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Effects of simulated benthic fluxes on phytoplankton dynamic and photosynthetic parameters in a mesocosm experiment (Bay of Brest, France)

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ABSTRACT

Benthic faunal activity and density play an important role in determining the rates of benthic nutrient fluxes, which enrich the water column and contribute to phytoplankton growth. The intensity of nutrient fluxes in the Bay of Brest depends on the density of the invasive gastropod, *Crepidula fornicata*. In order to study the impact of benthic fluxes on phytoplankton dynamics, realistic daily nutrient inputs simulating various densities of *C. fornicata* were added to six enclosures during three weeks. The increase in fertilization intensity influenced the phytoplankton biomass. A succession from *Chaetoceros* spp. to *Pseudo-nitzschia* spp. and *Leptocylindrus danicus* was observed in all enclosures, but the dynamics of successions were different. *Pseudo-nitzschia* spp. was favored in the three more fertilized enclosures, while *Chaetoceros* spp. persisted longer in less enriched enclosures. Despite an apparent nitrogen limitation, the quantum efficiency of PSII (F_v/F_m) was high (>0.5) and stable in all enclosures. The maximal photosynthetic capacity (P^Bmax) was also invariable and oscillated around an average value of 2.23 mg C (mg Chl a)$^{-1}$ h^{-1}. The stability of F_v/F_m and P^Bmax observed at different nutrient input intensities demonstrates that the daily inputs maintained the physiological balance of the microalgae. The maximal light utilization efficiency (α) and the light saturation parameter (E_k) were also quite stable after day 8, which reveals that photosynthetic parameters were driven by growth constraints due to nutrient availability and not by incident light or species successions. We suggest that our results correspond to an "E_k independent variation" regulation. We propose that such regulation of photosynthetic parameters appears when there are frequent nutrient additions which do not allow replete nutrient conditions to be reached but lead to physiological equilibrium.

Thanks to our results we can understand how even low benthic fluxes, by supporting their cellular physiological status, allowed diatoms to dominate the phytoplankton community in the six enclosures. These results confirm the importance of daily benthic inputs as much as the amount of nutrient inputs. However, we suggest that high benthic fluxes have a buffering effect on nutrient availability, thereby limiting the consequences of short-term events which can entail a sudden increase of nutrient input, and are known to promote Dinophyta bloom formation.

1. Introduction

Phytoplankton dynamics and production are controlled by physical, chemical and biological factors (Cloern, 1996). In shallow coastal ecosystems, the combination of mixing and nutrient inputs due to wind, tides, river discharges and benthic fluxes is known to influence the phytoplankton community structure and primary production (Del Amo et al., 1997; Azevedo et al., 2006; Jouenne et al., 2007; Pannard et al., 2008). These factors are highly variable at different time scales from short-term events to seasons (Pannard et al., 2008). Modifications of the phytoplankton community structure and photosynthetic parameters relating to sudden nutrient inputs (rainfall), wind or tides have been described (Ragueneau et al., 1996; Buyukates and Roelke, 2005; Spatharis et al., 2007; Jouenne et al., 2007; Pannard et al., 2007, 2008), while the influence of benthic fluxes are less documented (Del Amo et al., 1997; Fouillaron et al., 2007).

Many chemical, physical and biological processes influence the nutrient supply from the sediments to the pelagic zone (Sakamaki

et al., 2006). Chemical and physical processes consist of mineralization of organic matter, nitrification/denitrification, hydrodynamics, etc. (Sakamaki et al., 2006). Biological factors are mainly related to the microphytobenthos, commonly present in shallow coastal ecosystems (Ní Longphuirt et al., 2006, 2009; Leynaert et al., 2009) and to the benthic fauna, which drives bioturbation, bio-irrigation and bioaccumulation of organic material (Marinelli, 1994; Ragueneau et al., 2005). Ragueneau et al. (2002, 2005) showed that in the Bay of Brest the massive biodeposition of the invasive gasteropod *Crepidula fornicata* impacts the benthic fluxes of dissolved silicic acid (DSi). They demonstrated that the intensity of these DSi fluxes was dependent on the density of this gastropod and they suspected that these fluxes partly control the phytoplankton dynamic and limit the risk of Dinophyta harmful algal blooms events during summer months in this bay (Del Amo et al., 1997; Chauvaud et al., 2000; Ragueneau et al., 2002, 2005). The development of the Great Scallop (*Pecten maximus*) is deeply perturbed by *C. fornicate*. The removal of the gastropod was proposed by fishermen (Thouzeau et al., 2000), however, such action would probably lead to large variations in benthic fluxes. In this context, three sets of mesocosm experiments were conducted in spring, summer and autumn 2004 to study the response of phytoplankton community to various benthic fluxes of nutrients. Fouillaron et al. (2007) reported results from the spring experiment. They largely focused on diatoms and silicic acid uptake and observed that the increase of nutrient input changed only the magnitude of the diatom bloom. It did not result in any shift towards non-siliceous phytoplankton as one might have expected given the low fertilization rates in some of the mesocosms. In the present study we report results from the autumn experiment and try to understand further the role of benthic fluxes on primary production. We investigated particularly the effects of the magnitude of daily nutrient addition on phytoplankton dynamics and photosynthetic parameters.

Photosynthetic parameters are highly sensitive to nutrient stresses (Lippemeier et al., 1999; Behrenfeld et al., 2004), temperature (Claquin et al., 2008) and light (Anning et al., 2000). In addition to those parameters, Jouenne et al. (2007) pointed out the need to consider phytoplankton community structure changes when trying to understand the variability in primary production. The community structure and consequently species succession can play a dominant role in production variations (Falkowski and Owen, 1980; Shaw and Purdie, 2001; Jouenne et al., 2007).

This work investigates the influence of benthic macrofauna on phytoplankton dynamics, which is an important component of benthic/pelagic coupling in shallow coastal ecosystems. Besides confirming or not, at another season, the conclusion of Fouillaron et al. (2007) which showed that the increase of fertilization, at constant nutrients ratio, only changed the magnitude of the bloom without modification of the community structure, the aims of this paper are to evaluate:

(1) How do benthic fluxes impact the phytoplankton dynamics?
(2) How do various daily inputs influence the photosynthetic parameters and primary production of a natural phytoplankton community?
(3) What are the relationships between the phytoplankton community variations and the photosynthetic parameters?

