**Supplementary material**

**Ocean Alkalinity Enhancement does not cause cellular stress in phytoplankton in a mesocosm experiment.**

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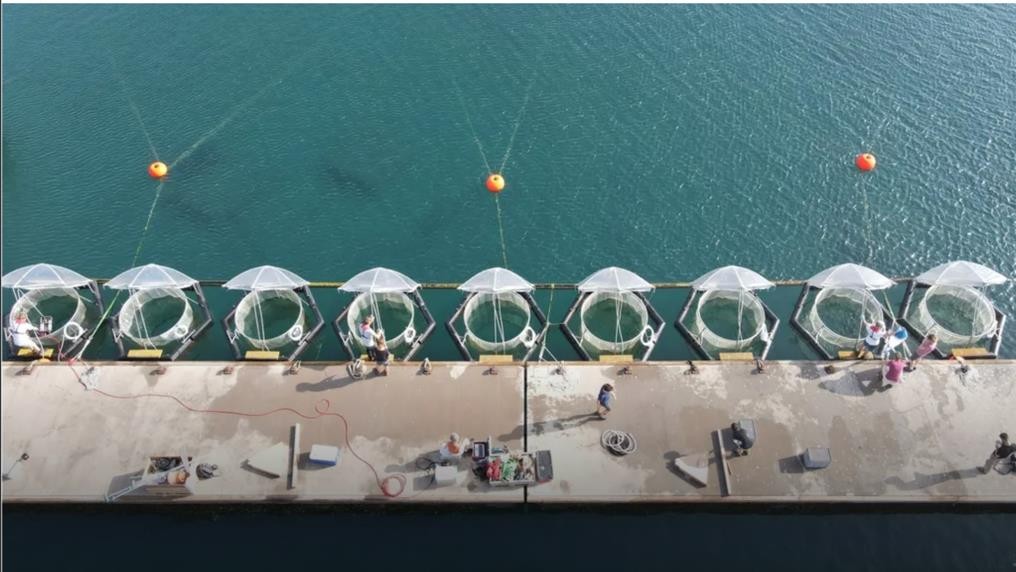
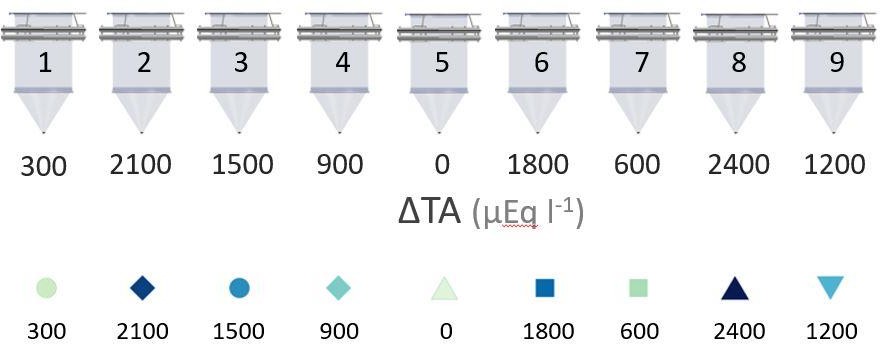
# A



**Gran Canaria**

**Sahara**

**B**



# C

**Figure S1.** A) Study site in the port of Taliarte, Gran Canaria (Spain). B) Operating mesocosms during the experiment (photograph by Peter Yeung, National Geographic ©). C) Mesocosms experimental design representingthe alkalinity gradient (OAE) (µmol·L-1) in the nine mesocosms: M5 (0), M1 (300), M7 (600), M4 (900), M9 (1200), M3 (1500), M6 (1800), M2 (2100), M8 (2400) (design by Silvan Goldenberg ©, GEOMAR).

