EGU: Biogeosciences

Supporting Information for

Stoichiometry of marine gels cycling in the North Pacific Subtropical Gyre: Insights on subsurface preformed nitrate anomalies

Stella Rowley[1,2], Kieran Curran[1], Kai Ziervogel[1], Tracy A. Villareal[3], and Robert T. Letscher[1,2]

[1]Ocean Process Analysis Laboratory, University of New Hampshire, Durham NH

[2]Earth Sciences, University of New Hampshire, Durham NH

[3]Marine Science Institute, University of Texas at Austin, Port Aransas, TX

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Text S1: The method of bacterial cell counts, and enzyme activities completed within the incubations.

For each incubation experiment, water was subsampled from the replicate carboys at each sampling time point and fixed with 0.01% glutaraldehyde and stored in a -80 °C freezer until they were measured at the UNH laboratory using a flow cytometer (Miltenyi BiotecMACSQuant VYB) with SYBR green as a DNA stain (Gasol & Del Giorgio, 2000). Measured cells/mL were then converted to cells/L and used to calculate the per-cell activity of the Glucosidase enzyme activity.

Alpha-glucosidase enzyme activity, which hydrolyzes the a-glycosidic bonds in polysaccharides, was determined using a distinct fluorogenic substrate proxy (Hoppe, 1983), as a proxy for carbohydrate degradation such as within TEP. At the 4-discrete incubation time points , the incubated water was subsampled and 25 µL of 1mM MUF-a-D-glucopyranoside solution was added and shaken. Over the next 24-hour-period fluorescence was measured at 4 time points (T0,T1,T2,T3). After a 24-hour period the fluorescence readings performed at an excitation of 365-395 nm and emission of 440-470 nm using a handheld Turner® TBS-380 mini UV fluorometer. The fluorescence readings were calibrated using the MUF standard solution which was prepared within the range 10 – 250 µM MUF, with the slope and time duration of the 4-point glucosidase incubation at each time used to convert to nM C hr^{-1} rates.

Text S2: The limited association between glucosidase rates and TEP consumption rates.

The association between glucosidase rates and TEP consumption rates at S(5m), S(125m) and, N(5m) was not statistically significant with p-values much greater then 0.05 (S(5m): Kendall τ = -0.33, p-value =1.0, n=3; S(125m): Kendall τ= -0.33, p value =0.75, n=4; Station: N(5m): no association) Although there was a notably strong negative association, at N(125) (Kendall τ =-0.73, p value 0.07, n=5). We found no evidence of a correlation of TEP formation (F treatment) and glucosidase activity throughout the incubations.

Text S3: Time intervals when TEP was consumed were correlated with time intervals when CSP was consumed and when TEP accumulated or CSP accumulated. There was no correlation between TEP and CSP consumption or production rates throughout the incubations:

CSP consumption rate vs TEP consumption rate (M&W treatments):

Station: S(5m): No time points correlated within the incubation
Station: S(125m): Kendall τ=-0.333, p-value=1.0, n=3
Station: N(5m): Only one time point correlated within the incubation
Station: N(125m) Only two time points correlated within the incubation

CSP formation rate vs TEP formation rate (F treatment) :

Station: S(5m): Only two time point correlated within the incubation
Station: S(125m): Only one time point correlated within the incubation
Station: N(5m): No time points correlated within the incubation
Station: N(125m): Only one time point correlated within the incubation

TextS4. The calculation of the O_2 remineralization of TOC

$$\sum \boldsymbol{O_2\ consumed} = \triangle \boldsymbol{TEP\_C} + \triangle \boldsymbol{DOC} + \triangle \boldsymbol{DOP}$$

We used the O_2: N (8.8:1) for POC and O_2: N (18.1) for DOC taken from Letscher & Villareal (2018) and the C: N (7.7:1) for POC and C:N for DOC (11:1) taken from Liang et al (2023) to get the O_2:C for both DOC (1.65) and POC (1.25). We then took a 50% DOC / 50% POC (representative for the shallow layers of the NPSG; Letscher & Villareal, 2018) weighted average to get the O_2:C for TOC (1.4).

