**Supplementary Material**

**Material and methods**

**2.4 eDNA isolation, amplicon sequencing and bioinformatics**

Modifications during eDNA isolation with DNeasy PowerSoil kit (Qiagen, Hilden, Germany) involved mechanical disruption by vortex on a VortexGenie 2 (Fisher Scientific, Waltham, MA, USA) for 15 min at maximum speed and incubation at 37 °C for 30 min with the addition of 2 µL of lysozyme (0.5 mg mL–1 solution). Extracted DNA yield and quality were measured by Qubit fluorometer (Thermo Fisher, USA), while the integrity of DNA was checked on 1% agarose gel. Novogene Co., Ltd. (Cambridge, United Kingdom) made PCR library for 16S and 18S rRNA genes as follows: The 16S rRNA V4-V5 variable region was targeted by PCR primers from Earth Microbiome Project 515F and 806R primers: (FWD:5'-GTGYCAGCMGCCGCGGTAA-3'; REV:5'-GGACTACNVGGGTWTCTAAT-3'), while the 18S rRNA gene V4 variable region was targeted by PCR primers Reuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and Reu-kREV3 modified (5'-ACTTTCGTTCTTGATYRATGA-3'). PCR amplification was performed by using the specific primers connecting with barcodes. The PCR program included a 28 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, with a final elongation step at 72 °C for 5 minutes. The PCR products of proper size were selected through 2% agarose gel electrophoresis. The same amount of PCR products from each sample was pooled, end-repaired, A-tailed, and further ligated with Illumina adapters. Libraries were checked with Qubit fluorescence and real-time PCR for quantification, while a bioanalyzer was used for size distribution detection and then sequenced on a paired-end Illumina platform (NovaSeq 5000) to generate 250 bp paired-end raw reads. Quality of raw reads was checked with FastQC version 0.11.5. [1] and visualized with MultiQC tool (reports available at PUH data storage and management service publication page (https://puh.srce.hr/s/ZSS2qz8ji5PStDj)) for all samples together [2], after which reads were processed using QIIME2 version 2021.11 [3]. Code for QIIME2 pipeline is available at PUH data storage and management service publication page (https://puh.srce.hr/s/ZSS2qz8ji5PStDj). To decontaminate datasets, taxa filtering was performed to exclude chloroplast, mitochondrial, Archaeal, Eukarya and unassigned sequences from 16S rRNA dataset and chloroplast, mitochondrial, *Embryophyceae*, fish (Teleostei and Chondrichtyes; families Hominidae, Felidae and Canidae, genus *Gallus* from 18S rRNA dataset; focusing only on Bacteria in 16S dataset and mesozooplankton in 18S dataset. Prior to alpha and beta-diversity analyses, phylogenetic trees were constructed by multiple-sequence-alignment of representative sequences of 16S and 18S ASVs given by DADA2 in MAFFT [4] followed by masking evolutionary uninformative or ambiguous sites. FastTree2 [5] was then used to infer rooted and unrooted trees. Alpha diversity indices (species richness and evenness within samples) were performed using core-metrics-phylogenetic workflow of QIIME2 producing Shannon, Pielou’s and Faith’s PD along with Kruskal-Wallis statistical test performed on 19 samples per gene with confidence level 95% (Supplement Data). Beta diversity analyses between sample groups were calculated by DEICODE [6] using a robust Aitchison distance by default settings (--p-min-feature-count 10 and --p-min-sample-count 500) and compositional biplots were visualized by EMPeror [7], while statistical differences between chosen sample groups were then calculated by ANOSIM and PERMANOVA tests implemented in QIIME2, performed on 19 samples per gene with confidence level 95%. Feature log-ratios and rankings were inspected with QURRO [8]. Features are classified either as ‘Denominator’ (samples collected during ITW phenomenon) or as ‘Numerator’ (samples collected after ITW) according to their log-fold changes in abundances across sample types. ‘Denominators’ and ‘Numerators’ contributing most to the Euclidian distance from center (eight features) are plotted on rPCA calculated from DEICODE. Taxa bar plots visualization and alpha-diversity indices were performed in RStudio version 1.2.1335, using qiime2R [9]) and ggplot2 [10] packages. Ecologically relevant bacterial function prediction was made using the FAPROTAX database [11]. OTU table (derived with clustering by SILVA138 database, 99% identity threshold) was overlapped with Python script collapse\_table.py in BIOM JSON format version 2.1.4. which generated a functional table (code available at PUH data storage and management service publication page (https://puh.srce.hr/s/ZSS2qz8ji5PStDj)). Visualization and inspection of the functional table was done according to ITW events using packages reshape2 [12], ggpubr [13] and ggplot2 [10].