*Gráfico

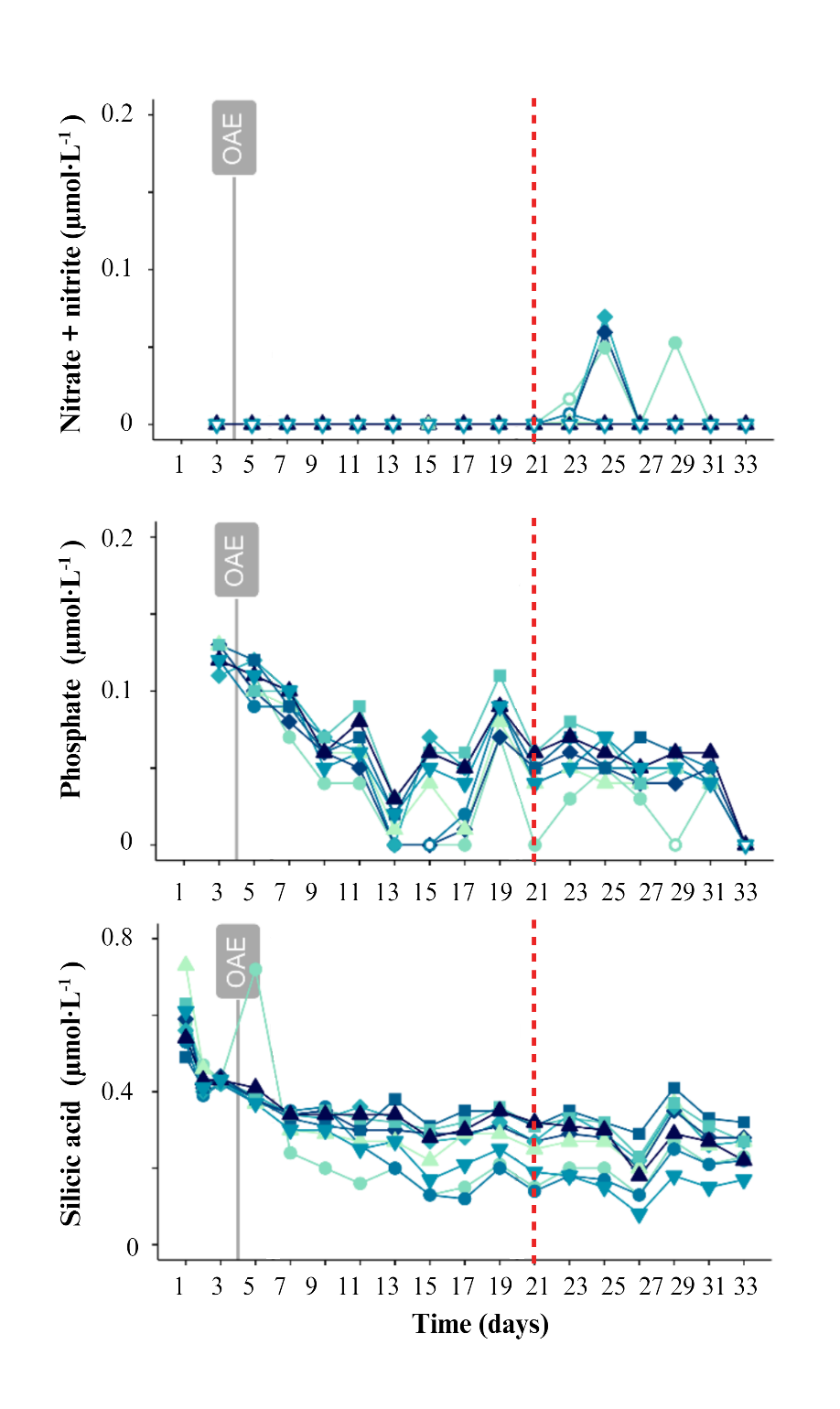
Descripción generada automáticamente*

**C**

**B**

**A**

**Figure S2.** A) Evolution of total alkalinity (TA) (µmolL-1); and B) dissolved inorganic carbon (DIC) (µmol·L-1), C) pH over time during the mesocosms experiment*. Marin -Samper et al. 2024. Reproduction with permission of Biogeoscience*



**C**

**B**

**A**

**Figure S3.** Nutrients concentration over time and in response to Ocean Alkalinity Enhancement OAE in µmol·L-1. A) Nitrate + Nitrite B) Phosphate C) Silicic acid. *Paul et al., (2024). Reproduction with permission of Biogeosciences*

