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Response of phytoplankton to organic enrichment and shrimp activity in tropical aquaculture ponds: a mesocosm study

Hugues Lemonnier[1,*], Sébastien Hochard[1], Kento Nakagawa[1,2], Claude Courties[3], Martine Rodier[4]

[1]IFREMER LEAD, BP 2059, 98846, Nouméa Cedex, New Caledonia
[2]LTMA, Applied Technology and Technology Transfer Laboratory, BP 2384, 98846 Nouméa Cedex, New Caledonia
[3]Sorbonne Universités, UPMC Univ. Paris 6, UMS 2348 Observatoire Océanologique, 66650, Banyuls/mer, France
[4]IRD, Ecosystèmes Insulaires Océaniens (EIO) UMR 241 (Université de la Polynésie française - Institut de Recherche pour le Développement - Institut Malardé-Ifremer), Centre IRD de Tahiti, BP529, 98713 Papeete, French Polynesia

ABSTRACT: We conducted a mesocosm study to investigate the combined effect of organic enrichment and sediment resuspension by shrimp on phytoplankton communities in shrimp aquaculture ponds. Hence, the factorial design included 2 factors: (1) shrimp density with a concomitant increase of feed input, resulting in organic enrichment, and (2) access of shrimp to the sediments. Increasing feed input in the system raised the eutrophication state of the environment, characterized by an increase in phytoplankton biomass. Bioturbation enhanced: (1) mineralization of organic matter via the microbial loop, resulting in faster nutrient recycling, (2) primary production and (3) buffering capacity against eutrophication consequences. The phytoplankton community showed both large temporal variations of its taxonomic composition and resilience to treatments. A shift in species dominance from diatoms + dinoflagellates to green algae was observed in all treatments and coincided with meteorological and N pool changes. Results suggest that algal production was primarily limited by phosphorus and light at low (i.e. low feeding) and high (i.e. high feeding) eutrophication states, respectively. Growth rate of species to be a major factor favoring their dominance in the phytoplankton community in this highly dynamic ecosystem. Consequences for water column management are discussed.

KEY WORDS: Aquaculture · *Litopenaeus stylirostris* · Phytoplankton communities · Eutrophication · Bioturbation

INTRODUCTION

Shallow marine environments are primarily defined by their high ratio of sediment surface area to water volume (McGlathery et al. 2007). These features add complexity to how the system responds to eutrophication particularly due to a strong benthic–pelagic coupling. In shallow eutrophic areas, organic matter loading can enhance sediment respiration and the release of nutrients into the overlying water column that sustains part of the primary producers' growth and production (Cloern 2001, Porter et al. 2010). Eutrophication may also promote the proliferation of certain species, some of which may often cause harm through the production of toxins (Heisler et al. 2008, O'Neil et al. 2012, Paerl & Otten 2013), a phenomenon known as harmful algal blooms (HAB). Even if the different processes and effects of eutrophication are well known and documented (e.g. Cloern 2001), the effects on the phytoplankton community are not easily predictable (Schmoker et al. 2016), especially in shallow environments. In these areas,

*Corresponding author: hugues.lemonnier@ifremer.fr

wind and storm events, tides, waves, wave currents and bioturbation are involved in the dispersal of sediment particles, leading to an increase in turbidity in the water column. Reduction of light availability may become a limiting factor for primary production, with possible consequences for phytoplanktonic communities and productivity (Rochelle-Newall et al. 2011). There is also evidence that all these physical processes enhance nutrient flux from sediment into the water column (e.g. Havens 1991), which may also affect phytoplankton biomass and composition (Havens 1993, Porter et al. 2010). Due to the complexity and the uncontrollable variability of the processes involved, it is difficult to discriminate the factors influencing water eutrophication within natural shallow ecosystems from *in situ* measurements (Porter et al. 2010).