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**Table S1.** Sample list during the experiment with accompanying times and depths of sampling.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Sampling time (UTC)** | **Depth (m)** | **Water column layer** | **Zooplankton nets** |  | **ITW** |
| **13.7.2022** | 5AM | 0, 12 | Surface (S) |  |  | + |
| 20, 30 | Thermocline (T) |  |  | + |
| 50, 65, 75 | Deep layer (D) |  |  | + |
| 11AM | 0, 12, 20 | Surface |  |  | + |
| 30, 50, 75 | Deep layer |  |  | + |
| **14.7.2022** | 5AM | 0, 12 | Surface |  |  | + |
| 20, 30, 40 | Thermocline |  |  | + |
| 75 | Deep layer |  |  | + |
| 11AM | 0, 12 | Surface |  |  | + |
| 20, 30 | Thermocline |  |  | + |
| 40, 50, 75 | Deep layer |  |  | + |
| 5PM | 0 | Surface |  |  | + |
| 8, 12, 20 | Thermocline |  |  | + |
| 30, 40, 65 | Deep layer |  |  | + |
| 11PM | 0 | Surface |  |  | + |
| 12, 20 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| **15.7.2022** | 5AM | 0, 12, 20  30 | Surface  Thermocline | 40-0 m (S) |  | +  + |
| 40, 65 | Deep layer | 80-40 m (D) |  | + |
| 5PM | 0, 12 | Surface |  |  |  |
| 20 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |
| **16.7.2022** | 5AM | 0, 12 | Surface |  |  |  |
| 20 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |
| 5PM | 0, 12 | Surface |  |  |  |
| 20 | Thermocline |  |  |  |
| 29, 65 | Deep layer |  |  |  |
| **17.7.2022** | 5AM | 0, 12, 20 | Surface |  |  | + |
| 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| 11AM | 0, 12 | Surface |  |  | + |
| 20, 29 | Thermocline |  |  | + |
| 65 | Deep layer |  |  | + |
| 5PM | 0, 12 | Surface |  |  | + |
| 20 | Thermocline |  |  | + |
| 29, 40, 65 | Deep layer |  |  | + |
| 11PM | 0, 12 | Surface |  |  | + |
| 20, 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| **18.7.2022** | 5AM | 0, 12, 20 | Surface | 40-0 m (S,T) |  | + |
| 29 | Thermocline |  | + |
| 40, 65 | Deep layer | 80-40 m (D) |  | + |
| 11AM | 0, 20 | Surface |  |  | + |
| 29 | Thermocline |  |  | + |
| 65 | Deep layer |  |  | + |
| 5PM | 0 | Surface |  |  | + |
| 12, 20 | Thermocline |  |  | + |
| 29, 40, 65 | Deep layer |  |  | + |
| 11PM | 0, 12 | Surface |  |  | + |
| 20 | Thermocline |  |  | + |
| 29, 40, 65 | Deep layer |  |  | + |
| **19.7.2022** | 5AM | 0, 12, 20 | Surface | 30-0 m (S,T) |  | + |
| 29 | Thermocline |  | + |
| 40, 65 | Deep layer | 80-30 m (D) |  | + |
| 11AM | 0, 20 | Surface |  |  | + |
| 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| 5PM | 0 | Surface |  |  | + |
| 12, 20 | Thermocline |  |  | + |
| 29, 40, 65 | Deep layer |  |  | + |
| 11PM | 0, 12 | Surface |  |  | + |
| 20 | Thermocline |  |  | + |
| 29, 40, 65 | Deep layer |  |  | + |
| **20.7.2022** | 5AM | 0, 20 | Surface |  |  | + |
| 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| 11AM | 0, 12 | Surface |  |  | + |
| 20, 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| 5PM | 0 | Surface |  |  | + |
| 12, 20, 29 | Thermocline |  |  | + |
| 40, 65 | Deep layer |  |  | + |
| **21.7.2022** | 5AM | 0, 12 | Surface | 35-0 m (S,T) |  |  |
| 20, 29 | Thermocline |  |  |
| 40, 65 | Deep layer | 80-35 m (D) |  |  |
| 5PM | 0, 12 | Surface |  |  |  |
| 20, 29 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |
| **22.7.2022** | 5AM | 0, 12 | Surface | 25-0 m (S,T) |  |  |
| 20, 29 | Thermocline |  |  |
| 40, 65 | Deep layer | 80-25 m (D) |  |  |
| 5PM | 0, 12 | Surface |  |  |  |
| 20, 29 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |
| **23.7.2022** | 5AM | 0, 12 | Surface |  |  |  |
| 20, 29 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |
| 5PM | 0, 12 | Surface |  |  |  |
| 20, 29 | Thermocline |  |  |  |
| 40, 65 | Deep layer |  |  |  |

**Table S2.** Two-way crossed ANOSIM (factors 'ITW' – 'no ITW', and 'Water layer') of picoplankton flow cytometry counts (cell mL-1), micro-, and nanophytoplankton microscropy counts (cell L-1), and nutrients (phosphate (PO4), nitrate (NO3), nitrite (NO2) and silicic acid (SiO4), µmol L-1). Level of significance *p* < 0.05. *Rho* = 0 similarty, *Rho* = 1 disimilarity, threshold *Rho* > 0.5, \*\*\*strongly significant; \*\*putatively significant.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Rho values (significance of sample statistics) | | | |
| Factors | Picoplankton ( < 2 µm) | Nanophytoplankton (2 - 20 µm) | Microphytoplankton (> 20 µm) | Nutrients |
| 'ITW' – 'no ITW' | 0.084 (*p* = 0.02) \*\* | 0.108 (*p* = 0.003)\*\*\* | 0.045 (*p* = 0.04)\*\* | 0.008 (*p* = 0.4) |
| Surface – Thermocline | 0.766 (*p* = 0.001)\*\*\* | 0.026 (*p* = 0.08) | 0.028 (*p* = 0.08) | 0.038 (*p* = 0.06) |
| Thermocline - Deep layer | 0.523 (*p* = 0.001)\*\*\* | - 0.004 (*p* = 0.4) | 0.006 (*p* = 0.3) | 0.249 (*p* = 0.001)\*\*\* |
| Surface - Deep layer | 0.971 (*p* = 0.001)\*\*\* | 0.001 (*p* = 0.3) | 0.032 (*p* = 0.04)\*\* | 0.36 (*p* = 0.001)\*\*\* |

