0.751 | **0.001** | 29 | Bacteria | 0.183 | 0.028 | 29 | - | - | - | - | - | - | - | - |
| DO | - | - | - | Geo. | 0.526 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| DO | - | - | - | Temp. | 0.036 | 0.285 | 29 | - | - | - | - | - | - | - | - |
| DO | - | - | - | COD | 0.682 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| DO | - | - | - | $SiO_3^{2-}$ | 0.670 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| DO | - | - | - | $NO_3^-$ | 0.702 | **0.001** | 28 | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COD | 0.484 | **0.001** | 29 | Bacteria | -0.021 | 0.612 | 29 | - | - | - | - | - | - | - | - |
| COD | - | - | - | Geo. | 0.317 | **0.001** | 29 | - | - | - | - | - | - | - | - |
| COD | - | - | - | Temp. | 0.329 | **0.003** | 29 | - | - | - | - | - | - | - | - |
| COD | - | - | - | DO | 0.246 | **0.002** | 29 | - | - | - | - | - | - | - | - |
| COD | - | - | - | $SiO_3^{2-}$ | 0.175 | 0.010 | 29 | - | - | - | - | - | - | - | - |
| COD | - | - | - | $NO_3^-$ | 0.221 | **0.003** | 28 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | 0.462 | **0.001** | 29 | Bacteria | -0.071 | 0.902 | 29 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | - | - | - | Geo. | 0.289 | **0.003** | 29 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | - | - | - | Temp. | 0.119 | 0.052 | 29 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | - | - | - | DO | 0.070 | 0.118 | 29 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | - | - | - | COD | 0.065 | 0.188 | 29 | - | - | - | - | - | - | - | - |
| $SiO_3^{2-}$ | - | - | - | $NO_3^-$ | 0.247 | **0.003** | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | 0.461 | **0.001** | 28 | Bacteria | 0.118 | 0.102 | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | - | - | - | Geo. | 0.380 | **0.002** | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | - | - | - | Temp. | 0.439 | **0.001** | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | - | - | - | DO | 0.290 | 0.009 | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | - | - | - | COD | 0.210 | 0.016 | 28 | - | - | - | - | - | - | - | - |
| $NO_3^-$ | - | - | - | $SiO_3^{2-}$ | 0.253 | 0.007 | 28 | - | - | - | - | - | - | - | - |
| pH | 0.351 | **0.001** | 29 | - | - | - | - | - | - | - | - | - | - | - | - |
| Salinity | 0.341 | **0.001** | 29 | - | - | - | - | - | - | - | - | - | - | - | - |
| $PO_4^{3-}$ | 0.143 | 0.017 | 29 | - | - | - | - | - | - | - | - | - | - | - | - |
| $NO_2^-$ | 0.111 | 0.079 | 29 | - | - | - | - | - | - | - | - | - | - | - | - |

Data in bold indicate significant correlations ($P < 0.05$). Bacteria, Bacterial community composition; Geo., geographic distance; Temp., water temperature; DO, dissolved oxygen; COD, chemical oxygen demand.

**Table S3.** Mantel tests demonstrating Spearman's correlations of the compositional variation of bacterial communities (based on Bray-Curtis dissimilarity) with variation in archaeal community composition (based on Bray-Curtis dissimilarity), environmental variables (based on Euclidean distance), and geographic distance (with 999 permutations) during the winter or summer cruise. Partial-Mantel tests demonstrating correlations between the compositional variation of bacterial communities and key significantly correlative factors (with Mantel $\rho > 0.4$) after controlled by each other. Three WYE_L samples, three WYE_M samples, and five WP_L samples of archaeal communities were excluded due to low read counts. Communities and environmental profiles with missing data were removed to generate a fully overlapping data set for each test.