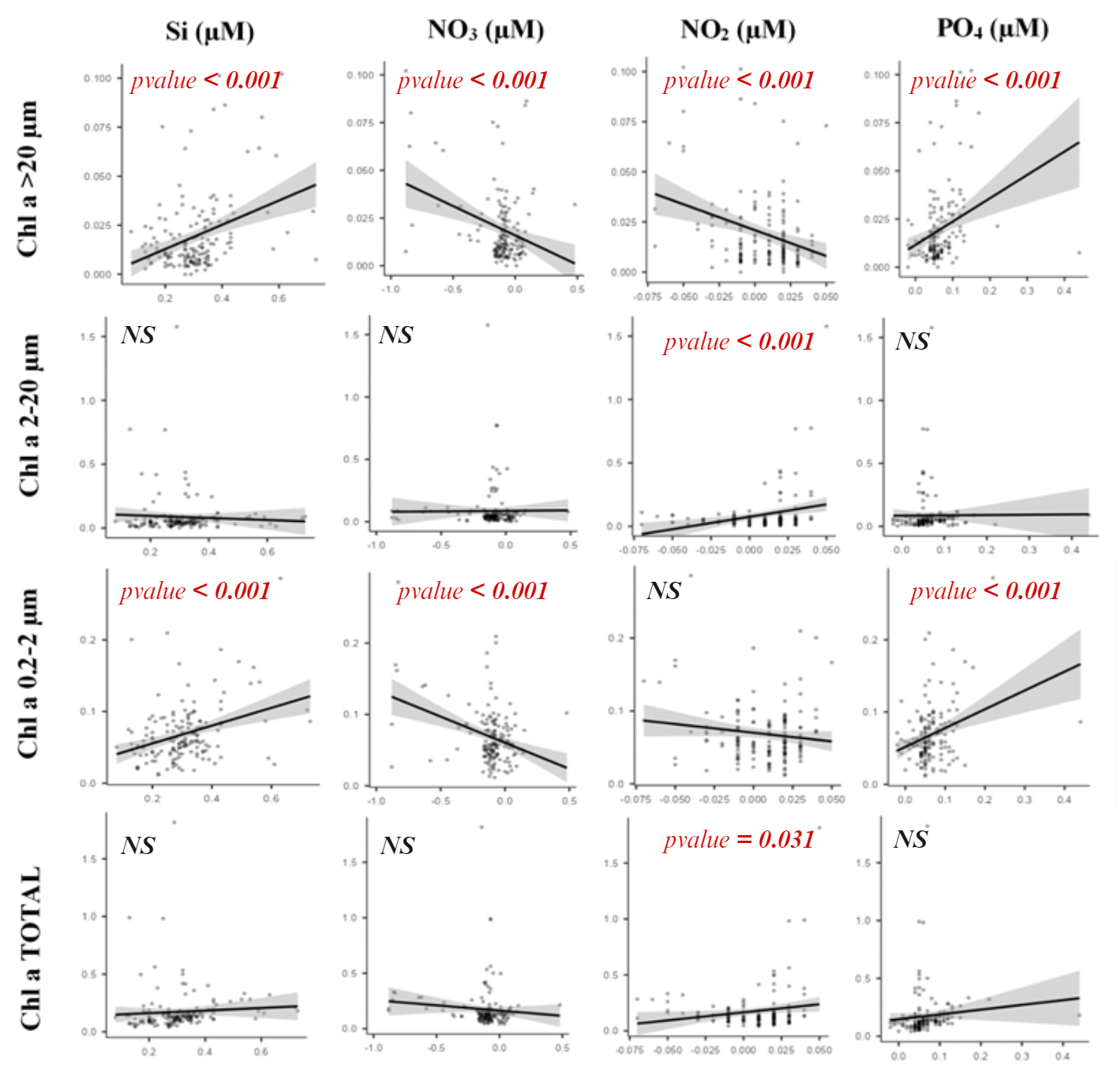
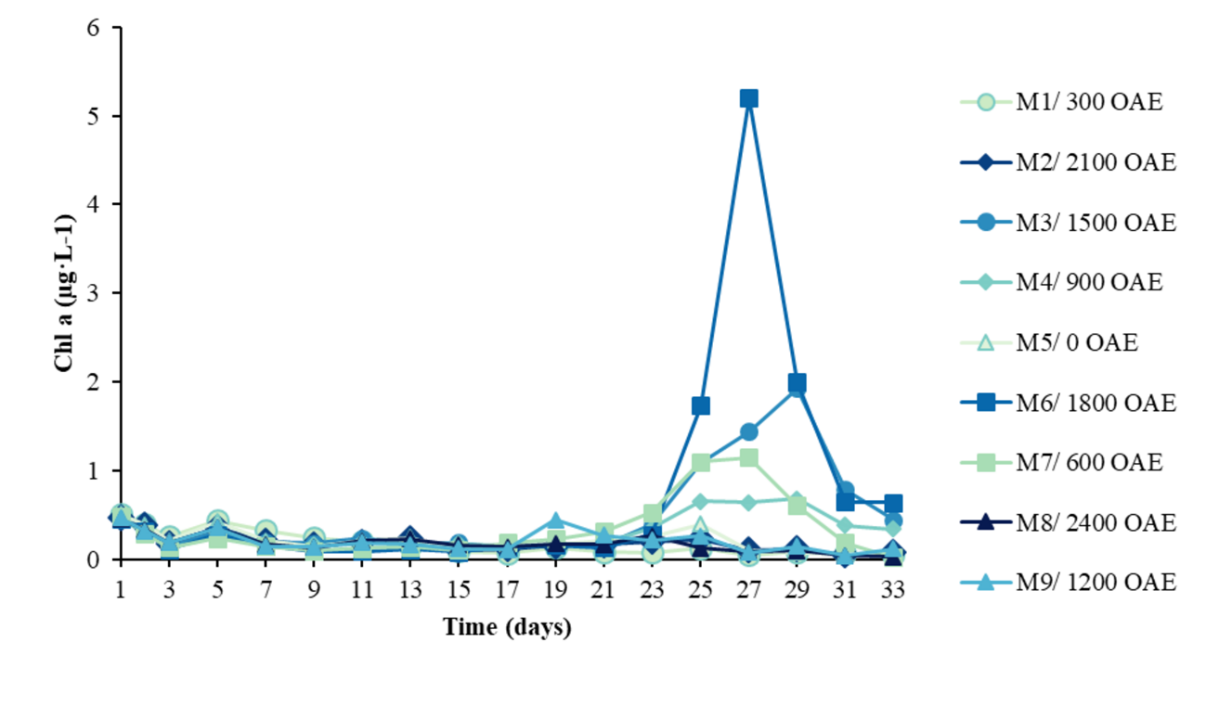
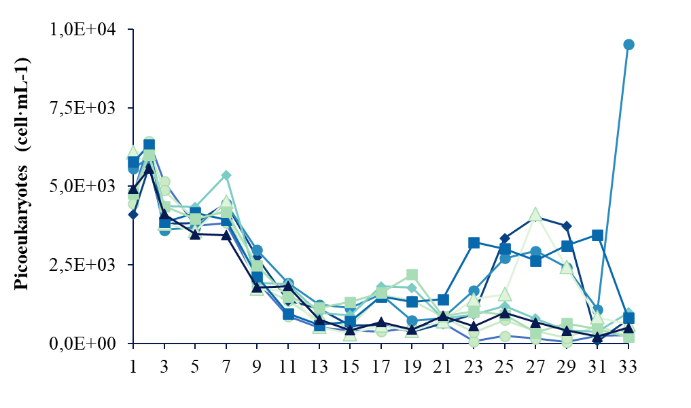