**Table S3.** Canonical correspondence analysis (CCA) results showing biplot scores of environmental variables, weighted average scores of species, constrained axes (CCA1, CCA2) eigenvalues and proportion of variability explained by each axis. Abbreviations: PPEs (Photosynthetic picoeukaryotes), Chl *a* (Chlorophyll *a*).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Nano-, and microphytoplankton** | | **Picophytoplankton** | |
|  | **CCA1** | **CCA2** | **CCA1** | **CCA2** |
| **NO2  (µmolL-1)** | 0.843 | 0.212 | 0.710 | -0.591 |
| **NO3 (µmolL-1)** | 0.889 | -0.156 | 0.537 | -0.777 |
| **PO4 (µmolL-1)** | -0.212 | -0.098 | -0.021 | -0.038 |
| **SiO4 (µmolL-1)** | 0.830 | -0.340 | 0.681 | -0.547 |
| **Temperature (°C)** | -0.772 | -0.416 | -0.994 | -0.099 |
| **Chl *a* [µgL-1]** | 0.600 | -0.149 | 0.432 | 0.107 |
| **Diatoms (micro) (cellL-1)** | 0.216 | 0.015 |  |  |
| **Dinoflagellates (micro) (cellL-1)** | -0.219 | 0.013 |  |  |
| **Dinoflagellates (nano) (cellL-1)** | -0.020 | -0.020 |  |  |
| **Coccolithophores (micro) (cellL-1)** | -0.137 | -0.163 |  |  |
| **Coccolithophores (nano) (cellL-1)** | 0.012 | -0.059 |  |  |
| **Cryptophyta (cellL-1)** | -0.093 | 0.229 |  |  |
| **Chlorophyceae (cellL-1)** | -0.169 | -0.012 |  |  |
| **Microphytoplankton (cellL-1)** | 0.116 | -0.007 |  |  |
| **Nanophytoplankton (cellL-1)** | 0.015 | 0.012 |  |  |
| **PPEs (cellmL-1)** |  |  | -0.031 | -0.016 |
| ***Synechococcus* (cellmL-1)** |  |  | -0.054 | 0.018 |
| ***Prochlorococcus* (cellmL-1)** |  |  | 0.138 | 0.002 |
| **Heterotrophic bacteria (cellmL-1)** |  |  | -0.035 | -0.007 |
| **Eigenvalues** | 0.016 | 0.007 | 0.006 | 0.000 |
| **Proportion explained** | 0.478 | 0.201 | 0.864 | 0.023 |

**Table S4.** Dominant mesozooplankton taxa collected at 06:00 (UTC+2) (sample time for molecular analyses) analyzed with SIMPER dissimilarity index corresponding to deep layers and surface layers of plankton net 200 µm mesh size. Average dissimilarity = 65.36.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Surface** | **Deep layer** |  |  |  |  |
| **Species** | Av.Abund | Av.Abund | Av.Diss | Diss/SD | Contrib% | Cum.% |
| ***Evadne spinifera*** | **134.27** | 15.34 | 14.09 | 1.86 | 21.55 | 21.55 |
| ***Paracalanus parvus parvus*** | **71.09** | 45.34 | 5.46 | 1.36 | 8.36 | 29.91 |
| ***Centropages typicus*** | 23.57 | **43.71** | 4.17 | 1.47 | 6.38 | 36.29 |
| ***Oikopleura (Coecaria) longicauda*** | 25.05 | **35.52** | 3.93 | 0.84 | 6.01 | 42.3 |
| ***Oithona similis*** | **30.49** | 16.4 | 2.73 | 1.45 | 4.18 | 46.48 |
| ***Temora stylifera*** | **23.53** | 2.25 | 2.41 | 1.42 | 3.69 | 50.17 |
| ***Pseudevadne tergestina*** | **19.46** | 2.81 | 2.34 | 1.01 | 3.58 | 53.74 |
| ***Ctenocalanus vanus*** | **21.35** | 6.6 | 1.88 | 0.91 | 2.88 | 56.62 |
| ***Bivalvia*** | **18.24** | 5.82 | 1.76 | 1.13 | 2.69 | 59.31 |
| ***Corycaeus spp.*** | **16.48** | 7.34 | 1.75 | 1.21 | 2.68 | 61.99 |

**Table S5.** Sample list of net tows and accompanying information about layer (depth), fraction and sequencing of two marker genes: 16S rRNA and 18S rRNA. Number of raw reads after sequencing and subsequent filtered, denoised and non-chimeric reads are shown for each sample.