Outdoor shrimp aquaculture ponds are artificial marine systems that are shallow (<2 m) and highly eutrophic, associated with elevated nutrient inputs. These systems show low diversity and simplified trophic chains, where phytoplankton play a pivotal role in the flow of energy and nutrients, due to their high abundance, efficient nutrient uptake and high productivity (Burford 1997). Phytoplankton cell proliferation, linked to increasing inputs of nutrients through feeding, has been widely analysed (e.g. Casé et al. 2008). Temporal changes of dominant species due to dynamic variations of growth factors such as light, temperature, substrate supply (inorganic and organic nutrients), predation and virus infection have also been reported (e.g. Burford 1997, Casé et al. 2008). According to the ecological theory of *r/K* selection, an unstable environment in a pond containing high nutrient levels is particularly favorable for fast-growing organisms as opportunistic pathogens (De Schryver & Vadstein, 2014). This lack of stability linked to an imbalance in the N:P ratio may promote the development of toxic algal species stressful for shrimp, playing a direct or indirect role in disease outbreaks in shrimp aquaculture ponds in New Caledonia (Lemonnier et al. 2006, 2010, 2016, Lucas et al. 2010).

However, while the role of phytoplankton in maintaining water quality in tropical shrimp ponds has been studied in the field by several authors (e.g. Burford 1997), few studies have been conducted to distinguish the role of the different factors involved in the control of the phytoplankton community and dynamics. 'Stocking density', defined as the number of animals per unit area, is one of the most important factors. Raising shrimp stocking density in ponds requires increased organic matter input for shrimp feeding. As a result, more waste — mainly faeces and unconsumed feed pellets — is produced, leading to an increase in the eutrophication level of the pond ecosystem (Martin et al. 1998, Bouwman et al. 2013). The nutrients, produced through organic matter degradation or excreted by shrimp, are rapidly assimilated by phytoplankton. This results in low concentrations of inorganic nutrients in the water column (Burford & Williams 2001, Burford et al. 2003). Moreover, shrimp act as bioengineers in ponds and have large physical and biogeochemical impacts on sediment through their bioturbation. Therefore, any modifications of shrimp stocking density will affect processes linked to the bioturbation (Ritvo et al. 1997, 2004, Joyni et al. 2011).

Due to the complexity of physical and biological processes in the field, it is difficult to distinguish the effect of each forcing variable on the phytoplankton community, function and dynamics. To overcome this difficulty, we set up highly controlled experiments in mesocosms. This paper reports the results of an experiment in which (1) different densities of shrimp with their corresponding amounts of feed inputs and (2) access/no access to sediment were studied according to a factorial approach to disentangle the effects of organic matter input by feeding from the effects of shrimp activity on sediment by bioturbation. Because few studies have addressed changes to perturbation in both composition and functionality, making the generalization of patterns difficult (Nogales et al. 2011), phytoplankton communities were followed through flow cytometry (FCM) and spectrofluorometry, in close relationship with the biogeochemical functioning of the water column. The aim of this work is to improve our knowledge of phytoplankton ecology in outdoor pond aquaculture and, more generally, in eutrophic ecosystems. By improving our knowledge in this field, this should facilitate the implementation of technical measures to increase efficiency in recycling waste produced during rearing and limit the risk of growth of harmful species (*Vibrio* spp., harmful algae, etc.).

MATERIALS AND METHODS

Experimental set-up

The mesocosm experiment was conducted over a 44-d period from 7 November to 21 December 2011 at the Saint-Vincent Aquaculture Research Centre, located on the west coast of New Caledonia (South Western Pacific – 21° 55' 36 S, 166° 05' 04 E). Sixteen

cylindrical polyethylene tanks, each with an effective volume of about 1600 l and a surface area of 1.72 m^2, were used in an outdoor area. The bottom of each mesocosm was filled with a 20 cm thick layer of dry and natural sediment. The sediment was collected from the intertidal zone (unvegetated with salt deposits) between mangroves and agricultural land, which is where shrimp farms are generally located in New Caledonia. Sediment grain size was predominantly within the silty clay fraction and sediment organic content was below 2%. Each tank was filled with seawater (0.8 m depth) pumped from the nearby bay (called the 'inaccessible' bay) and previously filtered through a 5 mm filter (Nortene Technologies).