2. Materials and methods

2.1. Study site and mesocosm experiment

3 sets of mesocosm experiments were conducted in spring, summer and autumn 2004, in the Bay of Brest (France), a temperate ecosystem on the Atlantic coast. Results presented herein are from the autumn study carried out during 3 weeks, between 15 September and 4 October, 2004. The design was the same as described in Fouillaron et al. (2007) for the spring experiments. Six enclosures made of 4 m deep polyethylene bags with a volume of 5.5 m^3 were used (Fig. 1b). They were filled in the morning of 15 September 2004 with seawater from the Bay of Brest by lowering them into the water column and lifting them up slowly. Nutrient additions were performed by daily evening inputs. Si was in the form of silicic acid (DSi), N as nitrate, and P as phosphate. Daily fertilizations were defined after consideration of the benthic fluxes measured *in situ* at the end of summer for different densities of *C. fornicata*, which gave the range of nutrient additions (Table 1) (Ragueneau et al., 2002). A gradient of additions was performed at a constant nutrient ratio, from enclosure 1 (M1) to enclosure 5 (M5). In M6, additional N was added to reach a Si/N ratio of 0.8. Removal of sedimentary material was ensured by pumping the bottom water out of the enclosures every 2 d.

Daily water profiles of salinity and temperature were performed using a HYDROLAB DS5 probe (USA). No stratification was observed during the whole experiment. Ten liters of seawater were sampled every day at dawn in each enclosure for nutrients and chlorophyll *a* (Chl *a*) concentrations, phytoplankton enumeration and identification. Photosynthetic parameters were measured at solar noon. At the end of the experiment, the total amount of water removed from the enclosures was less than 5% of the initial volume.

2.2. Nutrients, Chl a and phytoplankton analyses

Nitrate and DSi concentrations were measured immediately after sampling on a Technicon Auto Analyser II (Tréguer and Le Corre, 1975). For phosphate determination, 100 ml of seawater was immediately frozen until further analysis by the colorimetric method (Murphy and Riley, 1962). The Chl *a* concentration was measured by filtering 1 l on a Whatman GF/F glass-fibre filter. The filters were then ground in 10 ml of 90% acetone, extracted at 4 °C for 12 h in the dark, and consequently centrifuged. The extract fluorescence was measured before and after acidification with 1 N HCl using a Turner Design fluorometer. The concentrations of Chl *a* and phaeopigments were calculated according to Arar and Collins (1992). Samples of phytoplankton were collected every 2 d, fixed with lugol and cell counts of microphytoplankton were carried out using the method of Utermöhl (1958).

2.3. Photosynthetic measurements

2.3.1. ^{14}C incorporation

Simulated *in situ* incubations were conducted in a radial photosynthetron (Babin et al., 1994) for 40 min (Jouenne et al., 2005). Rapidly after sampling, the seawater was dispensed into culture flasks of 50 ± 0.2 ml and 50 μl of sodium bicarbonate, marked with ^{14}C (2 μ Ci) in aqueous solution, was added. 100 μl was immediately sampled from each flask and put in scintillation vials containing 250 μl of ethanolamine (Sigma), which prevents the radiolabelled inorganic CO_2 from escaping to the atmosphere. The flasks were placed in the photosynthetron and optical filters were inserted between the flasks in order to create a gradient of 9 light intensities (0; 12; 83; 162; 400; 570; 825; 1240; 1800 μmol photons $m^{-2} s^{-1}$), the last flask was kept in the dark to estimate the non-photosynthetic inorganic carbon incorporation. Light intensity was measured in the flask using a micro-spherical quantum sensor (US-SQS/L Walz, Effeltrich, Germany). The light was produced by a 1000 W Metal Halogene Powerstar® HQI lamp (OSRAM, Switzerland). The temperature of the photosynthetron was controlled by a seawater circuit. At the end of the incubation, 250 μl of 37% formaldehyde was
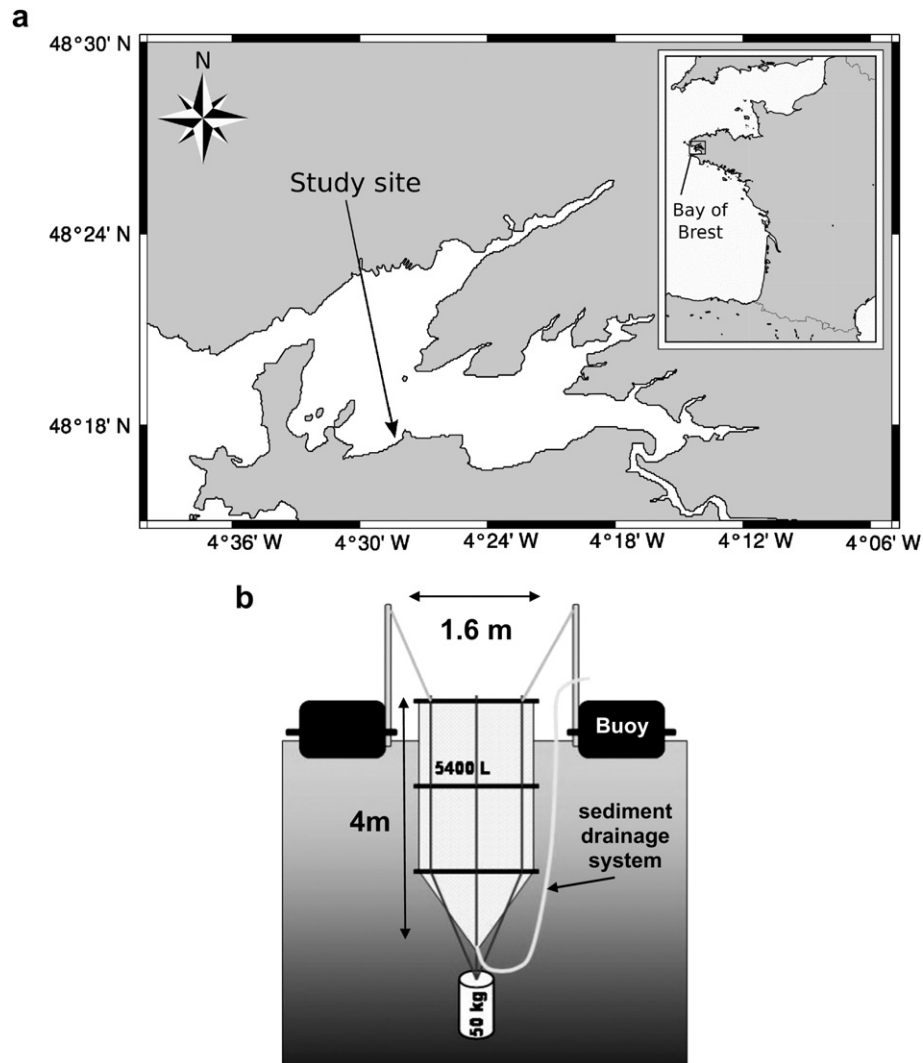
Fig. 1. a – Location of the study site. b – Schematic view of a mesocosm.

