

The following supplement accompanies the article

Marine bacterioplankton consortia follow deterministic, non-neutral community assembly rules

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Supplementary material

Table S1. Time-series samples amplified for 16S rRNA pyrosequencing as indicated by an X. The month of deepest mixing is indicated in **bold**; X indicate samples used for the analysis of residuals for non-normal distribution after seasonal differencing.

| Year | Month | BATS# | Surface | 200 | MLD(m) |
|------|-------|-------|---------|-----|------------|
| 1991 | 8 | 35 | X | X | 18 |
| | 9 | 36 | X | X | 38 |
| | 10 | 37 | X | X | 24 |
| | 11 | 38 | X | X | 56 |
| | 12 | 39 | X | X | 86 |
| 1992 | 1 | 40 | X | X | 132 |
| | 2 | 41 | X | X | 238 |
| | 3 | 42 | X | X | 32 |
| | 4 | 43 | X | X | 40 |
| | 5 | 44 | X | X | 20 |
| | 6 | 45 | X | X | 16 |
| | 7 | 46 | X | X | 22 |
| | 8 | 47 | X | X | 24 |
| | 9 | 48 | X | X | 16 |
| | 10 | 49 | X | X | 60 |
| | 11 | 50 | X | X | 64 |
| | 12 | 51 | X | | 84 |
| 1993 | 1 | 52 | X | | 104 |
| | 2 | 53 | X | X | 132 |
| | 3 | 54 | X | X | 210 |
| | 4 | 55 | X | X | 106 |
| | 5 | 56 | X | X | 12 |
| | 6 | 57 | X | X | 28 |
| | 7 | 58 | X | X | 10 |
| | 8 | 59 | X | X | 32 |
| | 9 | 60 | | | 30 |
| | 10 | 61 | X | X | 34 |
| | 11 | 62 | X | X | 56 |
| | 12 | 63 | X | X | 58 |
| 1994 | 1 | 64 | | X | 114 |
| | 2 | 65 | X | X | 132 |
| 1997 | 9 | 108 | X | X | 38 |
| | 10 | 109 | X | X | 32 |
| | 11 | 110 | X | X | 40 |
| | 12 | 111 | X | X | 102 |
| 1998 | 1 | 112 | X | X | 76 |
| | 2 | 113 | X | X | 144 |
| | 3 | 114 | X | X | 212 |
| | 3 | 114 A | X | X | 92 |
| | 4 | 115 A | X | X | 116 |
| | 5 | 116 | X | X | 38 |
| 6 | 117 | X | X | 16 | |

| | | | | | |
|------|----|-------|---|---|------------|
| | 7 | 118 | X | X | 16 |
| | 8 | 119 | | X | 22 |
| | 9 | 120 | X | X | 40 |
| | 12 | 123 | X | X | 76 |
| 1999 | 1 | 124 | X | X | 108 |
| | 2 | 125 | X | X | 128 |
| | 3 | 126 | X | | 122 |
| | 4 | 127 | | | 208 |
| | 5 | 128 | | | |
| | 6 | 129 | | | 26 |
| | 7 | 130 | | | 16 |
| | 8 | 131 | X | X | 30 |
| | 9 | 132 | X | X | 36 |
| | 10 | 133 | X | X | 48 |
| | 11 | 134 | X | X | 70 |
| | 12 | 135 | X | | |
| 2000 | 1 | 136 | X | X | 171 |
| | 2 | 137 | X | X | 138 |
| | 2 | 137 A | X | X | 247 |
| | 3 | 138 | X | X | 248 |
| | 4 | 139 | X | X | 46 |
| | 5 | 140 | X | X | 19 |
| | 6 | 141 | X | X | 23 |
| | 7 | 142 | X | | 26 |
| | 8 | 143 | X | X | 9 |
| | 9 | 144 | X | X | 32 |
| | 10 | 145 | X | X | 47 |
| | 11 | 146 | X | X | 76 |
| | 12 | 147 | X | X | 96 |
| 2001 | 1 | 148 | X | X | 146 |
| | 2 | 149 | X | X | 91 |
| | 2 | 149 A | X | X | 55 |
| | 3 | 150 | X | X | 127 |
| | 3 | 150 A | X | | 158 |
| | 4 | 151 | X | X | 52 |
| | 5 | 152 | X | X | 45 |
| | 6 | 153 | X | X | 8 |
| | 7 | 154 | X | X | 28 |
| | 8 | 155 | X | X | 22 |
| | 9 | 156 | X | X | 39 |
| | 10 | 157 | X | X | 38 |
| | 11 | 158 | X | X | 64 |
| | 12 | 159 | X | X | 88 |
| 2002 | 1 | 160 | X | X | |
| | 1 | 160 A | X | X | |
| | 2 | 161 | X | X | 158 |
| | 2 | 161 A | | | 128 |

