

## **Supplementary Materials for**

### **Fingerprints of Holocene climate on the functional trait diversity of Arctic marine biota**

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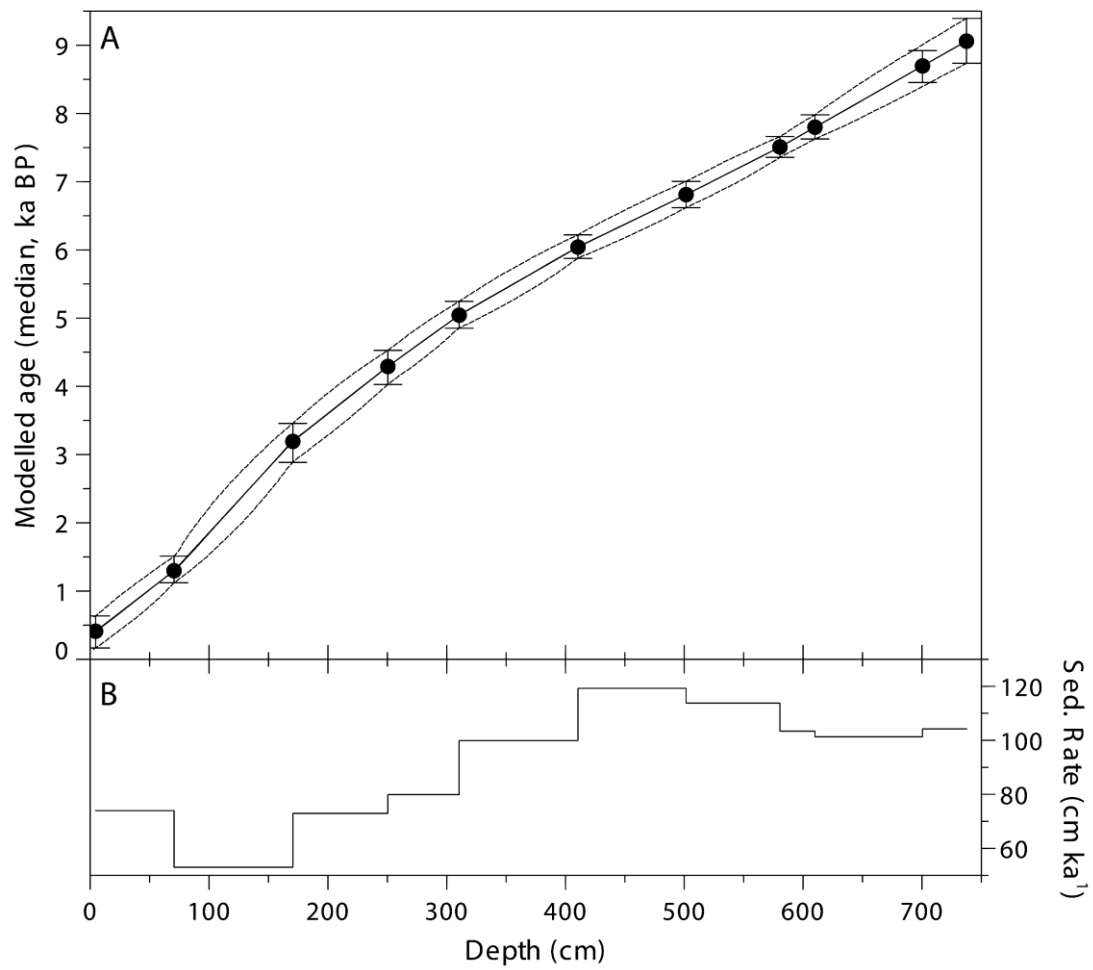
Email: [hz@geus.dk](mailto:hz@geus.dk); [sri@geus.dk](mailto:sri@geus.dk)

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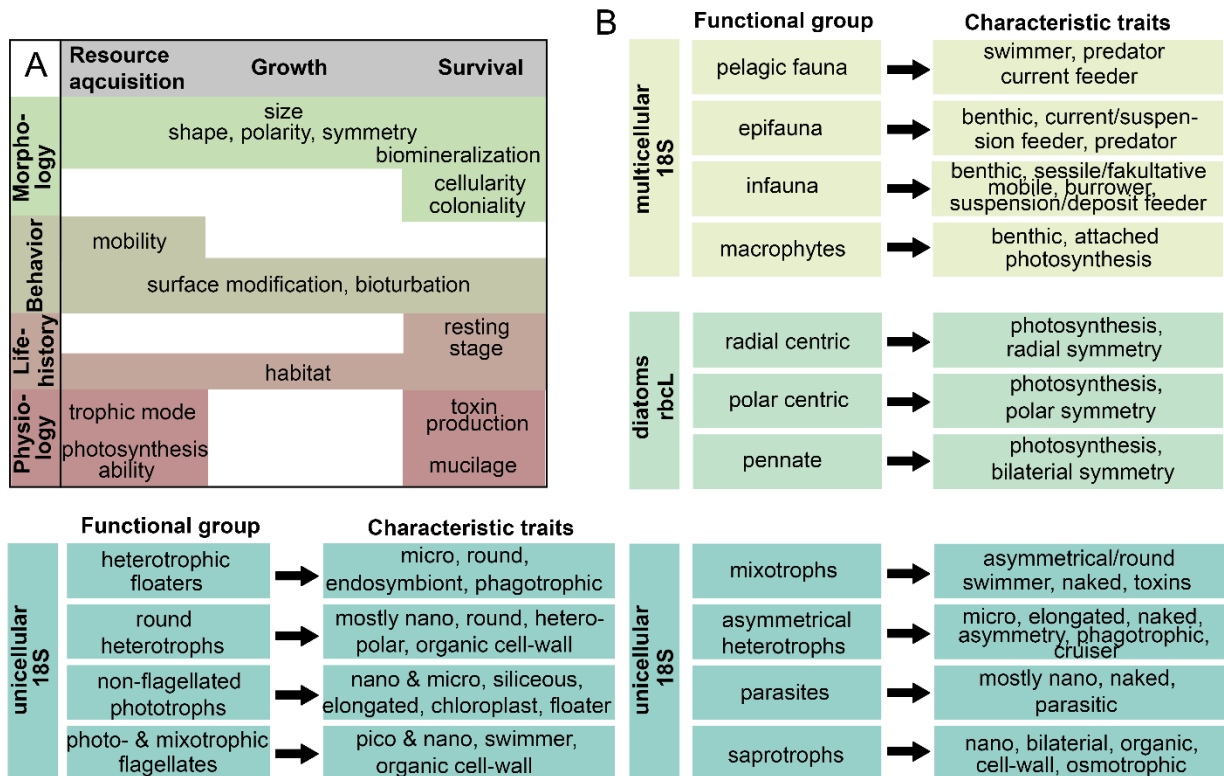
Supplementary Figs. 1 to 21

