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1 Coupling High frequency monitoring and bioassay experiments to investigate a Harmful algal
2 bloom in the Bay of Seine (French-English Channel)

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4 Léon Serre-Fredj^{a,b}, Franck Jacqueline^c, Maxime Navon^{a,b}, Guillaume Izabel^{a,d}, Léo Chasselin^{a,d},
5 Oriane Jolly^{a,d}, Michel Repecaud^c, Pascal Claquin^{a,b*}

6
7 a. Normandie Université, Université de Caen Normandie, Esplanade de la Paix, F-14032,
8 Caen, France

9 b. Unité Biologie des ORganismes et Ecosystèmes Aquatiques (BOREA, FRE 2030),
10 Sorbonne Université, Muséum National d'Histoire Naturelle, CNRS, Université Pierre et
11 Marie Curie, Université de Caen Normandie, IRD 207, Université des Antilles. Centre de
12 Recherches en Environnement Côtier (CREC) - Station Marine, BP49, 54, rue du Docteur
13 Charcot - 14530 Luc-sur-Mer, France

14 c. Ifremer LER/N, Avenue du Général de Gaulle, 14520, Port-en-Bessin, France

15 d. Centre de Recherches en Environnement Côtier (CREC) - Station Marine de l'Université de
16 Caen Normandie, BP49, 54, rue du Docteur Charcot - 14530

17
18
19 *Corresponding Author

20 Pascal Claquin

21 Email: pascal.claquin@unicaen.fr

22 Address: BOREA Biologie des Organismes et Ecosystèmes Aquatiques, FRE 2030 MNHN
23 CNRS SU UA UCN IRD 207, Université de Caen Normandie, Centre de Recherches en
24 Environnement Côtier (CREC) - Station Marine, BP49, 54, rue du Docteur Charcot - 14530
25 Luc-sur-Mer, France

26

1 Abstract

2

3 Coastal ecosystems are increasingly threatened by eutrophication and dystrophy. In this context, the
4 full pattern of a bloom dominated by the dinoflagellate, *Lepidodinium chlorophorum*, was
5 investigated by a high frequency monitoring buoy equipped with sensors allowing nutrients and
6 photosynthesis measurements. An increase of the N/P ratio affected phytoplankton physiology
7 leading to bloom collapse with a slight oxygen depletion. In parallel, enrichment experiments were
8 performed on the natural bloom population. After 5 days of incubation the community structure,
9 using flow cytometry and several physiological parameters were analysed. The data reveal a
10 potential N and P co-limitation and a decoupling between primary production and productivity in
11 fully enriched conditions. Under unbalanced N/P inputs, high level of alkaline phosphatase activity
12 and transparent exopolymeric particle production, which favour phytoplankton sedimentation, were
13 observed. Nutrient inputs and their stoichiometry control phytoplankton growth, the community
14 structure, physiological regulations, the fate of the bloom and consequences.

15

16 Keywords: *Lepidodinium chlorophorum* ; eutrophication ; FRRf, , Transparent exopolymeric
17 particles ; flow cytometry

1. Introduction

As the ocean's first frontier, coastal ecosystems are increasingly under threats that affect many essential benthic and pelagic habitats of marine species (Barbier et al., 2011). Eutrophication, which is due to excess nutrient inputs in coastal systems caused by human activities is one of the major environmental problems affecting coastal ecosystem in the world (Rabalais et al., 2009). These inputs affect both the concentration of nutrients and their stoichiometry (Martin et al., 2008; Watanabe et al., 2017). The growth and diversity of primary producers, like phytoplankton, which are at the base of the marine food web, are largely controlled by these inputs (Smith, 2006). Unbalanced nutrient inputs and dystrophy, both frequently linked with eutrophication, have several effects on phytoplankton: modification of communities (Leruste et al., 2019; Shen, 2001), alter the growth rate (Nwankwegu et al., 2020), produce allochemical-like toxins which, in turn, favour monospecific blooms (Granéli et al., 2008). Associated with other environmental parameters including light, temperature, water mass residence time, or river discharges, nutrient inputs can lead to phytoplankton blooms (Heisler et al., 2008) that may be classified as harmful algal blooms (HAB) (Anderson, 2009). HAB species are usually split into two groups, one of which is able to produce toxins or harmful metabolites that damage wildlife, or result in poisoning of human seafood, and the second in which high densities of non-toxic cells can harm the environment by producing scums or foams which lead to oxygen depletion (Anderson et al., 2002).

Nutrient inputs into the Bay of Seine (France) are mainly controlled by inputs from the Seine River (Aminot et al., 1998) although smaller rivers inputs also have local impacts (Lemesle et al., 2015). Over the last decades, many programmes for the management of nutrient inputs have reduced nutrient discharges but have mainly had an impact on phosphorus inputs, whereas N inputs remain high (Garnier et al., 2019). The stoichiometry of nutrient inputs have been broadly modified over the last decade and very high N/P ratio inputs are regularly measured in the Seine River and Seine estuary (Meybeck et al., 2018).

1 Although chlorophyll biomass has decreased in recent years in the English Channel (Gohin
2 et al., 2019), the relative proportion of the dinoflagellate group has increased (Hernández-Fariñas et
3 al., 2014; Widdicombe et al., 2010). In the Bay of Seine, several species of dinoflagellate like
4 *Karenia mikimotoi* and *Lepidodinium chlorophorum* are able to form large harmful algal bloom
5 (HAB) (Napoléon et al., 2014). *Lepidodinium chlorophorum* is known to produce large quantities
6 of transparent exopolymeric particles (TEP) (Claquin et al., 2008) and to be responsible for the
7 mortality of marine organisms due to oxygen depletion induced by decomposition of the population
8 (Sournia et al., 1992).

9 Many studies have shown that phytoplankton communities can respond within a few hours
10 after an environmental change (Lefort and Gasol, 2014; Thyssen et al., 2008) which stresses out the
11 necessity of implementing a high frequency sampling (Bouman et al., 2005). In the present work, a
12 late summer *L. chlorophorum* bloom event was studied in detail using high frequency
13 measurements made by an instrumented buoy called SMILE (0° 19.68', 49° 21.23'). In addition to
14 standard sensors (temperature, salinity, turbidity, oxygen, light and fluorescence), the SMILE buoy
15 is equipped with a fast repetition rate fluorimeter (FRRf), which enables photobiological and
16 physiological measurements of the phytoplankton communities, and two types of nutrient sensors,
17 which together provide new and more realistic pictures of bloom dynamics.

18 In addition to monitoring the bloom event, enrichment bioassay experiments were performed on
19 samples collected during the bloom in order to understand how nutrient inputs influence the
20 structure and physiological parameters of the communities. The specific objectives of our studies
21 were to:

22 1) Characterise bloom dynamics from an original point of view by including physiological,
23 productivity, and nutrients measurements at a high frequency.

24 2) investigate phytoplankton dynamics and the ecophysiology of a community dominated by *L.*
25 *chlorophorum* as a function of nutrient inputs.

26

1 **2. Material and Methods**

2

3 **2.1. High frequency measurements using an instrumented buoy**

4 The bloom event was monitored *in situ* using the SMILE buoy in the Bay of Seine (Fig. 1)
5 measuring since 2016 which belong the COAST-HF network (French Coastal ocean observing
6 system - High frequency). Different types of measurements were performed at different frequencies
7 by the buoy.

8 **2.1.1. List of measurements performed at high frequency by the buoy**

9 Conductivity and temperature (tetracon sensor, WTWTM, Germany), turbidity (Seapoint turbidity
10 meter, Seapoint Sensor, USA), oxygen (AADI Oxygen optode, Aanderraa, Norway) and in-vivo
11 fluorescence (Cyclops-6K, Turner Design, USA) sensors that measure fluorescent in fluorescein in
12 fluorescent units (FFU) are grouped in a multiparameter probe NKE instrument (MP7, NKE
13 Instrumentation ®, France), PAR (Photosynthetically active radiation) is measured with a Satlantics
14 sensor (Satlantics, Italy). All these parameters were measured at 20-min intervals (data available
15 doi.org/10.17882/53689). Photosynthetic parameters were determined at 2-h intervals using the
16 FastOcean FRRf3 sensor with the Act2 system (Chelsea Technologies, UK) with an excitation light
17 provided by a blue light-emitting diode (450 nm). Inorganic nutrients (NO_3^- , PO_4^{2-} , NH_3 , NO_2^-)
18 were measured by the chemical probe Wiz (SYSTEA S.p.A, Italy) once a day while an optical
19 sensor, OPUS (Trios, Germany), also measured NO_3^- at 20-min intervals. All measurements were
20 performed at one-meter depth.

21 **2.1.2. High-frequency measurements of inorganic nutrients**

22 The Wiz probe uses micro loop flow analysis (μLFA) for autonomous measurements of four
23 nutrients (NO_3^- , PO_4^{2-} , NO_2^-) using colourimetric methods and NH_3 with a fluorimetric method
24 (Azzaro, 2014; Moschetta et al., 2009). As the buoy has limited energy, measurements were only

1 performed once a day. The detection limits were $0.35 \mu\text{mol.l}^{-1}$ for NO_3^- and $9.2 \cdot 10^{-2} \mu\text{mol.l}^{-1}$ for
2 PO_4^{2-} . NO_3^- was also measured using an optical sensor (OPUS, Trios, Germany). The OPUS UV is
3 a spectral sensor equipped with a xenon flash lamp as light source and a high-end miniature
4 spectrometer (MMS, Zeiss, Germany) with 256 channels covering the spectral range from 200 to
5 360 nm as detector. This sensor measures NO_3^- at high frequency with low energy consumption. A
6 20-mm optical path allowed measurements from 1 to $357 \mu\text{mol.l}^{-1}$ with a precision of $0.18 \mu\text{mol.l}^{-1}$.

7 **2.1.3. High frequency photosynthetic measurements**

8 FLCs (fluorescent light curve) were measured *in situ* by the buoy using a FRRf-ACT2. The
9 samples were analysed after a 5 min period of dark incubation for the oxidation of the Quinone A
10 (Q_A). At the end of the dark period F_0 (minimum fluorescence) was measured by using non actinic
11 weak light. A single-turnover (ST) protocol consisting of 100 flashlets of $1 \mu\text{s}$ with $2 \mu\text{s}$ of interval
12 was applied for measuring F_m (maximum fluorescence in dark) (Kolber et al. 1998). The maximum
13 quantum efficiency of PSII (F_v/F_m) was calculated as (Genty et al., 1989):

$$15 \quad \frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

16 Samples were then exposed to 10 light steps of increasing PAR (from 0 to $1,500 \mu\text{mol photon.s}^{-1}.\text{m}^{-2}$)
17 for 30-sec each step. The effective quantum efficiency of PSII ($F_{q'}/F_{m'}$) was measured at each
18 light step as (Genty et al 1989):

$$20 \quad \frac{F_{q'}}{F_{m'}} = \frac{(F_{m'} - F')}{F_{m'}}$$

21 Where $F_{m'}$ is the maximum fluorescence under light and F' the steady state fluorescence under light.
22 The relative electron transport rate (rETR, relative unit) was calculated for each irradiance (E) as:

$$23 \quad rETR(E) = \frac{F_{q'}}{F_{m'}} \times E$$

1 The maximum relative electron transport rate ($rETR_{max}$) was estimated by fitting the FLC data to
 2 the model of Webb et al. (1974) modified by Boatman et al. (2019) using ACT2RUN (Chelsea
 3 Technologies, UK) software to estimate α and E_k with α , the initial slope of the FLC, and E_k the
 4 light saturation index:

$$5 \quad rETR(E) = \alpha \times E_K \times \left(1 - e^{-\frac{E}{E_K}}\right) - \beta \times E_{K\beta} \times \left(1 - e^{-\frac{E-E_K}{E_{K\beta}}}\right)$$

6 $rETR_{max}$ is calculated as:

$$7 \quad rETR_{max} = \alpha \times E_K$$

8 Using the absorption algorithm of Oxborough et al. (2012), the PSII electrons flux per unit volume
 9 is calculated as follows:

10

$$11 \quad a_{LHII} = \frac{F_m \times F_0}{F_m - F_0} \times K_a \times 10^{-6}$$

12 where K_a is an inherent constant of the FRRf. Finally, the PSII flux per unit volume ($JV_{II_{max}}$) is
 13 calculated as:

$$14 \quad JV_{II_{max}} = \frac{a_{LHII} \times rETR_{max} \times 3600}{24 \times 10^3}$$

15 where $JV_{II_{max}}$ is expressed in $\text{mmol e}^- \cdot \text{m}^{-3} \cdot \text{h}^{-1}$

16

17 The electron transport rate ($ETR_{II_{max}}$) is calculated as:

$$18 \quad ETR_{II_{max}} = \frac{JV_{II_{max}}}{[chla]}$$

19

20 where $ETR_{II_{max}}$ is expressed in $\text{mmol e}^- \cdot \text{mg chla}^{-1} \cdot \text{h}^{-1}$

21

22 **2.2. Enrichment experiment**

23 Bioassays were designed to assess the impacts of enrichment on phytoplankton assemblages
 24 and on physiological responses. The design was modified from Ly et al. (2014) to fit our specific

1 requirements. Seawater was sampled on the 29th of August 2019 at the SMILE buoy site during the
2 bloom event. The seawater was filtered just after sampling on a 100- μm mesh to remove grazers.
3 One hour after sampling, enrichments were applied to 500-ml sub-samples in polycarbonate bottles
4 to perform the bioassays. All the bioassays were incubated in a water bath incubator under natural
5 sunlight for 5 days. The water bath incubator was continuously fuelled with seawater pumped
6 directly from the sea to maintain the temperature. The seawater temperature and PAR were recorded
7 at 5-min intervals by respectively, a RBRsolo T logger and a RBR solo³ PAR logger connected to a
8 Li-COR “Underwater Quantum Sensor” LI-192. Five types of enrichments, each with five
9 replicates, were performed in the incubator: C (control, no enrichment), P (+P), N (+N), NSI
10 (+N+Si) and PNSI (+P+N+Si). The enrichments applied to the bioassays were defined by the
11 maximum value of N, P and Si measured over the year 2018 in the Bay of Seine by the National
12 Network for Environmental Survey SOMLIT (<https://www.somlit.fr/> since 2007 at this site) as
13 respectively 50 $\mu\text{mol.l}^{-1}$ for N, 3 $\mu\text{mol.l}^{-1}$ for P and 50 $\mu\text{mol.l}^{-1}$ for Si.
14 After 5 days, after homogenisation, 25 ml were sampled from all the bioassay bottles to measure
15 photosynthetic parameters, and phytoplankton functional types and alkaline phosphatase activity
16 (APA). At day 5, supplementary measurements of TEP, nutrients and Chla concentration were
17 performed. Only the data obtained at day 5 are shown here. All the measurements were also made
18 just before enrichment at day 0 (D0).

19 **2.2.1. Photosynthetic measurements**

20 Photosynthetic measurements were performed with a second FRRf-ACT2. The same protocol as the
21 one described above for *in-situ* measurements was applied to the samples for incubation. A 5-ml
22 aliquot of sample was placed in the measuring chamber of the FRRf-ACT2 and FLC were
23 performed after a 5-min period of dark adaptation.

24 **2.2.2. Flow cytometry**

25 . Unfixed sample were analysed within one hour of sampling with the CytoSense (Cytobuoy b.v.,
26 Netherland) equipped with a blue laser (488 nm, 50 mW) and a green laser (552 nm, 50 mW). This

1 produces pulse shapes based on the inherent optical properties of the particle when they cross the
2 laser: sideward angle scatter (SWS), forward scatter (FWS), red (FLR, 668-734 nm), orange (FLO,
3 601-668 nm) and yellow fluorescence (FLY, 536-601 nm). The threshold was set at 16 mV to
4 reduce data acquisition concerning non-photosynthetic particles triggered on FLR, for each sample
5 (5 per enrichment) 380 μl were analysed with a speed of $2.0 \mu\text{l}\cdot\text{s}^{-1}$. The CytoSense can analyse
6 chains, cells, or colonies between 1 and 800 μm in diameter, microspheres measuring between 1 μm
7 (Yellow-Green fluorescent, FluoSpheres®) and 1.6 μm (non-fluorescent, provided by Cytobuoy)
8 were used to calibrate size recording (daily use). In order to improve the clustering a mix of bead
9 were analysed with the 1 μm and 1.6 μm and supplementary beads after this experiment (2 μm , 6
10 μm , 10 μm , 20 μm , Fluoresbrite® YG microsphere, Polyscience) to establish size regression (2-6
11 μm and 6-20 μm) and discriminate picoeukaryotes, nanoeukaryotes and microphytoplankton. To
12 distinguish the phytoplankton (Fig., 2), five clusters were determined using the cells' optical
13 properties and attributed to *Synechococcus* spp., picoeukaryotes, nanoeukaryotes and
14 microphytoplankton, Cryptophytes and *Lepidodinium chlorophorum* (Elbrächter and Schnepf,
15 1996; Hansen et al., 2007). The *Synechococcus* spp. cluster has the smallest FWS signal and a high
16 orange fluorescence (FLO) signal which matches very small cells with a high concentration of
17 phycoerythrin.

18 To accurately define the cytometric signature of *L. chlorophorum*, this species was isolated from the
19 water sample during the bloom event. After a few days in culture, the isolated strains of *L.*
20 *chlorophorum* were analysed on the CytoSense to match the signature. Picoeukaryotic cells are small
21 cells ($< 2 \mu\text{m}$) and produce low FLR and FWS signals. Nanoeukaryotics and microphytoplankton
22 cells were differentiated from picoeukaryotic cells using the amplitude of the FLR signal and the
23 bead signal. Cryptophyte clusters have higher FLO than *Synechococcus* due to the high
24 concentrations of phycoerythrin in their cells and an FWS equivalent to the nanoeukaryotic and
25 microphytoplankton cells (Olson et al., 1989; Thyssen et al., 2014).