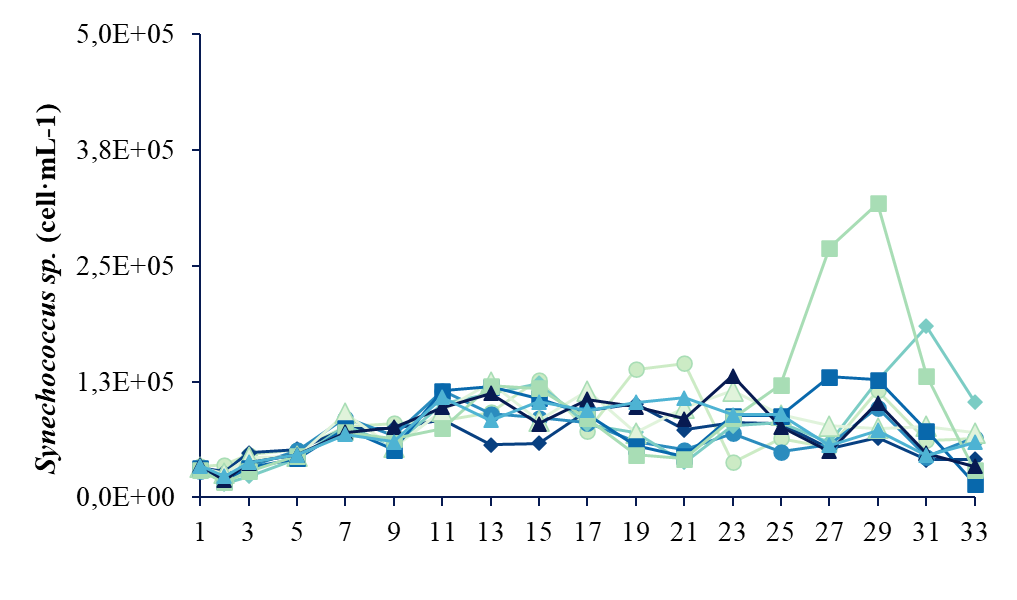
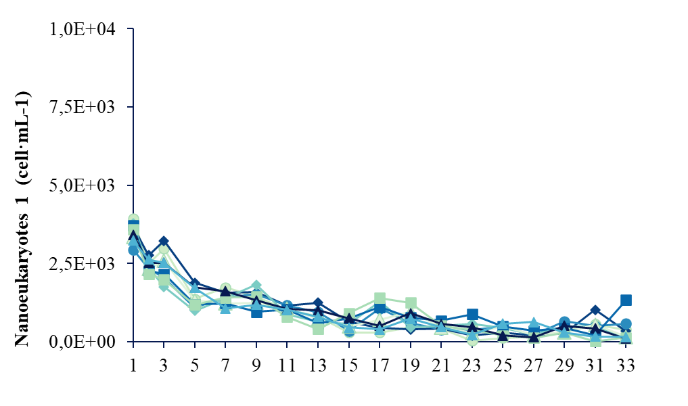