added in order to stop photosynthetic activity (Jouenne et al., 2005). The incubated sub-samples were then filtered on Whatman GF/F, 25 mm filters and the filters were placed in 20 ml scintillation vials. HCl with 1 N (250 µl) was added to each filter in order to degas any radioactive inorganic carbon. 20 ml of scintillation cocktail (Hionic Fluor, Perkin Elmer Life Sciences) was added to the vials. From disintegrations *per* minute (DPM) counts, carbon incorporation was calculated. The value for the dark incorporation was subtracted from all others and results obtained were standardized according to the Chl *a* biomass in order to obtain estimates of primary productivity (P^B). P^B was expressed in mg C (mg Chl *a*)$^{-1}$ h^{-1} or in mmol C (mg Chl *a*)$^{-1}$ h^{-1}.

Table 1
Daily nutrient pulse additions to the six mesocosms. Additions and the nutrient ratios were defined to benthic nutrients fluxes measured *in situ* in the Bay of Brest.

Mesocosm	M1	M2	M3	M4	M5	M6
Si added (µM d^{-1})	0.40	0.55	0.70	0.85	1.00	1.00
N added (µM d^{-1})	0.31	0.42	0.54	0.65	0.77	1.25
P added (µM d^{-1})	0.025	0.034	0.044	0.053	0.063	0.063
Si:N	1.30	1.30	1.30	1.30	1.30	0.8
Si:P	16	16	16	16	16	16
N:P	12	12	12	12	12	20

2.3.2. P vs E curves

Primary productivity was plot against light. As no significant photoinhibition was observed, the P *vs* E model of Webb et al. (1974) was applied to the P^B.

$$P^B(E) = P^B max\left(1 - \exp\left(-\alpha E/P^B max\right)\right),$$

where, $P^B max$ is photosynthetic capacity (mg C (mg Chl *a*)$^{-1}$ h^{-1}), and α (mg C (mg Chl *a*)$^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$) is the initial slope of the P *vs* E curve, or maximal light utilization efficiency. The model was applied to the data by using Sygmaplot 10 software (SPSS, USA). The light saturation parameter E_k (µmol photons m^{-2} s^{-1}) was calculated as

$$E_K = P^B max/\alpha$$

From the parameters ($P^B max$ and α) and light measurements in the enclosure, the daily depth-integrated gross primary production (Pz) was then calculated (mg C m^{-2} d^{-1}) (Falkowski and Raven, 1997). Light in the enclosures was determined using a spherical underwater quantum sensor Li-193 connected to a dataLogger LI-1400 (LI-COR USA) at the surface (E0) and at different depths in the enclosures. These measurements allowed calculation of the light

extinction coefficient, k, and the mean light in the enclosures, E_m (μmol photons m^{-2} s^{-1}).

2.3.3. PAM fluorometry

The maximum energy conversion efficiency, or quantum efficiency of PSII charge separation (F_v/F_m), was measured using a WATER/B – PAM (Walz, Effeltrich, Germany) (Schreiber et al., 1986). After a dark adaptation of 15 min, a 3 ml sub-sample was placed in a darkened measuring chamber. The sample was excited by a weak blue light (1 μmol m^{-2} s^{-1}, 470 nm, frequency 0.6 kHz) and fluorescence was detected at wavelengths above 695 nm. The blank was performed on filtered water from each mesocosm (0.2 μm polycarbonate membrane filter). The quantum efficiency of PSII (F_v/F_m) was calculated by (Genty et al., 1989),

$$F_v/F_m = (F_m - F_0)/F_m$$

where, F_0 is the minimal fluorescence, and F_m is the maximum fluorescence (during a saturating light pulse, 0.6 s, 470 nm, 1700 μmol m^{-2} s^{-1}), of a dark-adapted sample.

2.4. Statistical analyses

The relationships between the biomass of the main phytoplankton taxa and the physical, chemical and biological parameters were examined by using a Canonical Correspondence Analysis (CCA) (software CANOCO 4.5 (Ter Braak and Smilauer, 2002)). CCA was performed on log transformed data [ln(Ax + B); A = 1, B = 1] (Ter Braak and Smilauer, 2002).

3. Results

3.1. Biomass evolution and species succession

The biomass (Chl a) increased in all mesocosms during the course of the experiments (Fig. 2) as a function of the daily input of nutrients (Table 1). M1 and M2 presented the lowest increase of biomass while M5 and M6 showed the highest (Fig. 2). The highest concentration reached in M1 was 4.13 μg Chl a l^{-1} on day 18, while in M6 it was 7.89 μg Chl a l^{-1} on day 17.

The system was largely dominated by diatoms (90.8%). Other micro- or nanoplankton taxa remained at very low concentrations in all mesocosms during the whole experiment and were composed of Dinophyta (6.0%) and other flagellates (3.2%).
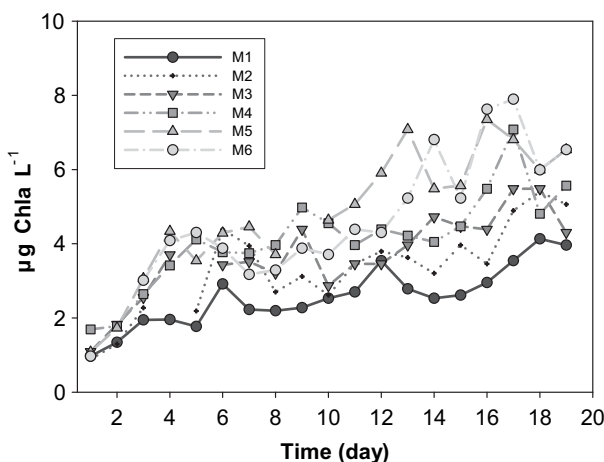
Diatom cell count increased as a function of enrichments from less than 3×10^5 cells l^{-1} at the beginning of the experiment in all enclosures, to a maximum of 2×10^6 in M1, and 12×10^6 cells l^{-1} in M6.