26 **2.2.3. Measurements of inorganic nutrients (NO_3^- , PO_4^{3-} , $\text{Si}(\text{OH})_4$)**

1 Water samples were collected and filtered through a cellulose acetate filter (ClearLine, CA, 33 mm,
2 0.45 μm) in 50 ml falcon tubes and immediately frozen ($-20\text{ }^{\circ}\text{C}$), with the exception of $\text{Si}(\text{OH})_4$
3 ($4\text{ }^{\circ}\text{C}$). Analyses were conducted using a Seal Analytical AA-3 system (Aminot and K  rouel, 2007).
4 The limits of quantifications were $0.02\text{ }\mu\text{mol.l}^{-1}$ for PO_4^{3-} and $0.05\text{ }\mu\text{mol.l}^{-1}$ for NO_3^- , NO_2^- , $\text{Si}(\text{OH})_4$.
5 N/P ratio was calculated as $(\text{NO}_2^- + \text{NO}_3^-) / \text{PO}_4^{3-}$.

6 **2.2.4. Chlorophyll-a measurements**

7 Water samples (250 ml) were filtered through a glass-fiber filter (Whatman, GF/F, 47 mm) and
8 immediately frozen ($-20\text{ }^{\circ}\text{C}$) until analysis. Ten ml of 90% acetone (v/v) were added to extract the
9 pigment and the samples were then left in the dark at $4\text{ }^{\circ}\text{C}$ for 12 h. After being centrifuged for 5
10 min at 1,700 g twice, the Chl a concentration of the extracts was measured using a Trilogy
11 fluorimeter (Turner Designs, Sunnyvale, USA) according to the method of Strickland and Parsons
12 (1972).

13 **2.2.5. Transparent exopolymeric particles (TEP)**

14 Water samples (150-200 ml) were filtered through polycarbonate membrane filters
15 (Millipore, $0.4\text{ }\mu\text{m}$) and immediately frozen ($-20\text{ }^{\circ}\text{C}$) until analysis. According to Claquin et al.
16 (2008) adapted from Passow and Alldredge (1996), the filters were stained with a solution 0.02%
17 Alcian blue (Sigma) with 0.06% acetic acid (pH: 2.5). Excess dye was removed by adding water
18 before centrifugation at 3,000 g, $19\text{ }^{\circ}\text{C}$ for 15 min. This washing cycle was repeated twice, then 6
19 ml of 80% H_2SO_4 were added. After 2 h, measurements were conducted using a spectrometer
20 (Ultrospec 1000, Pharmacia Biotech) at 787 nm. Calibration were done using a xanthan gum (10-
21 700 μg) as standard, as described by Claquin et al. (2008). After being divided by the Chla
22 concentration, TEP concentrations are expressed in $\mu\text{g Xanthan eq.}\mu\text{g chl}^{-1}$.

23 **2.2.6. Alkaline phosphatase activity (APA)**

24 APA, as the potential maximum activity per chlorophyll unit, was measured according to
25 Hrustic et al, (2017). Samples (3920- μl) were placed in an UV-cuvette and 80 μl of 4-
26 Methylumbelliferyl phosphatase (MUF-P) 500 μM substrate were added. While incubating at room

1 temperature, the samples were measured at hourly intervals over a total period of 7 h.
2 Measurements were taken with a RF-6000 spectrofluorophotometer (Shimadzu, Japan). APA was
3 calculated as the slope of the linear regression of the evolution of concentration while incubating.
4 Using a MUF standard curve, the results are expressed in concentration units per hour divided by
5 the concentration in Chl *a* to normalize ($\text{nM}\cdot\text{h}^{-1}\cdot\mu\text{g chl}a^{-1}\cdot\text{l}^{-1}$).

6 **2.3. Statistical and data analysis**

7 Analyses were conducted using R software (R-project, CRAN) version 3.6.1. As the
8 incubation data did not follow a normal law, a Kruskal-Wallis test was performed using “stats”
9 package and a Conover-Iman pairwise test was performed using the “conover.test” package. Only
10 significant tests with a p-value < 0.05 were accepted.

1 **3 Results**

2 **3.1. Bloom time series**

3 High frequency measurements provided by the SMILE buoy are presented in Fig.3. An
4 increase in FFU and oxygen saturation on the 24th of August indicated the beginning of the bloom
5 and remained high until the end of the bloom event on the 30th of August, 2019 (Fig. 3 A, B). The
6 highest dissolved O₂ concentration and FFU were measured on the 27th of August with respectively
7 16.5 mg O₂ .l⁻¹ and 165 FFU. This highest value of FFU was also reached the 28th, 29th and 30th
8 August because of a saturation of the FFU sensor. The lowest oxygen concentration ever recorded
9 in 2 years (data available) was measured the 31th August with a value of 5.9 mg.l⁻¹. Based on the
10 variation in O₂ and FFU, the bloom lasted six days. The temperature increased from 19.3 °C on the
11 20th of August to reach a maximum of 23.0 °C on the 27th of August and decreased to 19.6 °C on the
12 3rd of September (Fig. 3 C). This short rise in temperature occurred after a drop in temperature
13 starting on the 27th of August. Turbidity increased on the 27th of August, three days after the
14 beginning of the bloom, to reach 120 NTU before dropping on the 29th of August (Fig. 3 D).
15 Between the 25 and 30th of August, high freshwater input events were observed, leading to a drop in
16 salinity from 33.7 to 31.4 on the 29th of August (Fig. 3 E). The light pattern appeared to be quite
17 stable between days (with a maximum of 1,600 μmol photons.m⁻².s⁻¹) with a decrease at mid-bloom
18 (Fig. 3 F).

19 Nutrients N, P, Si followed the typical summer trend observed at this site in the English
20 Channel (see Data SOMLIT, the French Coastal Monitoring Network; <https://www.somlit.fr/> since
21 2007 at this site) with low stocks of nutrients at the beginning of August followed by nutrient inputs
22 at the end of the month (Napoleon et al., 2012). An increase in PO₄³⁻ and NO₃⁻ concentrations was
23 observed on the 21st of August just before the bloom began (Fig. 4 B, C, D). After the beginning of
24 the bloom, NH₃ increased from 2.3 μmol.l⁻¹ to 5.5 μmol.l⁻¹ on the 4th of September (Fig. 4 A).
25 During the bloom, a decrease was observed in the concentration of PO₄³⁻ from 1.7.10⁻¹ to 0.2.10⁻¹

1 $\mu\text{mol.l}^{-1}$ (Fig. 4 D) whereas there was an increase in NO_3^- which reached $1.5 \mu\text{mol.l}^{-1}$ and increased
2 the N/P ratio to 71 (Fig. 4 E) on the 26th of August. The OPUS sensor enabled nitrate concentrations
3 to be monitored at a higher frequency (20-min intervals) than the WIZ sensor (24-h intervals). Both
4 sensors showed the same general trend for NO_3^- with an increase at the beginning of the bloom
5 event followed by a decrease on the 29th of August (Fig. 4 B, C). The high frequency data provided
6 by the OPUS sensor revealed a dramatic drop in NO_3^- concentrations on the 26th of August, two
7 days after the beginning of the bloom (Fig. 4 C). The concentration on Si(OH)_4 on the 30th of
8 August was $13.16 \mu\text{mol.l}^{-1}$ according to the SOMLIT database and the Si/N and Si/P ratios were
9 respectively 8.7 and 329.0.

10 Fv/Fm displayed a daily cyclic pattern, the overall decrease of Fv/Fm the first day (from 20th
11 to 26th August) is less than 0.03. Daily variation display more variation than this overall decrease,
12 the mean value for this 6-days period is 0.57 ± 0.01 (n=60). The Fv/Fm dropped on the 26th of
13 August and reached a minimum value of 0.30 on the 28th of August. Finally, Fv/Fm increased to
14 reach a value of 0.55 on the 1st of September (Fig. 5 A). JV_{IImax} showed a daily cycle based on the
15 daily light cycle. JV_{IImax} values showed a steadily rising trend that reached a peak of 89.51 mmol e^-
16 $\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ on the 28th of August. After this peak, a dramatic drop in JV_{IImax} values was observed. The
17 JV_{IImax} value reached a value of $89.5 \text{ mmol e}^- \cdot\text{m}^{-3}\cdot\text{h}^{-1}$ on the 27th of August (Fig. 5 B). $\text{ETR}_{\text{IImax}}$
18 values also showed a daily cycle, in which the values increased steadily before a dramatic drop on
19 the 28th of August. The $\text{ETR}_{\text{IImax}}$ reached a value of $29.4 \text{ mmol e}^- \cdot\text{mg chla}^{-1}\cdot\text{h}^{-1}$ on the 3rd of
20 September (Fig. 5 C).

21 **3.2. Incubation results**

22 During the 5-day incubation period, the mean value of the water temperature was $19.8 \text{ }^\circ\text{C}$
23 and the maximum value of the PAR was $2112 \mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with an overall mean value
24 during daily light periods of $441 \mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

1 The initial inorganic nutrient pool was low in the D0 sample with 3.0 $\mu\text{mol.l}^{-1}$ of nitrate and
2 a phosphate value below the detection limit ($< 0.02 \mu\text{mol.l}^{-1}$). After incubation, nitrate
3 concentrations in N and NSi enrichments decreased from 53 $\mu\text{mol.l}^{-1}$ (D0 value + enrichment) to
4 $31.5 \pm 7.65 \mu\text{mol.l}^{-1}$ in each enrichment (Fig 6. A), silica was not consumed in the NSi enrichment
5 (mean = $54.28 \mu\text{mol.l}^{-1}$) (Fig 6. C). In the P enrichment, the phosphate concentration decreased
6 from 3 $\mu\text{mol.l}^{-1}$ (D0 value + enrichment) to $1.9 \pm 0.15 \mu\text{mol.l}^{-1}$ (Fig 6. D). Finally, in the NPSi
7 enrichment, both nitrate and phosphate were nearly fully consumed with $3 \pm 0.07 \mu\text{mol.l}^{-1}$ of nitrate
8 and $0.09 \pm 0.05 \mu\text{mol.l}^{-1}$ of phosphate left while only $19 \pm 2.14 \mu\text{mol.l}^{-1}$ of silica remained at the
9 end of the incubation period. After 5 days of incubation, the concentration of Chl *a* decreased in the
10 control, P, N, and NSi enrichments compared to the D0 value of 8.51 ± 1.04 (n=3) whereas in the
11 NPSi Chl *a* concentration (Fig. 7 A) increased significantly (mean = $22.16 \mu\text{g.l}^{-1}$, Kruskal-Wallis: $\chi^2 = 13.6$;
12 $p_{\text{value}} = 8.6 \cdot 10^{-3}$, Conover: mean $p_{\text{value}} = 1.9 \cdot 10^{-3}$).

13 Some common patterns regarding cells concentration were observed in the cytometric
14 phytoplankton groups in all bioassays with enrichment. In the group of smaller cells:
15 *Synechococcus*, the number of cells dropped after 5 days of incubation with no significant
16 difference between enrichments (Fig. 7 B). Regarding picoeukaryotes, the mean concentration of
17 cell in the P enrichment ($2.0 \cdot 10^4 \pm 3.8 \cdot 10^3 \text{ cells.cm}^{-3}$) was higher than the control, and the N and NSi
18 enrichments, with mean values of respectively, $1.8 \cdot 10^4$, $8.6 \cdot 10^3 \pm 2.8 \cdot 10^3$ and $7.3 \cdot 10^3 \pm 2.3 \cdot 10^3$
19 cells.cm^{-3} . NPSi enrichment showed the highest concentration of picoeukaryotes (mean = $5.4 \cdot 10^4 \pm$
20 $1.5 \cdot 10^4 \text{ cells.cm}^{-3}$) (Kruskal-Wallis: $\chi^2 = 19.0$, $p_{\text{value}} = 7.9 \cdot 10^{-4}$) (Fig. 7 C). Nanoeukaryotes also
21 showed the highest concentration for NPSi enrichment with $9.9 \cdot 10^4 \pm 3.4 \cdot 10^4 \text{ cells.cm}^{-3}$ (Kruskal-
22 Wallis: $\chi^2 = 13.3$, $p_{\text{value}} = 1.2 \cdot 10^{-2}$, Conover: mean $p_{\text{value}} = 1.7 \cdot 10^{-3}$). Regarding other treatments,
23 controls contained higher concentrations of nanoeukaryotes ($2.4 \cdot 10^3 \pm 8.8 \cdot 10^2 \text{ cells.cm}^{-3}$) than other
24 enrichments with respectively $2.1 \cdot 10^3 \pm 4.5 \cdot 10^2$, $1.8 \cdot 10^3 \pm 1.0 \cdot 10^3$ and $1.9 \cdot 10^3 \pm 9.1 \cdot 10^2 \text{ cells.cm}^{-3}$
25 with P, N and NSi enrichments (Fig. 7 D). Cryptophytes exhibited the same trend as nanoeukaryotes
26 with a higher concentration in the control than in P, N and NSi enrichments (Fig. 7 E) while NPSi

1 enrichment showed the significant higher concentration ($\chi^2 = 17.5$, $p_{\text{value}} = 1.4 \cdot 10^{-3}$, Conover: mean
2 $p_{\text{value}} = 4.2 \cdot 10^{-3}$), but the value may be too low to draw conclusion. Finally, with an initial
3 concentration of $389 \pm 166 \text{ cells.cm}^{-3}$ *L. chlorophorum* decreased in all clusters except for the NPSi
4 enrichment. The lowest concentration of $127 \pm 26 \text{ cells.cm}^{-3}$ was measured in controls while the
5 highest was observed in NPSi enrichment with $1,876 \pm 1,496 \text{ cells.cm}^{-3}$. The difference in NPSi
6 enrichment observed in this species was highly significant (Kruskall-wallis: $\chi^2 = 18.5$, $p_{\text{value}} =$
7 $9.7 \cdot 10^{-4}$, Conover: mean = $2.2 \cdot 10^{-4}$) compared to in all the other enrichments (Fig. 7 F). The values
8 measured for *L. chlorophorum* in the incubation are consistent with the maximum concentration
9 value observed in august 2010, in the Bay of Seine by the French Observation and Monitoring
10 program for Phytoplankton and Hydrology in coastal waters (REPHY , DOI: 10.17882/47248) with
11 $418.3 \text{ cells.cm}^{-3}$.

12 Low values of APA $1.2 \cdot 10^{-3} \pm 1.5 \cdot 10^{-4} \text{ nM.h}^{-1} \cdot \text{mg chla}^{-1} \cdot \text{l}^{-1}$ were measured at D0 just after
13 sampling. After incubation, APA increased in all enrichments by a factor of 15, 19; 24.8; 44.6; 4.4
14 respectively, in the control, P, N, NSi and NPSi enrichments compared to at D0. The minimum
15 value of $6.6 \cdot 10^{-3} \pm 3.9 \cdot 10^{-3} \text{ nM.h}^{-1} \cdot \text{mg chla}^{-1} \cdot \text{l}^{-1}$ was observed in the NPSi cluster (Fig. 7 G) but no
16 significant differences were found among enrichments (Kruskall-Wallis test). As observed for APA,
17 the concentration of TEP increased in all treatments except in the NPSi enrichment where TEP
18 decreased. The concentration of TEP increased by a factor of 3 in the P enrichments ($0.3 \pm 0.05 \mu\text{g}$
19 Xanthan $\text{equ.}\mu\text{g chla}^{-1}$), by a factor 1.9 in both the N and NSi enrichments ($0.5 \pm 0.21 \mu\text{g}$ Xanthan
20 $\text{equ.}\mu\text{g chla}^{-1}$). NPSi enrichment presented the lowest concentration ($0.043 \pm 0.03 \mu\text{g}$ Xanthan
21 $\text{equ.}\mu\text{g chla}^{-1}$) but no significant differences were found among the treatments because of the high
22 variability of the results between replications (Fig. 7 H).

23 Fv/Fm had a significantly high value in the NPSi enrichment with a mean of 0.46 ± 0.03
24 (Kruskall-Wallis: $\chi^2 = 10.6$, $p_{\text{value}} = 3.0 \cdot 10^{-2}$, Conover: mean $p_{\text{value}} = 1.2 \cdot 10^{-2}$) (Fig 8. A) which is
25 higher than the D0 value (0.41). The highest maximum production capacity (JV_{IImax}) was also
26 measured in the NPSi enrichments (Fig 8. B) with a value of $162 \pm 77 \text{ mmol e}^{-} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ revealing a

1 significant difference from that in the other treatments (Kruskall-Wallis : $\chi^2 = 9.7$, $p_{\text{value}} = 4.0 \cdot 10^{-2}$,
2 Conover: mean $p_{\text{value}} = 3.6 \cdot 10^{-2}$) and again higher than the D0 value ($6.1 \text{ mmol e}^- \cdot \text{m}^{-3} \cdot \text{h}^{-1}$). The
3 highest electron transport rate ($\text{ETR}_{\text{IImax}}$) was measured in the P and NSi enrichments (Fig 8. B)
4 with values of respectively, 15.5 ± 5.2 , $15.3 \pm 6.5 \text{ mmol e}^- \cdot \text{mg chl} \cdot \text{h}^{-1}$. A value of $8.1 \pm 3.1 \text{ mmol}$
5 $\text{e}^- \cdot \text{mg chl} \cdot \text{h}^{-1}$ was measured in the NPSi enrichment but no significant differences were found
6 between this and the other treatments (Kruskall-Wallis : $\chi^2 = 10.6$, $p_{\text{value}} = 3.0 \cdot 10^{-2}$), Conover: mean
7 $p_{\text{value}} = 7.6 \cdot 10^{-2}$) (Fig 8. C), the value is however lower than the D0 value ($21.7 \text{ mmol e}^- \cdot \text{mg chl} \cdot$
8 h^{-1}).