| | | | | | |
|------|----|-------|---|---|------------|
| | 3 | 162 | X | X | 152 |
| | 3 | 162 A | X | X | |
| | 4 | 163 | X | X | 49 |
| | 5 | 164 | X | X | 27 |
| | 6 | 165 | X | X | 25 |
| | 7 | 166 | X | | 19 |
| | 8 | 167 | X | X | 13 |
| | 9 | 168 | X | X | 48 |
| | 10 | 169 | X | X | 35 |
| | 11 | 170 | X | X | 65 |
| | 12 | 171 | X | X | 75 |
| 2003 | 1 | 172 | X | X | 112 |
| | 2 | 173 | X | X | 215 |
| | 2 | 173 A | X | X | |
| | 3 | 174 | X | X | 90 |
| | 3 | 174 A | X | X | 104 |
| | 4 | 175 | X | X | 31 |
| | 5 | 176 | X | X | 47 |
| | 7 | 177 | X | X | 19 |
| | 7 | 178 | X | X | 17 |
| | 8 | 179 | X | X | 25 |
| | 9 | 180 | X | X | 26 |
| | 10 | 181 | X | X | 53 |
| | 12 | 183 | X | X | 99 |
| 2004 | 1 | 184 A | X | X | 193 |

Depths (Surface and 200) are in meters below the surface. MLD = Mixed Layer Depth, calculated as the depth where sigma-t was equal to sea surface sigma-t plus an increment in sigma-t equivalent to a 0.2°C temperature decrease (Sprintall & Tomczak 1992). The month of deepest mixing is indicated in bold font. Red X's indicate samples used for the analysis of residuals for non-normal distribution after seasonal differencing. PERMANOVA analyses of the period before and after the 1994-1997 gap for both surface and 200 m samples did not show convincing evidence for sample composition differences compared to other tests of equal sized samples from different time periods although differences in NTU distributions appeared to drive some sample separation.

Table S2. NTUs with the greatest number of connections.

| Network | NTU | Number of Connections | Phylogenetic group |
|---------|-----|-----------------------|---------------------------|
| Surface | 519 | 243 | Gammaproteobacteria |
| | 521 | 215 | Gammaproteobacteria |
| | 933 | 203 | Alphaproteobacteria |
| | 791 | 200 | Gammaproteobacteria |
| | 524 | 191 | Gammaproteobacteria |
| | 497 | 188 | Gammaproteobacteria/SAR86 |
| | 420 | 184 | Gammaproteobacteria/SAR86 |
| | 455 | 184 | Gammaproteobacteria/SAR86 |
| 200 m | 455 | 268 | Gammaproteobacteria/SAR86 |
| | 457 | 268 | Gammaproteobacteria/SAR86 |
| | 467 | 255 | Gammaproteobacteria/SAR86 |
| | 456 | 249 | Gammaproteobacteria/SAR86 |
| | 420 | 235 | Gammaproteobacteria/SAR86 |
| | 418 | 223 | Gammaproteobacteria/SAR86 |
| | 792 | 209 | Gammaproteobacteria |
| | 415 | 208 | Gammaproteobacteria/SAR86 |

NTU (nodal taxonomic unit) refers to nodes on the reference phylogenetic tree to which sequences were binned. Nodes were numbered sequentially so values closer together have greater phylogenetic relatedness.