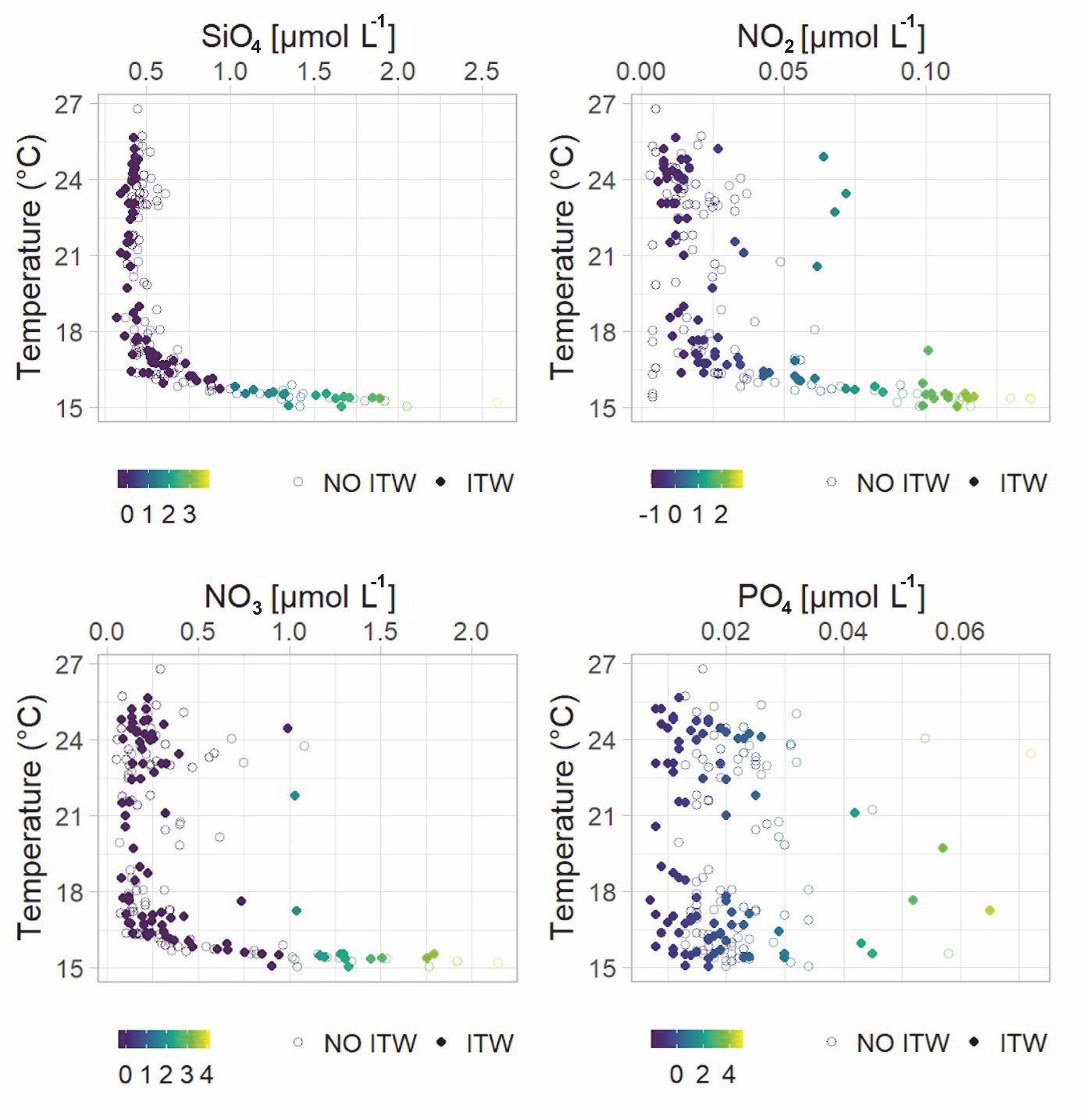
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Date** | **Depth (m)** | **Fraction (µm)** | **Gene** | **raw reads** | **filtered** | | **denoised** | | **merged, non-chimeric** | **% non-chimeric** | **ASV** | **ITW** |
| **N1** | **15.7.2022** | 40-0 | 200 | 16S rRNA | 135,194 | 117,022 | 116,458 | | 113,391 | | 83.87 | 257 | + |
| 18S rRNA | 136,381 | 111,668 | 109,803 | | 90,246 | | 66.17 | 239 |
| **N2** | 80-40 | 53 | 16S rRNA | 134,162 | 82,118 | 80,731 | | 57,657 | | 42.98 | 137 | + |
| 18S rRNA | 134,695 | 112,652 | 111,681 | | 96,667 | | 71.77 | 175 |
| **N3** | 40-0 | 53 | 16S rRNA | 133,116 | 86,901 | 85,515 | | 62,552 | | 46.99 | 112 | + |
| 18S rRNA | 140,739 | 107,123 | 105,607 | | 74,073 | | 52.63 | 171 |
| **N4** | 80-40 | 200 | 16S rRNA | 136,757 | 122,202 | 121,229 | | 82,824 | | 60.56 | 158 | + |
| 18S rRNA | 143,371 | 112,733 | 111,717 | | 99,518 | | 69.41 | 192 |
| **N5** | **18.7.2022** | 40-0 | 53 | 16S rRNA | 141,737 | 29,157 | 28,632 | | 18,206 | | 12.8 | 121 | + |
| 18S rRNA | 134,898 | 106,566 | 105,062 | | 86,668 | | 64.25 | 189 |
| **N6** | 80-40 | 200 | 16S rRNA | 135,389 | 94,417 | 92,962 | | 69,040 | | 50.99 | 116 | + |
| 18S rRNA | 134,426 | 99,559 | 98,676 | | 81,369 | | 60.53 | 145 |
| **N7** | 40-0 | 200 | 16S rRNA | 135,261 | 80,425 | 79,003 | | 61,183 | | 45.23 | 135 | + |
| 18S rRNA | 135,634 | 113,095 | 112,383 | | 93,898 | | 69.23 | 132 |
| **N8** | 80-40 | 53 | 16S rRNA | 135,730 | 86,707 | 84,656 | | 48,921 | | 36.04 | 195 | + |
| 18S rRNA | 132,290 | 106,445 | 105,150 | | 74,358 | | 56.21 | 154 |
| **N9** | **22.7.2022** | 25-0 | 53 | 16S rRNA | 134,712 | 86,182 | 83,436 | | 61,940 | | 45.98 | 283 |  |
| 18S rRNA | 132,375 | 77,341 | 76,115 | | 63,456 | | 47.94 | 180 |
| **N10** | 25-0 | 200 | 16S rRNA | 140,547 | 83,579 | 81,731 | | 65,574 | | 46.66 | 181 |  |
| 18S rRNA | 135,857 | 107,427 | 106,269 | | 76,121 | | 56.03 | 192 |
| **N11** | 80-25 | 53 | 16S rRNA | 134,149 | 45,380 | 43,981 | | 34,763 | | 25.91 | 132 |  |
| 18S rRNA | 135,559 | 104,633 | 102,652 | | 77,008 | | 56.81 | 212 |
| **N12** | 80-25 | 200 | 16S rRNA | 136,273 | 73,327 | 72,316 | | 47,577 | | 34.91 | 133 |  |
| 18S rRNA | 135,664 | 81,273 | 79,727 | | 50,923 | | 37.54 | 189 |
| **N13** | **19.7.2022** | 80-30 | 200 | 16S rRNA | 141,135 | 127,029 | 125,722 | | 84,604 | | 59.95 | 118 | + |
| 18S rRNA | 132,255 | 103,386 | 101,601 | | 79,827 | | 60.36 | 239 |
| **N14** | 30-0 | 200 | 16S rRNA | 134,709 | 119,975 | 117,993 | | 81,203 | | 60.28 | 153 | + |
| 18S rRNA | 136,443 | 93,421 | 92,710 | | 82,133 | | 60.2 | 175 |
| **N15** | 80-30 | 53 | 16S rRNA | 132,112 | 115,310 | 113,700 | | 76,203 | | 57.68 | 126 | + |
| 18S rRNA | 135,318 | 98,102 | 96,906 | | 86,055 | | 63.59 | 134 |
| **N16** | **21.7.2022** | 35-0 | 200 | 16S rRNA | 135,460 | 120,539 | 119,099 | | 93,409 | | 68.96 | 115 |  |
| 18S rRNA | 136,011 | 90,565 | 89,555 | | 71,050 | | 52.24 | 184 |
| **N17** | 35-0 | 53 | 16S rRNA | 132,735 | 119,588 | 117,682 | | 80,573 | | 60.7 | 162 |  |
| 18S rRNA | 134,502 | 99,301 | 98,056 | | 64,811 | | 48.19 | 146 |
| **N18** | 80-35 | 53 | 16S rRNA | 133,655 | 119,420 | 117,810 | | 90,668 | | 67.84 | 192 |  |
| 18S rRNA | 136,538 | 88,050 | 87,412 | | 70,319 | | 51.5 | 134 |
| **N19** | 80-35 | 200 | 16S rRNA | 134,794 | 116,503 | 115,238 | | 84,285 | | 62.53 | 122 |  |
| 18S rRNA | 135,426 | 104,663 | 104,102 | | 92,443 | | 68.26 | 107 |
|  | ***Total*** | | | 16S rRNA | 2,577,627 | 1,825,781 | 1,797,894 | | 1,314,573 | | 51.00 | 1,821 |  |
| 18S rRNA | 2,578,382 | 1,918,003 | 1,895,184 | | 1,510,943 | | 58.60 | 3,353 |