Different genera of diatoms bloomed over the time-course of the experiments. *Chaetoceros* spp. first dominated the population, constituting up to 50% of the total diatom cells, along with *Pseudo-nitzschia* spp. (20–30% of total diatom cells). The dynamic of the
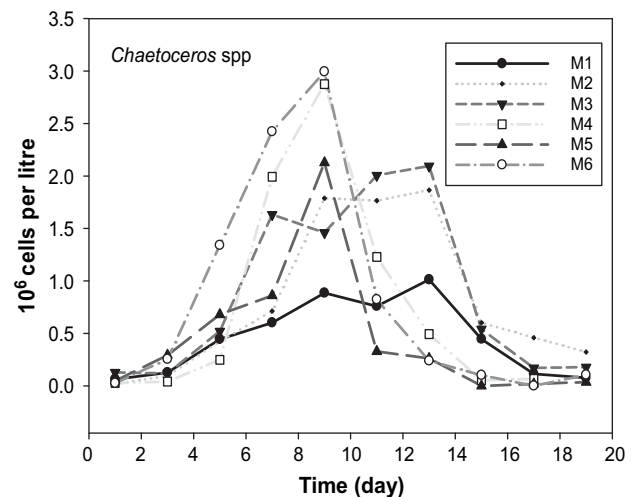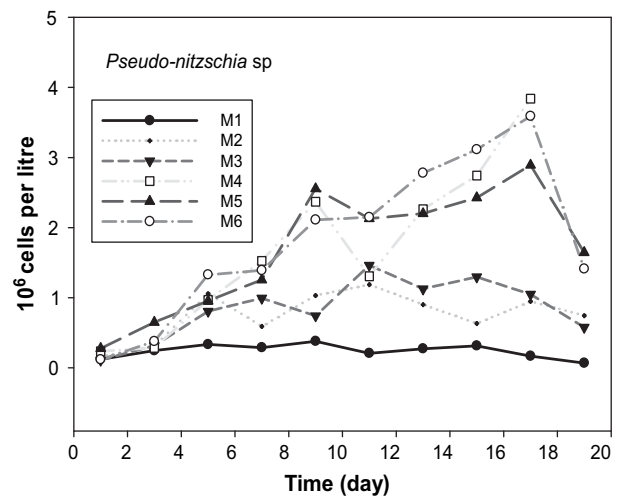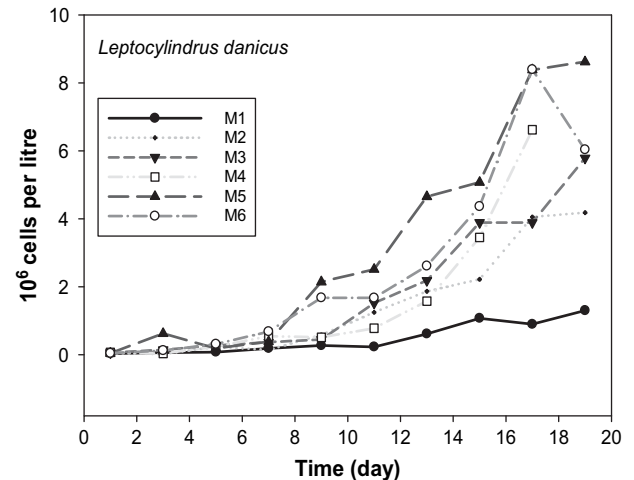

Fig. 3. Evolution of the three dominant genus, *Chaetoceros* spp., *Pseudo-nitzschia* spp., *Leptocylindrus danicus*, in the six mesocosms.

Fig. 2. Variation of Chl a biomass in the six mesocosms.

dominant diatom species then shifted in all the enclosures. In the three more fertilized enclosures (M4–M6) the biomass of *Chaetoceros* spp. failed after 8 d, while the growth of *Pseudo-nitzschia* spp. increased. In the three less enriched enclosures (M1–M3) the biomass of *Chaetoceros* spp. remained at its highest value longer, till day 14, while *Pseudo-nitzschia* spp. maintained the same concentration. In parallel, after week one of the experiment, *Leptocylindrus danicus* started to grow and became dominant in all mesocosms by the end of the experiment (Fig. 3).

3.2. Nutrient status

Initial experimental DIN concentrations in all enclosures were around 0.12 μM. Directly after the first enrichments (from 0.31 to 1.25 μM NO_3), which occurred in the evening of day 1, the DIN concentration increased in the six mesocosms (data not shown). The highest concentrations were found in M5 and M6 and respectively reached 0.53 and 0.50 μM. Despite daily nutrient additions, DIN concentrations then decreased and were more or less stable in all enclosures after 3 or 4 d, oscillating around 0.10 μM. The average value of DIN between the days 4 and 19 in the six enclosures was 0.10 ± 0.40 μM. For DSi, the initial concentration was 1 μM. DSi concentrations increased in all enclosures immediately after the first additions accordingly to the amounts added (from 0.4 to 1 μM of DSi) but then decreased rapidly until day 5 (data not shown). After day 5 and despite daily enrichments, concentrations remained low in all mesocosms, around 0.4 μM (± 0.1). During the first 4 d the PO_4^{3-} concentrations showed the same trend as DIN and DSi (data not shown), subsequently the concentrations were very low in all enclosures, which were frequently under the detection limit. The average PO_4^{3-} concentration measured in all enclosures during the experiment was 0.015 ± 0.015 μM. Nutrient Si:N:P ratios were compared to Redfield (1934) and Brzezinski (1985) ratios (Fig. 4) in order to characterize which nutrient was the most likely to become limiting. DIN appeared to be the most limiting nutrient in all enclosures. We noticed that potential PO_4^{3-} limitations were occasionally observed while DSi was never limiting.