Supplementary Tables 1 to 2

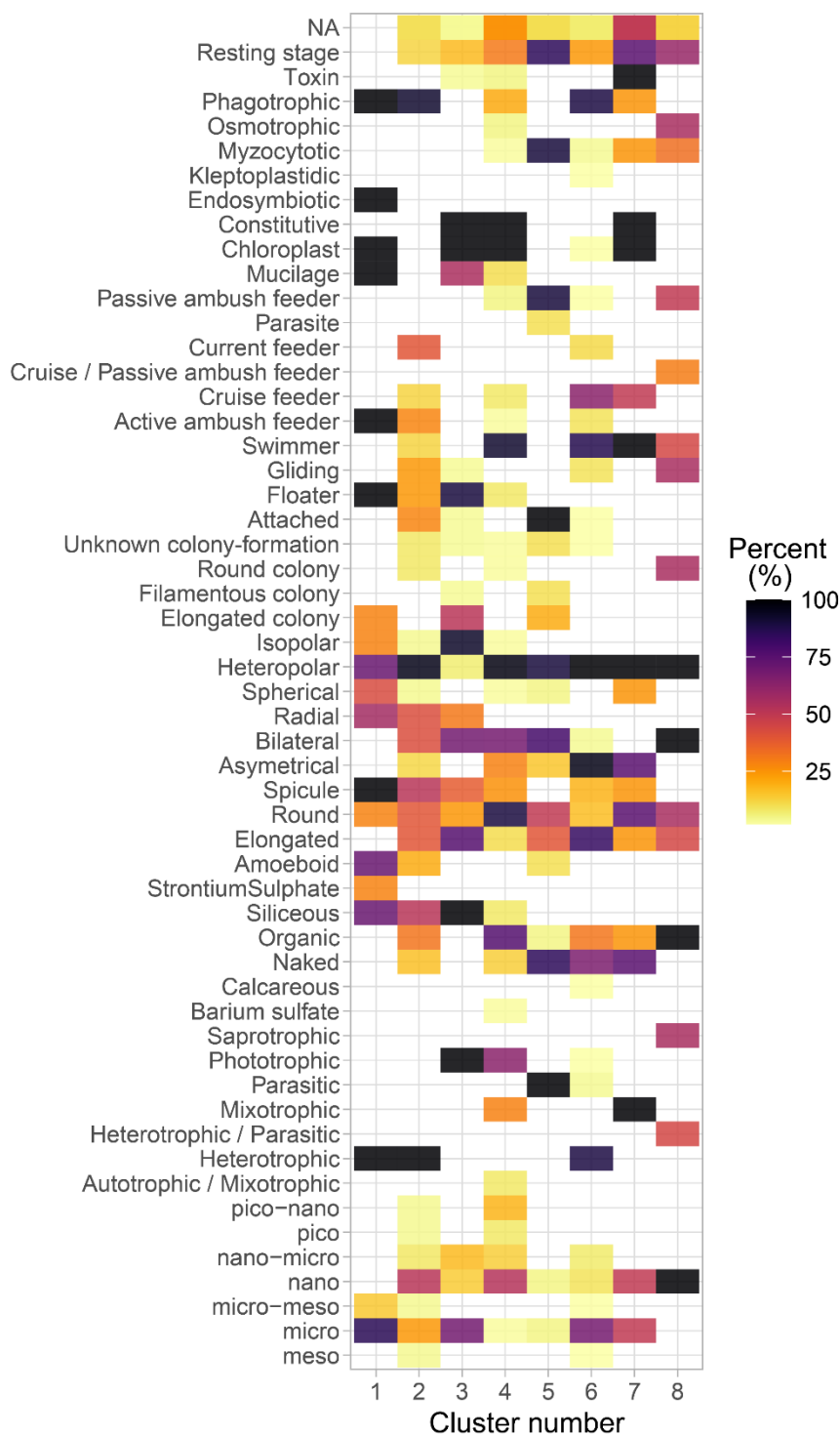
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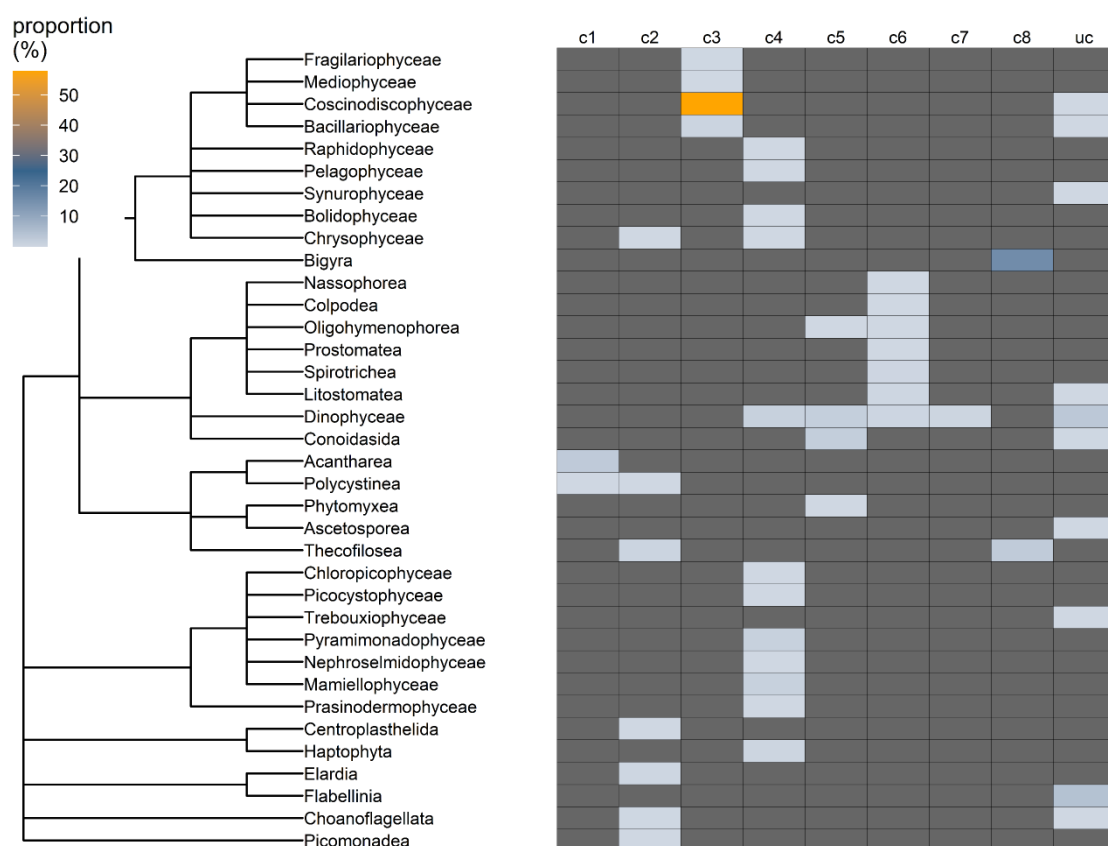
**Supplementary Figure 1.** Age-depth model (A) and resulting sedimentation rates (B) for AMD14-204. The age-depth model was updated using the marine20 calibration curve<sup>1</sup> and a local reservoir correction of  $-93 \pm 111$  years<sup>2</sup>.



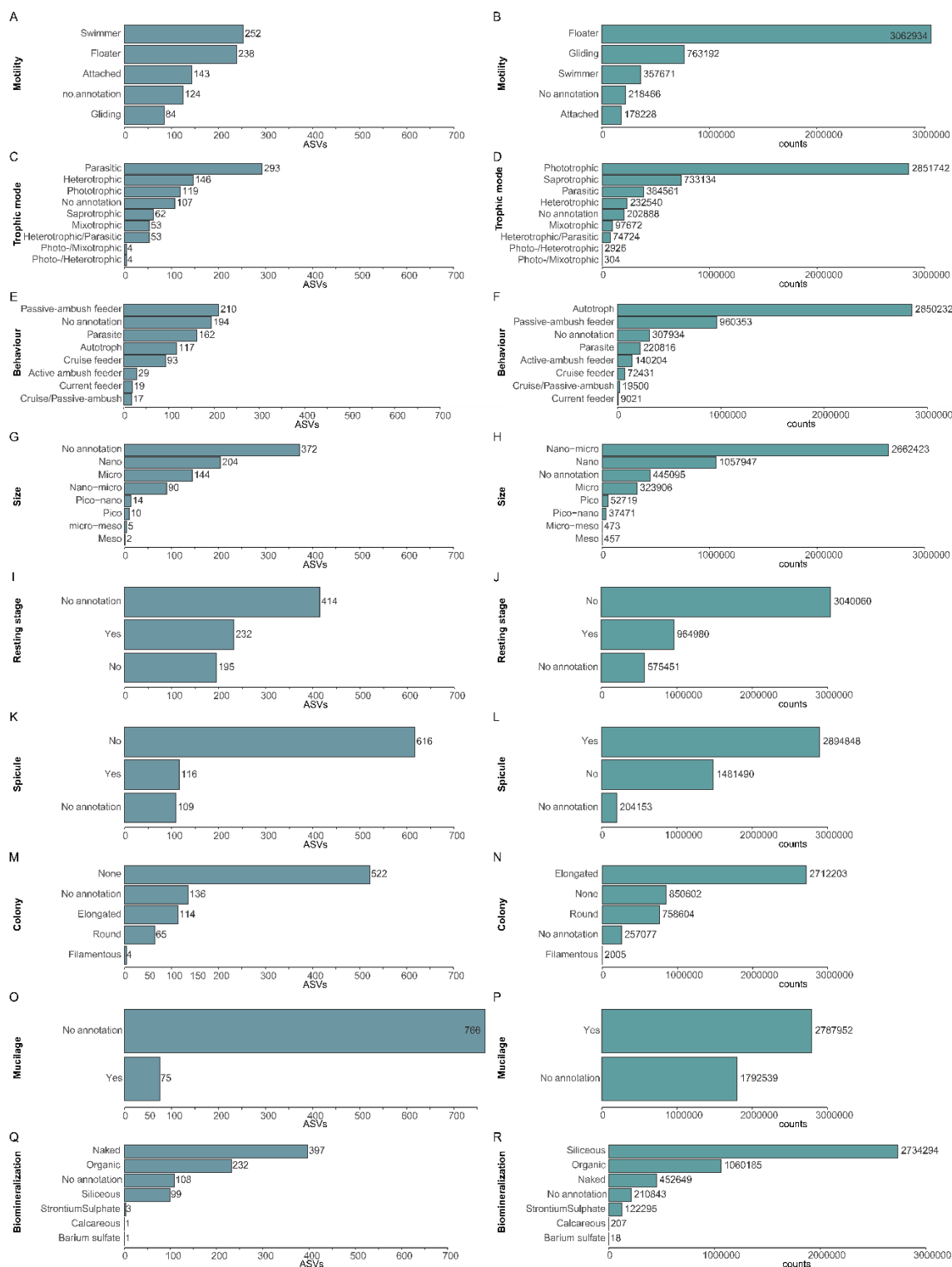
**Supplementary Figure 2.** Functional trait framework. (A) Overview of functional traits modified after Martini et al.<sup>3</sup>. (B) Overview of assigned functional groups, sorted by level of cellularity, and the relevant trait modalities that are characteristic for these groups according to agglomerative clustering (18S unicellular). Multicellular taxa (18S) were grouped by habitat. Only “shape” was considered for the rbcL dataset.