**Table S6.** (A) List of the most contributing prokaryotic features to sample clustering in RPCA plots along axis 1 (ITW axis). List of features were extracted from RPCA ordinations using the QURRO plugin by filtering top and bottom features (5%). Taxonomic annotation was added from classification results using the SILVA v. 138 reference database. 'Denominator' features correspond to 'ITW' sample group; 'Numerator' features correspond to 'no ITW' sample group. (B) T-test results of CLR values of prokaryotic features (Table S6A) between sample groups 'ITW' – 'no ITW' and fractions (large = 200 µm; small = 53 µm).

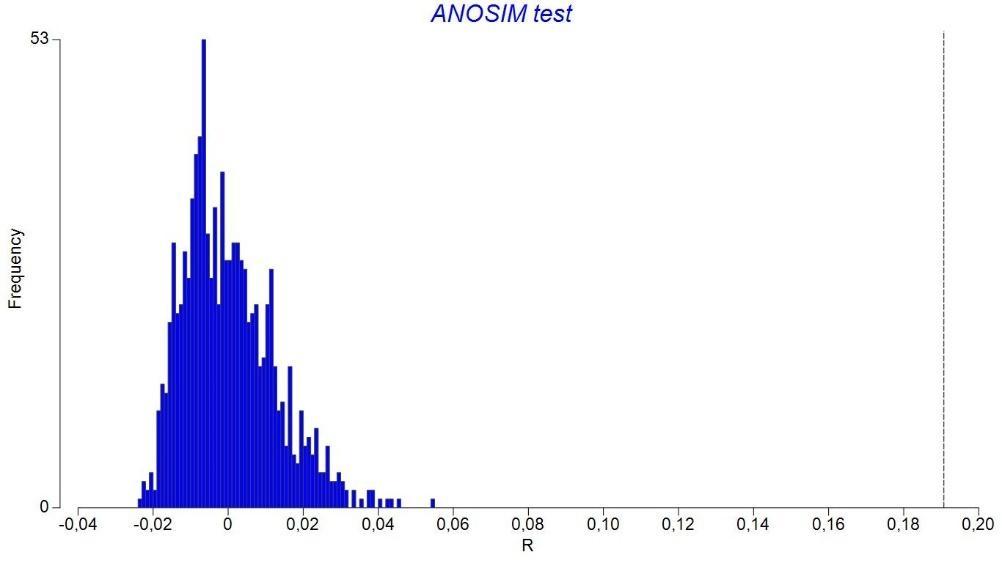
***Note:*** *this table is provided as a supplementary xlsx file.*