9

10 **4. Discussion**

11 The high frequency data provided by the instrumented buoy SMILE alerted us to the
12 occurrence of a HAB bloom dominated by the Dinophyceae *Lepidodinium chlorophorum* in late
13 August 2019, in the Bay of Seine. *L. chlorophorum* contained green chloroplasts probably derived
14 from a prasinophyte endosymbiont (Hansen et al., 2007). Dinophyceae species with green
15 chloroplasts are rare (Hansen et al., 2007) and to our knowledge *L. chlorophorum* is the only “green
16 Dinophyceae” that has been identified in the English Channel (Sournia et al., 1992 and REPHY data
17 base), so we were able to define a specific cytometric signature to characterise this species in our
18 samples. The cytometric signature was confirmed in a strain isolated during the bloom thus
19 allowing us to be extremely confident in our determination and the counting of *L. chlorophorum* in
20 the present study. Although *L. chlorophorum* was first described in the English Channel in 1982
21 (Sournia et al., 1992), its ecology is still poorly documented. It is described as an euryhaline
22 species, able to produce dinocysts (Elbrächter and Schnepf, 1996; Honsell and Talarico, 2004), with
23 an optimum growth temperature of $22 \text{ }^\circ\text{C}$ established by Claquin et al. (2008) using a *L.*
24 *chlorophorum* strain isolated in the English Channel. The conditions at the end of August 2019 were
25 suitable to trigger a major *L. chlorophorum* bloom. The monitoring data from the SMILE buoy
26 revealed the influence of nearby river outflows which, in summer, in the Bay of Seine, are defined

1 as warmer and more turbid freshwater inputs enriched in nutrients (Morelle et al., 2018; Napoléon
2 et al., 2012). As already reported in many studies, these factors favour Dinophyceae blooms
3 (Glibert, 2016), whose *L. chlorophorum* (Pozdnyakov et al., 2017) underlined the importance of
4 rises in temperature while (Karasiewicz et al., 2020; Siano et al., 2018) pointed to the role of a drop
5 in salinity during *L. chlorophorum* bloom events. Beside this general behaviour, which is widely
6 described in the literature, our high-frequency data allowed us to monitor both the bloom and
7 nutrient dynamics and the physiological state of the community in detail. The combination of a rise
8 in temperature, a drop in salinity and nutrient inputs characteristic of estuarine waters triggered a
9 bloom event in the bay which lasted six days on the monitoring site. After six days, the level of
10 fluorescence dropped to its original value, marking the end of the bloom. The end of a bloom event
11 can be triggered by different processes, such as a predatory (Tiselius and Kuylenstierna, 1996),
12 virus event (Schroeder et al., 2003) or nutrient depletion (Walve and Larsson, 2007). In the present
13 study, the high N/P ratio points to phosphate depletion. Thus, P appears to be a limiting nutrient and
14 can be assumed to be the main driver of the decline in the bloom. Previous studies demonstrated
15 that a bloom collapse can be explained by phosphate limitation (Walve and Larsson, 2007). The
16 hypothesis of a P-limited bloom is supported in this study by the Fv/Fm value, which underwent a
17 dramatic drop associated with a decline in biomass estimated by FFU one day after this drop. A
18 value of 0.56 was measured for the Fv/Fm during the first three days of the bloom event, then
19 decreased to 0.29 at mid bloom. This decrease could be partly due to a non-photochemical
20 quenching mechanism (Harrison and Smith, 2013) but in our case, the minimum value appeared to
21 be tightly linked to the increase in the N:P ratio. In the literature, a drop in the maximum quantum
22 yield has been associated with nutrient limitation (Behrenfeld et al., 2004; Claquin et al., 2010),
23 rapidly rising temperatures (Zhang et al., 2012), a change in salinity (D'ors et al., 2016), or even a
24 parasitic event (Park et al., 2002). As *L. chlorophorum* tolerate a rise in sea temperature and a drop
25 in salinity (Pozdnyakov et al., 2017; Siano et al., 2018), in our case, we assume that the decline in
26 the maximum quantum yield was most likely caused by the phosphate limitation. P limitation has

1 been shown to significantly reduce the Fv/Fm in natural communities, in the field (Napoléon et al.,
2 2012), in bioassays (Ly et al., 2014), and in lab cultures (Napoléon et al., 2013; Tan et al., 2019;
3 Wongsansilp et al., 2016; Wu et al., 2012). Besides, the maximum quantum yield decreased at mid
4 bloom; the $JV_{II_{max}}$ remained high because of the high level of biomass, and then dropped
5 dramatically with the collapse of the bloom.

6 High-frequency sampling enabled by the SMILE buoy provided evidence for a link between
7 P limitation and the collapse of a HAB bloom of *L. chlorophorum*. In a more general context,
8 dinoflagellates have been associated with eutrophication since industrial development (Kim et al.,
9 2018) and a recent shift has been observed (Fischer et al., 2020; Klais et al., 2013; Spilling et al.,
10 2018) at global scale comprising an increase in the dinoflagellate/diatom ratio. Wasmund (2017)
11 pointed to the relationship between an increase in the dinoflagellate/diatom ratio and a decrease in
12 the quality indicator of environmental status assessment. The same trend has been described in the
13 western English channel (Widdicombe et al., 2010) and in the Bay of Somme (Hernández-Fariñas et
14 al., 2014). High-frequency monitoring allowed to understand and characterise short term processes
15 and the factors which can trigger a large-scale bloom event and its fate. A bioassay enrichment
16 experiment was performed during the bloom event to more deeply explore the behaviour and
17 possible, control of bloom dynamics. Studying enrichments in a phytoplankton community involved
18 in a natural bloom event is quite rare, and this enrichment bioassay enabled us to test other
19 outcomes and their effects on phytoplankton communities as well as on several physiological
20 indicators. However, the results obtained during the incubation have to be carefully consider in
21 comparison with *in situ* data because the daily dynamics was not followed during the five days and
22 many factors like hydrodynamics or grazing for example were removed. Beyond drawing a parallel
23 between our microcosm experiments and our observations of a natural bloom, this second step of
24 our study allowed us to investigate experimentally shifts in phytoplankton diversity during a HAB
25 of *L. chlorophorum* and its effect on ecological functions such as primary productivity as a function
26 of nutrient inputs. The relationship between primary productivity and biodiversity drives ecosystem

1 biomass production and is therefore a major question of ecological functioning and ecosystem
2 management (Hodapp et al., 2019).

3 The first outcome of this experiment was the remarkable behaviour of NPSi enrichment in
4 comparison with other treatments. NPSi enrichment resulted in higher concentrations of almost all
5 cytometric populations apart from *Synechococcus*. Bloom communities were not sustained by
6 partial enrichment in N, or P or NSi. Only NPSi enrichment was able to trigger growth of the
7 phytoplankton community. Unfortunately, an NP enrichment could not be performed because only
8 five conditions could be tested into our incubator. Despite the absence of NP enrichment conditions,
9 the experiment highlighted an apparent N, P co-limitation in this bioassay. N and P had to be added
10 to significantly stimulate growth. Furthermore, it is important to note that in the NPSi enrichment,
11 unlike Si, N and P were depleted. Such co-limitations have been extensively studied and discussed
12 in coastal ecosystems (Davidson and Howarth, 2007) and NP inputs are known to promote
13 dinoflagellate growth (Reynolds, 2006). During our experiments, the growth of all cytometric
14 eukaryotic phytoplankton communities was stimulated under NPSi enrichment. Our results showed
15 that the growth of *L. chlorophorum* was only stimulated in the fully replete condition.

16 A higher primary production capacity ($JV_{II_{max}}$) and a higher physiological status (Fv/Fm)
17 were observed in the NPSi enrichment while lower productivity capacity ($ETR_{II_{max}}$) was measured
18 in comparison with other treatments. The decoupling of production ($JV_{II_{max}}$) and productivity
19 ($ETR_{II_{max}}$) in the NPSi enrichment was unexpected. This decoupling can be attributed to the size
20 community structure, as differences in productivity between picoeucaryote, nanoeucaryote and
21 microphytoplankton have been reported (Lefebvre et al., 2012; Li et al., 2011; Zhu et al., 2019) and
22 may be one consequence of photoacclimatation processes. The increase in biomass in the NPSi
23 enrichment reduced light penetration and increased self-shading (Huisman and Weissing, 1994)
24 leading to an increase in the concentration of Chla in the photosystem antenna (Falkowski and
25 LaRoche, 1991; MacIntyre et al., 2002). Such regulation has been associated with an internal
26 increase in pigment self-shading, thereby reducing the pigment-specific light absorption coefficient

1 (σ_{PSII}) (Finkel et al., 2004). In our study, the lowest specific light absorption coefficient (σ_{PSII}) at
2 440 nm (Kolber et al., 1998) was measured in the NPSi enrichment (See supplementary data).
3 Therefore, the self-shading mechanism makes it possible to explain the uncoupling observed
4 between $JV_{I_{max}}$ and $ETR_{I_{max}}$ in these optimal growth conditions.

5 APA (as the potential maximum activity per chlorophyll unit) was quantified in order to
6 characterise the P limitation during our experiment (Kuenzler and Perras, 1965; Lin et al., 2016;
7 Tanaka et al., 2006). Alkaline phosphatase enables cells to access organic phosphate but the process
8 is energy consuming (Falkowski and Raven, 1998; Lin et al., 2016). Dinoflagellates can make
9 efficient use of dissolved organic phosphate (Wang et al., 2011), and quantifying APA is one way to
10 assess this use when P is limited. According to the APA, the enrichment conditions applied in this
11 bioassay can be gathered in three groups: (i) high P limitation for N and NSi, enrichments; (ii)
12 medium P limitation for control and P and finally (iii) low P limitation for NPSi enrichment. The
13 lack of any significant difference between controls and P enrichment despite the addition of P is
14 surprising. P enrichment is N limited, which notably affects cell physiology. Previous studies
15 showed that other limitations, like N (Kuenzler and Perras, 1965) or Si (Fuentes et al., 2014), can
16 lead to an increase in APA. Changes in the community structure may also influence APA (Lin et al.,
17 2015; Yuan et al., 2017). The observed differences in the picoeukaryote community under P replete
18 conditions could alter nutrient uptake behaviour because picoeukaryotes are known to be very
19 efficient in assimilating phosphorus. We hypothesise that this community produces more alkaline
20 phosphatase than the other communities observed in the other enrichments. In addition,
21 picoeukaryotes appeared to be favoured by P enrichment. This result showed that picoeukaryote
22 was able to grow under N limitations, which are frequently reported in oligotrophic areas (MacKey
23 et al., 2009; Siokou-Frangou et al., 2010) but rarely in eutrophic areas like the Bay of Seine where
24 N limitation is only observed in summer (Napoléon et al., 2012; Thorel et al., 2017). Our results
25 also showed that *Synechococcus* was unable to grow under any of the conditions we tested, which is

1 probably a bias introduced by our incubation methodology. The closed system could create greater
2 predatory pressure leading to a higher death rate of *Synechococcus* (Agawin et al., 2000).

3 Si depletion did not occur either during the bloom event or during the bioassay, but even if
4 Si was not limited during the course of this experiment, diatoms were not able to take over
5 domination from dinoflagellates. In the literature, dinoflagellate dominance over diatoms is
6 frequently reported to be driven by Si depletion (Liang et al., 2019; Zhou et al., 2017). Moreover,
7 previous works showed that both in a natural bay (Lie et al., 2011) and in a model (Tett and Lee,
8 2005), when the N:Si ratio increases, the dinoflagellate/diatom ratio also increases. This supports
9 our field measurements, which showed that environmental status (temperature and salinity)
10 combined with an input of N and P triggered the dinoflagellate bloom even though Si was not
11 limiting. Inputs of unbalanced nutrient water would not have triggered the bloom, as shown by the
12 absence of growth in the experiment when only P or N were added.

13 TEP is widely associated with carbon excretion by phytoplankton (Claquin et al., 2008;
14 Klein et al., 2011) even if bacteria can also promote TEP precursors (Li et al., 2016, 2015).
15 Moreover, in the literature, TEP production is associated with N limitation (Beauvais et al., 2003;
16 Corzo et al., 2000; Deng et al., 2016) or high N/P ratio (Mari et al., 2005). In our study, P, N, NSi
17 enrichments displayed higher TEP concentrations per Chla unit. As mentioned previously, in all
18 these experimental conditions, phytoplankton growth was limited in P or in N. Conversely, under
19 non-limited growth conditions, i.e., the NPSi treatment, TEP production was reduced, which is
20 physiologically coherent. Indeed, TEP can be considered as an overflow of carbon. As nutrient
21 stresses do not allow proper carbon assimilation in the different metabolic pathways, carbon is
22 accumulated as carbohydrates and part is excreted as TEP precursors. *L. chlorophorum* is described
23 in the literature as a high TEP producer (Claquin et al., 2008; Sournia et al., 1992). Claquin et al.,
24 (2008) showed under replete conditions that *L. chlorophorum* was able to produce 10 times more
25 TEP than other seven species tested which belonged to the main phytoplankton phyla. Such high
26 TEP production favours the formation of aggregates and the sinking rate (Thornton, 2002) which

1 can lead to anoxia events that affect the benthos community (Passow, 2002). In the present study, an
2 O₂ depletion was observed at the end of the bloom; but although rare, this event was far from
3 anoxia.

4

5. Conclusions

In this study, the entire temporal pattern of a HAB species, *L. chlorophorum*, identified by microscopy and flow cytometry, was monitored. We showed that the high-frequency measurements of physical-chemical and biological parameters provided by the SMILE buoy allowed to detect the triggers of the bloom and its fate. There were not flow cytometer on the buoy, but our study pointed out that such a measurement associated with the others parameter available at high frequency would represent a great opportunity to deeper explorer phytoplankton dynamics and manage HAB events. The bioassay experiments performed during this bloom event allowed to explore physiological regulation at a community scale. The regulation of photosynthetic parameters was investigated as a function of nutrient inputs and of community structure, which made it possible to characterise uncoupling of production and productivity in some circumstances. The bioassay experiments also provided evidence of potential P and N co-limitation in this ecosystem and revealed that unbalanced inputs favour high TEP production with a population dominated by *L.chlorophorum*, which, in turn, can lead to massive bloom, sedimentation, and local oxygen depletion. Management of the nutrient inputs ratio has to be considered both in connection with the triggering - but also with the fate of blooms.