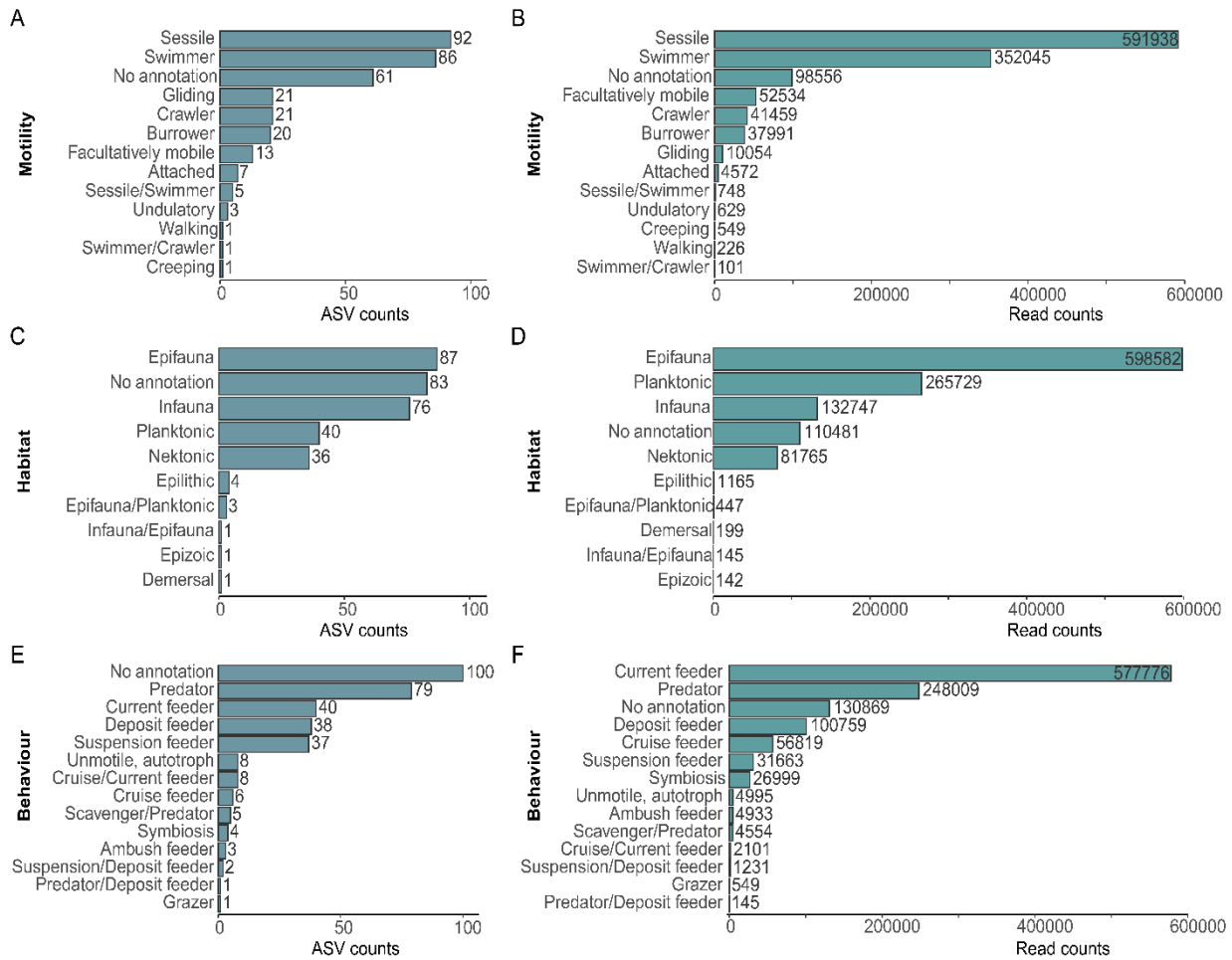
**Supplementary Figure 3.** Distribution of protist trait modalities across the eight clusters for the 18S data derived by agglomerative clustering. The proportional contributions show each factor level in the relative abundance of cluster members colored from yellow (low percentage) to black (high percentage).



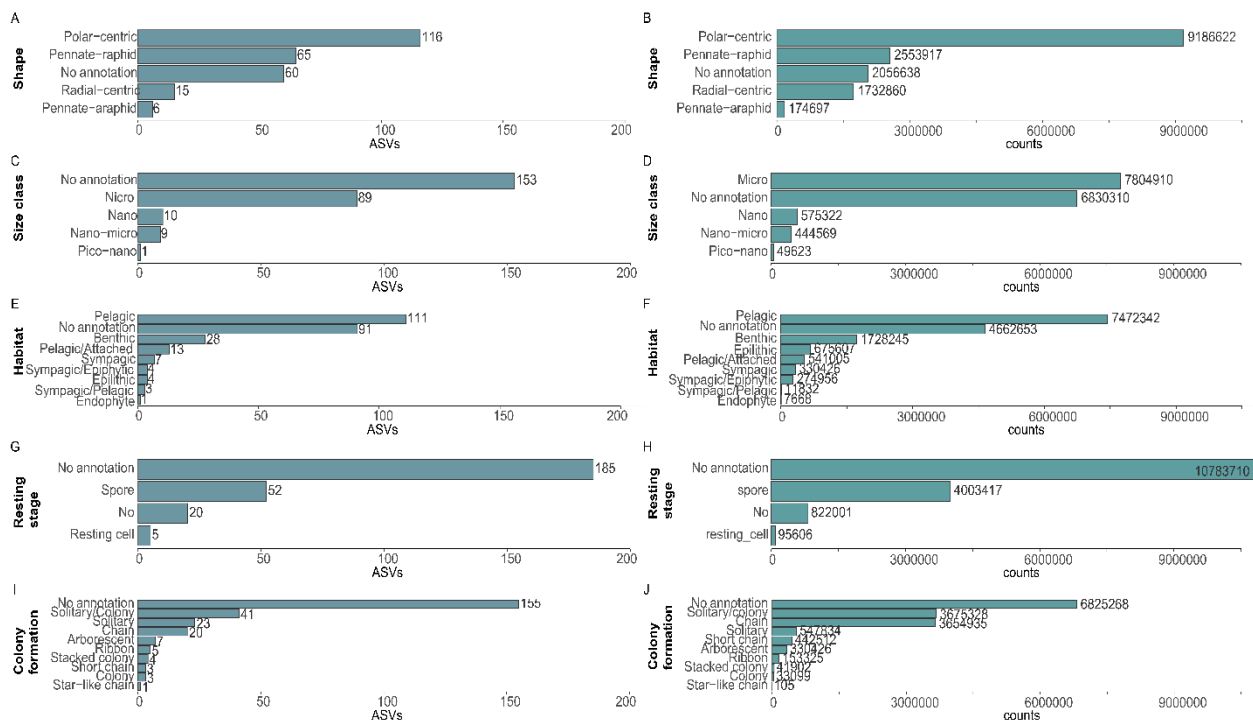
**Supplementary Figure 4.** Phyloheatmap showing the proportions of unicellular classes (retrieved with the 18S marker) in the protist functional groups. The proportions of classes lacking trait annotations are indicated as unclustered (uc). High proportions (in percent) are indicated by orange color, while from medium-to-low proportions, the colors transition from dark to light blue. Absence in a sample is indicated in grey. Clusters were named according to defining traits of Supplementary Figure 10: c1 = heterotrophic floaters; c2 = organic-walled, round heterotrophs; c3 = non-flagellated, siliceous phototrophs; c4 = photo- and mixotrophic flagellates; c5 = parasites; c6 = asymmetrical heterotrophs; c7 = mixotrophs including toxin producers; c8 = saprotrophs.



**Supplementary Figure 5.** Overview on traits and their modalities for unicellular taxa (18S) regarding the numbers of ASVs in blue (A, C, E, G, I, K, M, O, Q) and read counts in turquoise (B, D, F, H, J, L, N, P, R). If no information for a given trait was found, it was marked as “no annotation”.

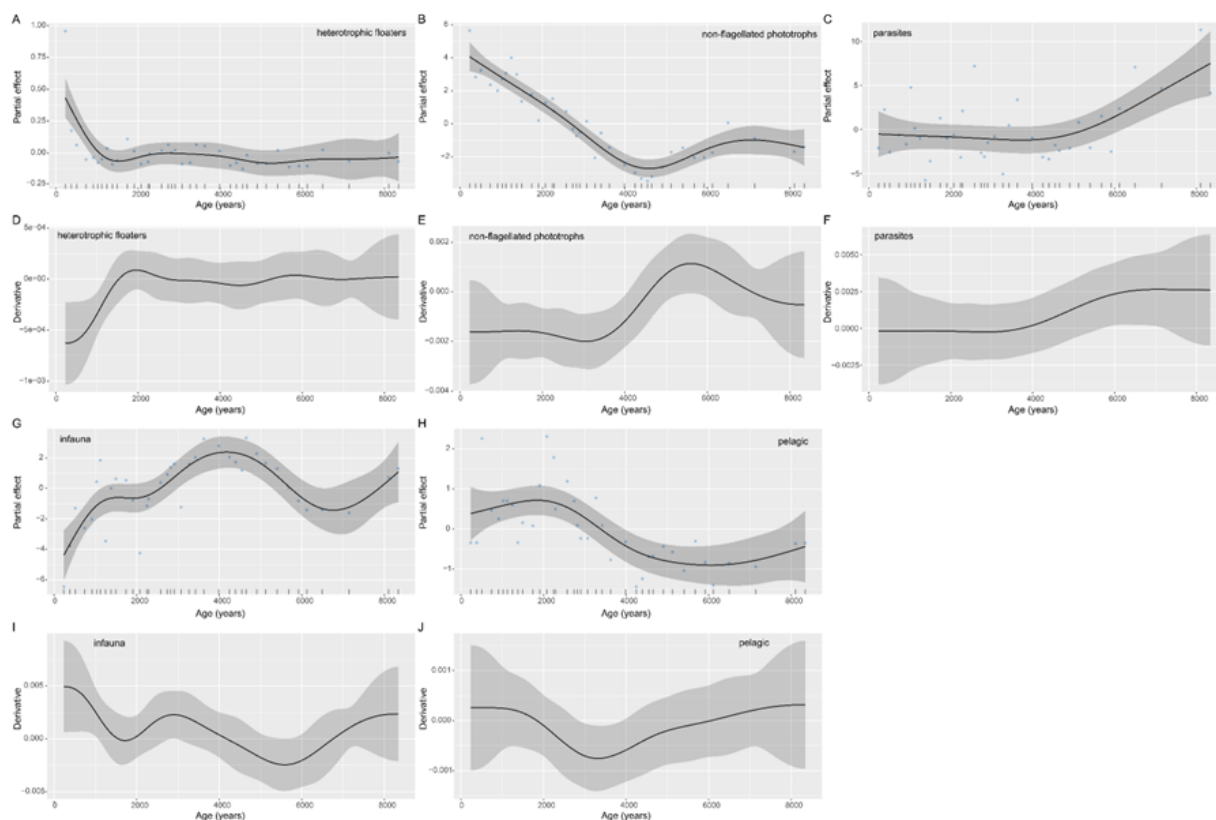


**Supplementary Figure 6.** Overview on traits and their modalities for multicellular taxa (18S) regarding the numbers of ASVs in blue (A, C, E) and read counts in turquoise (B, D, F). If no information for a given trait was found, it was marked as “no annotation”.