Text S5. The residual preform nitrate tracer equation ($rPreNO_3^-$) as defined by Letscher & Villareal (2018), which separates contributions to AOU from POM and DOM.

$$PreNO_3^- = NO_3^- measured - \left(fDOM \cdot \frac{AOU}{rDOM}\right) - \left(fPOM \cdot \frac{AOU}{rPOM}\right)$$

Text S6. To calculate the percent gel contribution on diminishing the preform anomaly in the upper 200m.

$$\%\ \text{gel contribution} = \text{abs}((preNO_3^- - rpreNO_3^-)/preNO_3^-) * 100$$

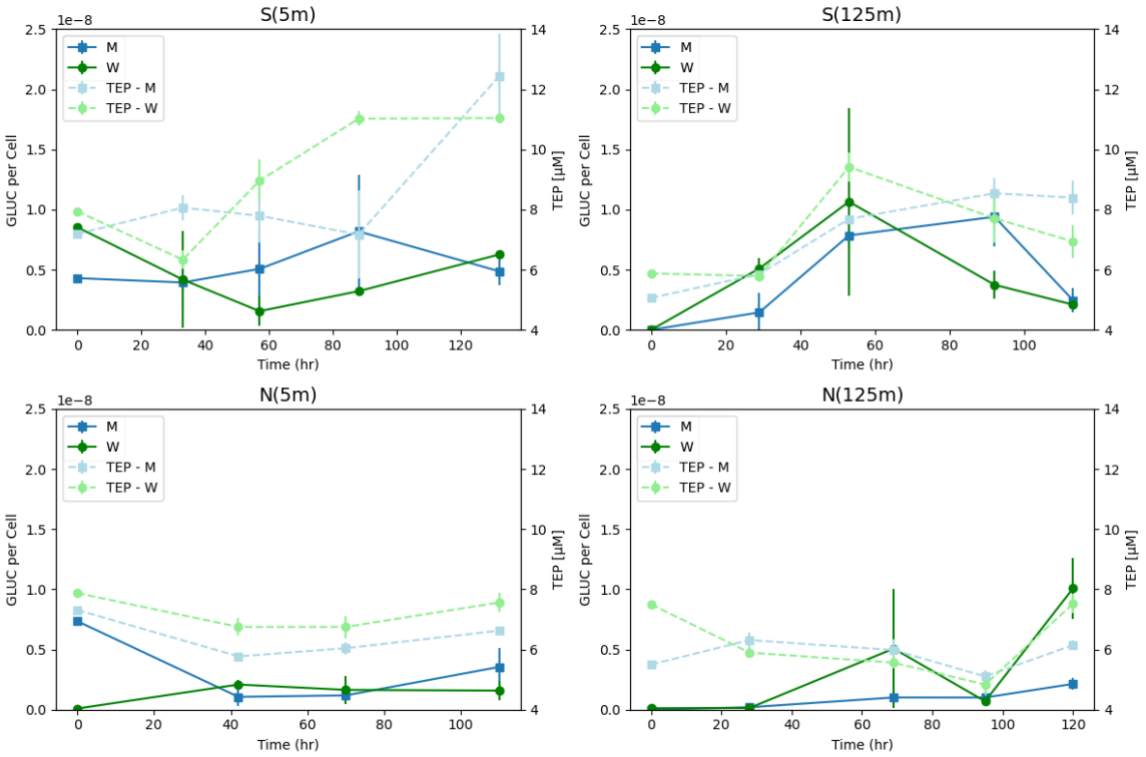
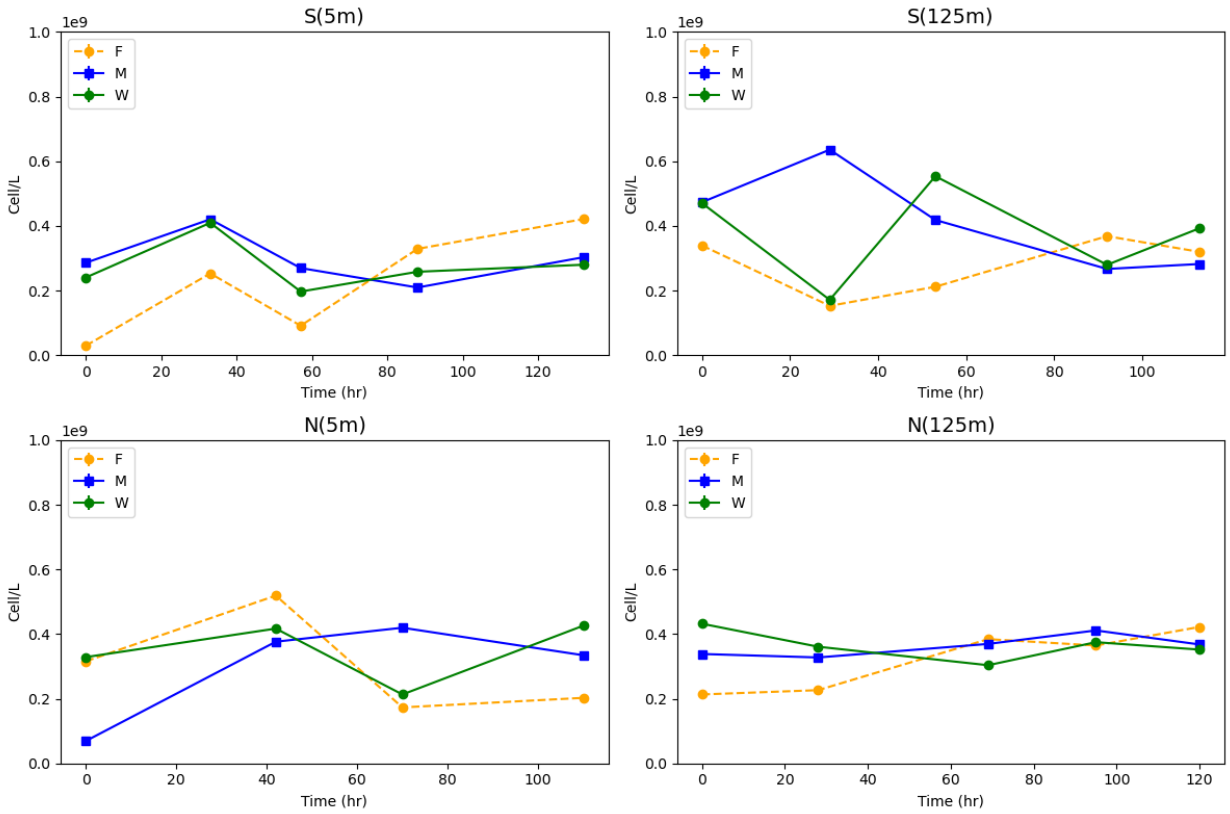