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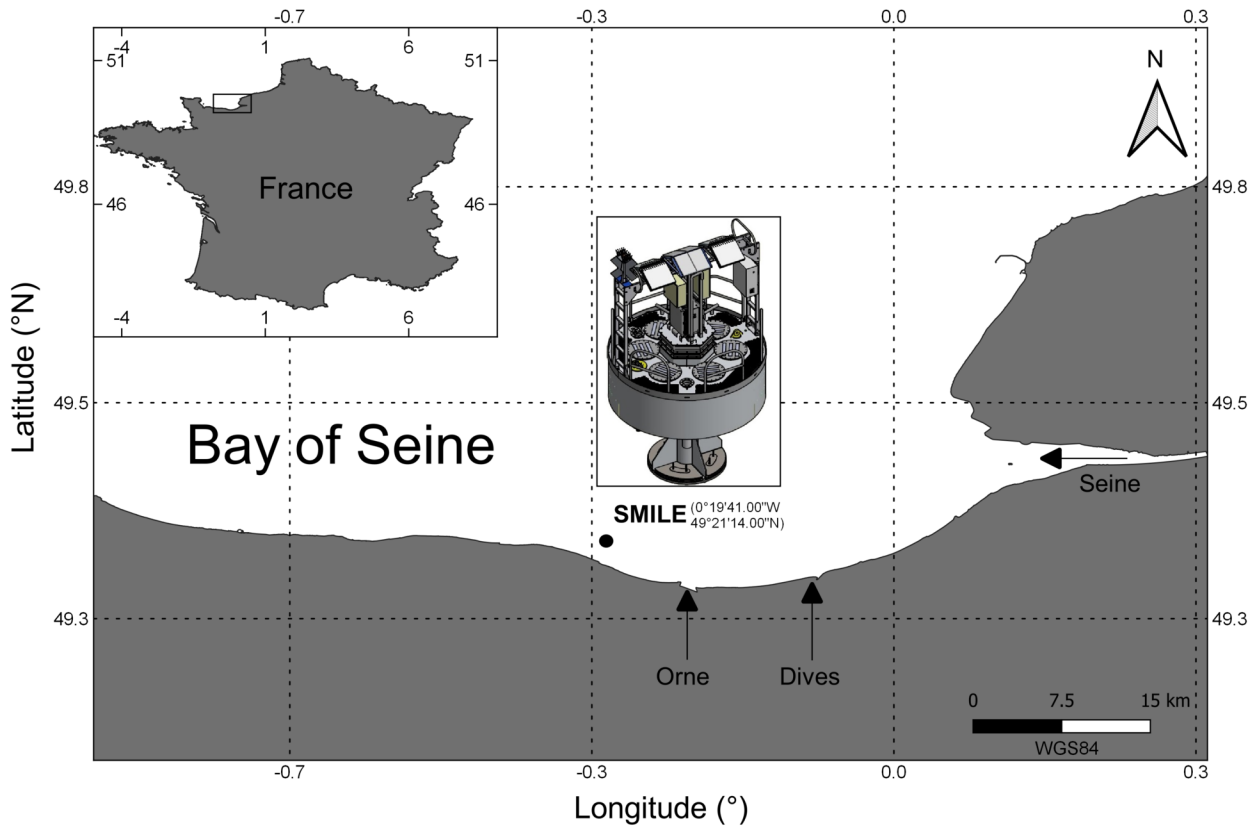
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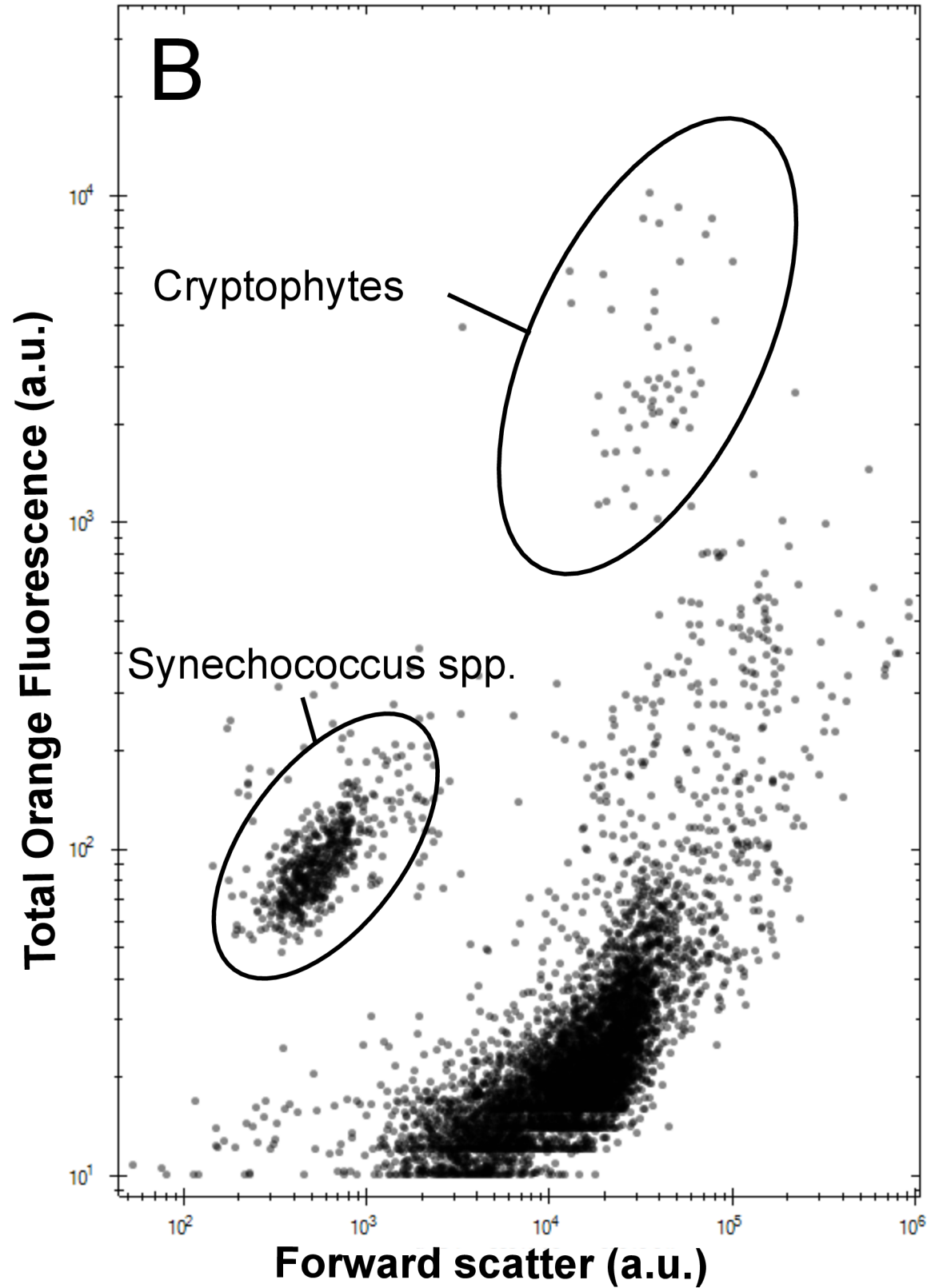
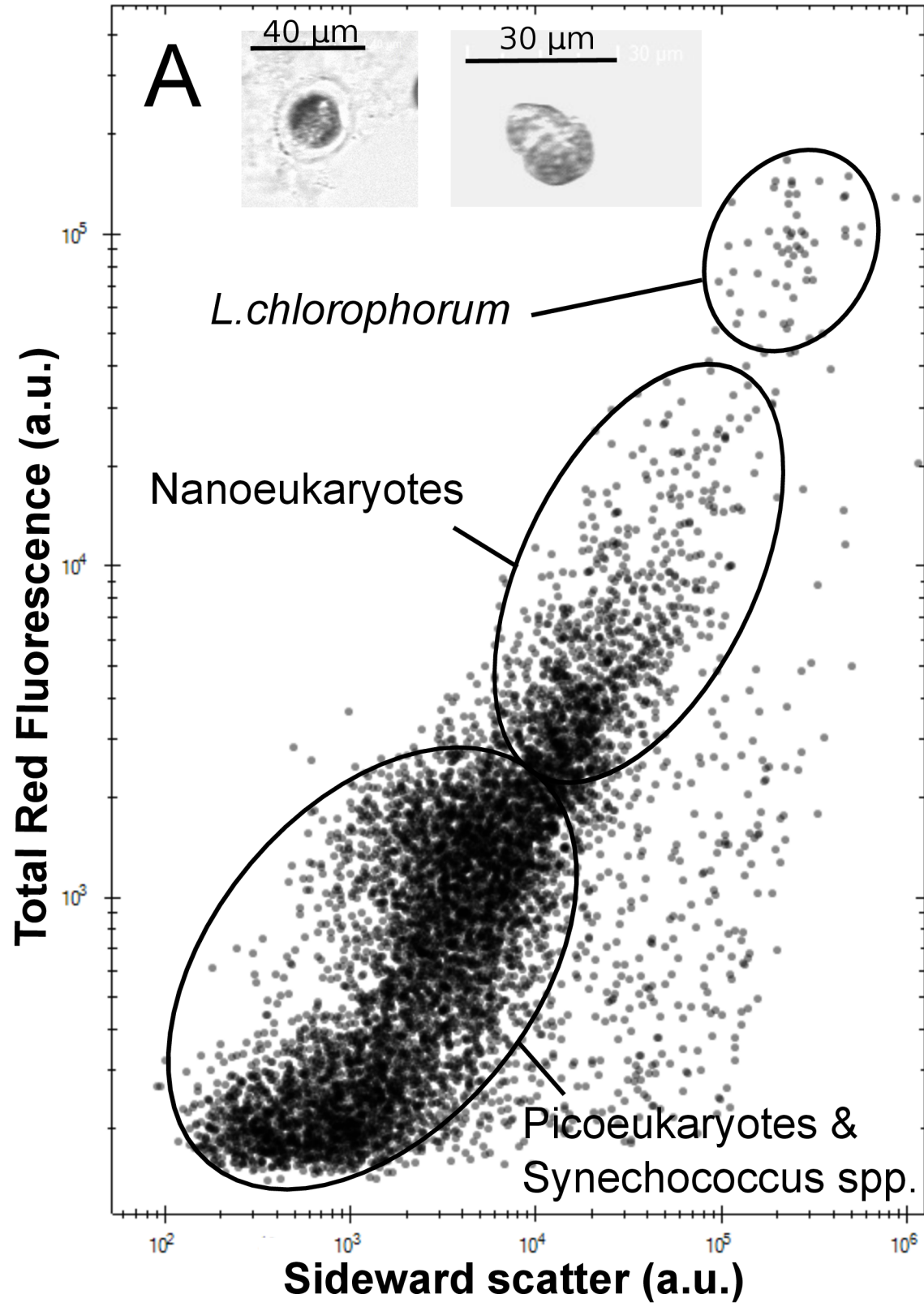
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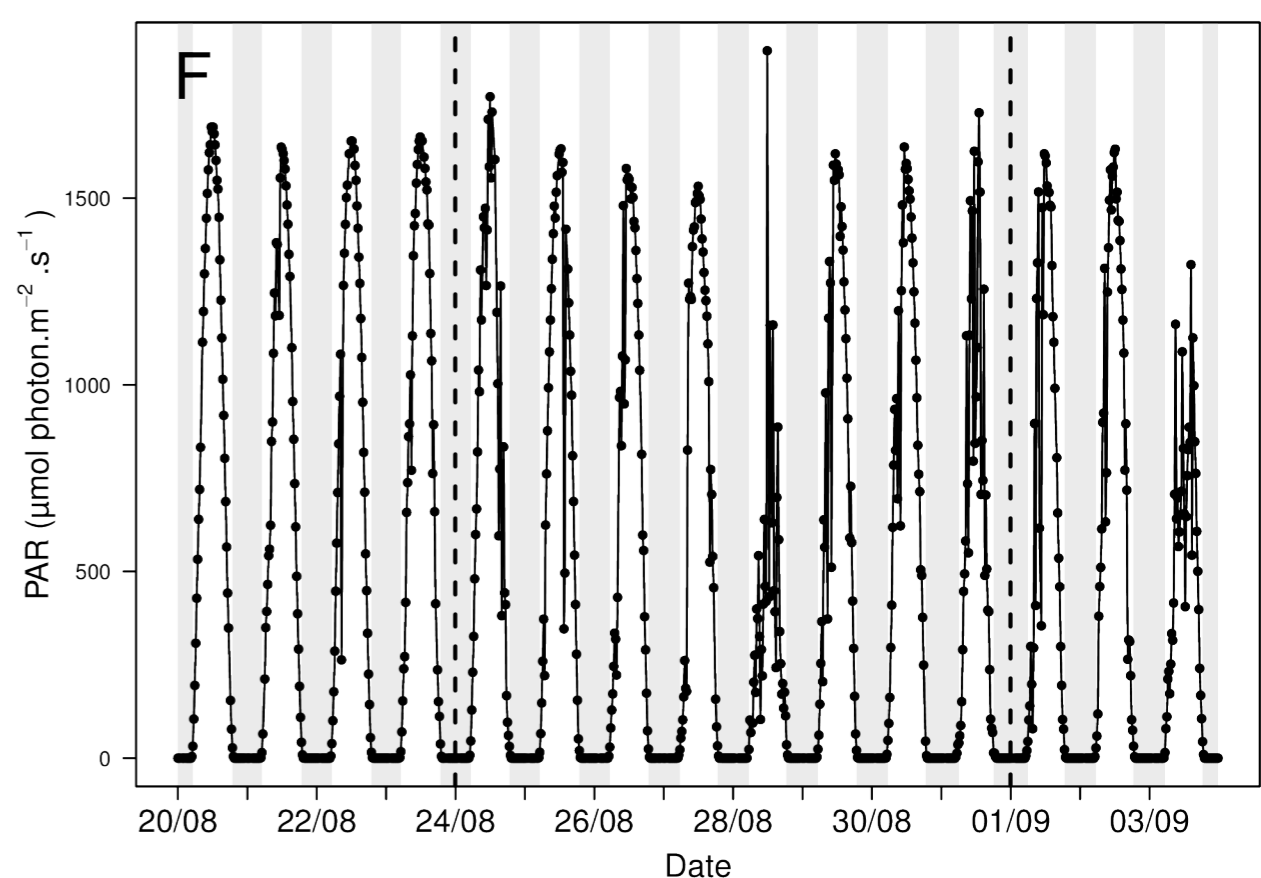
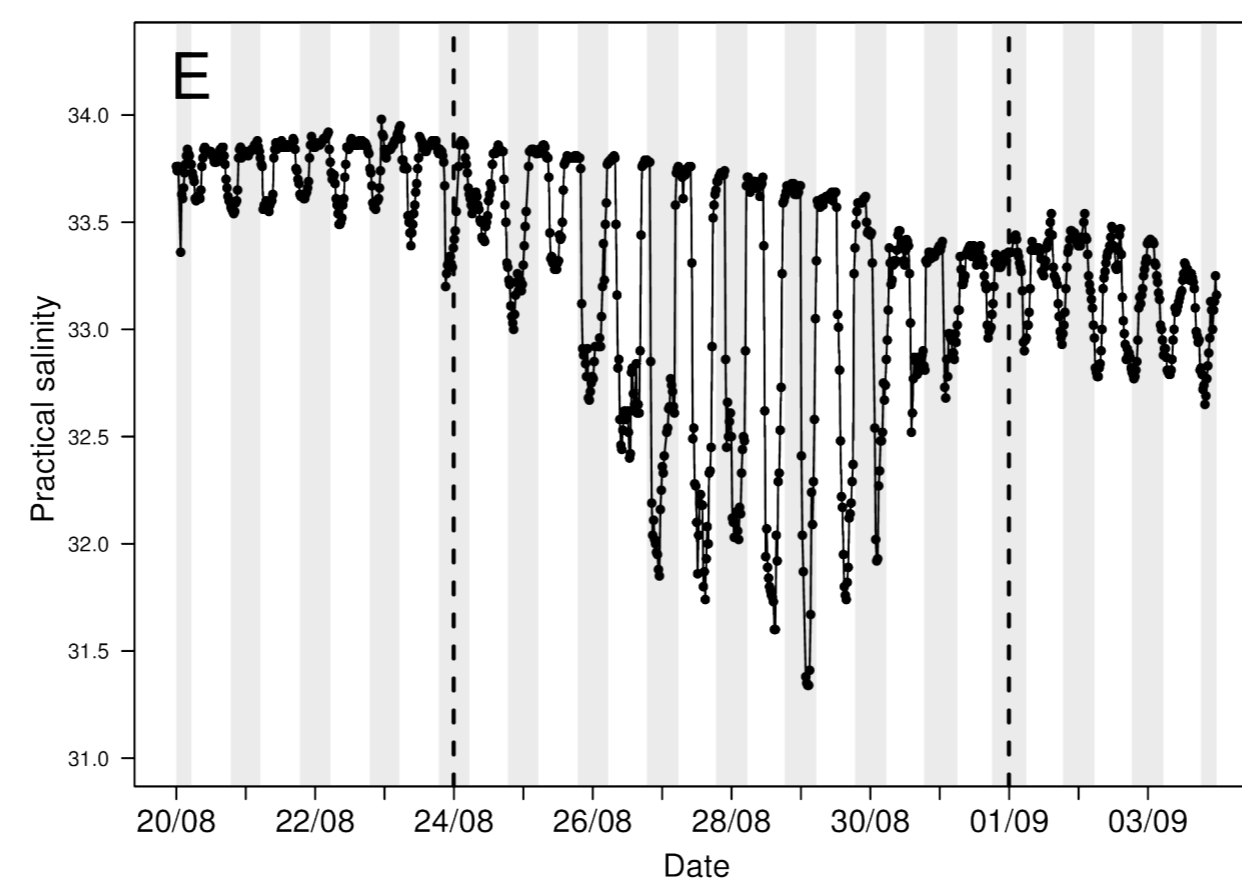
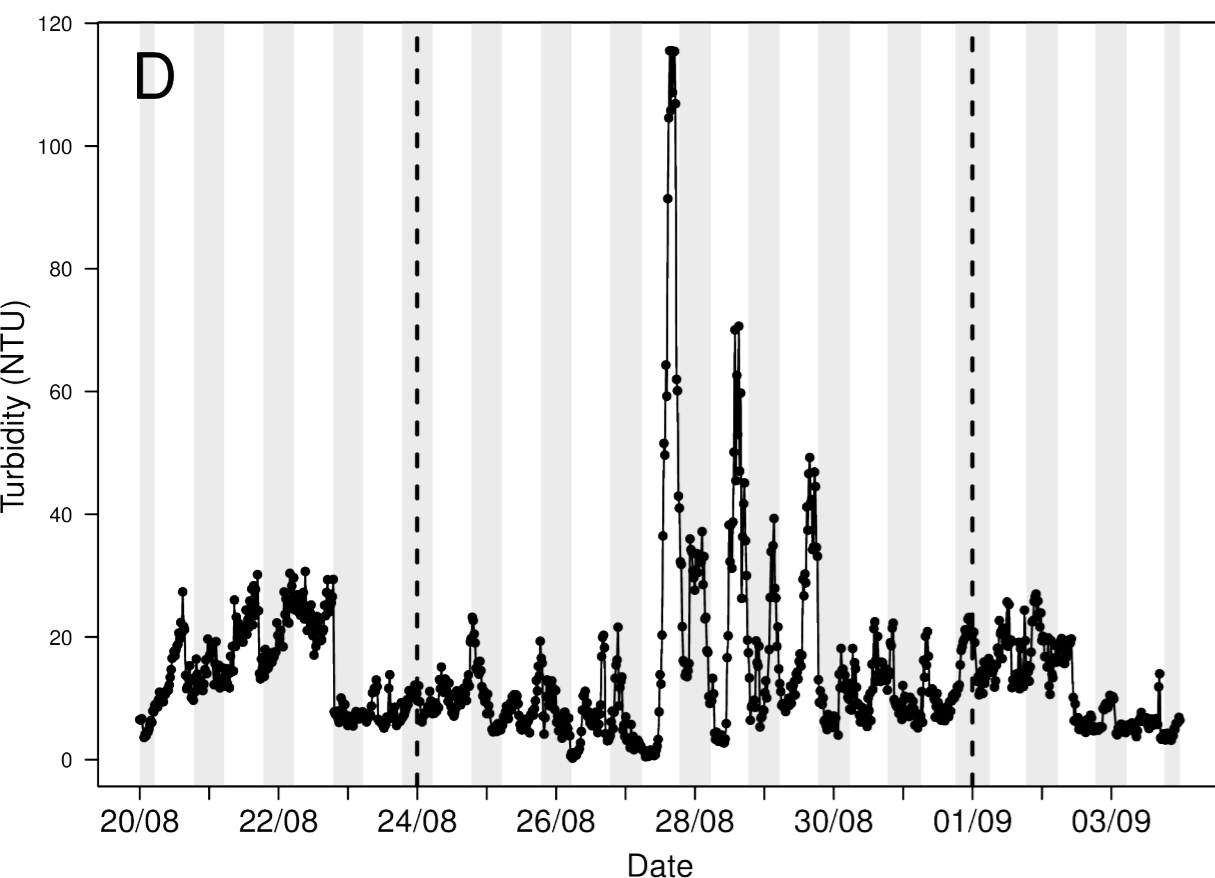
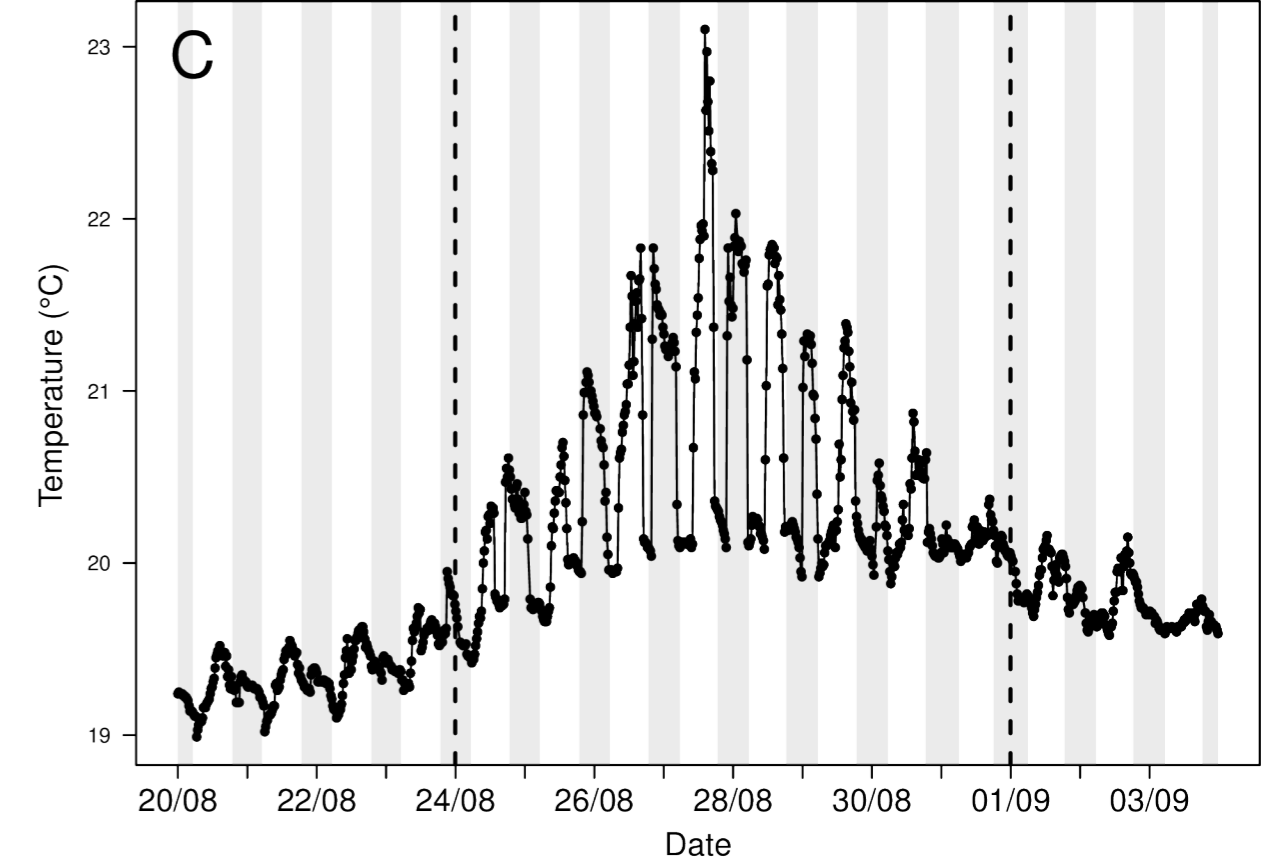
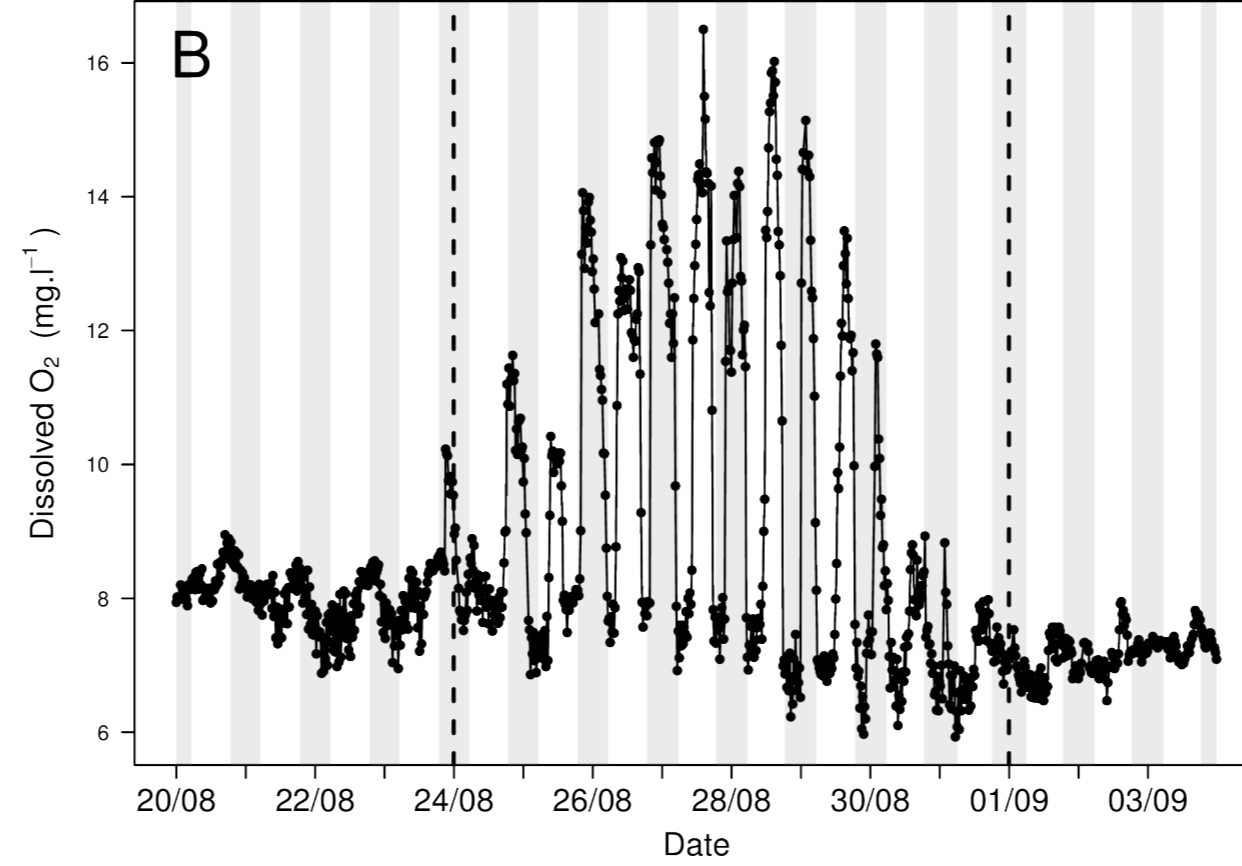
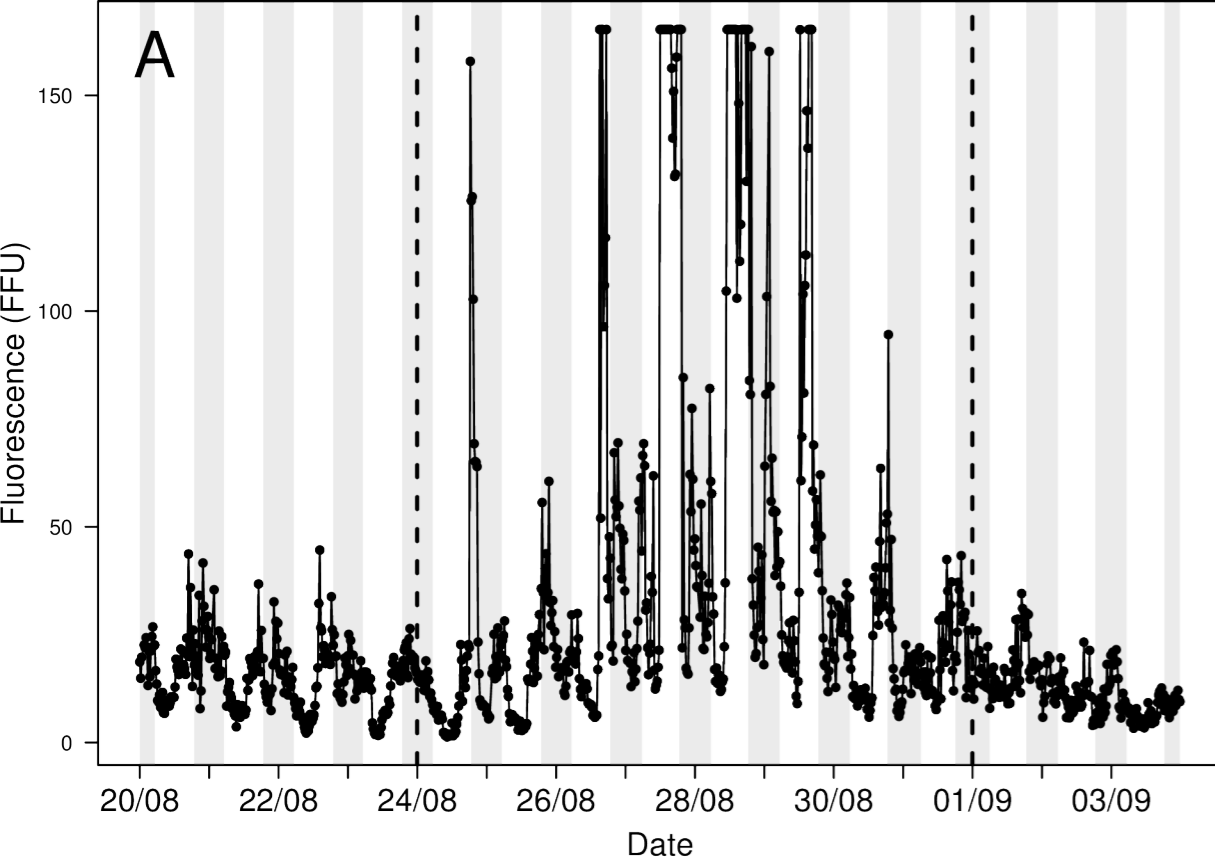