3.3. Photosynthetic parameters

The F_v/F_m variations followed the same trend in the six enclosures during the whole experiment (Fig. 5a). F_v/F_m values were always higher than 0.5, indicating a satisfactory physiological acclimation to the growth conditions (Parkhill et al., 2001). P^Bmax values showed
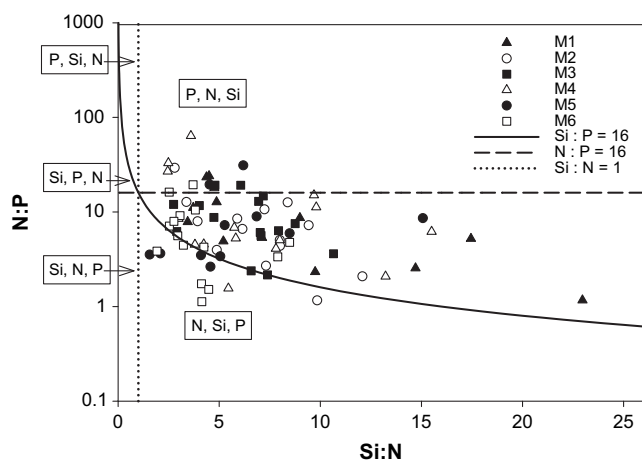

Fig. 4. Si:N:P ratios measured in the water column in the six mesocosms during the experiment. In each area, delimited by Redfield (1934) and Brzezinski (1985) ratios, nutrients are given in order of their potential limitation.
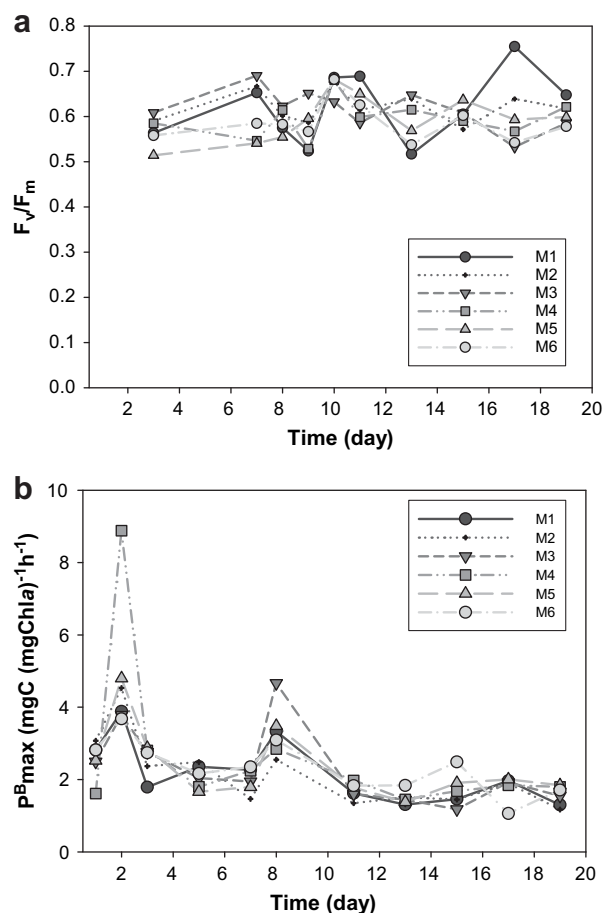

Fig. 5. Variation of the quantum efficiency of PSII (F_v/F_m) (a) and the maximal photosynthesis capacity (P^Bmax) (b) in the six mesocosms.

the same evolution in all mesocosms (Fig. 5b). Except for an increase of P^Bmax in the six enclosures on days 2 and 8 the values of P^Bmax were also almost identical in the six mesocosms and oscillated around an average value of 2.23 ± 0.85 mg C (mg Chl a)$^{-1}$ h^{-1}. The lowest P^Bmax was measured on day 1 in M4 (1.61 mg C (mg Chl a)$^{-1}$ h^{-1}), and the highest on day 2 also in M4 (8.88 mg C (mg Chl a)$^{-1}$ h^{-1}). This highest value does not seem realistic in comparison with the other data and should probably not be considered.

During the whole experiment α varied from 0.039 in M6 (day 1) to 0.01 mg C (mg Chl a)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$ in M4 (day 5) (Fig. 6a). During the first 7 d α tended to decrease in all enclosures and then it increased on day 8. After day 8, except for a few exceptions, α was quite stable in M2–M6 and varied around 0.026 mg C (mg Chl a)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$. The values of α calculated for M1 from day 8 were lower than the others, and were almost constant around 0.015 mg C (mg Chl a)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$. A significant linear relationship (p < 0.001) was found between P^Bmax and α in all enclosures between day 8 and day 19 (Fig. 7), while this relationship was not significant before day 8 (data not shown). Large variations of E_k, between 56 and 266 μmol photons m^{-2} s^{-1}, were observed during the first days and then all mesocosms followed roughly the same pattern (Fig. 6b). The E_k variations during this period appeared to be related to E_m. After day 8, E_k was quite stable in the enclosures, while E_m decreased from 280 to 81 μmol photons m^{-2} s^{-1}. During this period the average of E_k was 70.5 ± 14.3 μmol photons m^{-2} s^{-1} for the five enclosures from M2 to M6, and was 100.7 ± 13.6 μmol photons m^{-2} s^{-1} for M1. The E_k/E_m ratio increased during the first days and then it decreased

a

b

c
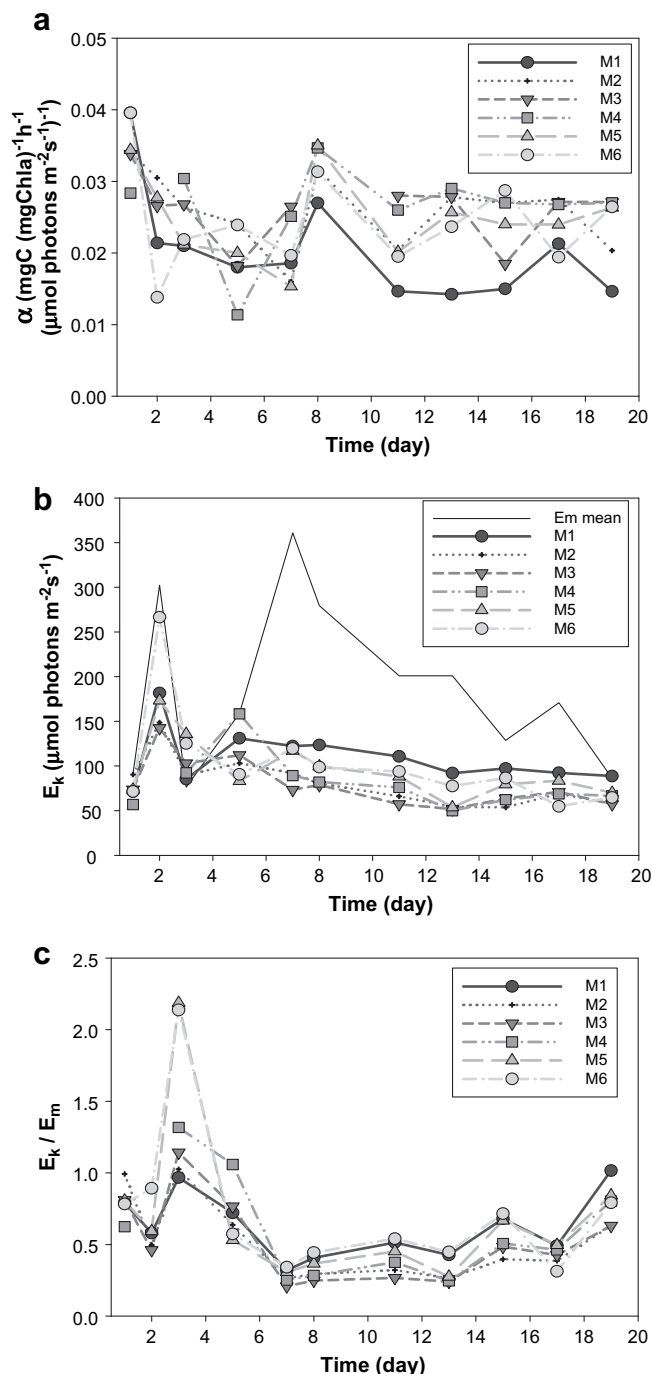
Fig. 6. Variation of the maximal light utilization efficiency (α) (a), of the light saturation parameter (E_k) (b) and of the photoacclimation index (E_k/E_m) (c) in the six mesocosms.