Table S3. PERMANOVA results for phylogenetic groups within the δ matrix

| Network | Phylum/Class | | Clade | |
|---------|-----------------------|-----------------------|----------|----------|
| | Pseudo-F ^a | Pairwise ^b | Pseudo-F | Pairwise |
| Surface | 5.74 | 129/231 | 10.4 | 34/36 |
| 200 m | 1.04 | 121/210 | 1.07 | 36/36 |

a – Pseudo-F statistic from the main test comparison. All pseudo-p values are < 0.001.

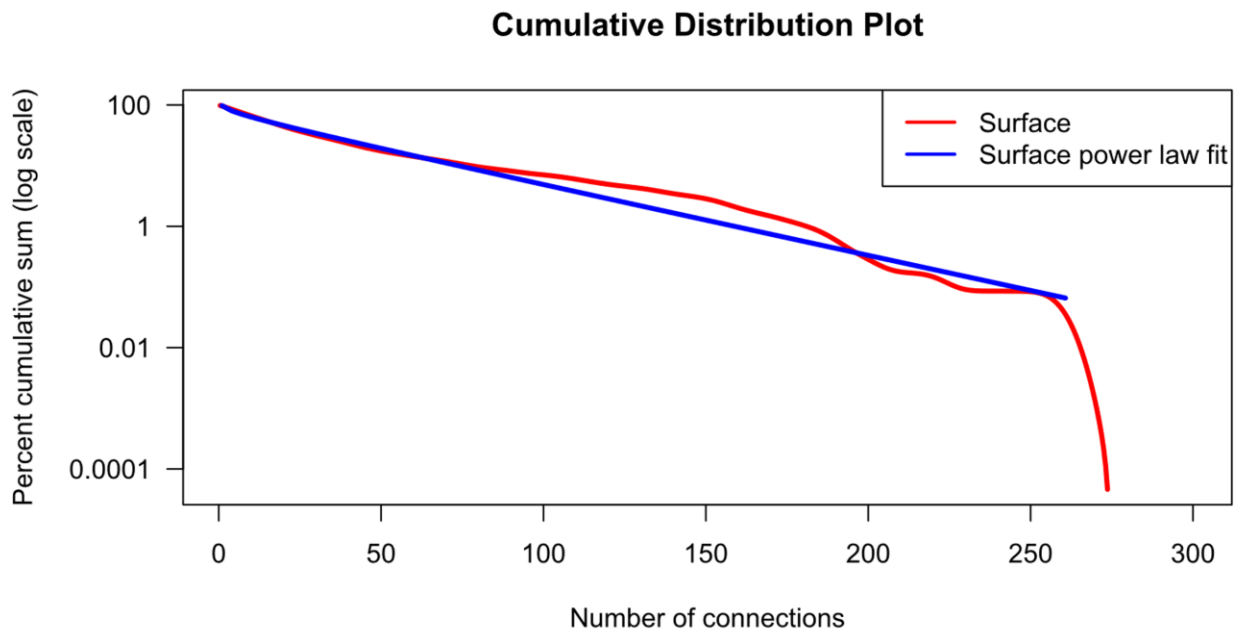
b – Number of significant pairwise tests compared to the total number of tests.

Table S4. Phylogenetic classifications for NTU's shown in Figure 1.