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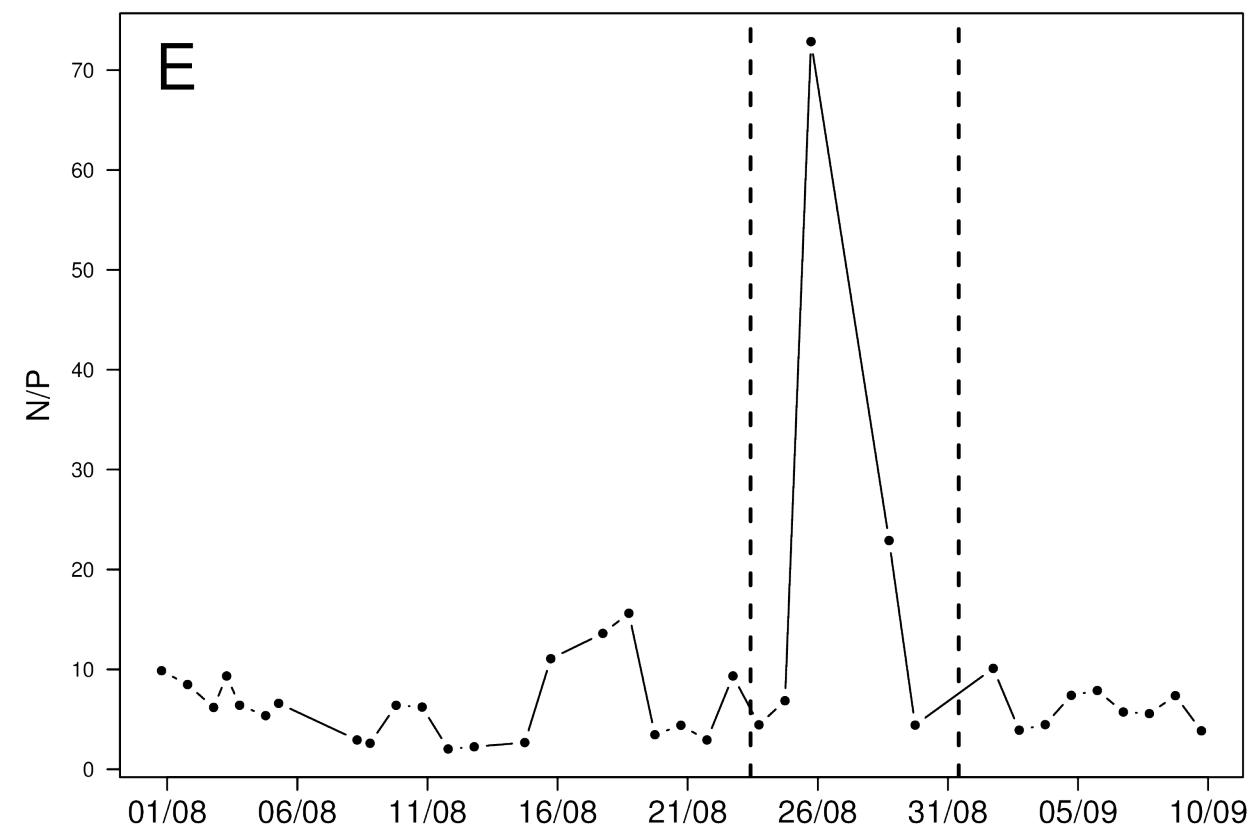
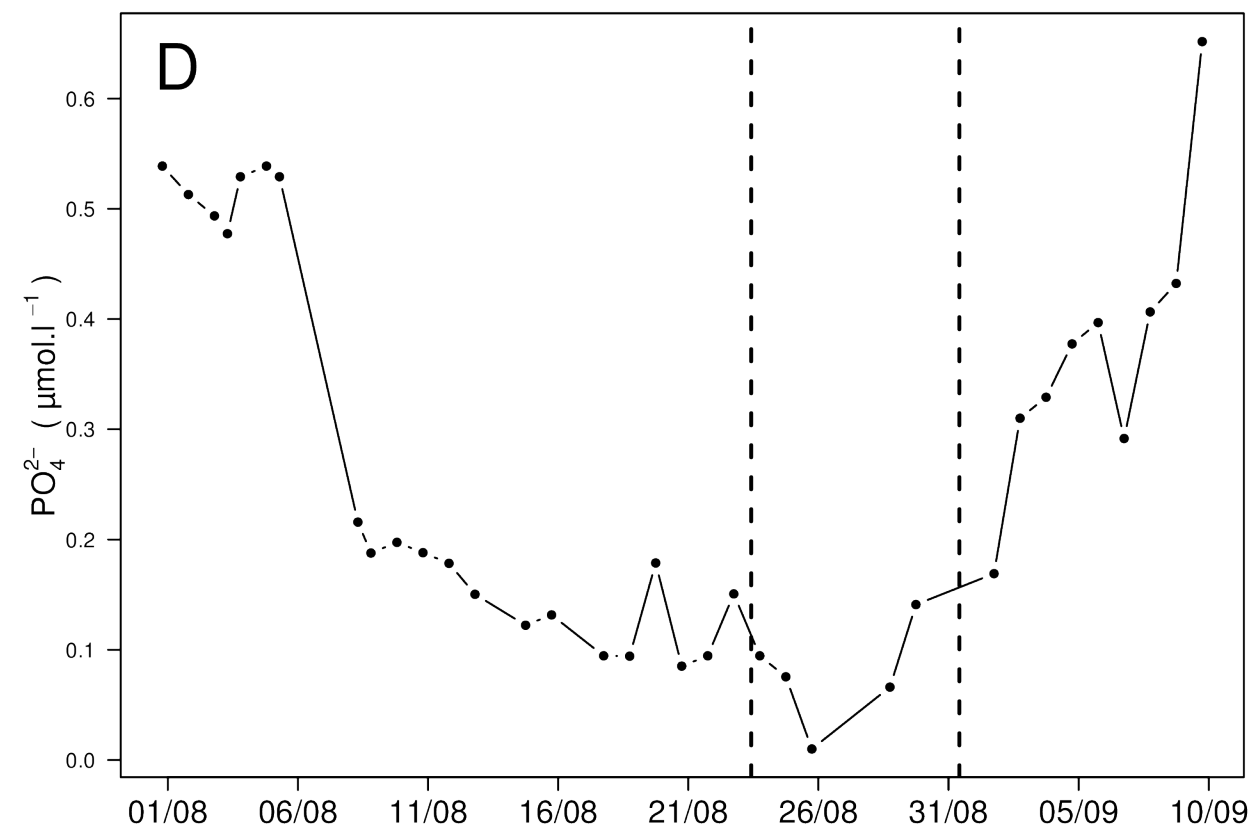
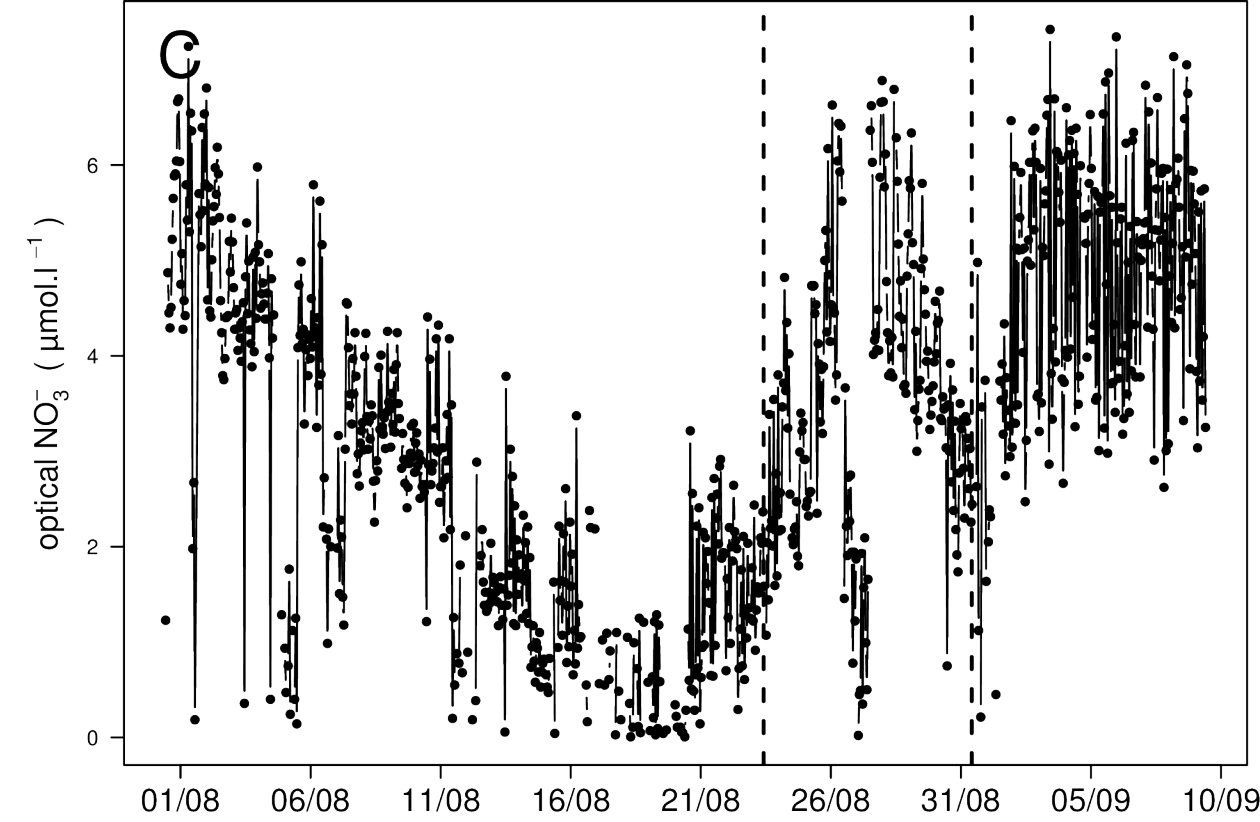
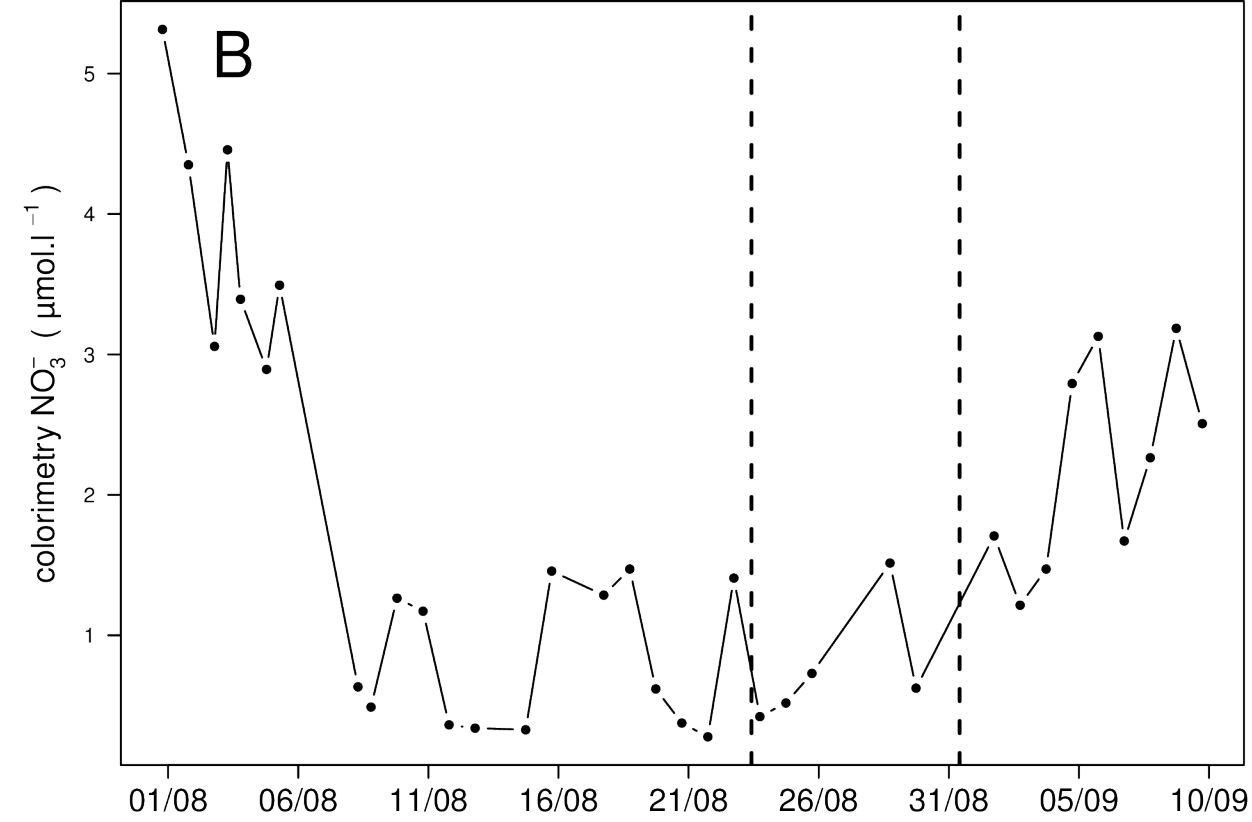
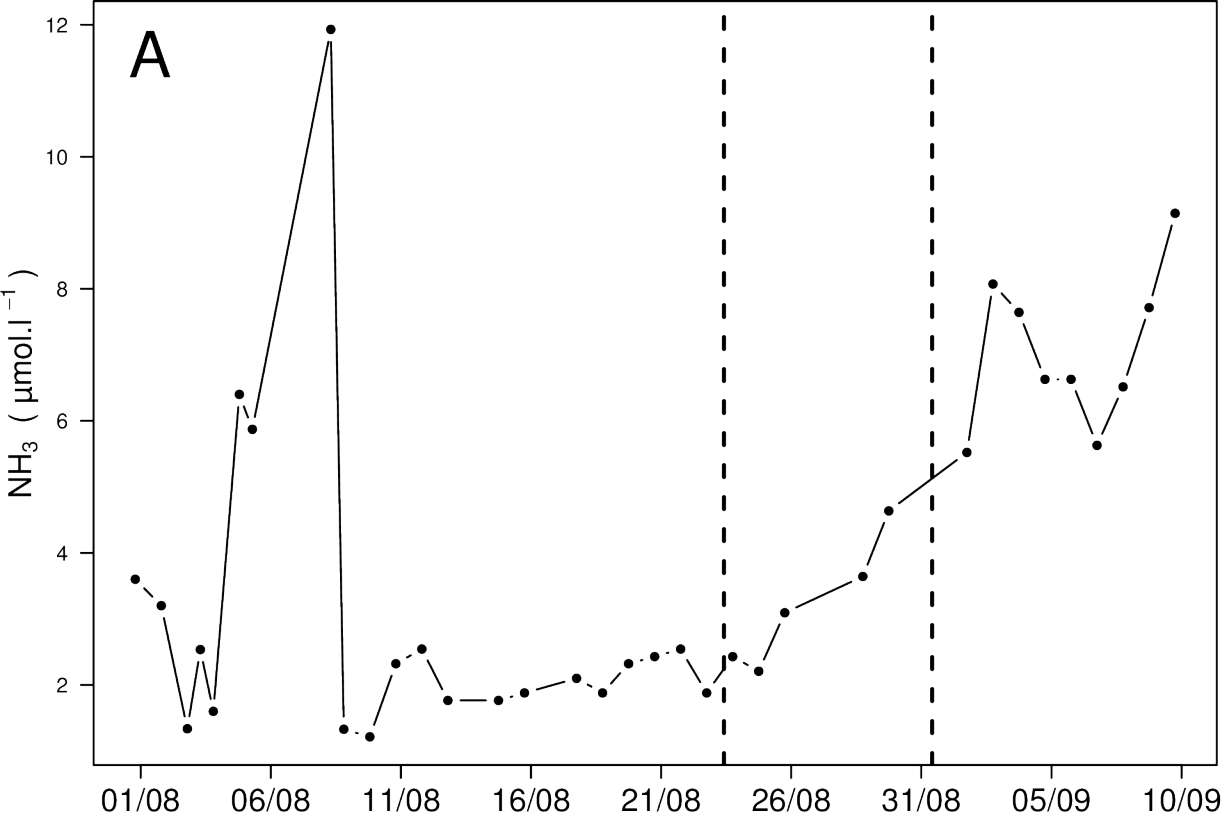
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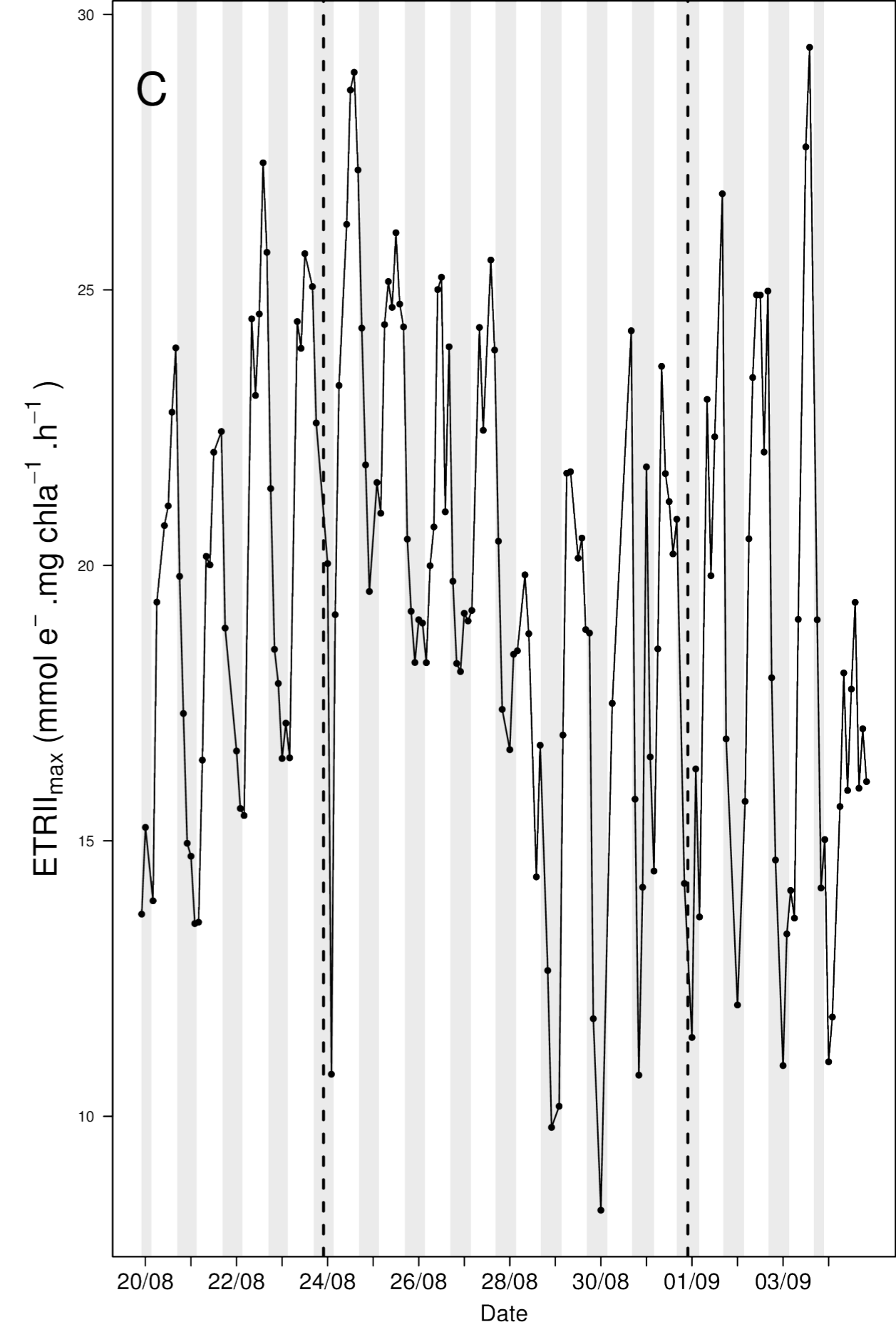
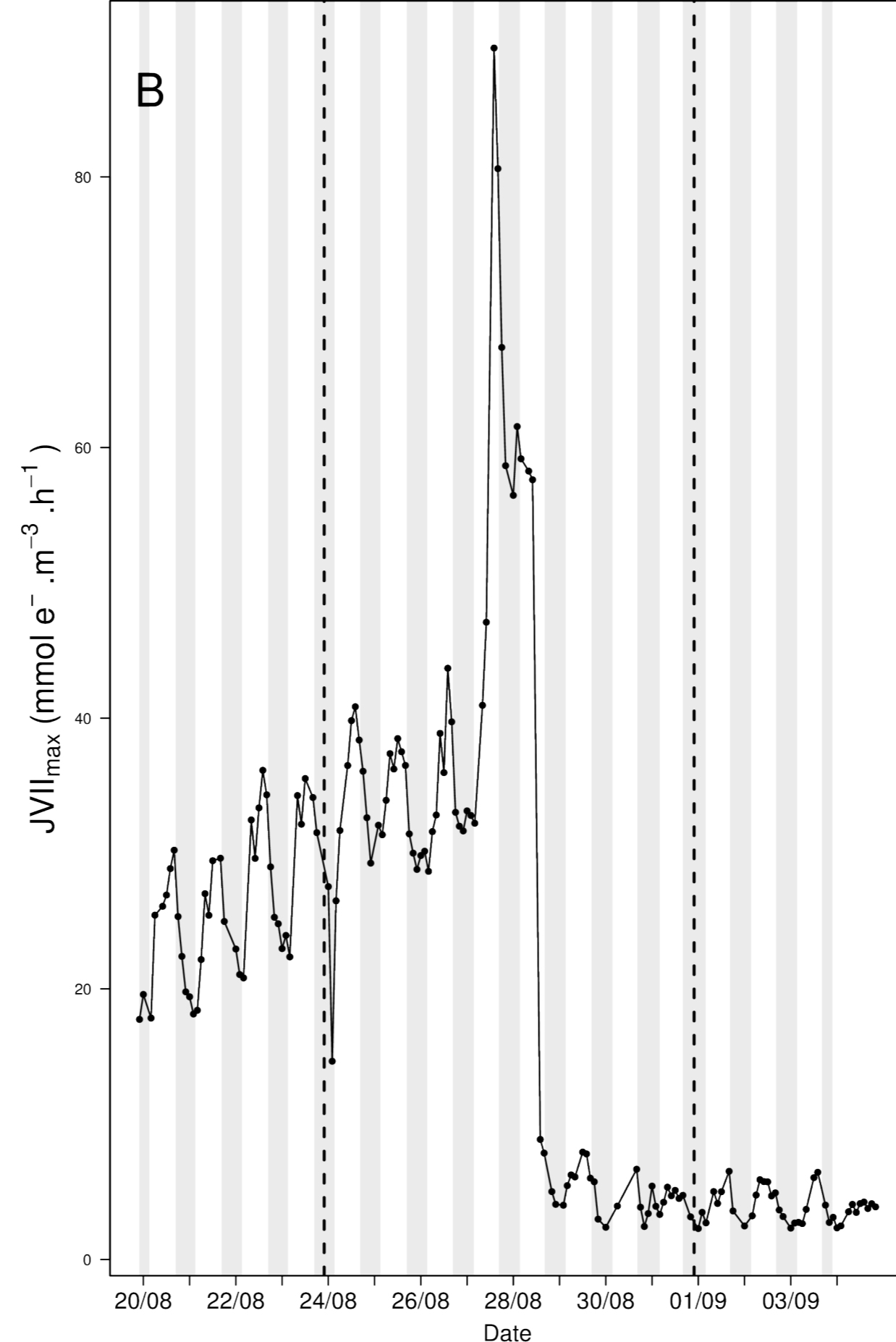
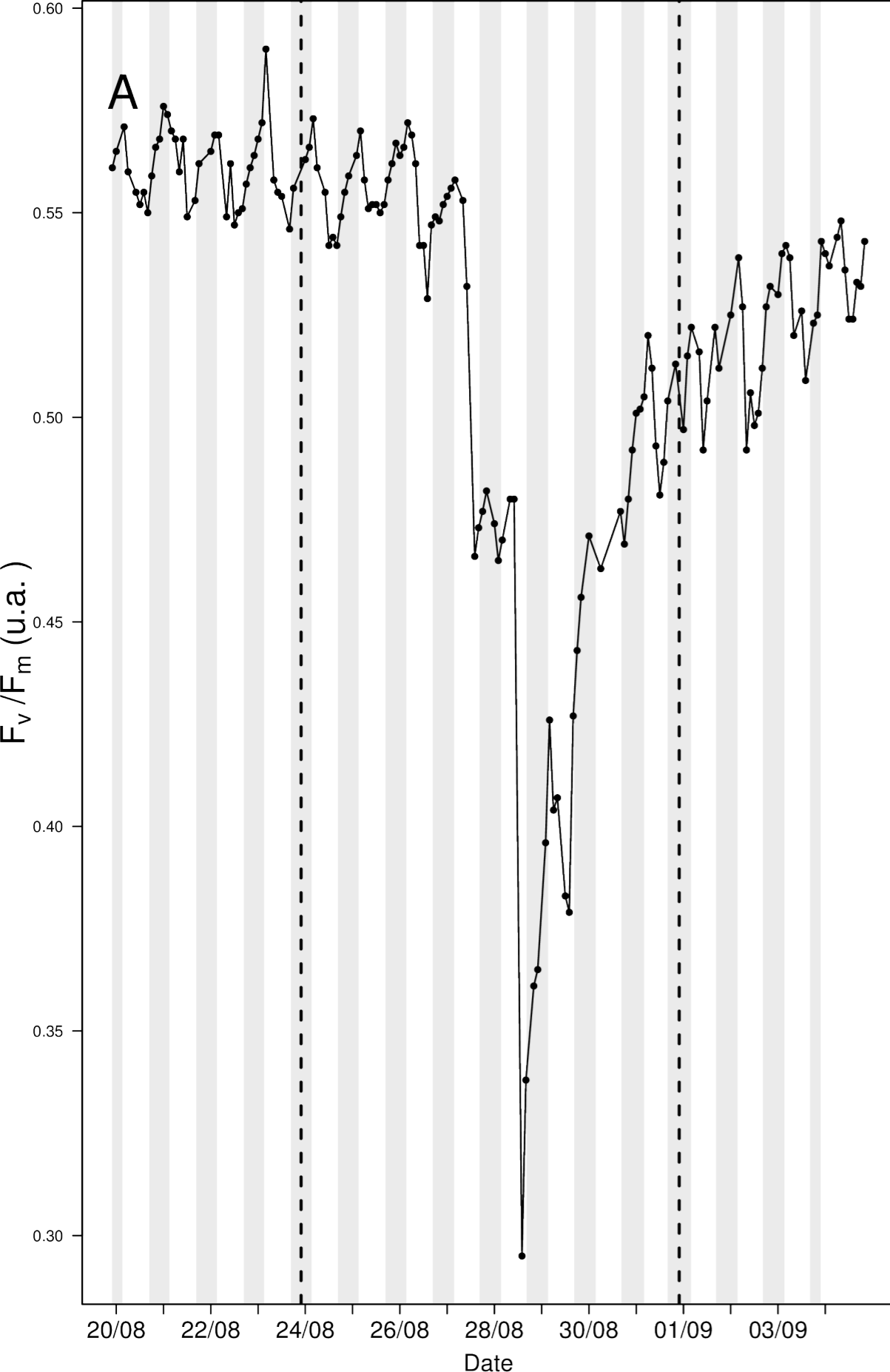
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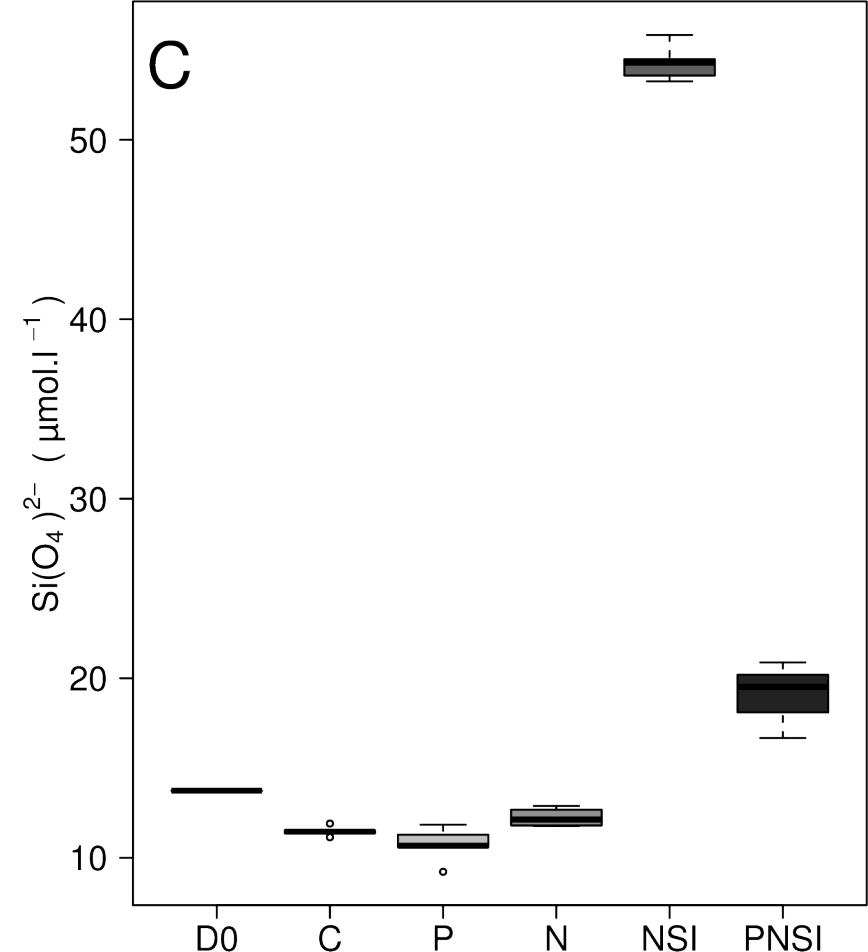
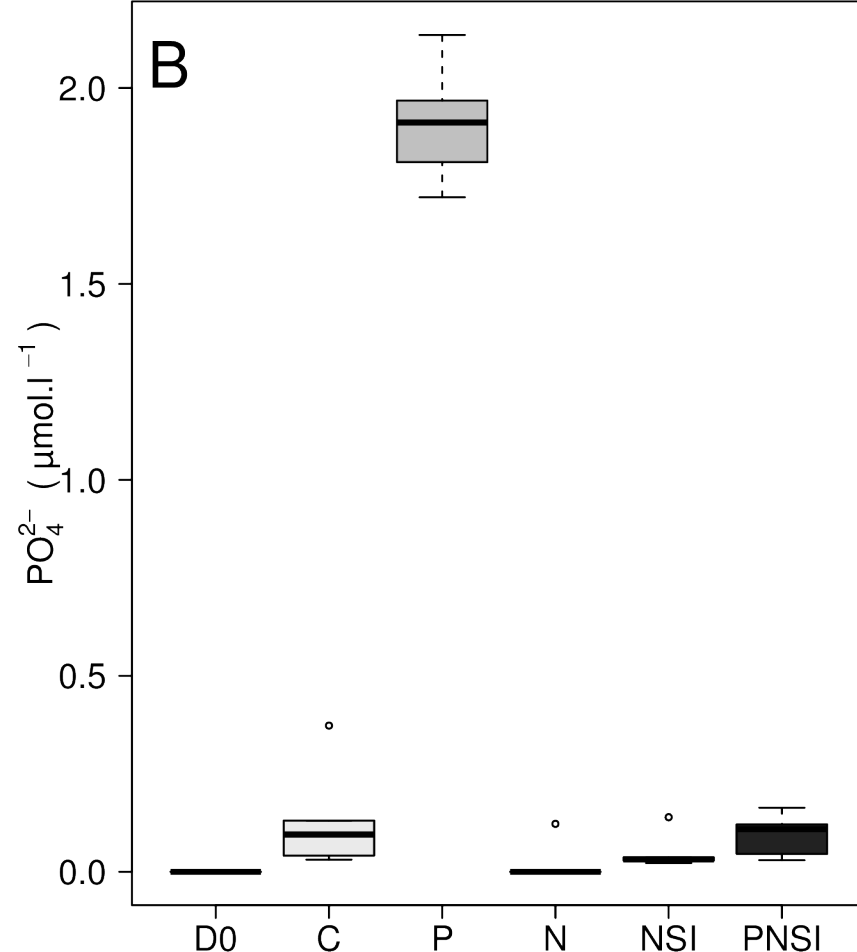
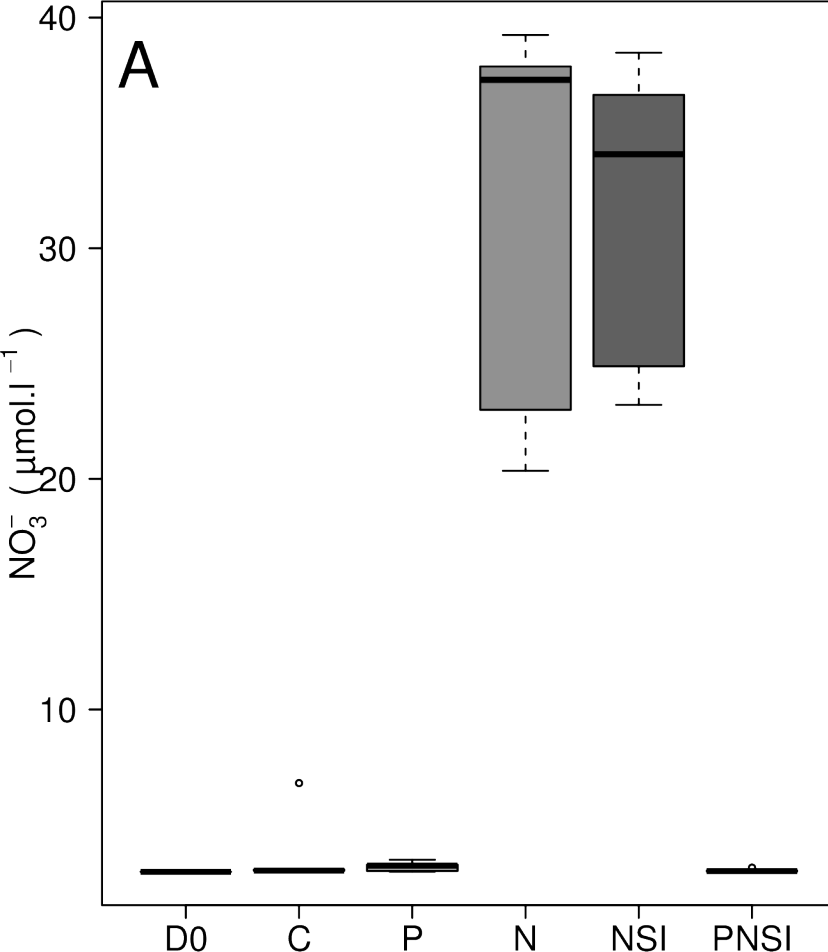