below 1 and was quite stable in all enclosures between day 7 and day 13. After day 13, due to the decrease of E_m, the ratio increased slightly (Fig. 6c).

3.4. Primary production

The variations in Pz were the result of Chl a, the photosynthetic parameters, k and the daily light values. Pz roughly increased in all enclosures until day 17 and dropped concurrently for all of them on the final day because of a low light availability (Fig. 8). The highest value reached on day 17 was 498 mg C m^{-2} d^{-1} in M4 and M5, and
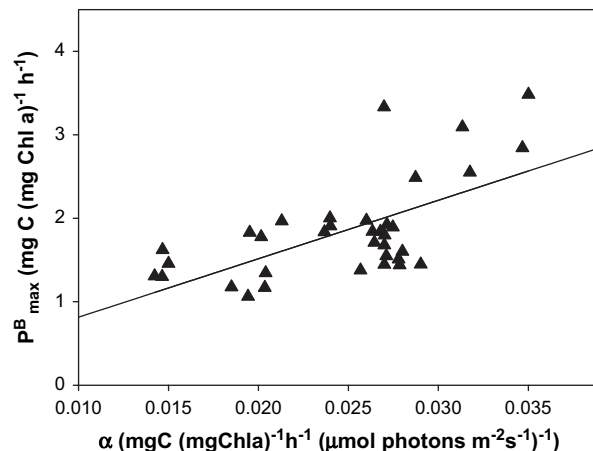

Fig. 7. Relationships between the maximal photosynthesis capacity (P^Bmax) and the maximal light utilization efficiency (α) measured in all enclosures between day 8 and day 19. $y = 70.03x + 0.11$; $r^2 = 0.40$; $p < 0.0001$.

the lowest value was 216 mg C m^{-2} d^{-1} in M1. Due to a high estimation of P^Bmax on day 8 in M3 a very elevated Pz value was calculated (557 mg C m^{-2} d^{-1}), which should be considered with caution. The mean Pz values (Table 2) showed that the daily estimated production integrated from the surface to the bottom of enclosures was higher in M5 and M4, respectively 260 and 235 mg C m^{-2} d^{-1}, and was twice as high as the production found in M1. The production in M1 was significantly lower than M6, M5 and M4 (Anova, tukey test $p < 0.05$), but was not significantly different from M2 and M3. No significant differences appeared between M2, M3, M4, M5 and M6.

3.5. Canonical correspondence analysis

CCA was used to relate the biomass of the main phytoplankton taxa determined in the six enclosures to environmental variables measured in the enclosures during the whole experimental period (3 weeks) (Fig. 9). The two first axes explained 94.4% of the variance in the species–environment relation (axis 1: 72.2% and axis 2: 22.2%). Monte Carlo permutation tests (499 permutations) showed that the first axis alone ($p = 0.006$) and all the canonical axes ($p = 0.002$) were statistically significant. Consequently, the descriptions and the discussion of the CCA analyses can be based on the first two axes. Based on the intersect correlations, nutrient
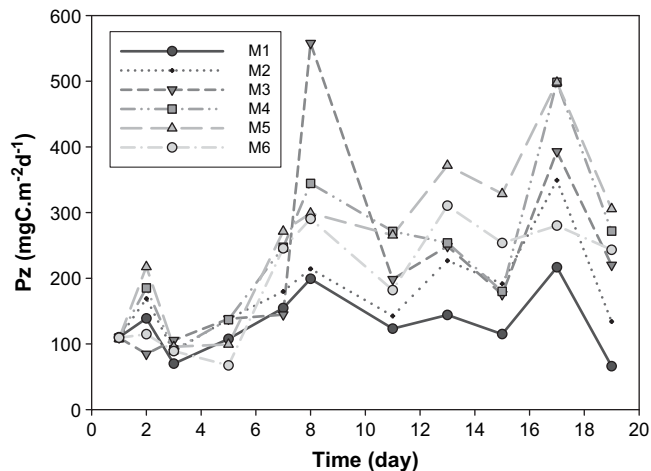
Fig. 8. Variation of the depth-integrated gross primary production Pz in the six mesocosms.

Table 2
Mean values of Pz integrated between the surface and the bottom of the enclosures during the course of the experiment. S.D. Standard deviation, n number of data. The highest measurement in M3 on day 8 was not taken into consideration.

	Pz (mgC m^{-2} d^{-1})		
	Mean	S.D	n
M1	131	46	11
M2	176	71	11
M3	181	86	10
M4	235	116	11
M5	260	124	11
M6	198	89	11

inputs (N, P, Si) and Chl *a* were largely related to the first axis while nutrient stocks (DSi, DIN and PO$_4$) and k were respectively positively and negatively related to the second axis. By analysing the CCA, we observed that there was no relationship between nutrient inputs (N, P, Si) and nutrient stocks (DSi, DIN and PO$_4$). All diatoms, except *Chaetoceros* spp., were positively related to nutrient inputs (N, P, Si), while Dinophyta and Cryptophyta biomass appeared related to nutrient stocks. Furthermore, P^{B}max was not related to the magnitude of the nutrient inputs or to the Chl *a* biomass.