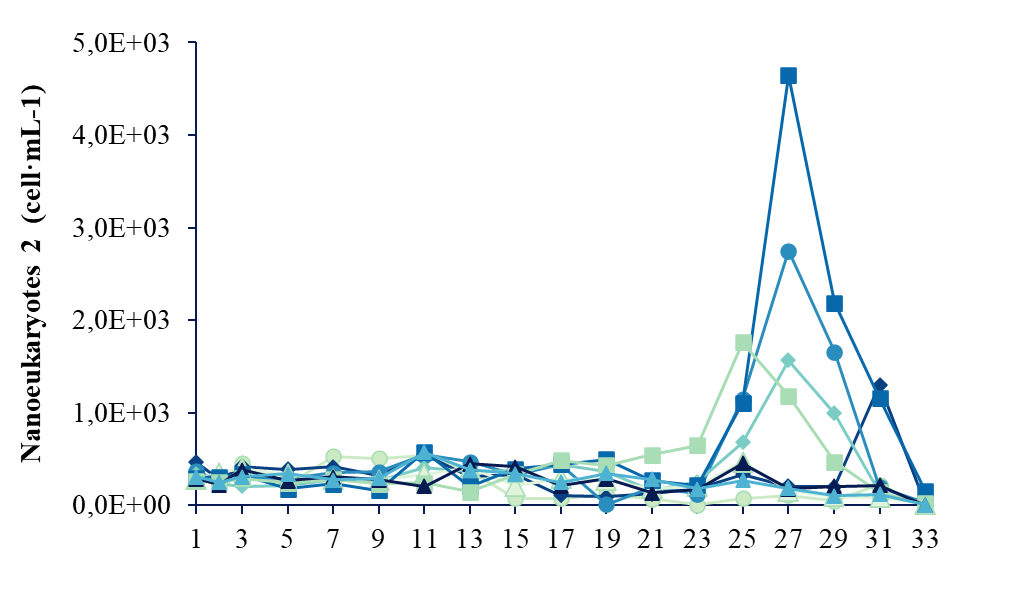
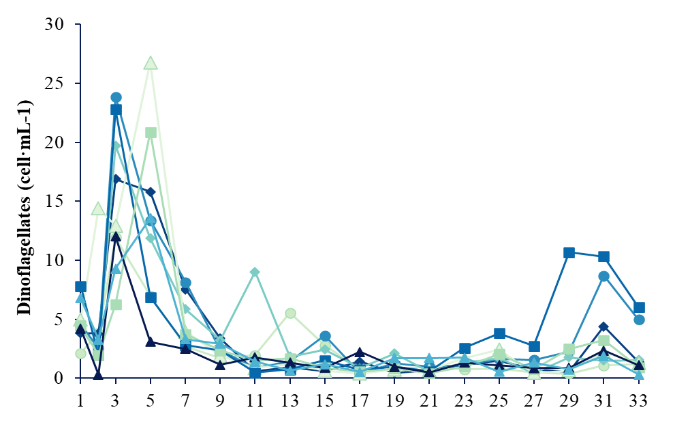
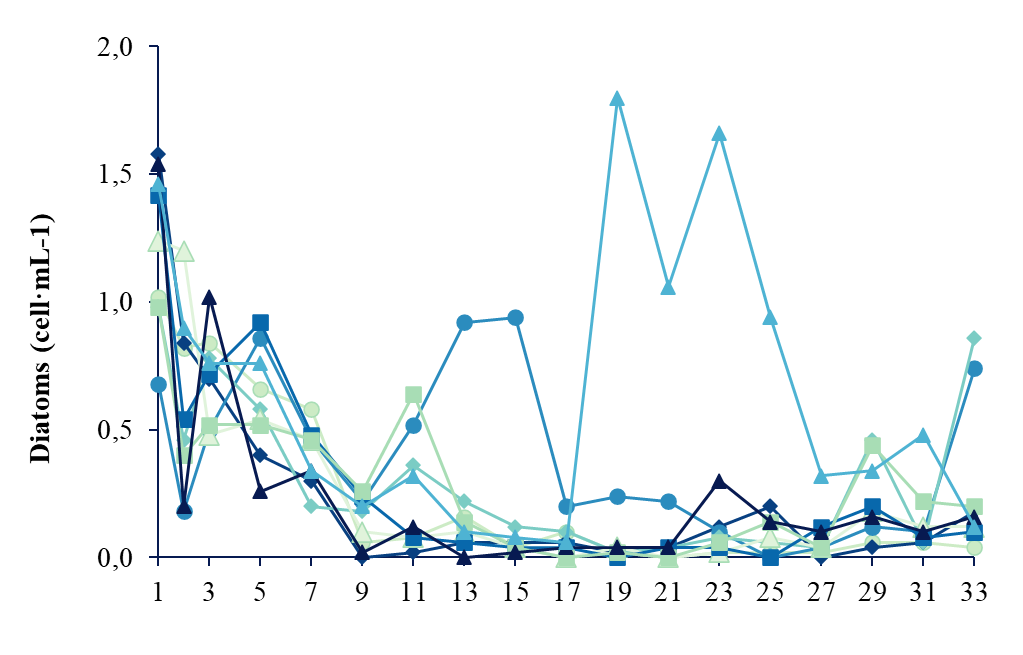