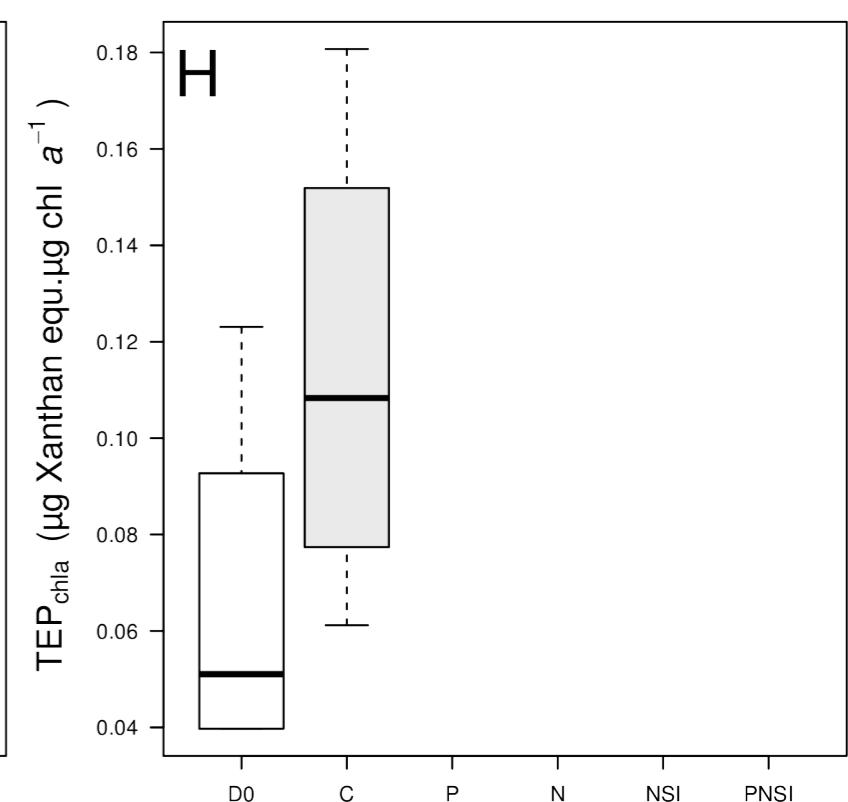
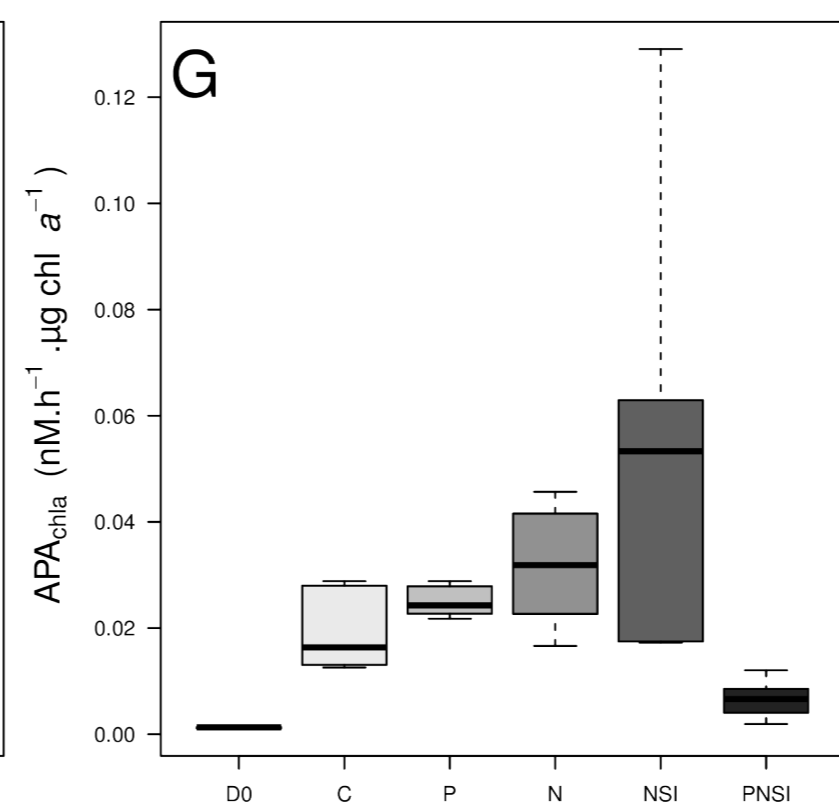
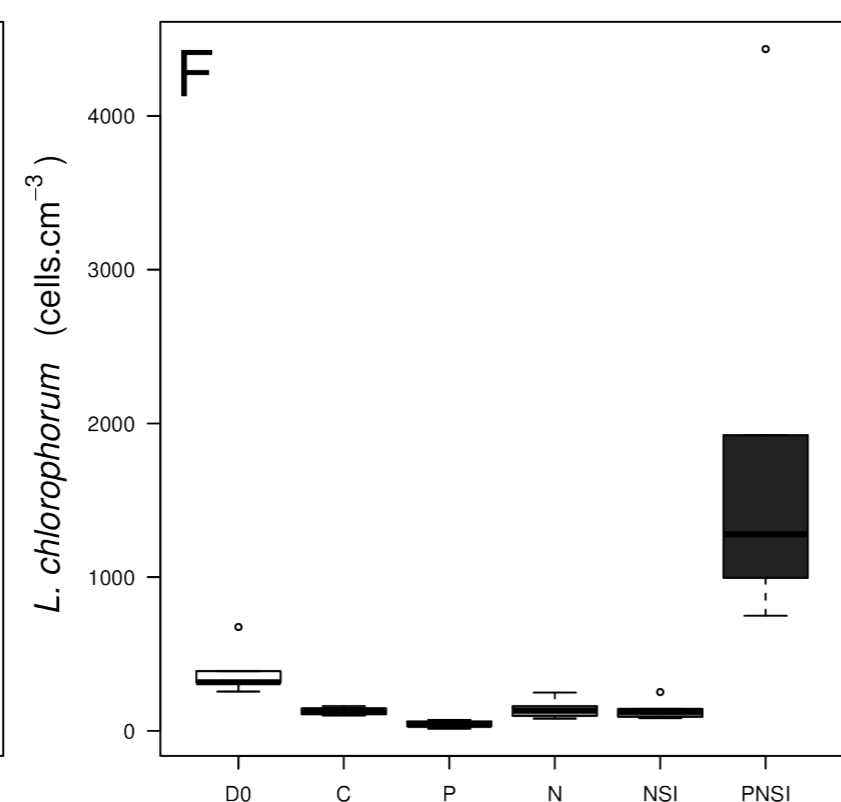
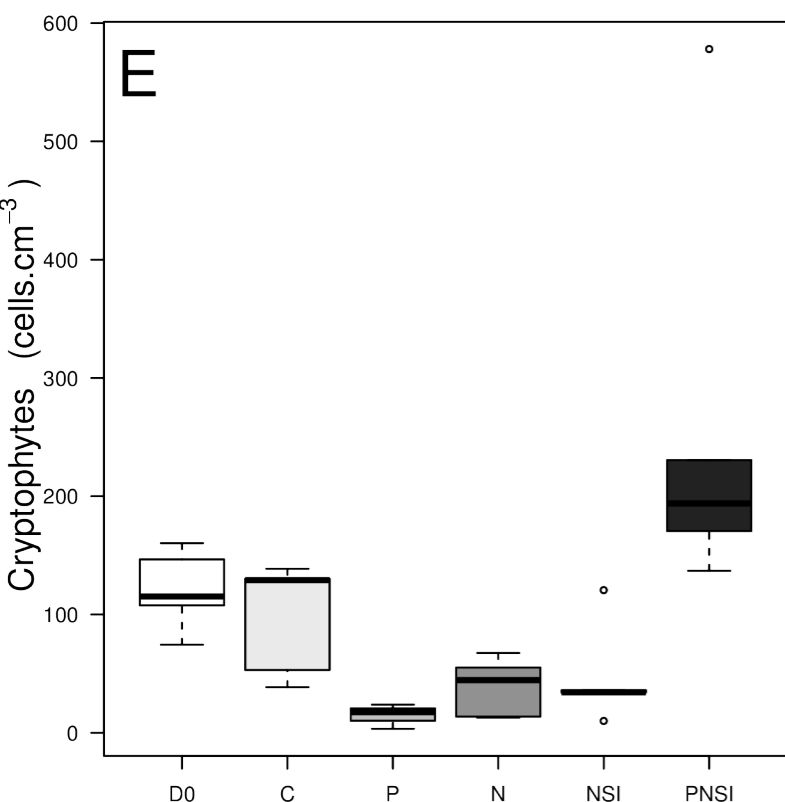
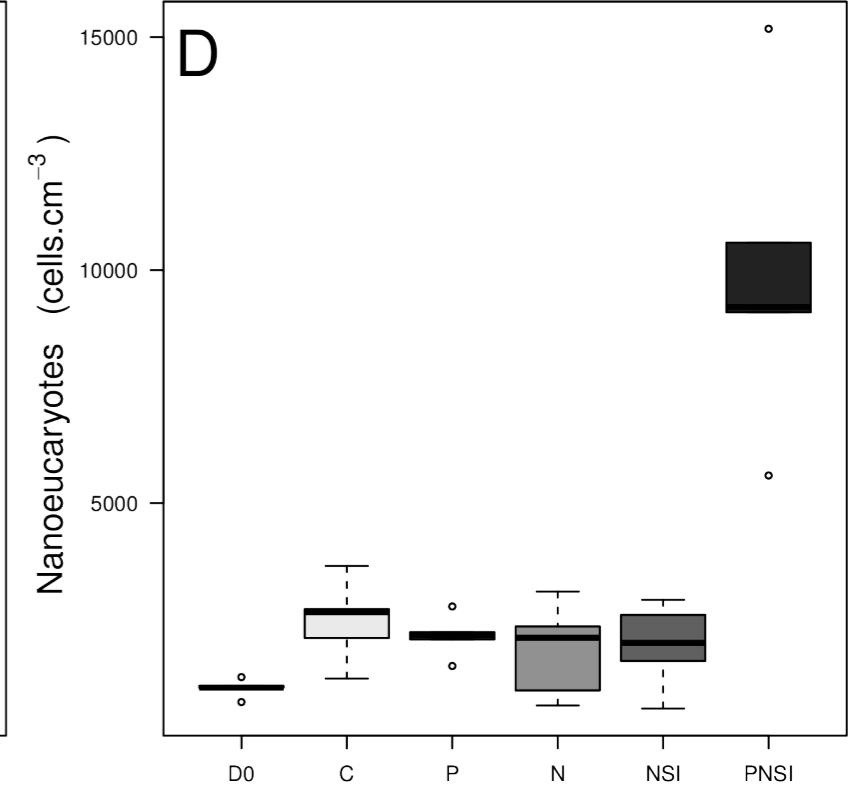
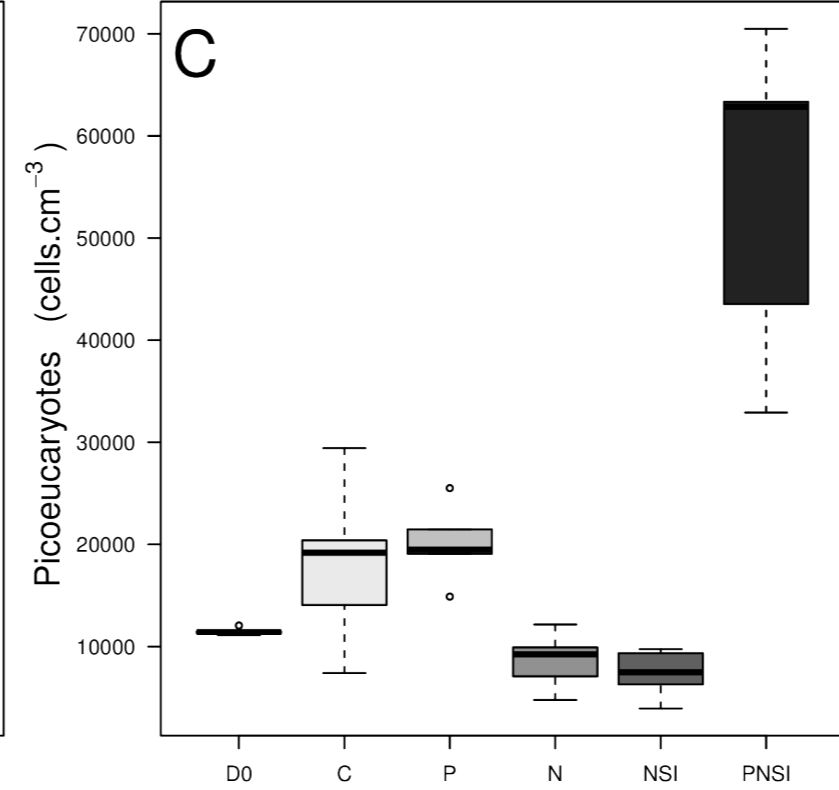
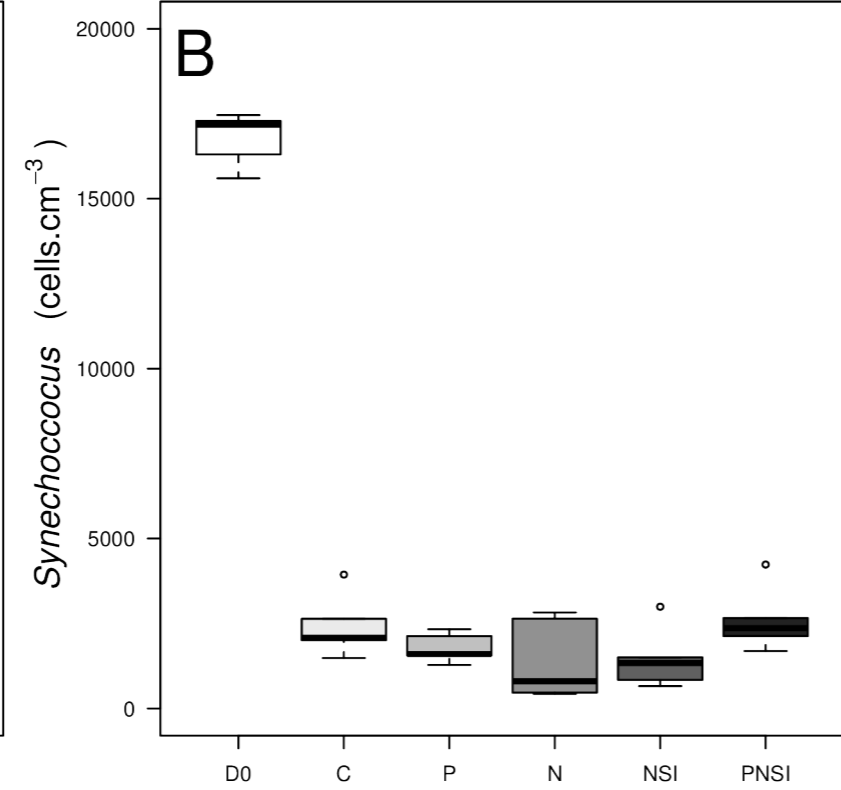
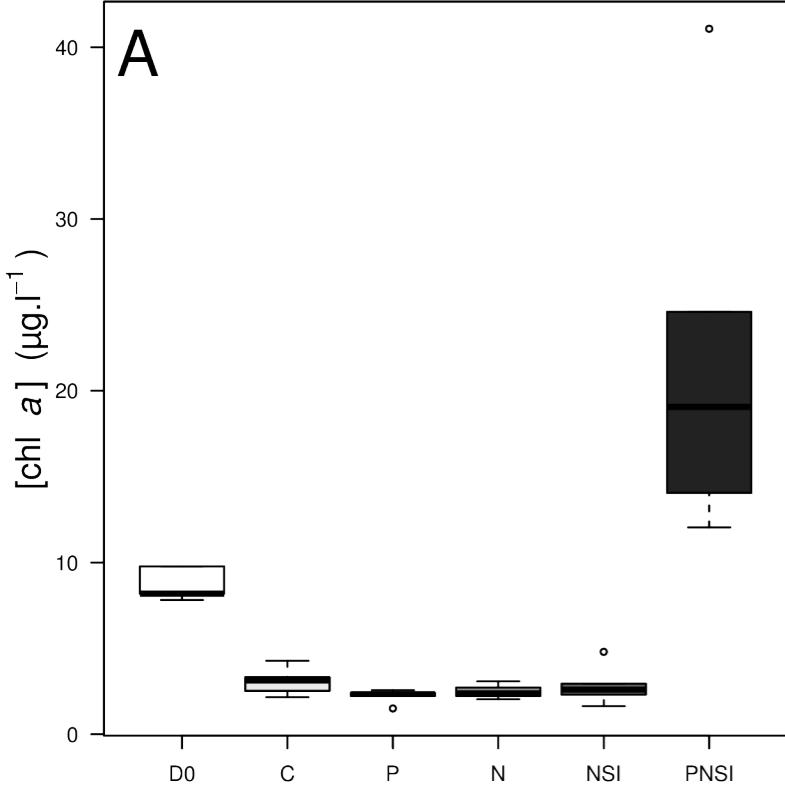