Figure S1. Glucosidase enzyme activity per cell for the Mixed (M) and Whole (W) treatments for each experiment, compared to the TEP activity throughout the incubations in the hatched boxes, plotted with SEM error bars. There is no clear correlation between TEP and Glucosidase activity throughout the incubations.



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124 **Figure S2.** Cells/L are the same order of magnitude as other studies done in Station ALOHA.

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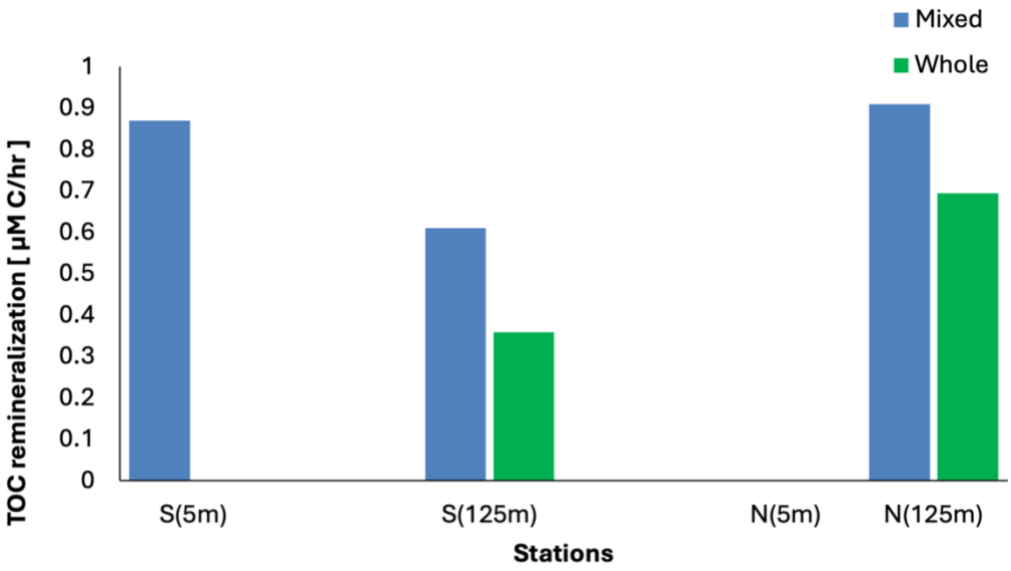

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127 **Figure S3.** The net TOC consumption (C µM/hr) over the time intervals within the incubations
128 where TEP was also exhibiting consumption.