**Table S7.** (A) List of the most contributing eukaryotic features to sample clustering in RPCA plots along axis 1 (ITW axis). List of features were extracted from RPCA ordinations using the QURRO plugin by filtering top and bottom features (10%). Taxonomic annotation was added from classification results using the PR2 reference database. 'Denominator' features correspond to 'ITW' sample group; 'Numerator' features correspond to 'no ITW' sample group. (B) T-test results of CLR values of eukaryotic features (Table S7A) between sample groups 'ITW' – 'no ITW' and fractions (large = 200 µm; small = 53 µm).

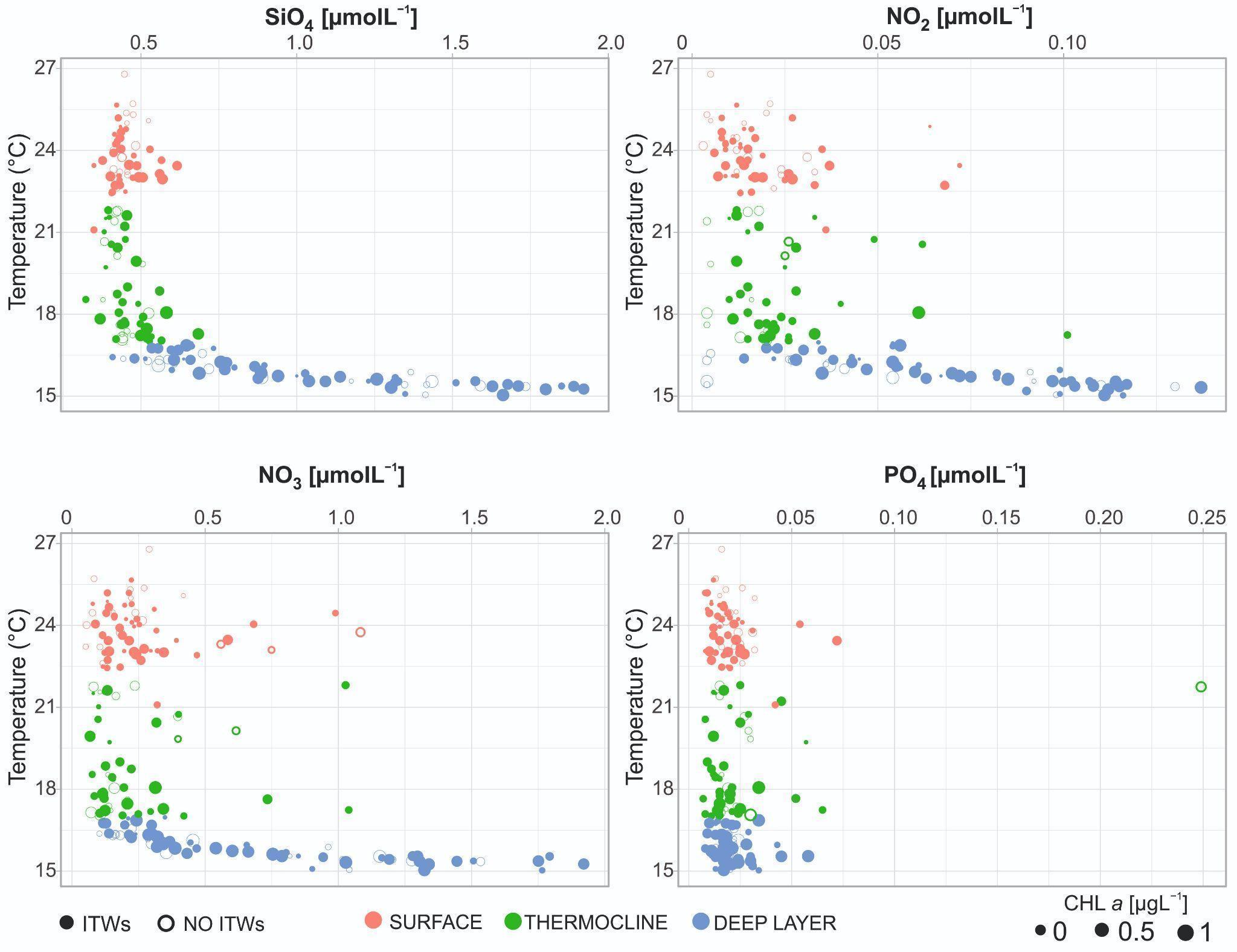
***Note:*** *this table is provided as a supplementary xlsx file.*