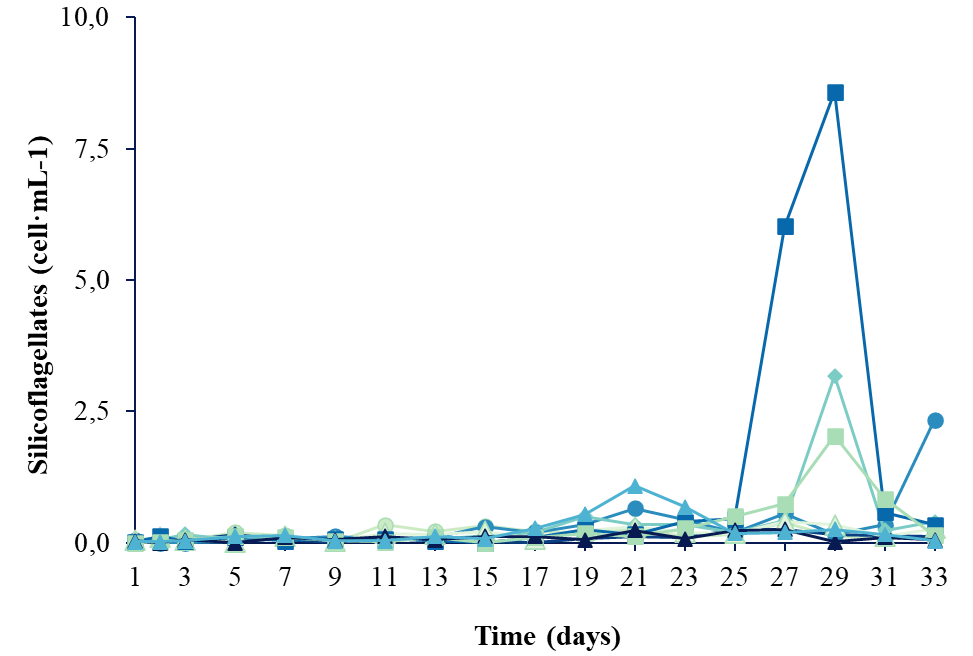
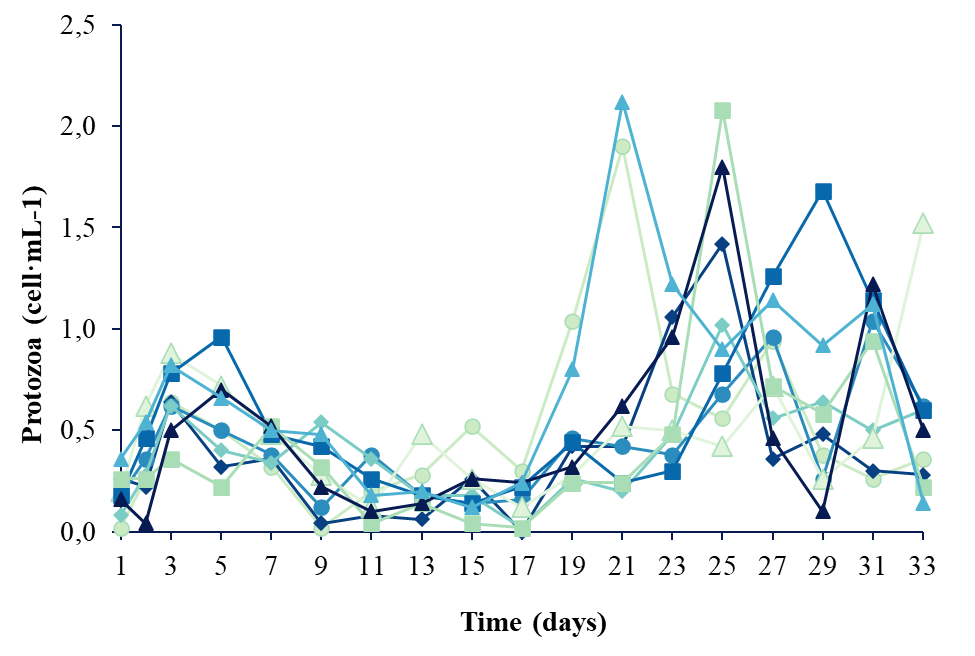