| Winter | | | | | | | | Summer | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mantel | | | Controlled by | Partial-Mantel | | | Variable | Mantel | | | Controlled by | Partial-Mantel | | |
| | ρ | ***P*** | n | | ρ | ***P*** | n | | ρ | ***P*** | n | | ρ | ***P*** | n |
| Archaea | 0.880 | **0.001** | 29 | Temp. | 0.637 | **0.001** | 29 | Archaea | 0.597 | **0.001** | 40 | $NO_2^-$ | 0.799 | **0.001** | 30 |
| Archaea | - | - | - | DO | 0.706 | **0.001** | 29 | Archaea | - | - | - | Salinity | 0.530 | **0.001** | 40 |
| Archaea | - | - | - | Geo. | 0.731 | **0.001** | 29 | Archaea | - | - | - | $SiO_3^{2-}$ | 0.795 | **0.001** | 30 |
| Temp. | 0.749 | **0.001** | 40 | Archaea | 0.344 | **0.001** | 29 | $NO_2^-$ | 0.572 | **0.001** | 30 | Archaea | 0.412 | **0.001** | 30 |
| Temp. | - | - | - | DO | 0.316 | **0.001** | 40 | $NO_2^-$ | - | - | - | Salinity | 0.471 | **0.001** | 30 |
| Temp. | - | - | - | Geo. | 0.455 | **0.001** | 40 | $NO_2^-$ | - | - | - | $SiO_3^{2-}$ | 0.396 | **0.001** | 30 |
| DO | 0.719 | **0.001** | 40 | Archaea | 0.424 | **0.001** | 29 | Salinity | 0.509 | **0.001** | 40 | Archaea | 0.550 | **0.001** | 40 |
| DO | - | - | - | Temp. | 0.093 | 0.038 | 40 | Salinity | - | - | - | $NO_2^-$ | 0.429 | 0.002 | 30 |
| DO | - | - | - | Geo. | 0.450 | **0.001** | 40 | Salinity | - | - | - | $SiO_3^{2-}$ | 0.568 | **0.001** | 30 |
| Geo. | 0.717 | **0.001** | 40 | Archaea | -0.077 | 0.889 | 29 | $SiO_3^{2-}$ | 0.429 | **0.001** | 30 | Archaea | 0.298 | **0.001** | 30 |
| Geo. | - | - | - | Temp. | 0.350 | **0.001** | 40 | $SiO_3^{2-}$ | - | - | - | $NO_2^-$ | 0.299 | **0.001** | 30 |
| Geo. | - | - | - | DO | 0.446 | **0.001** | 40 | $SiO_3^{2-}$ | - | - | - | Salinity | 0.549 | **0.001** | 30 |
| pH | 0.375 | **0.001** | 40 | - | - | - | - | pH | 0.366 | **0.001** | 40 | - | - | - | - |
| $SiO_3^{2-}$ | 0.371 | **0.001** | 40 | - | - | - | - | Geo. | 0.350 | **0.001** | 40 | - | - | - | - |
| COD | 0.344 | **0.001** | 40 | - | - | - | - | $NO_3^-$ | -0.066 | 0.858 | 24 | - | - | - | - |
| $NO_3^-$ | 0.319 | **0.001** | 34 | - | - | - | - | DO | -0.074 | 0.927 | 35 | - | - | - | - |
| Salinity | 0.244 | **0.001** | 40 | - | - | - | - | Temp. | -0.076 | 0.964 | 40 | - | - | - | - |
| $NO_2^-$ | 0.199 | **0.003** | 40 | - | - | - | - | COD | -0.116 | 0.968 | 30 | - | - | - | - |
| $PO_4^{3-}$ | -0.028 | 0.779 | 40 | - | - | - | - | $PO_4^{3-}$ | -0.425 | 0.978 | 9 | - | - | - | - |

Data in bold indicate significant correlations ($P < 0.05$). Archaea, Archaeal community composition; Temp., water temperature; DO, dissolved oxygen; Geo., geographic distance; COD, chemical oxygen demand.

**Table S4.** Summary of the features of microbial co-occurrence networks detected by FlashWeave. Topological features were calculated after removing indirect and environment-related edges. Archaeal-related topological features were calculated for archaeal co-occurrence networks. Only direct edges (with percentage in all edges in parentheses) were visualized in the chord diagrams (Fig. 5). Archaeal nodes (with percentage in all microbial nodes in parentheses), archaeal edges (with percentage in all microbial edges in parentheses), and positive edges (with percentage in all archaeal edges in parentheses) were shown.

| **Estuary** | **Season** | **Raw ZOTUs** | | **ZOTUs selected for network calculations (Reads ≥ 100, Sample coverage ≥ 15%)** | | **FlashWeave Result** | | | **Archaeal-related topological features** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **ZOTUs** | **Reads** | **ZOTUs** | **Reads (%)** | **Variables** | **indirect & direct edges** | **direct edges (%)** | **Nodes (%)** | **Edges (%)** | **Positive edges (%)** | **Modularity** | **Clustering coefficient** | **Network diameter** | **Average path length** | **Average degree** |
| Yellow River estuary | Winter | 4679 | 196991 | 1211 | 128190 (65.07%) | 1214 | 7514 | 595 (7.91%) | 19 (2.50%) | 10 (1.69%) | 8 (80.00%) | 1.018 | 0 | 2 | 1.091 | 1.053 |
| | Summer | 7908 | 414878 | 2293 | 333434 (80.37%) | 2296 | 48272 | 1567 (3.24%) | 110 (5.74%) | 68 (4.35%) | 57 (83.82%) | 1.173 | 0 | 4 | 1.441 | 1.236 |
| Yangtze River estuary | Winter | 9459 | 175969 | 2056 | 116284 (66.08%) | 2059 | 53209 | 1604 (3.01%) | 229 (12.92%) | 164 (10.26%) | 117 (71.34%) | 1.539 | 0.034 | 10 | 2.895 | 1.432 |
| | Summer | 76 55 | 441389 | 2415 | 400187 (90.67%) | 2418 | 77720 | 1733 (2.22%) | 218 (10.85%) | 150 (8.68%) | 118 (78.67%) | 1.282 | 0.083 | 6 | 1.68 | 1.376 |
| Qiantang River estuary | Winter | 9328 | 189518 | 2040 | 137465 (72.53%) | 2043 | 36964 | 1499 (4.05%) | 215 (11.92%) | 144 (9.63%) | 109 (75.69%) | 1.412 | 0 | 5 | 1.743 | 1.340 |
| | Summer | 8840 | 441814 | 2239 | 358172 (81.07%) | 2242 | 213318 | 2270 (1.06%) | 300 (14.19%) | 222 (9.8%) | 184 (82.88%) | 1.203 | 0.037 | 7 | 1.988 | 1.480 |
| Pearl River estuary | Winter | 6882 | 213057 | 2221 | 167453 (78.60%) | 2224 | 68600 | 1692 (2.46%) | 275 (14.55%) | 218 (12.93%) | 207 (94.95%) | 0.897 | 0 | 6 | 1.944 | 1.585 |
| | Summer | 6528 | 436220 | 2250 | 389455 (89.28%) | 2253 | 210171 | 1960 (0.93%) | 178 (8.64%) | 132 (6.76%) | 102 (77.27%) | 1.276 | 0.076 | 6 | 1.844 | 1.483 |