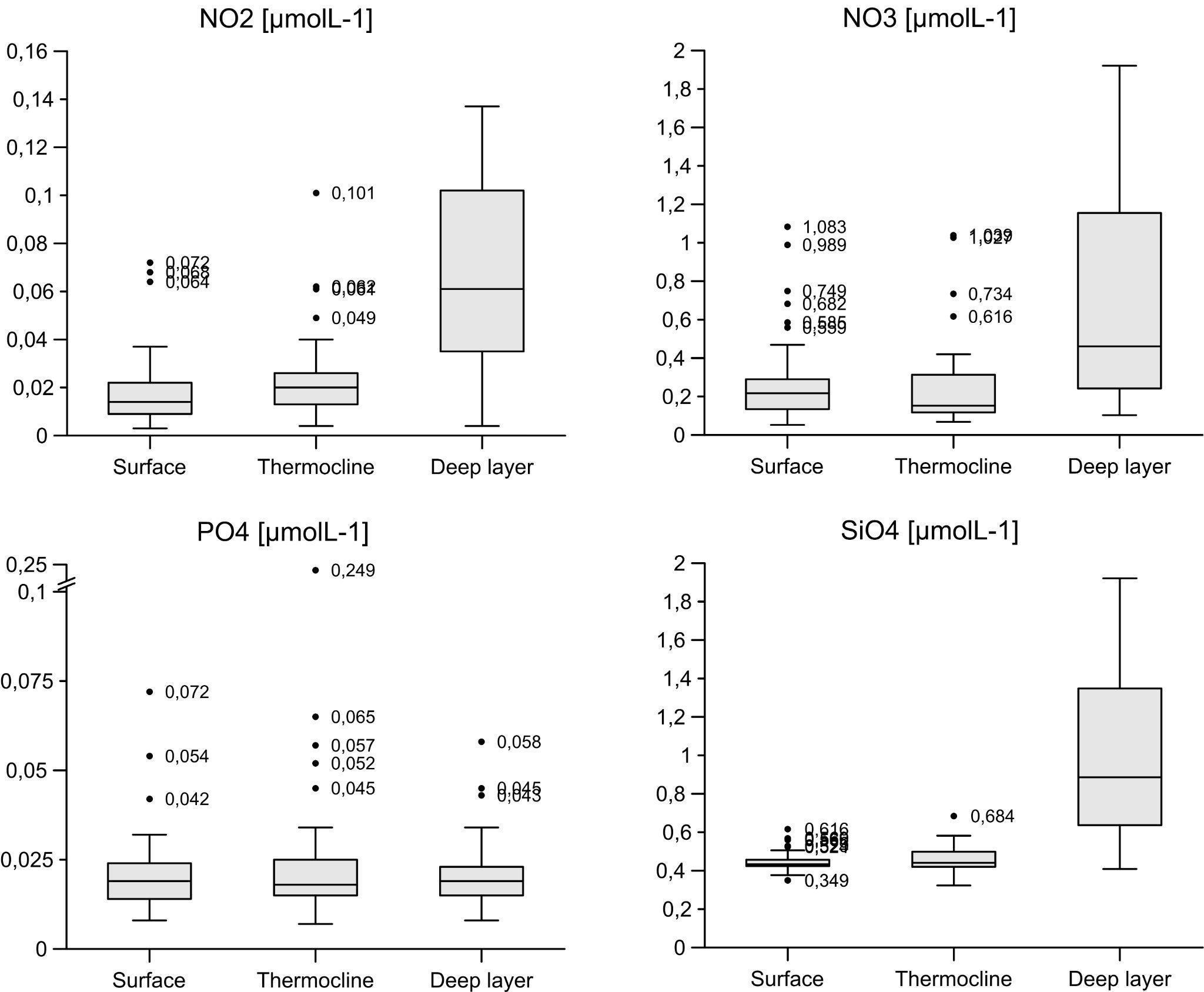
**Figure S1.** Z score analysis of all nutrients (silicic acid (SiO4), nitrite (NO2), nitrate (NO3), and phosphate (PO4), µmol L-1) to check the significance of extreme values. Data with z score > +/- 3 were omitted from further analysis.



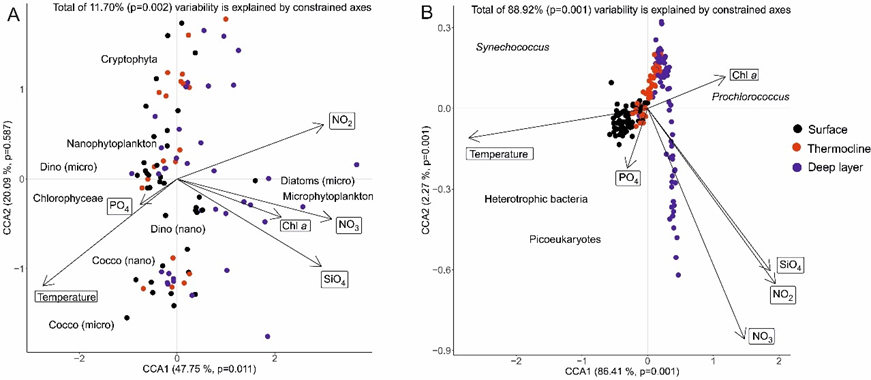
**Figure S2.** Analysis of similarities (ANOSIM) test done on Euclidean distance resemblance matrices of log-transformed and normalized nutrient concentrations (µmol L-1), log-transformed abundances of micro-, and nanophytoplankton (cell L-1), and picophytoplankton (cell mL-1) to test differences among sampling groups before and after ITWs within the stratified water layer.