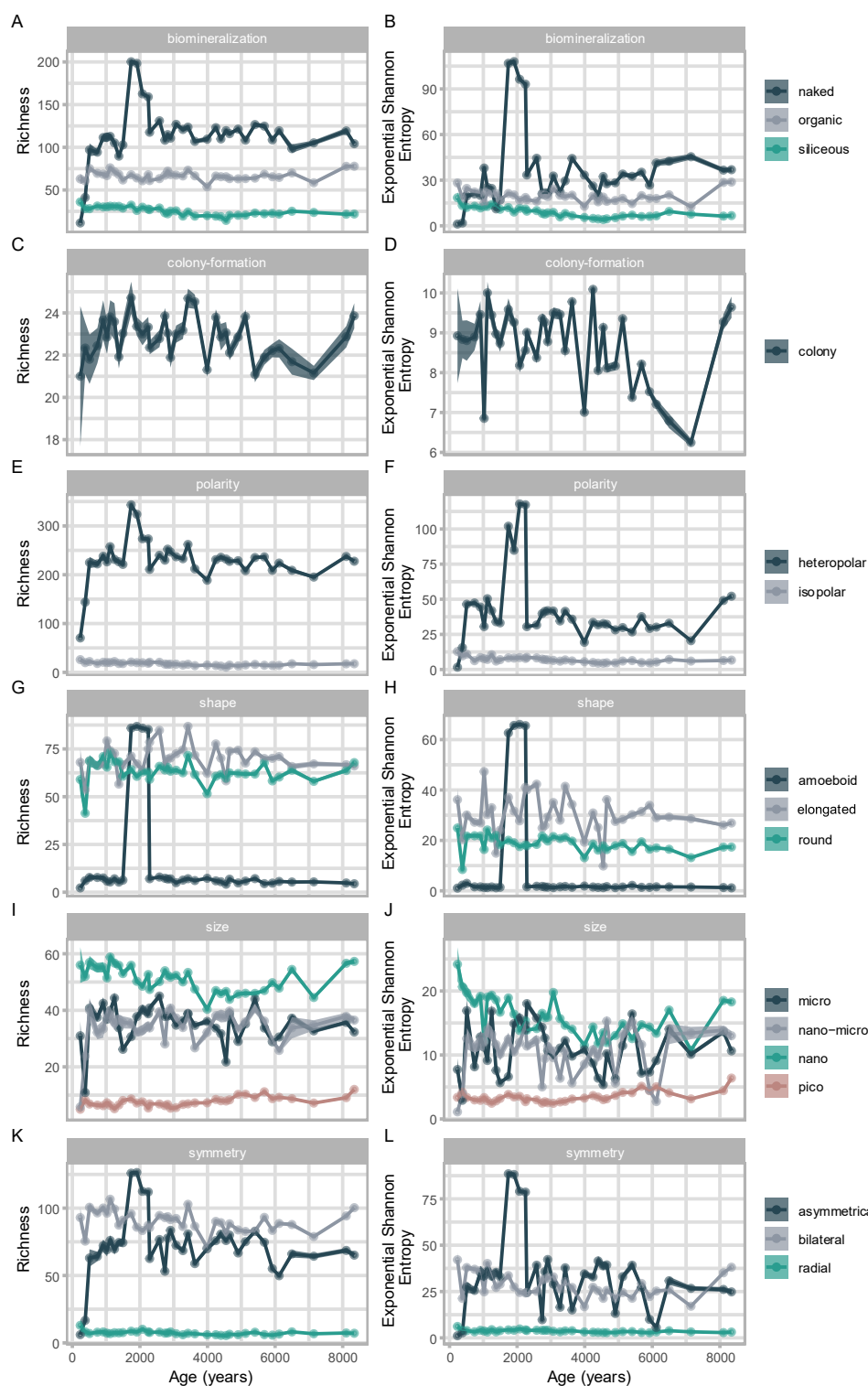


**Supplementary Figure 7.** Overview on traits and their modalities for diatom taxa (rbcL) regarding the numbers of ASVs in blue (A, C, E, G, I) and read counts in turquoise (B, D, F, H, J). If no information for a given trait was found, it was marked as “no annotation”.

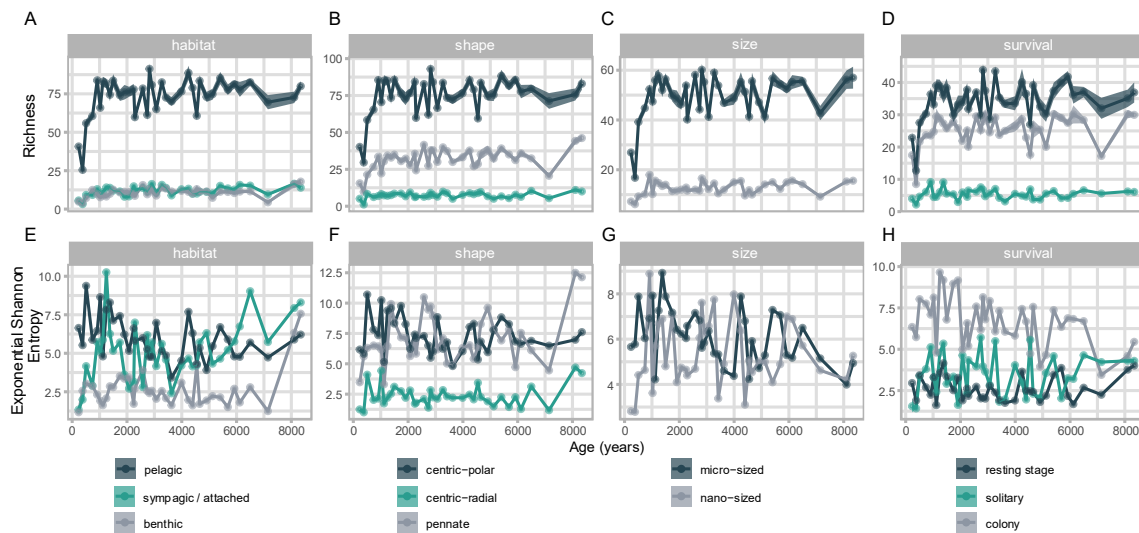




**Supplementary Figure 8.** Generalized additive models (GAMs) and their first derivatives of the functional groups showing a significant temporal change. GAMs (black line) and their corresponding 95% confidence intervals (grey ribbon) were fitted to the Exponential Shannon Entropy time-series of (A) heterotrophic floaters, (B) non-flagellated phototrophs, (C) parasites, (G) infauna, and (H) pelagic fauna (all 18S) using the *gratia* R package using REML smoothness<sup>4</sup>. The first derivatives (black line in D, E, F, I, J) are estimated to detect significant temporal changes, which is indicated by an interval not including zero in the 95% confidence interval (grey ribbon)<sup>5</sup>.



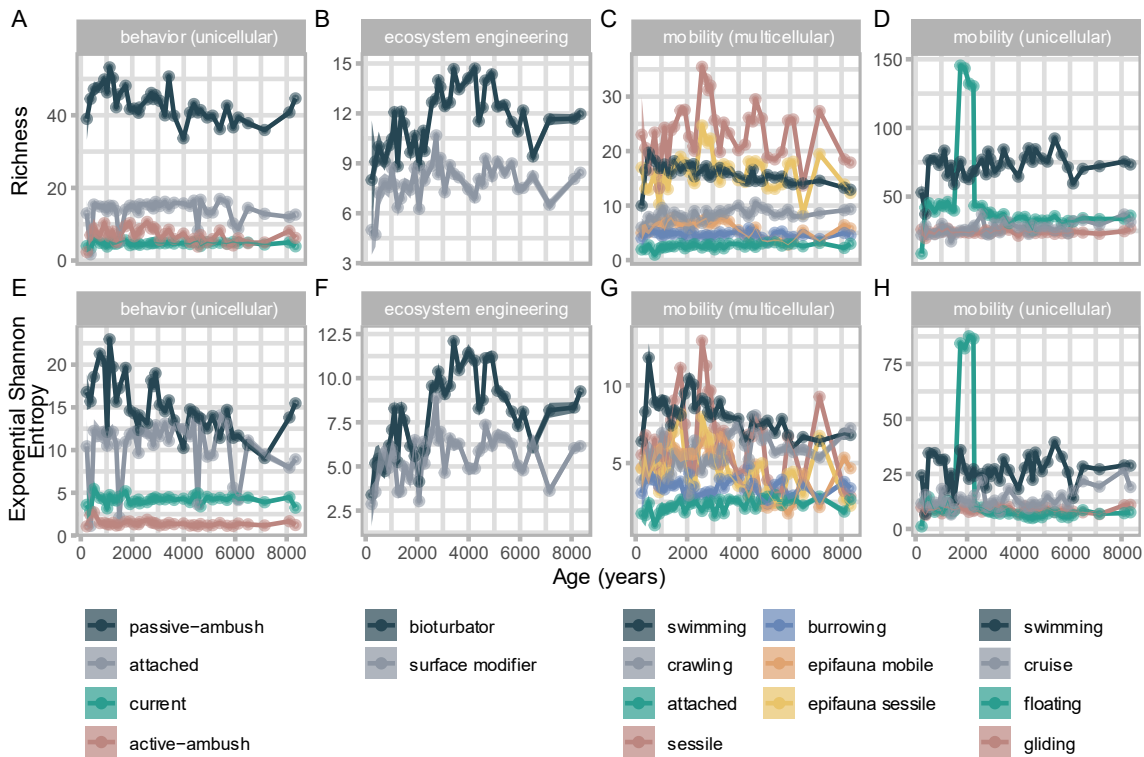
**Supplementary Figure 9.** Diversity estimates for the different traits among morphological strategies (A-L) of unicellular taxa derived by the broad eukaryote marker 18S\_allshorts. The ribbon marks the upper and lower confidence interval of the diversity estimates.