| Figure 1 panel | NTU number (Range from 1 to 2840) | Clade | Phylum (Class for Proteobacteria) |
|----------------|-----------------------------------|-------------------------|-----------------------------------|
| A | 440 | SAR86 IIIa | Gammaproteobacteria |
| A | 822 | Roseobacter Oct lineage | Alphaproteobacteria |
| A | 1085 | SAR116 Ib | Alphaproteobacteria |
| A | 1135 | SAR116 IIIa | Alphaproteobacteria |
| A | 1257 | SAR11 Ib | Alphaproteobacteria |
| A | 1531 | Undesignated | Bacteroidetes |
| A | 1611 | Undesignated | Bacteroidetes |
| A | 1786 | Undesignated | Bacteroidetes |
| B | 132 | Undesignated | Gammaproteobacteria |
| B | 510 | Undesignated | Gammaproteobacteria |
| B | 934 | Undesignated | Alphaproteobacteria |
| B | 1015 | Undesignated | Alphaproteobacteria |
| B | 1292 | SAR11 | Alphaproteobacteria |
| B | 1843 | SAR406 | Deltaproteobacteria |
| B | 1935 | Undesignated | Verrucomicrobia |
| B | 2499 | Undesignated | Clostridiales |
| C | 534 | Undesignated | Gammaproteobacteria |
| C | 721 | OM43 | Betaproteobacteria |
| C | 1069 | SAR116 Ib | Alphaproteobacteria |
| C | 1132 | SAR116 IIIa | Alphaproteobacteria |
| C | 1583 | Undesignated | Bacteroidetes |
| C | 1625 | Undesignated | Bacteroidetes |
| C | 1651 | Undesignated | Bacteroidetes |
| C | 1956 | Stramenopiles | Cyanobacteria |
| D | 336 | Undesignated | Gammaproteobacteria |
| D | 343 | Undesignated | Gammaproteobacteria |
| D | 522 | Xanthomonadales | Gammaproteobacteria |
| D | 746 | Xanthomonadales | Gammaproteobacteria |
| D | 936 | Rhizobiales | Alphaproteobacteria |
| D | 1230 | SAR11 Ia | Alphaproteobacteria |
| D | 1918 | Planctomyces | Planctomyces |
| D | 1989 | Plastid | Cyanobacteria |
| E | 358 | OM182 | Gammaproteobacteria |
| E | 455 | SAR86 | Gammaproteobacteria |
| E | 535 | Undesignated | Gammaproteobacteria |
| E | 908 | Undesignated | Alphaproteobacteria |
| E | 1125 | SAR116 IIIb | Alphaproteobacteria |
| E | 1232 | SAR11 Ia | Alphaproteobacteria |
| E | 1272 | SAR11 IIa | Alphaproteobacteria |
| E | 1545 | Undesignated | Bacteroidetes |
| F | 785 | Rhodocyclales | Betaproteobacteria |
| F | 930 | Undesignated | Alphaproteobacteria |
| F | 1170 | Undesignated | Alphaproteobacteria |

| | | | |
|---|------|-----------------|-----------------------|
| F | 1259 | SAR11 Ic | Alphaproteobacteria |
| F | 1460 | SAR276 | Deltaproteobacteria |
| F | 1511 | Undesignated | Deltaproteobacteria |
| F | 1893 | Undesignated | Unclassified Bacteria |
| F | 1919 | Undesignated | Planctomyces |
| G | 342 | Undesignated | Gammaproteobacteria |
| G | 594 | Undesignated | Betaproteobacteria |
| G | 894 | Roseobacter | Alphaproteobacteria |
| G | 906 | Roseobacter | Alphaproteobacteria |
| G | 1016 | Rhodobacterales | Alphaproteobacteria |
| G | 1165 | Undesignated | Alphaproteobacteria |
| G | 1430 | SAR324 I | Deltaproteobacteria |
| G | 1817 | Undesignated | Bacteroidetes |
| H | 409 | SAR86 II | Gammaproteobacteria |
| H | 497 | SAR86 SPOTS | Gammaproteobacteria |
| H | 852 | Roseobacter | Alphaproteobacteria |
| H | 874 | Roseobacter | Alphaproteobacteria |
| H | 1071 | SAR116 Ib | Alphaproteobacteria |
| H | 1093 | SAR116 Ia | Gammaproteobacteria |
| H | 1104 | SAR116 IIb | Alphaproteobacteria |
| H | 1240 | SAR11 Ia | Alphaproteobacteria |

A.



B.