Figure S4. Correlation plots and data distribution among nutrients concentration (Si, NO3, NO2, PO4 µM) and fractioned chlorophyll (dependent variable) on size ranges Chl a >20 µm, Chl a 2-20 µm, Chl a 0.2-2 µm and Chl a total. Significant values presented in red color (p value); non-significant values presented like *NS.*

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**Figure S5.** Temporal evolution of chlorophyll-*a* concentration in µg·L-1 during the mesocosms experiment. *Marin -Samper et al. 2024.* *Reproduction with permission of Biogeosciences.*

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**H**

**G**

**E**

**F**

**C**

**A**

**D**

**B**

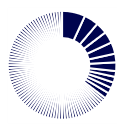
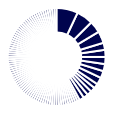
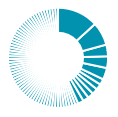
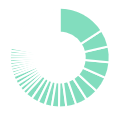
**Figure S6.** Cell abundance of phyto- and microphytoplankton in cell·ml-1. A) Picoeukaryotes <2 µm; B) Synechococcus spp. < 2 µm,;C) Nanoeukaryotes-1 2-20 µm; D) Nanoeukaryotes-2 >20 µm; E) Diatoms; F) Dinoflagellates; G) Silicoflagellates; H) Protozoa *A to D repoduced from Marin -Samper et al. 2024, with permission of Biogeosciences.*