We crossed different densities of shrimp (D) with access or no access to sediment (S^+/S^-) in a 2 × 2 factorial experiment run in triplicate. Enclosures preventing access to the sediment were constructed with netting (mesh: 1.5 cm) and were positioned in tanks about 20 cm from the sediment. These enclosures prevented shrimp from accessing the sediment and thus from feeding on the benthic community and acting as bioturbators of the sediment. However, they did not alter sedimentation or water–sediment nutrient exchanges. The tanks were stocked with juvenile *Litopenaeus stylirostris* shrimp, the species commonly reared in New Caledonia, at 4 shrimp m^{-2} (D4 for low density) or at 12 shrimp m^{-2} (D12 for high density). The mass of each individual was 9.0 ± 1.5 g and the stocking density at the beginning of the experiment in D4 and D12 tanks was about 37 and 110 g m^{-2}, respectively. D12 was designed to mimic the density generally found in shrimp aquaculture ponds in New Caledonia. Throughout the experiment, shrimp were fed twice a day using feeding trays with commercial pellets with 35–40% protein content. The mean daily feed input was 1.3 and 3.8 g m^{-2}, representing a total feed input over the 44-d experiment of 50 and 150 g m^{-2} for D4 and D12, respectively.

Four combinations were thus tested in triplicate, allocated randomly to the 12 tanks: (1) high shrimp density + access to the sediment ($D12S^+$), (2) high density – access ($D12S^-$) (3) low density + access ($D4S^+$) and (4) low density – access ($D4S^-$). Four other tanks without shrimp were used as controls, 2 without enclosure ($D0S^+$) and 2 with enclosure ($D0S^-$). The water was renewed daily in each tank by pumping water from the nearby bay (named the 'inaccessible' bay), at 20 ± 3% of the total volume as generally applied in shrimp farms. The inner walls of the tanks were cleaned twice weekly to reduce the effect of periphyton biomass and production on the experimental system (Chen & Kemp 2004). At the end of the experiment, all shrimp were harvested so as to calculate the survival rate, mean final mass and biomass produced in each tank.

Sampling and *in situ* measurements

Photosynthetically active radiation (PAR; ~400–700 nm) was measured 1 m above the mesocosms with a 1 s step and averaged each day using a LI-COR quantum sensor and LI-COR Li1400 data logger. Daily rainfall data were measured near the experimental facilities (<100 m) and obtained from the local weather forecasting service (Météo-France, Nouméa). Water temperature was automatically and continuously monitored with a 1 h step randomly in 5 tanks using thermo buttons (Proges plus). In 8 tanks (2 per treatment $D12S^+$, $D12S^-$, $D4S^+$ and $D4S^-$), an automatic system connected to a YSI 6600 probe provided continuous and hourly measurement of different parameters in the water column (salinity, temperature, fluorescence and turbidity). Discrete measurements of salinity, dissolved oxygen (DO) and temperature were also made in each tank every morning (08:00 h) using a portable conductivity meter (WTW cond330i) and an oxygen meter (WTW oxi330i). Twice a week in the morning (08:00 h), tank water was sampled at 50 cm below the water surface using a 2-l HCl-rinsed black polyethylene bottle to monitor the phytoplankton communities and analyse various parameters including pH, fluorescence, turbidity, nutrients and organic matter. Due to the shallowness of the tanks, this single water sample was representative of the entire water body, as tested in a preliminary experiment (H. Lemonnier & S. Hochard unpubl. data).

Laboratory analyses

Immediately after water sampling, pH, fluorescence and turbidity were measured in sub-samples using a WTW pH315i pH meter and an Aquafluor TM Handheld fluorometer (Turner Design). Water samples were then filtered through the ~0.7 µm pore size GF/F Whatman filter and sub-sampled for the different analyses.

Chemical analyses

Ammonium (NH_4^+) and soluble reactive phosphorus (SRP) analyses were carried out immediately on fresh water samples, while the other nutrients were meas-

ured on frozen samples. Ammonium was analysed following the method of Grasshoff & Johannsen (1972). SRP was measured in accordance with the molybdenum blue reaction described by Murphy & Riley (1962). Nitrate and nitrite [(NO_2^-+NO_3^-)–N] (NO_x) were determined using standard colorimetric techniques on a Bran + Luebbe AutoAnalyser III (Raimbault et al. 1990). Silicates were analysed only 5 times (Days 1, 11 18, 25 and 39) during the experiment, as previously described by Mullin & Riley (1955). Dissolved organic nitrogen (DON) was analysed following oxidation procedures described by Raimbault et al. (1999). Pre-oxidation dissolved inorganic nitrogen (DIN) concentrations were subtracted from the post-oxidation total dissolved nitrogen (TDN) concentration to derive the DON concentrations.