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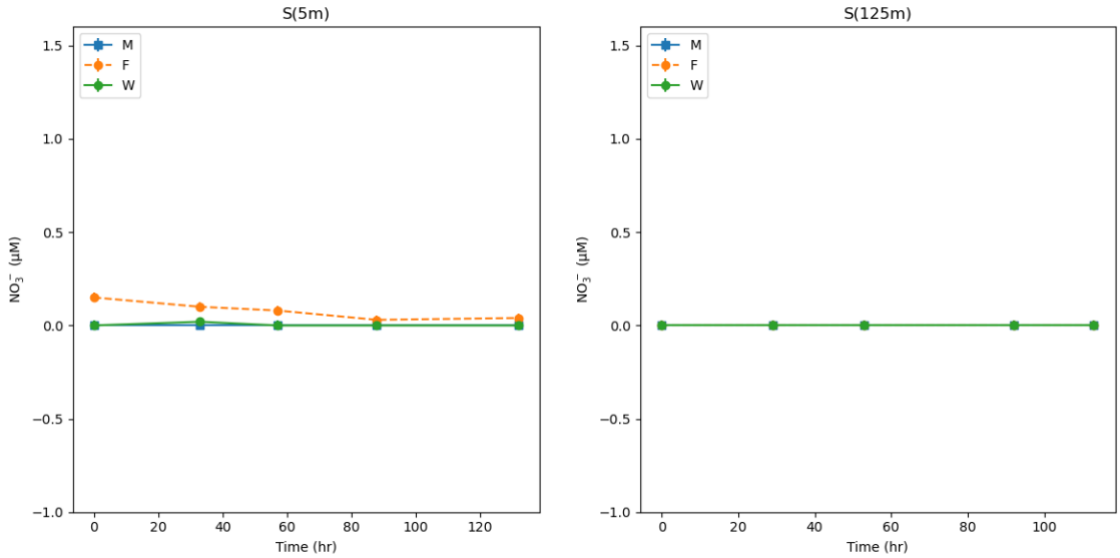
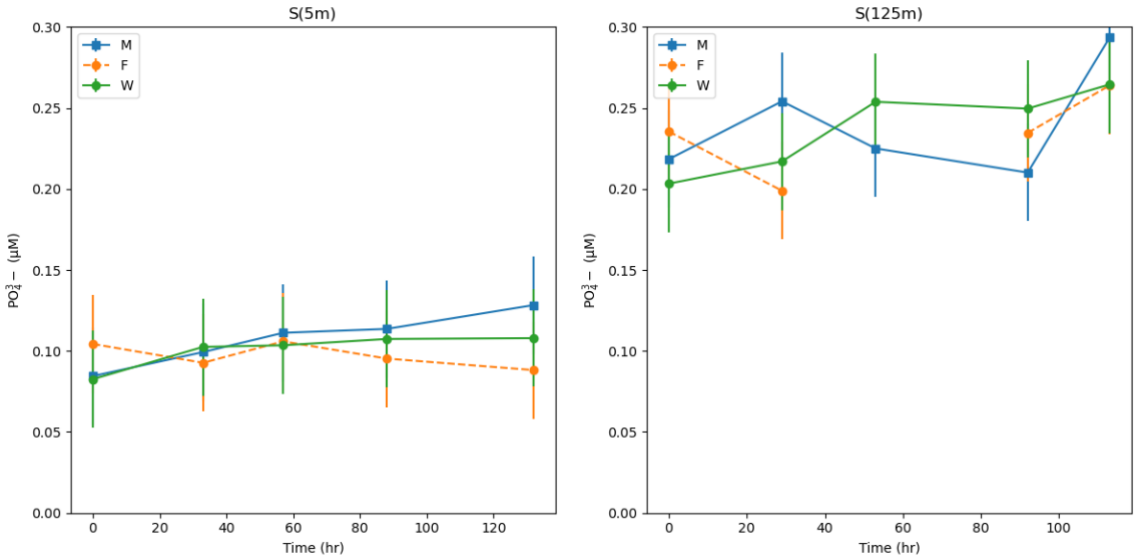