R2=0.188 *p*=0.366

**Figure S7.** Linear regressions among the percentage of stained cells and OAE treatments in picoeukaryotes and *Synechococcus spp*. A) Cell viability (FDA) in picoeukaryotes in phase I; B) Cell viability in picoeukaryotes in phase II; C) Oxidative stress (ROS) in picoeukaryotes in phase I; D) Oxidative stress in picoeukaryotes in phase II; E) Cell viability in *Synechococcus* in phase I; F) Cell viability in *Synechococcus* in phase II; G) Oxidative stress in *Synechococcus* in phase I; H) Oxidative stress in *Synechococcus* in phase II.

**B**

**Figure S8.** Linear regressions among the percentage of stained cells and OAE treatments in nanoeukaryotes 1(20 µm) and nanoeukaryotes 2 (>20 µm). A) Cell viability (FDA) in nanoeukaryotes 1 in phase I; B) Cell viability in nanoeukaryotes 1 in phase II; C) Oxidative stress (ROS) in nanoeukaryotes 1 in phase I; D) Oxidative stress in nanoeukaryotes 1 in phase II; E) Cell viability in nanoeukaryotes 2 in phase II; F) Oxidative stress in nanoeukaryotes 2 in phase II; G) Cell viability in microphytoplankton in phase II; H) Oxidative stress in microphytoplankton in phase II.



Downregulated protein abundances

Upregulated protein abundances

∆TA μmol ·L-1 L-1

**Figure S9.** Ring-Sector plots categorized by absolute abundances of significantly upregulated and downregulated protein abundances normalized to ∆TA 0 µmol. L-1 and housekeeping proteins. By ring-around each sector, the protein that has changed its abundance will appear, attending to its contribution amongst all the up/down-regulated dataset.

Interfaz de usuario gráfica, Gráfico, Gráfico de dispersión

Descripción generada automáticamente

**B**

**Figure S10.** Principal component analysis (PCA) of the variables related to cellular stress during the experiment. Yellow color: nutrients (Nitrate, nitrite, phosphate, silicate); pink: Pigments (Chla, DD, DT, perinidin, prasinoxanthan, fucoxanthin,violaxanthin, zeaxanthin); lilac: cell viability (FDA); purple: Oxidative Stress (ROS); green: PSII efficiency, Fv/Fm. State variables (OAE), are differentiated into Control (0 OAE), Low (300, 600, 900 OAE) , Medium (1200, 1500, 1800 OAE) and High (2100, 2400 OAE).

The arrows represent the included original variables such as nutrients, pigments, cell viability (FDA), oxidative stress (ROS), and cell abundances. The direction and length of the arrows indicate their contribution and correlation with the principal components. The points or symbols represent the different experimental conditions i.e. OAE treatemnts: Control, Low, Medium and High organized according to the loading factors of the variables. F1 (46.85%) and F2 (22.71%) together explain approximately 69.56% of the total variance, indicating that these two principal components capture most of the variability in the data. Thus, F1 is the component that explains the most variance, followed by F2. The relationship between variables that PCA has captured are Control is close to the origin, indicating that these samples are not significantly affected by the variables. The Low conditions cluster in the negative regions of F1 and F2, showing a greater impact of oxidative stress and low cell viability. Pigments (Fuco, DD, DT, Chla) and other variables related to cell abundances (e.g., dinoflagellates, diatoms) are associated with the Medium conditions both positive and negative in F1 and F2. Despite that Medium is more dispersed this precisely explains the major changes occurring during the experiment. The High treatment is well-defined in the positive region of F1, where abundances of certain pigments and cell groups predominate.

The experimental conditions are clearly differentiated in the PCA new axis and representation, suggesting that the measured variables are effectively discriminated depending on the treatments (Control, Low, Medium, High).