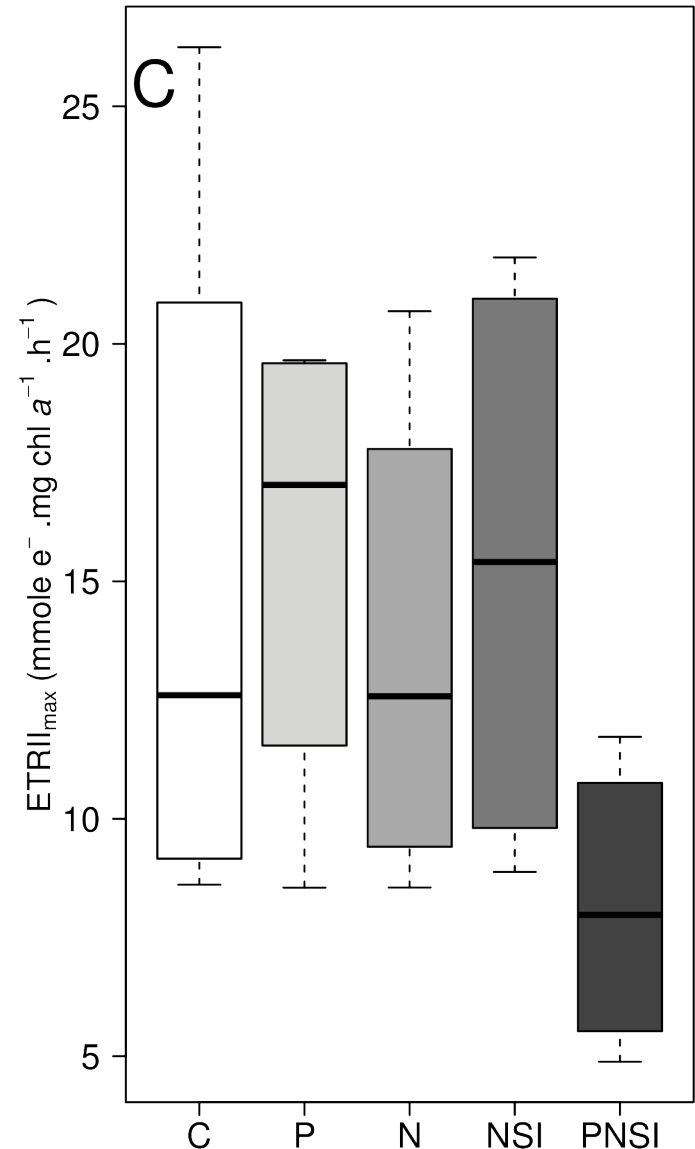
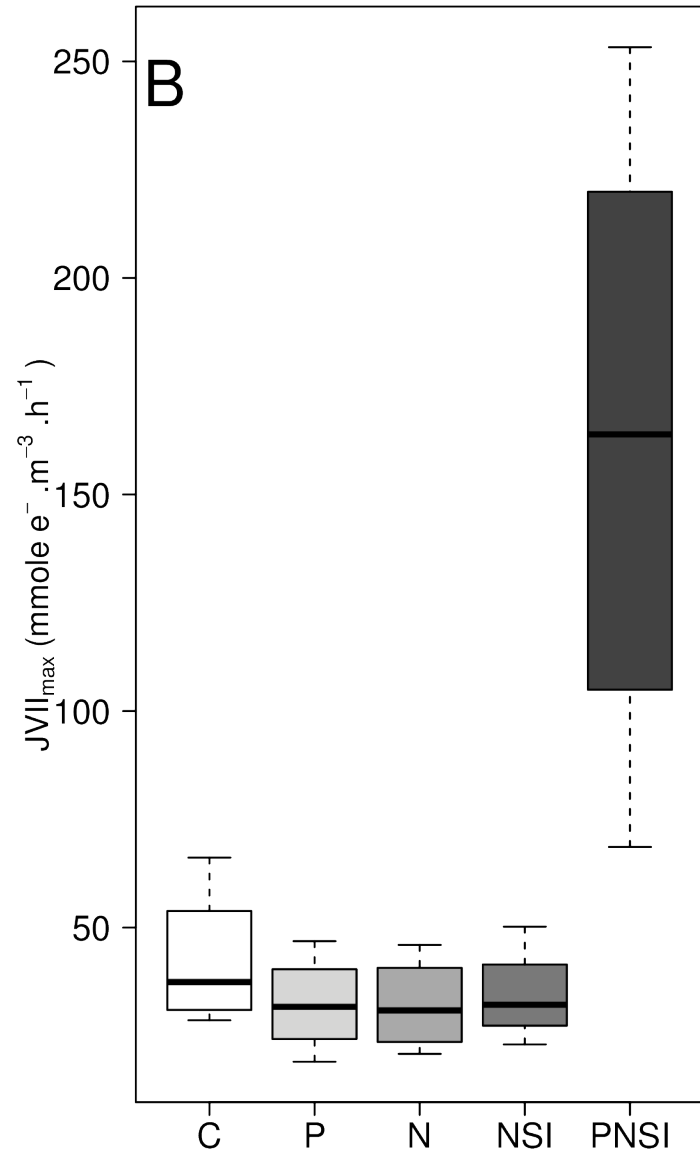
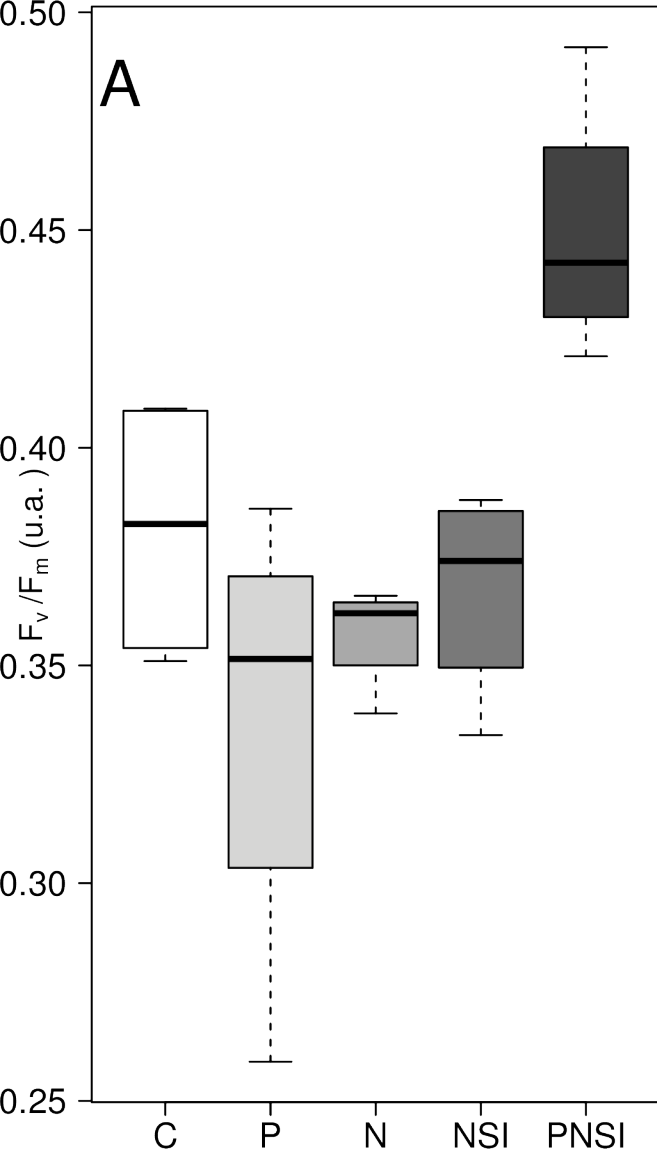