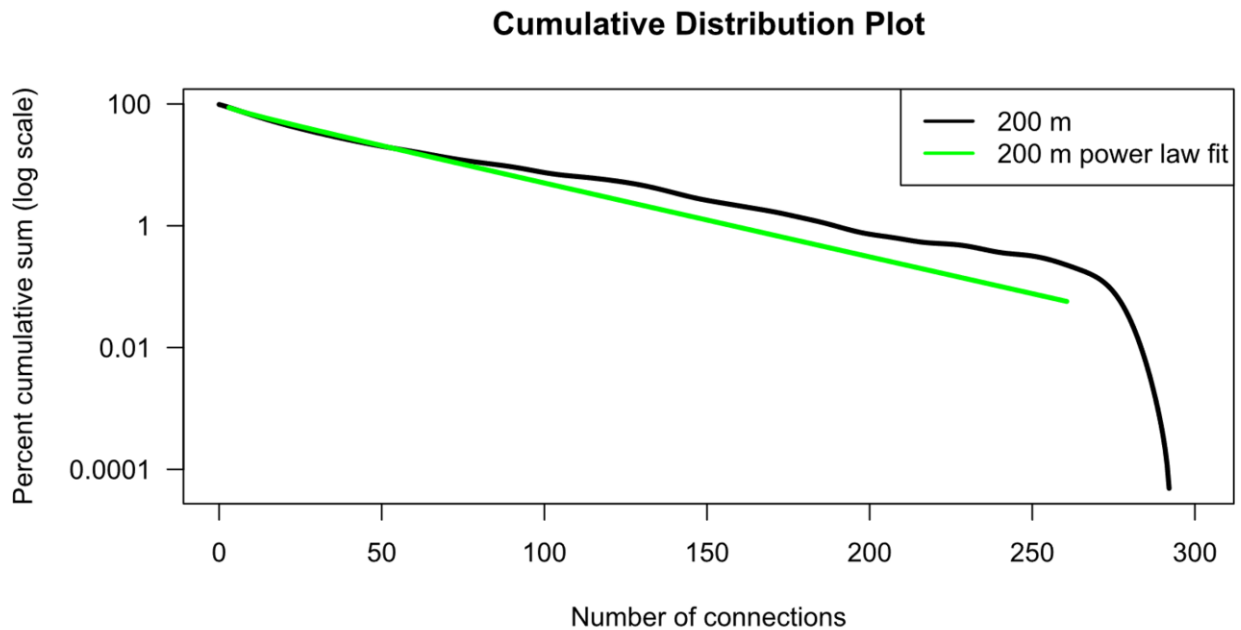


Figure S1. Cumulative distribution plots for the number of connections for each NTU (X axis). Cumulative percent is shown on the Y axis. Surface network (A) NTUs are shown in red and 200 m network (B) NTUs are shown in black. Truncated power law fits for surface (blue) and 200 m (green) are included. Power law fits were calculated from $F(x) = x^{-a}e^{-bx} + c$.

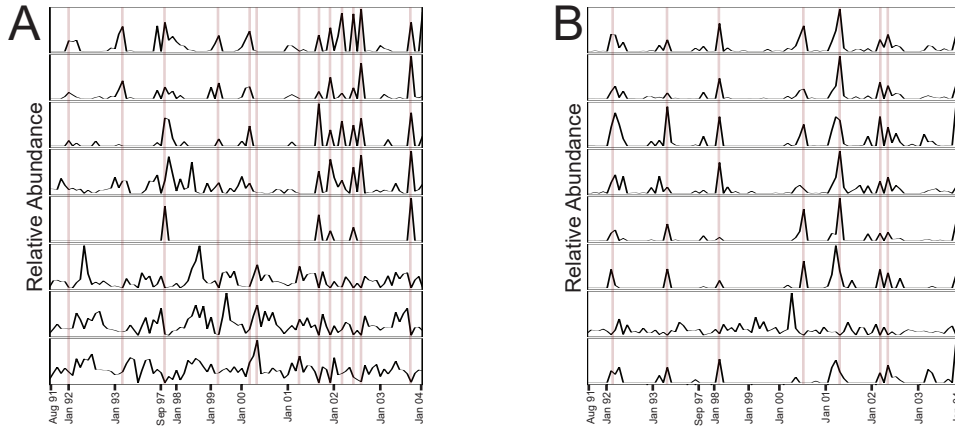


Figure S2. Relative abundance plots for the eight NTUs with the greatest number of connections at the surface (A) and 200 m (B). The most connected NTUs are frequently present in the system, especially during deep mixing events. Relative abundance of amplicon sequences (Y axis) for individual NTU's over the entire concatenated time series (X axis) are shown. The first months for each calendar year are marked on the X axis. The rose colored lines indicate periods of relative abundance for several NTUs