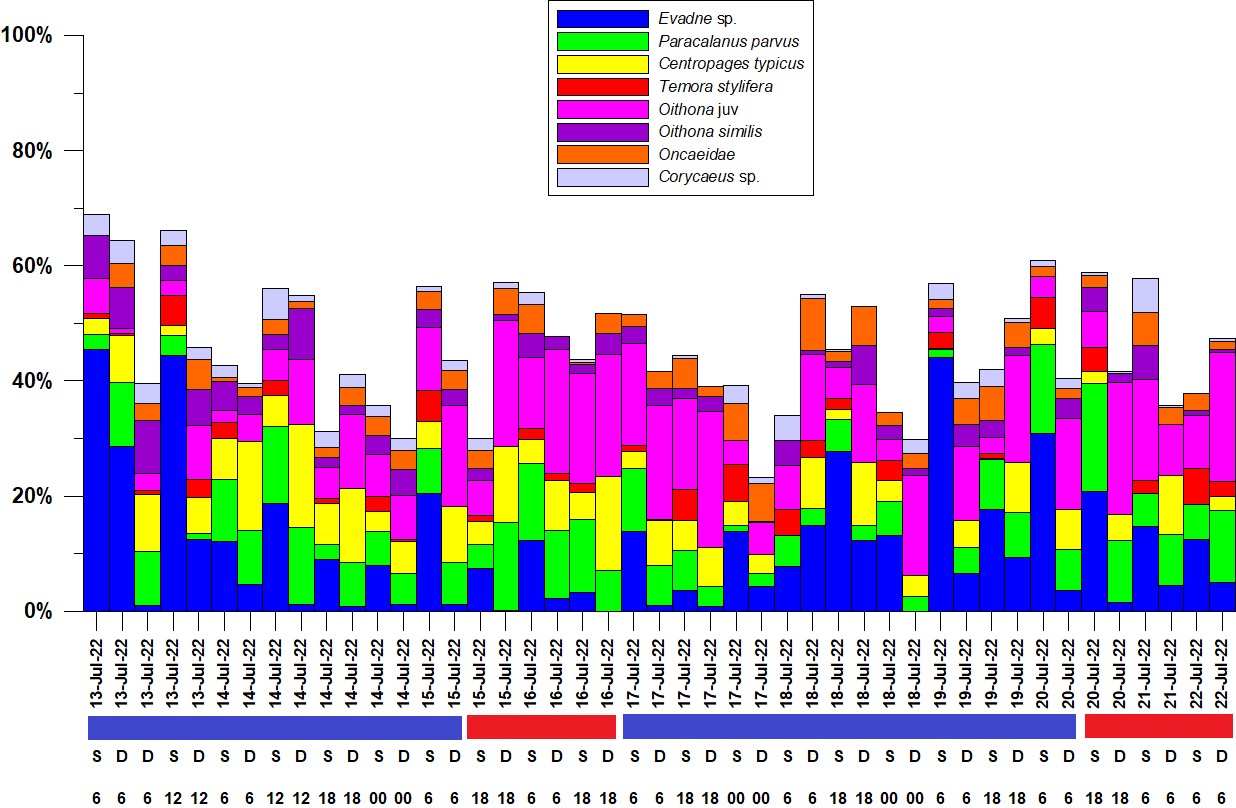
**Figure S3**. Nutrient (silicate (SiO4), nitrite (NO2), nitrate (NO3), phosphate (PO4)) concentrations correlated with temperature. Chlorophyll *a* concentrations gradient, distinct water layers and ITW events are superimposed on samples collected at Station S1 in the period between 13 and 23 July 2022.



**Figure S4.** Box whiskers plot with dataset range, mean, and outliers of nutrient concentrations (nitrite (NO2), nitrate (NO3), phosphate (PO4), silicate (SiO4)) measured at Station S1 in the period between 13 and 23 July 2022.



**Figure S5.** Canonical correspondence analysis (CCA) of environmental variables (temperature, phosphate (PO4), nitrate (NO3), nitrite (NO2), silicate (SiO4) and Chl *a*) as constrained variables on micro- and nanophytoplankton (A), and picophytoplankton (B) community. Symbols represent samples (*N* = 180) weighted scores (weighted averages of species) colored by factor “water layer” (surface, thermocline, and deep layer). Superimposed vectors (arrows) represent biplot scores of constrained environmental variables. Abbreviations: Dino (Dinoflagellates), Cocco (Coccolithophorids), Chl *a* (Chlorophyll *a*).

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**Figure S6.** Barplot showing abundances (%) of dominant mesozooplankton taxa during and after ITW events. S – surface layers; D – deep layers: 0, 6, 12, 18 – hour of sampling during a day (00:00, 06:00, 12:00 and 18:00 UTC+2).

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**Figure S7.** Alpha diversity indices calculated from 16S rRNA gene dataset with Kruskal-Wallis statistical test comparing sample groups 'ITW' – 'no ITW'. Upper panel: scatter plot with each sample value. Bottom panel: sample groups jitter barplots.

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**Figure S8.** Alpha diversity indices calculated from 18S rRNA gene dataset with Kruskal-Wallis statistical test comparing sample groups 'ITW' - 'no ITW'. Upper panel: scatter plot with each sample value. Bottom panel: sample groups jitter barplots.