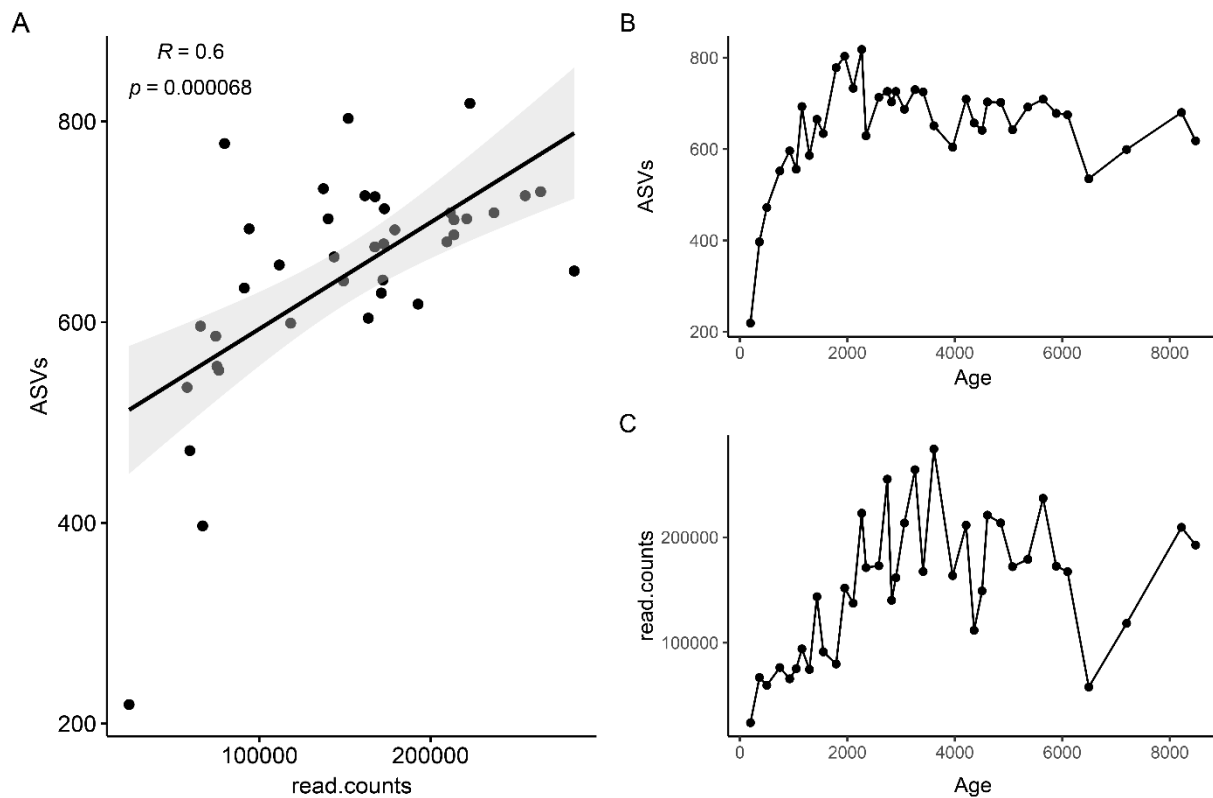
**Supplementary Figure 10.** Diversity estimates for the different traits among ecological strategies of the diatom-specific rbcL dataset (A-H). The ribbon marks the upper and lower confidence interval of the diversity estimates.



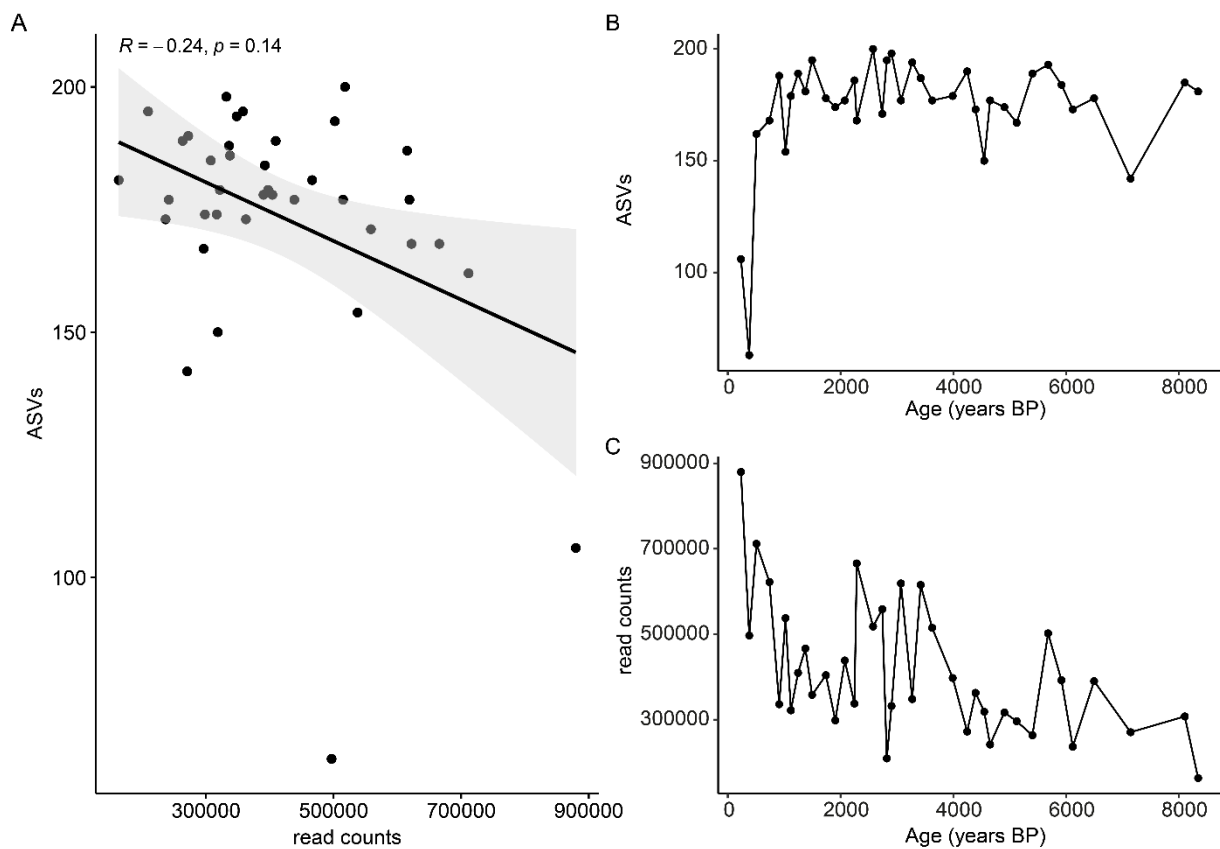
**Supplementary Figure 11.** Diversity estimates for the different traits among physiological strategies (A-F) derived by the broad eukaryote marker 18S\_allshorts. The ribbon marks the upper and lower confidence interval of the diversity estimates.



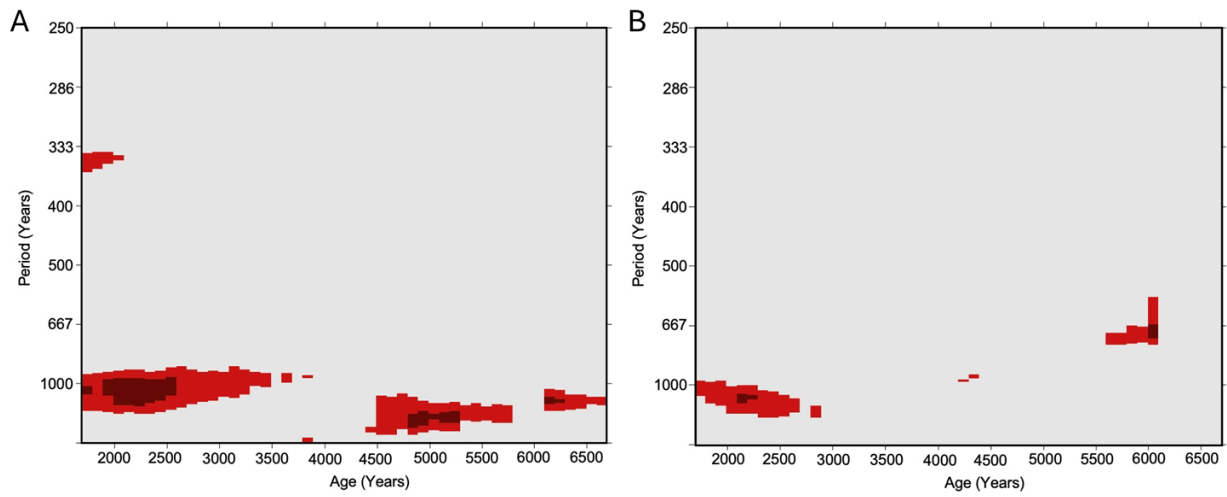
**Supplementary Figure 12.** Diversity estimates for the different traits among behavioral and life history strategies of uni- (A, D, E, H) and multicellular taxa (B, C, F, G) derived by the broad eukaryote marker 18S\_allshorts. The ribbon marks the upper and lower confidence interval of the diversity estimates.