S(5m)
S(125m)
M
F
W
NO_3^- (μM)
Time (hr)


S(5m)
S(125m)
M
F
W
PO_4^{3-} (μM)
Time (hr)

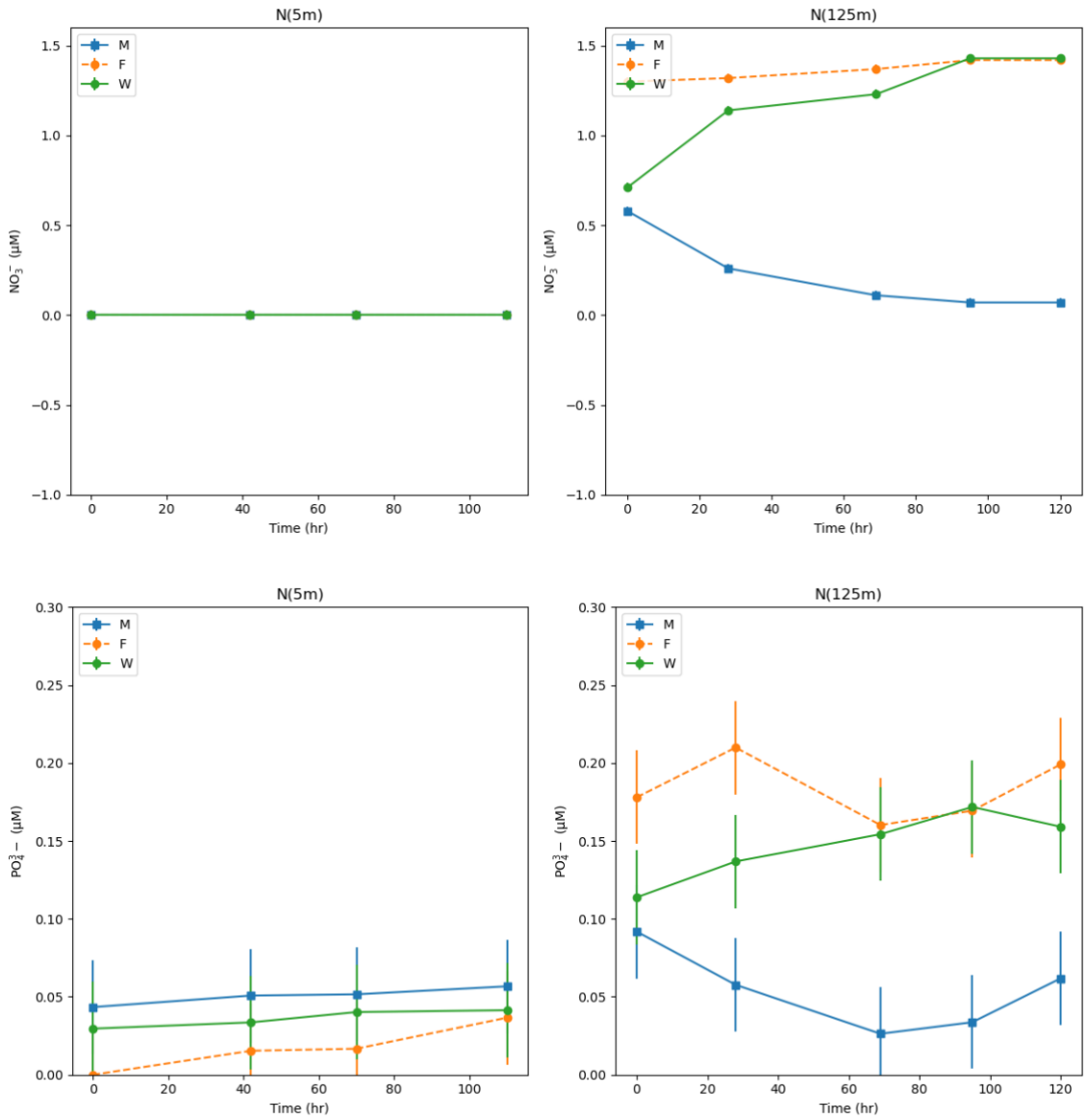


Figure S4. The NO_3^- and PO_4^{3-} concentrations [µM] variability within the incubations at all stations (S(5m),S(125m),N(5m), N(125m)) to identify possible cases of DOM remineralization. At S(125m) and N(5m) the NO_3^- concentrations were below detection. Error bars are the analytical error of NO_3^- determinations via the chemiluminescence method on a NOx Analyzer (ThermoFisher iQ Series 42 NO-NO_2-NO_x Analyzer) of 25 nM. The analytical error of PO_4^- is 30 nM when running seawater samples on a SEAL AQ300 Discrete Analyzer via the colorimetric molybdenum blue method.

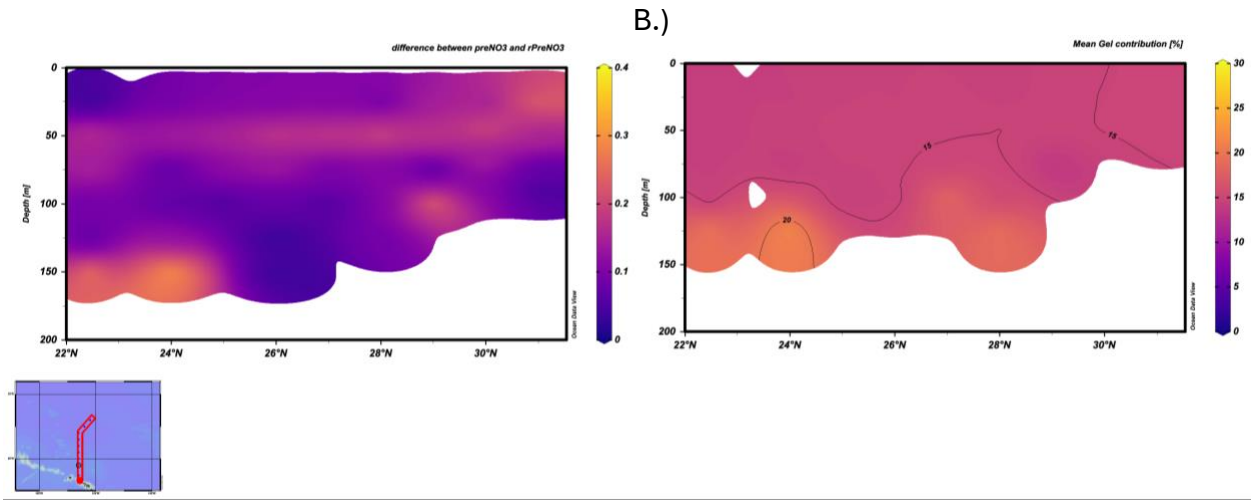


Figure S5. Meridional depth profiles of the June 2021cruise transect (from 22.75 to 31° N along ~ 158° W) A.) Visualizes the difference between the absolute values of the $PreNO_3^-$ tracer [µM] as defined by Letscher & Villareal, (2018) and revised $rPreNO_3^-$ (mean) [µM]. B.) The percent contribution of the gel pool to the preformed nitrate anomaly. Both plots A and B are interpolated with a weighted average.

Table S1 . Exopolymer Mean Consumption Rates (nM/hr), standard deviation of the mean, and number of instances (n) from the filtrate treatment (F).

Station	Mean TEP consumption (nM C/hour)	SEM	n	Mean CSP Consumption (nM N/hour)	SEM	n
S(5m)	-29.0	26.4	2	-0.39	N/A	1
S(125m)	-39.1	36.3	2	-0.14	N/A	1
N(5m)	-8.4	N/A	1	-0.12	0.11	2
N(125)	-13.2	3.6	2	-1.91	0.69	2

*For N(125m) removing the whole treatment.

Table S1. The Mean TEP and CSP consumption rates when isolating the Filtrate (F) treatment are significantly lower than the production rates of the F treatment, which allows for the assumption that the filtrate treatment represents TEP/CSP production/aggregation.