Supplementary methods for “Network pruning using diagnostic filtering to remove potentially spurious correlations”

In a linear regression model for the distributions of two NTU's, the residuals should be independent and normally distributed. The distribution of the residuals may not be normal if there is seasonality or other structure in the distribution of the NTU. To simulate the extent of this non-normality, we used an R simulation (written by Charlotte Wickham, Oregon State University, and used with her permission). Two random data series are generated. The `arma.sim` function in the `stats` package can impose structure on the data by inputting values other than 0 for auto-regression or moving average. The command `order = c(0,0,0)` produces white noise. If X and Y are both white noise, repeating the simulation 1000 times usually results in fewer than 50 rejections (p less than 0.05) of the null hypothesis when the null hypothesis is false (type I error). ARIMA values for one data series generally still have a p value less than 0.05. However, structure in both series results in much higher p values and may result in higher confidences for spurious correlations.

```
#both white noise
one_sim <- function(){
  x <- arima.sim(list(), n = 100)
  y <- 1 + arima.sim(list(), n = 100)
  fit <- arima(y, order = c(0, 0, 0), xreg = x)

  abs( fit$coef["x"] /
    sqrt(diag(fit$var.coef)["x"]) ) 1.96
}
```

```
reject <- replicate(1000, one_sim())
table(reject)
reject
FALSE TRUE
954 46
```

```
#one white noise, one structured
one_sim <- function(){
  x <- arima.sim(list(order = c(1,0,0), ar = 0.7), n = 100)
  y <- 1 + arima.sim(list(), n = 100)
  fit <- arima(y, order = c(0, 0, 0), xreg = x)

  abs( fit$coef["x"] /
    sqrt(diag(fit$var.coef)["x"]) ) 1.96
}
```

```
reject <- replicate(1000, one_sim())
table(reject)
reject
FALSE TRUE
950 50
```

```
#both structured
one_sim <- function(){
  x <- arima.sim(list(order = c(1,0,0), ar = 0.7), n = 100)
  y <- 1 + arima.sim(list(order = c(1,0,0), ar = 0.9), n = 100)
  fit <- arima(y, order = c(0, 0, 0), xreg = x)

  abs( fit$coef["x"] /
    sqrt(diag(fit$var.coef)["x"]) ) 1.96
}

reject <- replicate(1000, one_sim())
table(reject)
reject
FALSE TRUE
663 337
```

Supplementary Text

Explanation of Phylogenetically Weighted Connectivity (∂)

To understand this strategy, consider the following example. Suppose a network of university professors was made based on who knows whom. All professors have a list of professors that they know (also known as a profile). Suppose we wanted to calculate similarities between lists. A faculty member in microbiology who studies marine microorganisms works with oceanographers, and a particular oceanographer (“X”) is on their list. Now consider a faculty member in physics who studies ocean wave dynamics and works with another oceanographer (“Y”). When we calculate a similarity between the microbiologist and the physicist (who are not necessarily connected), the oceanographers (“X” and “Y”) are not a match but we can assign a weight to the relationship because they are both in the same department. Thus, the similarity between the microbiologist and physicist is higher because both have connections to oceanographers. By analogy, we infer that they play more similar roles in the university ecosystem because they both study oceanographic topics, although the overall similarities may not be high because they may not work with the same people. Subsequent analyses may use department affiliations as categories in the same way that phylogenetic classifications are used for the BATS network.

Explanation of the spectral clustering strategy

To understand this approach, and to contrast it with the previous weighted approach, again consider the hypothetical university professor network. In this case, only the similarity of direct connections is considered. A cluster may be formed from the microbiologist, oceanographer “X”, a zoologist who studies coral disease, a fisheries biologist who studies marine fish ecology, and an ecologist who studies fungal interactions. This cluster could be described as a life science community, whereas the physicist might be in a separate physical science community. Both communities might include members from many different departments, and the communities would function in parallel to each other.