**Supplementary Figure 13.** Overview of ASVs and read counts per sample for the 18S marker. (A) There is a significant ( $p < 0.001$ ) and positive relationship (Spearman rank correlation coefficient  $R = 0.6$ ) between the number of read counts and the number of ASVs. (B) Numbers of ASVs, and (C) number of read counts per sample ordered by age. The low diversity signal in the youngest samples (past ~1000 years in the record) may imply that diversity over the last 1000 years in the record may be underestimated.

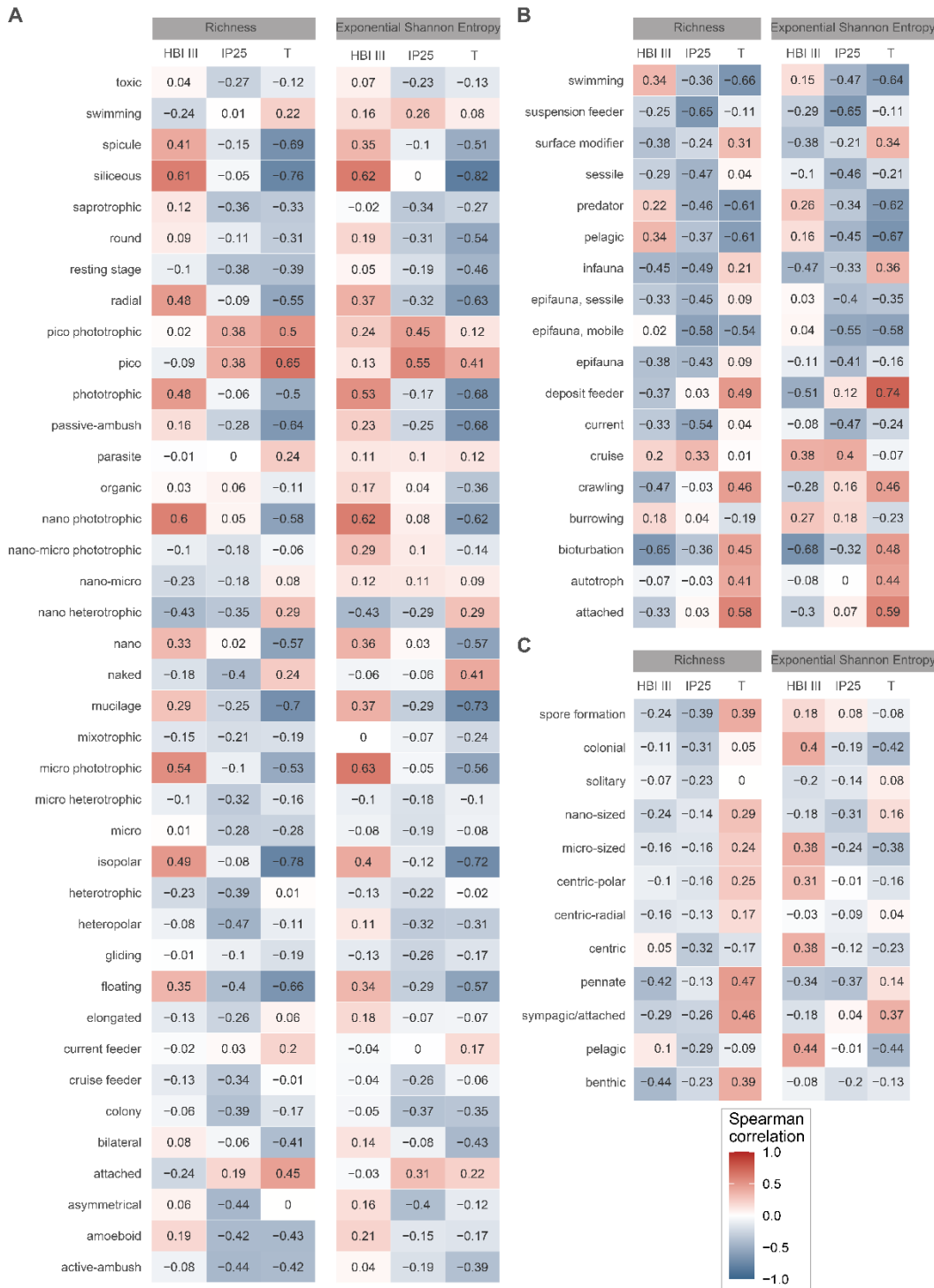


**Supplementary Figure 14.** Overview of ASVs and read counts per sample for the *rbcL* marker. (A) There is a weak, but statistically insignificant ( $p$ -value = 0.14) relationship (Spearman rank correlation coefficient  $R = -0.24$ ) between the number of read counts and the number of ASVs. (B) Numbers of ASVs and (C) number of read counts per sample ordered by age. In contrast to the 18S record, the low diversity signal in the youngest samples (past ~1000 years in the record) manifests despite overall highest read counts.

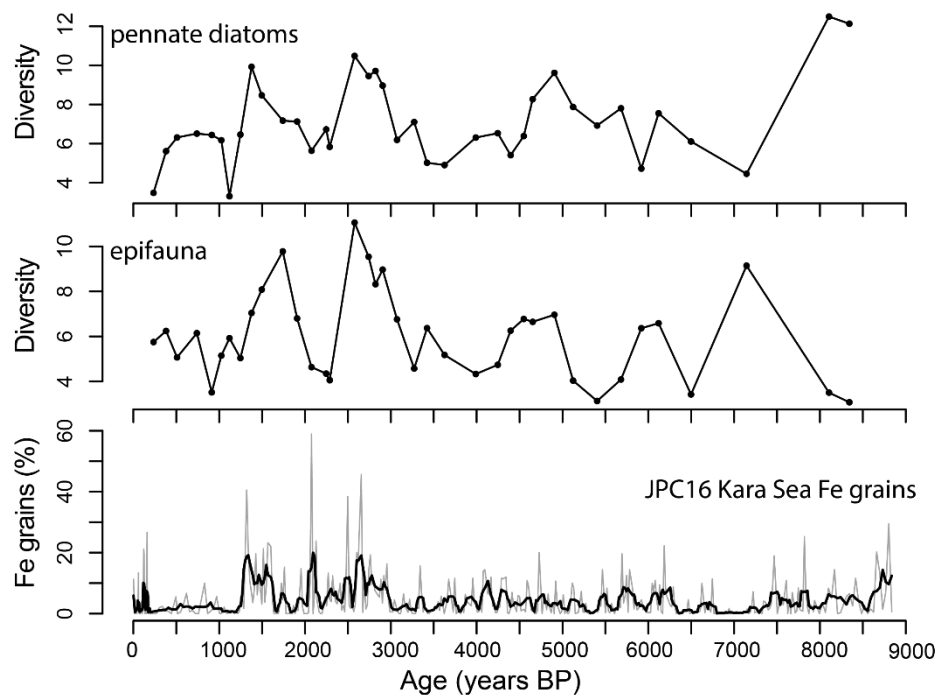


**Supplementary Figure 15.** Lomb-Scargle Periodograms for rarefied Shannon diversity of (A) epifauna (18S), and (B) pennate diatoms (18S) in 3000-year moving windows. The red and dark red colors denote the <95% and >99% false-alarm level respectively.