4. Discussion

The daily inputs applied in this study were intended to simulate enrichment of the water column by benthic fluxes. As previously mentioned the amounts of daily nutrient inputs performed during this experiment were realistic and were realized in accordance with the measurements performed in the Bay of Brest as a function of *C. fornicata* densities (Ragueneau et al., 2002). The daily rhythm of nutrient inputs applied in this study is also in accordance with previous studies performed on benthic fluxes. In two recent studies conducted in a tropical lagoon and in the Bay of Brest, fluxes of silicic acid out of the sediment were found to be up to 5 times higher during earlier morning periods than during the remainder of the diurnal period (Ní Longphuirt et al., 2009; Clavier et al., 2007).
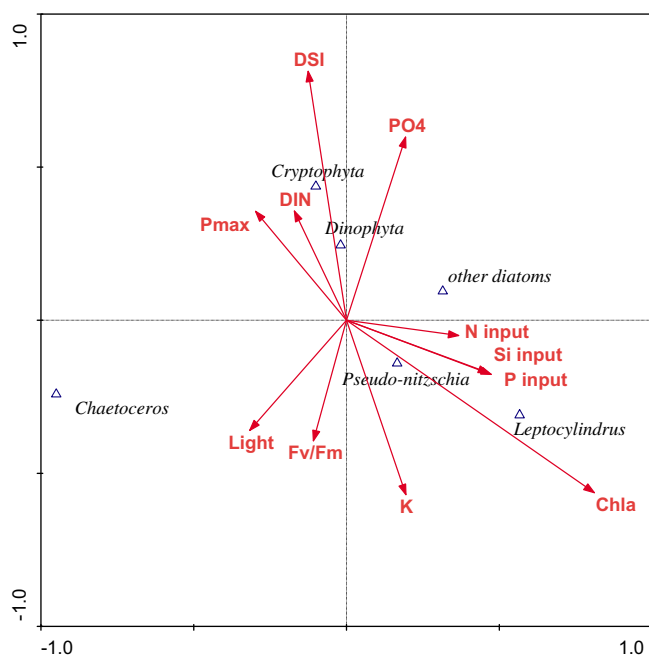
Fig. 9. Canonical correspondence analysis (CCA) ordination diagram showing the relationships between the biomass of the main phytoplankton taxa and the environmental variables determined in the six mesocosms.

4.1. Phytoplankton response to nutrient enrichment in autumn: dynamic and photosynthetic parameters

In all enclosures a succession between *Chaetoceros* spp., which dominated the population during the first days, *Pseudo-nitzschia* spp. and *L. danicus* were observed but the dynamics of the species succession were not the same. We observed that the development of *Pseudo-nitzschia* spp. was largely favored in the three more fertilized mesocosms, while *Chaetoceros* spp. was advantaged in the less fertilized mesocosms (Fig. 3). We can also note that no non-siliceous species were advantaged by the increased N input in M6 (Table 1). On the contrary, according to nutrient ratios the highest potential DIN limitation was observed in M6. In all enclosures DSi was never limiting, which explained the large dominance of diatoms. According to the CCA analyses (Fig. 9), the dynamics of *L. danicus* and *Pseudo-nitzschia* spp. were quite similar and related to the nutrient inputs while *Chaetoceros* spp. did not seem to be related to nutrient inputs.

In order to appreciate the photosynthetic and the physiological status of the phytoplankton populations various photosynthetic parameters were measured. The ratio F_v/F_m is often used as an indicator of nutrient stress. It is maximal (around 0.6) for nutrient replete cultures and declines with nutrient starvation. However, Parkhill et al. (2001) have shown that it can remain high and constant under nutrient stress when phytoplankton is acclimated to nutrient limitation. According to the extremely low nutrient concentration measured in the enclosures (Fig. 4), the high and stable values of the F_v/F_m measured in all enclosures (Fig. 5a) probably resulted from the daily inputs of nutrients, which maintained the good physiological status of the cells despite the apparent DIN limitation. This maintenance, which was observed at different degrees of nutrient inputs during the three weeks, suggests a high physiological acclimation capacities of this phytoplankton community (mainly diatoms) and showed that even the lowest daily enrichment level (M1) ensured a balance in the cellular physiology of the microalgae.

The support of the physiological status by the daily inputs was confirmed by the stability and the level of the P^{B}max values (Fig. 5b). In spite of the species successions, the maximal photosynthetic capacity (P^{B}max) and the maximal quantum efficiency of PSII (F_v/F_m) remained constant over the course of the experiment. These results showed that each successive species developed the same production capacities. Such results imply that these species are ecophysiologically close, particularly *L. danicus* and *Pseudo-nitzschia* spp. (Fig. 9). This idea of a balanced physiological status supported by daily fertilization strengthens the observation performed during the spring experiments (Fouillaron et al., 2007). Fouillaron et al. (2007) observed that whatever the nutrient inputs, diatoms dominated the phytoplankton community under apparent DSi limitation, and did not entail flagellate algae species development. Only the magnitude of the diatom bloom changed. Our results showed that the daily inputs maintained the physiological status and the photosynthetic capacity of the diatoms community, thus allowing them to dominate the phytoplankton community in all six enclosures. Svensen et al. (2002) also observed that in a mesocosm study continuous supply simulated by continuous input or frequent pulses resulted in a stable system while the simulation of extreme nutrient enrichments entrained the domination of one species, the dinoflagellate *Gyrodinium aureolum*. The stability of α and E$_k$ (Fig. 6a and 6b) was also observed after day 8 in all enclosures. During the first week those parameters were more variable than P^{B}max and F_v/F_m, but the stability observed during the second and the third week confirmed the balance of the physiological status of the phytoplankton community. Mangoni et al. (2009) observed that all photosynthetic parameters (P^{B}max,