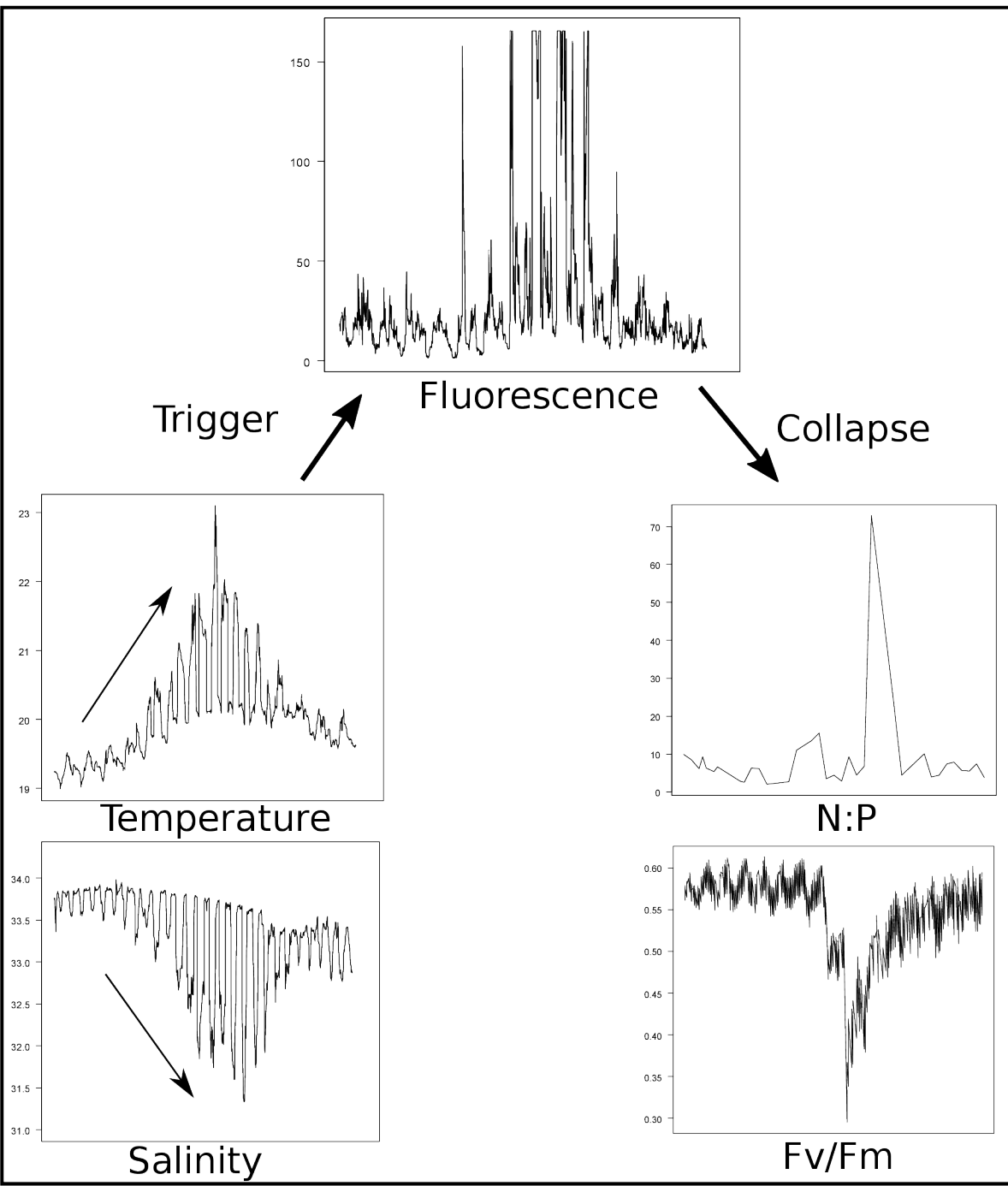








Bloom



Bioassay