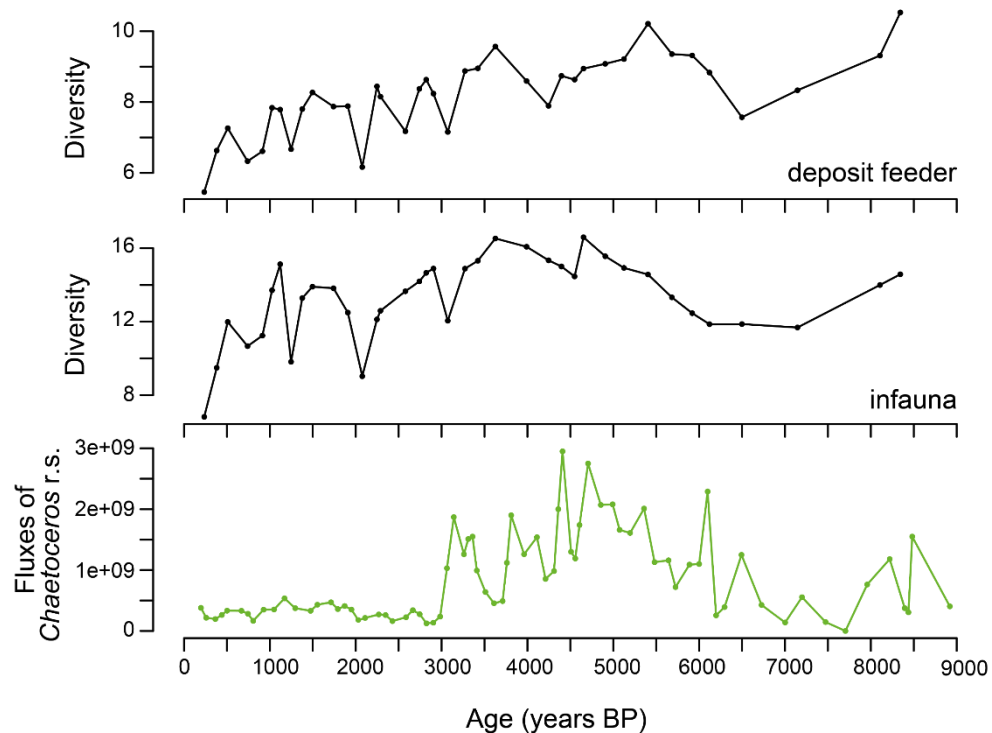




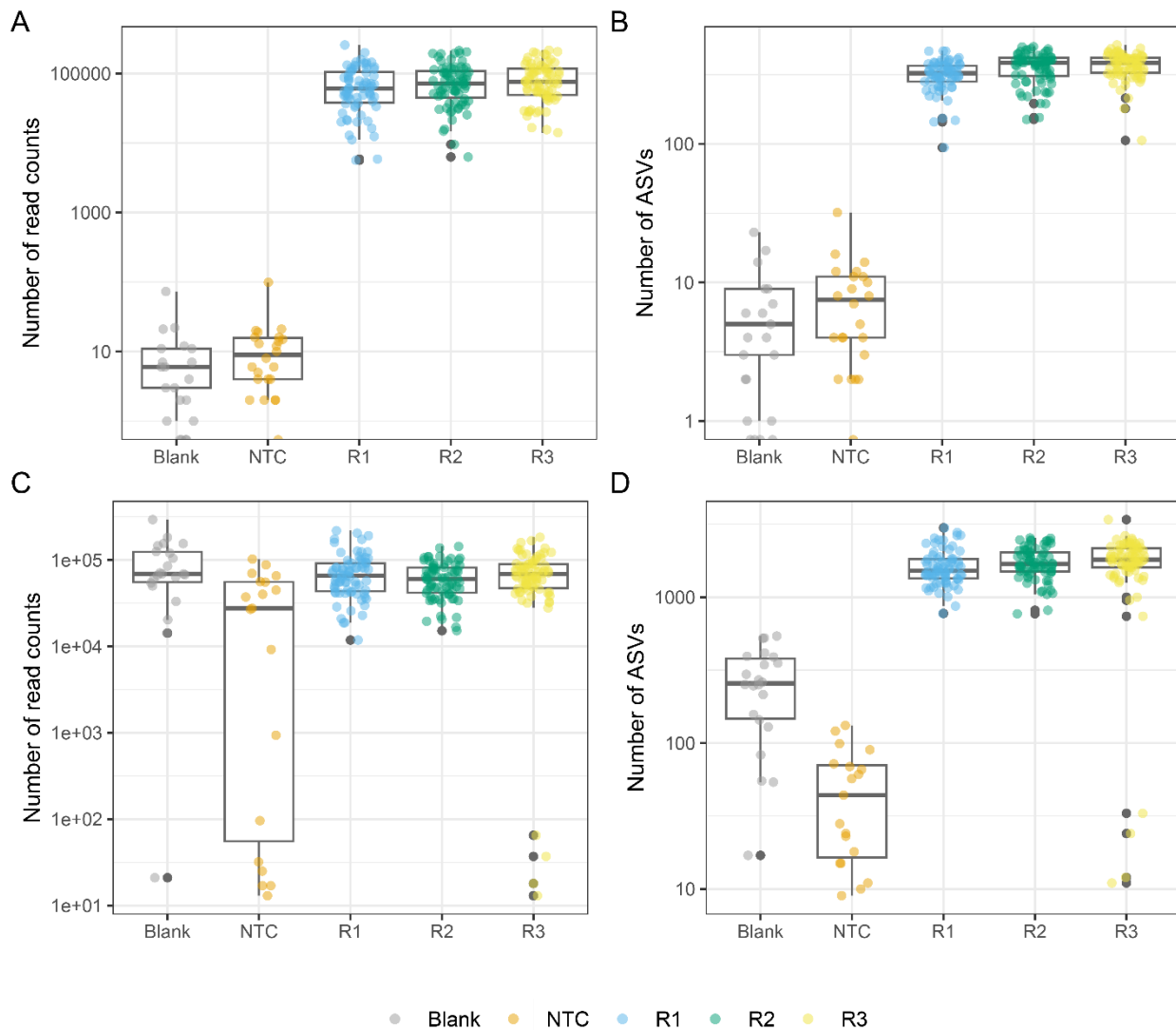
**Supplementary Figure 16.** Within-trait diversity-proxy relationships. Spearman rank correlation coefficients for rarefied richness and Exponential Shannon Entropy for (A) unicellular taxa, (B) multicellular taxa, and (C) diatoms (positive indicated in red, negative in blue) between biomarker records (HBI III, IP<sub>25</sub><sup>6</sup>) and mean surface temperature (T) of the 60-90°N latitude band<sup>7</sup>.



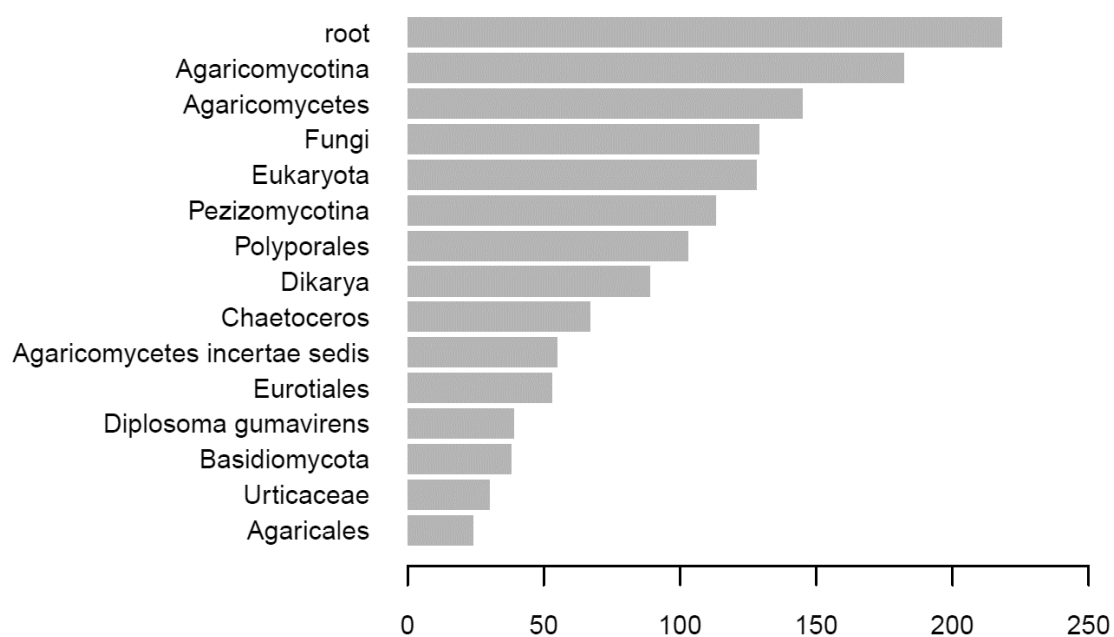
**Supplementary Figure 17.** Plot of the pennate diatoms and epifauna Exponential Shannon Entropies (Diversity) compared to of the Kara Fe grain weighted percentage in core JPC16 off North Alaska (grey) with the 100-year running average (bold black curves)<sup>8</sup>.



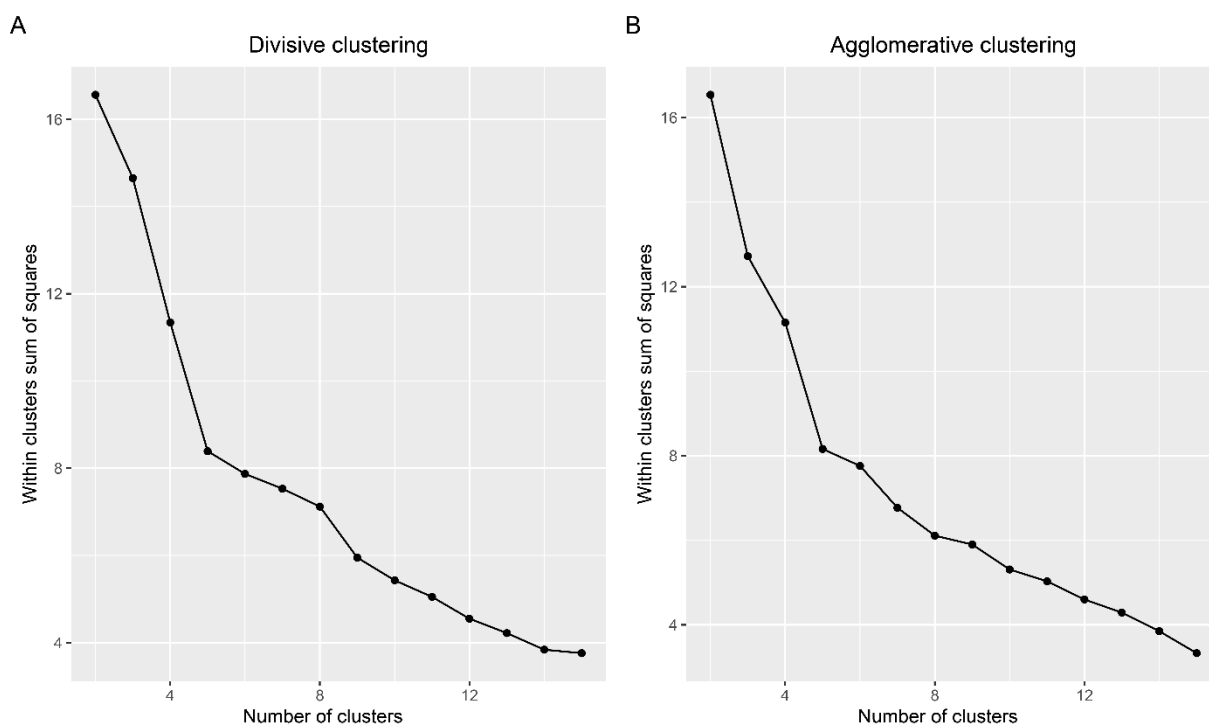
**Supplementary Figure 18.** Plot of the deposit feeder and infauna Exponential Shannon Entropy (Diversity, black) compared to the flux of *Chaetoceros* resting spores (green) from the microfossil record of this core as indicator for primary productivity<sup>6</sup>.