Fluorometry

To analyse the chlorophyll *a* (chl *a*) concentration in the water column, 25 to 50 ml water samples were filtered through Whatman GF/F filters and then stored frozen (–20°C) until they were analysed. Chl *a* and pheophytin *a* concentrations were determined in methanol extract before and after acidification using a fluorometer (model TD700, Turner Designs) using the method described by Herbland et al. (1985).

Flow cytometry

Water column subsamples (1.5 ml) were preserved with 1% glutaraldehyde (final concentration) and stored in liquid nitrogen pending flow cytometric analysis (Vaulot et al. 1989). Samples were defrosted at ambient temperature and subsequently analysed using a FACScan flow cytometer (BD-Biosciences) equipped with an air-cooled argon laser (488 nm, 15 mW). Methodology and data analysis used in this study are fully described in Lemonnier et al. (2016). Based on cellular right-angle light scatter (RALS) and fluorescence properties, several populations were conventionally distinguished (e.g. Lucas et al. 2010): 2 distinct *Synechococcus* populations and 5 eukaryote groups. The 2 picocyanobacteria populations (Syn) were pooled in a single group.

Spectrofluorometry

Water samples (0.2 l) were filtered onto 47 mm GF/F filters and stored at –80°C until analysis. Chlorophyll pigments were extracted in 95% acetone and analysed by spectrofluorometry with a Perkin Elmer LS55 spectrofluorometer following the method described in Neveux & Lantoine (1993), and using qualitative and quantitative improvements described in Ténorio et al. (2005) and Neveux et al. (2010). Determination of the different pigments was implemented from the 31×26 excitation–emission matrix by the PARAFAC method (Luciani et al. 2008) adapted for pigments by C. Chevalier (pers. comm.). Concentrations (in µg l^{-1}) for chl *a*, chl *b*, chl (c_1+c_2), chl *c*3 and divinyl-chl *a* (dv-chl *a*) were estimated using external standards provided by DHI® Water and Environment (Denmark). Chl *a* was used as proxy for phytoplankton bulk, and the other chlorophylls provided information on the composition of phytoplankton communities (Roy et al. 2011).

Contribution of each taxa to total chl *a*

The chl *a* associated with picocyanobacteria (chl $a_{picocyano}$) was estimated using FCM counts and a chl *a* content of 0.94 pg $cell^{-1}$ (Lemonnier et al. 2016). The chl *a* associated with chromophytes and green algae (chl a_{c+b}) was calculated by subtracting chl $a_{picocyano}$ from total chl *a* (Tchl *a*). A strong correlation (r = 0.99, n = 132) was observed between chl a_{c+b} and chl (c_1+c_2) for samples containing less than 0.5 µg l^{-1} of chl *b*, leading to a mean chl (c_1+c_2)/chl a_{c+b} ratio of 0.183 (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a080p105_supp.pdf). Using this ratio and the concentration of chl (c_1+c_2), the chl *a* associated with chromophytes (chl a_c) was estimated in all the samples. Chl *a* associated with diatoms and dinoflagellates (chl $a_{diat+dino}$) was calculated as chl a_c minus chl *a* linked to cryptophytes and haptophytes. Since pelagophytes and chrysophytes have never or rarely been described in environments such as shrimp ponds in New Caledonia (Lemonnier et al. 2016), these taxa were not taken into account in our calculations. The contribution of haptophytes (chl a_{hapto}) was calculated using a chl c_3/chl *a* ratio of 0.161 recently found for shrimp ponds (Lemonnier et al. 2016), while chl *a* associated with cryptophytes (chl a_{crypt}) was estimated using FCM counts and a chl *a* content of 1.35 pg $cell^{-1}$ (Lafarga-De la Cruz et al. 2006, da Silva et al. 2009). Finally, the chl *a* attributed to green algae (chl a_{chloro}) was equal to chl a_{c+b} minus chl a_c, as calculated above.