α and E_k) decreased in parallel with nutrient depletion, in a meso-cosm experiment without regular nutrient additions, confirming the importance of nutrients availability on those parameters. In nutrient replete conditions, photosynthetic parameters P^Bmax, α and E_k are known to be largely dependent on incident light (Behrenfeld et al., 2004). Variations of those parameters are due to photoacclimation mechanisms, which are particularly efficient in microalgae (Falkowski and Raven, 1997). Photosynthetic parameters were measured at solar noon but incident light and then E_m changed each day. The E_k/E_m ratio can be used as an index of photoacclimation (Fig. 6c). A low E_k/E_m ratio indicates light saturation and an imbalance between light-harvesting and downstream photosynthetic reactions (Anning et al., 2000, 2001), while a ratio around 1 indicates optimization of light-harvesting with photosynthetic metabolism as a function of the incident light. In the present study, it appears that, except for few events during the first week and the last day, the E_k/E_m exhibited values below 1 revealing a poor photoacclimation at sampling time. However, this ratio is highly dependent on the light history of microalgae and of the nutrient status, which may partly explain those results (Behrenfeld et al., 2004). The apparent stability of E_k that was observed can be associated with the "E_k independent variation" described by Behrenfeld et al. (2004). No significant relationships were found between E_m and E_k (data not shown). This "E_k independent variation" reveals a P^Bmax and α co-variation but no clear physiological explanation is forthcoming to explain such a relationship. Behrenfeld et al. (2004) proposed a few potential explanations implicating pigment variability, nutrient availability, or taxonomy. A significant and linear co-variation between P^Bmax and α was only found in enclosures after day 8 (Fig. 7). We can notice that a stability of P^Bmax and α also lead to a constant E_k. According to the conceptual model proposed by Behrenfeld et al. (2004) explaining the "E_k independent variation", nutrient limitations, particularly nitrogen limitation, would entail a decrease in the reductants required for carbon fixation. The values of the photosynthetic parameters are then driven by growth constraints due to nutrient availability and not by incident light (Behrenfeld et al., 2004), which is in accordance with our data.

Some studies pointed out the importance of taxa on photosynthetic parameters variations (Côté and Platt, 1983; Jouenne et al., 2007; Mangoni et al., 2009) while others pointed out mainly environmental factors (Behrenfeld et al., 2004). In this work, despite different dynamics of successions between three less and three more enriched mesocosms, no differences of the trend followed by the photosynthetic parameters appeared. The photosynthetic parameters remained stable during the *L. danicus* and *Pseudo-nitzschia* spp. transition. Only a few field experiments support the "E_k independent variation" model proposed by Behrenfeld et al. (2004) but none of them permit a clear explanation of this regulation (Platt and Jassby, 1976; Côté and Platt, 1983; Jouenne et al., 2007). Our study highlights that such a regulation of photosynthetic parameters appears when there are frequent nutrient additions which do not allow replete nutrient conditions to be reached but lead to physiological equilibrium. These physiological effects of nutrient fluxes on photosynthetic parameters may help to simulate and understand better the impacts of benthic fluxes on the primary production dynamics in shallow coastal ecosystems.

As expected, the increase of nutrient inputs resulted in a biomass augmentation and consequently to an increase in the depth-integrated gross primary production Pz (Fig. 8). The measured values of Pz were in accordance with previous values found in a Western European coastal macrotidal ecosystem (Jouenne et al., 2007) and with the values measured by Svensen et al. (2002) in enclosures controlled by continuous or frequent inputs. By enriching enclosures with one or two massive pulses of nutrients, Svensen et al. (2002)

observed that Pz could reach high values of around 600–900 mg C m^{-2} d^{-1}. The estimation of the Pz values allows an estimation of the bay's production capacity as a function of benthic fluxes which is a key value for characterizing a coastal ecosystem (Grangeré et al., 2009) and for ecosystem management (Azevedo et al., 2006, 2008).

4.2. Environmental implications: influence of benthic fluxes on the ecosystem

Benthic faunas have an important role to play in regulating benthic fluxes (Marinelli, 1994; Ragueneau et al., 2005). One species, *C. fornicata*, was largely studied in the Bay of Brest. Studies have hypothesized that the intensity of nutrients recycling by *C. fornicate*, and particularly Si at the sediment–water interface was strongly involved in diatom dominance in this Bay (Ragueneau et al., 2002, 2005). Our results point out that, in regard to the benthic fluxes simulated in this study, the daily nutrient additions are probably more significant than the quantitative values of the fertilization. According to our findings, we can speculate that hydrodynamic and biological factors, which largely control the rhythm of fluxes between sediment and water column, would then play a major role in addition to the *C. fornicata* covering. The equilibrium resulting from these daily benthic nutrient pulses would consequently reduce the risk of non-siliceous bloom events, but as mentioned previously, it is important to remember that the increased fertilization entailed an increase in *Pseudo-nitzschia* spp. (harmful genus) in all enclosures. It can be suggested that the nutrient loadings were not strong enough to destabilize the phytoplankton community structure and species composition, however, the aim of the study was more specifically to simulate a real potential variation of benthic fluxes in the context of *C. fornicata* eradication from the bay of Brest as called for by local fishermen and exposed in Introduction section. Moreover, even if the present study and the work by Fouillaron et al. (2007) point out the importance of the daily nutrient inputs instead of the magnitude of the nutrients additions, we can suppose that high benthic fluxes (i.e. high densities of *C. fornicata*) have a buffering effect on the nutrient availability of the bay and limit the consequences of short-term events like wind or rain which can entail a sudden and high nutrient concentration input which are known to promote Dinophyta potentially toxic bloom formation (Svensen et al., 2002; Spatharis et al., 2007). Pannard et al. (2008) exposed how such short-term events can influence the dynamic of the phytoplankton communities and can modify the photosynthetic capacities. A simulation of sudden high nutrient inputs as a function of various magnitudes of benthic fluxes would allow us to have a better understanding of the real impact of those fluxes on the resistance of the Bay of Brest against Dinophyta toxic bloom formation.

5. Conclusion

(1) This study highlights the effects of daily inputs on phytoplankton dynamics and shows the importance of benthic pulse rhythms as much as the amount of nutrients input to understand phytoplankton successions which largely confirmed the conclusion by Fouillaron et al. (2007).

(2) In addition, we clearly showed and explain how photosynthetic parameters can be driven by nutrient availability and not by incident light or species successions.

(3) The stability and the high level of photosynthetic capacities that we observed despite apparent DIN limitation should be considered in models of primary production in shallow coastal ecosystem where benthic fluxes are important.

(4) Regarding the management of the Bay, we pointed out the possible apparition of *Pseudo-nitzschia* spp. linked with an

increase of fertilization, which reveals the new sanitary problem due to ASP toxins produced by some *Pseudo-nitzschia* which have been appearing in the North-West French coast from Normandy to Brittany, including the Bay of Brest, since 2004 (Nezan et al., 2006). Consequently, high densities of *C. fornicata* would allow the avoidance of Dinophyta toxic bloom formation but would promote *Pseudo-nitzschia* development.

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