**Supplementary Figure 20.** Comparison of read counts and ASVs between rbcL and 18S\_allshorts datasets. The negative controls of the rbcL dataset (a, b) show minimal contamination compared to samples, with replicate R1 exhibiting slightly fewer ASVs on average than R2 and R3. In contrast, the negative controls of the 18S\_allshorts dataset (c, d), particularly extraction blanks, reveal significant contamination, with higher read counts observed in extraction blanks than in samples on a logarithmic scale. The sample replicates R1-R3 demonstrate a comparable number of read counts and ASVs, with a slight increase in mean ASVs observed from R1 to R3. However, some PCR-products from R3 have lower read counts, resulting in fewer ASVs.



**Supplementary Figure 21.** Top contaminants and their numbers of ASVs in negative controls of the 18S\_allshorts dataset. None of the ASVs above (including *Chaetoceros* ASVs and reads counts) were part of the subsequent analyses.



**Supplementary Figure 21.** Within-cluster sum-of-squares resulting from (A) divisive clustering and (B) agglomerative clustering of protist trait modalities derived from the 18S marker. We chose eight clusters, as with higher number of clusters the sum of squares is not changing substantially.

**Supplementary Table 1.** Processing and filtering steps in the bioinformatic and statistical workflow, with number of read counts kept after each step for the 18S\_allshorts and diat\_rbcL76 markers.

| <b>Program</b> | <b>processing step</b>           | <b>read counts 18S</b> | <b>read counts rbcL</b> |
|----------------|----------------------------------|------------------------|-------------------------|
|                | original number of read pairs    | 24066878               | 26423891                |
| OBITools       | illumina pairedend               | 24066878               | 26423891                |
| OBITools       | aligned                          | 23778718               | 26370412                |
| OBITools       | assigned                         | 21085742               | 20845776                |
| OBITools       | unassigned                       | 2692976                | 5524636                 |
| R              | contaminant removal              | 12389989               | NA                      |
| uchime3        | chimera detection and removal    | 12085864               | NA                      |
| R              | exclude if internal in > 60%     | 11525689               | 16890818                |
| R              | at least 3 occurrences           | 11453157               | 16769064                |
| R              | at least 100 counts              | 11256181               | 16727053                |
|                | remove fungi, land plants, root, |                        |                         |
| R              | high level taxa                  | 5772210                | 15686061                |

**Supplementary Table 2.** Spearman rank correlation coefficients and p-values between biomarker records (HBI III, IP<sub>25</sub><sup>6</sup>) and mean surface temperature (T) of the 60-90 degree latitude band<sup>7</sup> and the richness and the exponential Shannon entropy estimates of the functional groups.

| functional group                | proxy            | correlation<br>coefficient<br>(richness) | p-value<br>(richness) | correlation<br>coefficient (Shannon) | p-value<br>(Shannon) |
|---------------------------------|------------------|------------------------------------------|-----------------------|--------------------------------------|----------------------|
| pennate                         | T                | 0.48045                                  | 0.00227               | 0.23486                              | 0.15579              |
| centric-polar                   | T                | 0.42296                                  | 0.00815               | -0.21925                             | 0.18598              |
| centric-radial                  | T                | 0.28746                                  | 0.08012               | 0.00620                              | 0.97053              |
| heterotrophic floater           | T                | -0.48938                                 | 0.00182               | -0.43357                             | 0.00654              |
| heterotrophic round             | T                | 0.47080                                  | 0.00285               | 0.38284                              | 0.01768              |
| non-flagellated phototrophs     | T                | -0.78868                                 | 0.00000               | -0.86131                             | 0.00000              |
| photo-mixotrophic<br>flagellate | T                | 0.43921                                  | 0.00580               | 0.05021                              | 0.76468              |
| parasite                        | T                | 0.35096                                  | 0.03074               | 0.23090                              | 0.16310              |
| heterotrophic asymmetric        | T                | 0.32857                                  | 0.04400               | 0.27426                              | 0.09565              |
| mixotroph                       | T                | -0.04727                                 | 0.77808               | -0.03207                             | 0.84843              |
| saprotroph                      | T                | -0.45600                                 | 0.00401               | -0.38352                             | 0.01746              |
| infauna                         | T                | 0.29852                                  | 0.06869               | 0.44772                              | 0.00482              |
| epifauna                        | T                | 0.05908                                  | 0.72457               | -0.14342                             | 0.39034              |
| pelagic                         | T                | -0.52292                                 | 0.00076               | -0.57584                             | 0.00016              |
| macrophyte                      | T                | 0.47879                                  | 0.00236               | 0.48325                              | 0.00212              |
| pennate                         | IP <sub>25</sub> | -0.41982                                 | 0.00869               | -0.29392                             | 0.07327              |
| centric-polar                   | IP <sub>25</sub> | -0.41903                                 | 0.00883               | -0.06531                             | 0.69686              |
| centric-radial                  | IP <sub>25</sub> | -0.30387                                 | 0.06363               | -0.13600                             | 0.41555              |
| heterotrophic floater           | IP <sub>25</sub> | 0.26534                                  | 0.10739               | 0.36274                              | 0.02521              |
| heterotrophic round             | IP <sub>25</sub> | -0.39878                                 | 0.01314               | -0.30174                             | 0.06561              |
| non-flagellated phototrophs     | IP <sub>25</sub> | 0.08583                                  | 0.60840               | 0.13300                              | 0.42601              |
| photo-mixo. flagellate          | IP <sub>25</sub> | -0.01958                                 | 0.90711               | 0.07998                              | 0.63311              |
| parasite                        | IP <sub>25</sub> | 0.11947                                  | 0.47495               | 0.34081                              | 0.03627              |
| heterotrophic asymmetric        | IP <sub>25</sub> | -0.51109                                 | 0.00104               | -0.49310                             | 0.00166              |
| mixotroph                       | IP <sub>25</sub> | -0.14963                                 | 0.36993               | -0.14122                             | 0.39770              |
| saprotroph                      | IP <sub>25</sub> | 0.01922                                  | 0.90881               | 0.19557                              | 0.23932              |
| infauna                         | IP <sub>25</sub> | -0.57852                                 | 0.00014               | -0.48204                             | 0.00218              |
| epifauna                        | IP <sub>25</sub> | -0.40327                                 | 0.01206               | -0.26806                             | 0.10370              |
| pelagic                         | IP <sub>25</sub> | -0.41421                                 | 0.00973               | -0.33993                             | 0.03679              |
| macrophyte                      | IP <sub>25</sub> | -0.03754                                 | 0.82294               | -0.00025                             | 0.99880              |
| pennate                         | HBI III          | -0.38944                                 | 0.01566               | -0.36753                             | 0.02321              |
| centric-polar                   | HBI III          | -0.21183                                 | 0.20169               | 0.33896                              | 0.03737              |
| centric-radial                  | HBI III          | -0.11614                                 | 0.48743               | 0.00465                              | 0.97789              |
| heterotrophic floater           | HBI III          | 0.33227                                  | 0.04153               | 0.34194                              | 0.03562              |
| heterotrophic round             | HBI III          | -0.45655                                 | 0.00396               | -0.42411                             | 0.00796              |
| non-flagellated phototrophs     | HBI III          | 0.64831                                  | 0.00001               | 0.68061                              | 0.00000              |
| photo-mixo. flagellate          | HBI III          | -0.18031                                 | 0.27869               | 0.24928                              | 0.13123              |
| parasite                        | HBI III          | -0.25115                                 | 0.12826               | -0.02301                             | 0.89092              |
| heterotrophic asymmetric        | HBI III          | -0.26321                                 | 0.11034               | -0.21415                             | 0.19669              |
| mixotroph                       | HBI III          | -0.03229                                 | 0.84739               | -0.03181                             | 0.84964              |
| saprotroph                      | HBI III          | 0.32326                                  | 0.04774               | 0.20639                              | 0.21378              |
| infauna                         | HBI III          | -0.49500                                 | 0.00158               | -0.55079                             | 0.00034              |
| epifauna                        | HBI III          | -0.46299                                 | 0.00342               | -0.14115                             | 0.39794              |
| pelagic                         | HBI III          | 0.30460                                  | 0.06296               | 0.24031                              | 0.14614              |
| macrophyte                      | HBI III          | -0.13313                                 | 0.42554               | -0.11438                             | 0.49412              |



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