Water column metabolism and daily N budget

Metabolism

Oxygen fluxes in the water column were measured in light and dark incubation bottles. These bottles (300 ml) were made of borosilicate glass and had been soaked in dilute HCl (1% vol/vol) for several days before the measurements. The bottles were rinsed and filled with water taken from the tanks at 50 cm below the surface. Incubation was carried out in 8 tanks at mid-depth (2 per treatment for $D12S^+$, $D12S^-$, $D4S^+$ and $D4S^-$). The oxygen sensor spot method was used to follow oxygen as described in Warkentin et al. (2007). SP-PST3-PSUP-YOP-D5 oxygen sensor spots, also known as planar optodes (Presens GmbH), and a fibre-optic oxygen meter (Fibox 3: Presens GmbH) were used for this study. Oxygen fluxes were assessed from the time course of oxygen between 09:00 and 13:00 h with a 1 h time step. Total respiration (R) and net primary production (NPP) rates were deduced from the DO variations in the dark and light bottles, respectively (Bender et al. 1987). Gross primary production (GPP) represented the sum of the rate of R and NPP:

$$\text{GPP}\ (\mu\text{mol}\ O_2\ l^{-1}\ h^{-1}) = R + \text{NPP} \quad (1)$$

Daily N budget

To calculate the total phytoplankton N demand, a stoichiometry of 6.6 between daily GPP and daily phytoplankton N demand was used following Stumm & Morgan (1996):

$$\text{N demand}\ (\mu\text{mol N}\ l^{-1}\ d^{-1}) = (\text{GPP} \times 12)/6.6 \quad (2)$$

We hypothesize that phytoplankton daily N demand was supported (1) by the N pool excreted by shrimp and (2) by the internal N pool produced through mineralization processes in the water column, assuming that the nitrogen input by renewed water was negligible.

(1) Daily N input excreted by shrimp was derived from food, following Ebeling et al. (2006):

$$\text{Daily N input}\ (\mu\text{mol}\ l^{-1}\ d^{-1}) = [(\text{daily feed input} \times \text{PC} \times 0.144)\ /14]/V \quad (3)$$

where daily feed input is in μg N m^{-2} d^{-1}, PC represents protein content in feed (45% in this study) and V is the volume of the tank.

(2) Potential N mineralization (N_{min}) was assessed using daily heterotrophic respiration (R_{het}), deduced from total respiration (R) and corrected from phytoplankton basal respiration. Phytoplankton basal respiration was fixed at 20% of daily GPP following Langdon (1993):

$$R_{het}\ (\mu\text{mol}\ l^{-1}\ d^{-1}) = R \times 24 - 0.2 \times (\text{GPP} \times 12) \quad (4)$$

Heterotrophic respiration was used to obtain the potential N mineralization following a stoichiometry of 6.6 between O_2 consumption and N mineralization (Chapelle et al. 2000):

$$N_{min}\ (\mu\text{mol}\ l^{-1}\ d^{-1}) = R_{het}/6.6 \quad (5)$$

Daily N budgets refer to the difference between the assimilation by phytoplankton and production by shrimp excretion and potential mineralization. A correction was applied to this budget to take into account the daily water exchange:

$$\text{Daily N budget}\ (\mu\text{mol}\ l^{-1}\ \text{day}^{-1}) = (\text{Daily N input} + N_{min} - \text{N demand}) - 0.2 \times [NH_4^+] \quad (6)$$

Statistical analyses

Results are presented as means ± SD. Statistical comparisons of experimental data were carried out using XLStat software 2011 (Addinsoft), at each sampling date by a 2-way ANOVA, with shrimp density (D; 2 or 3 fixed levels – function of the data set) and access to the sediment (S; 2 fixed levels) as major sources of variance (Scherrer 1984). Data were first checked for a normal distribution and homogeneity of variance using the Shapiro–Wilk and Bartlett's tests, respectively. If data were not normally distributed, they were transformed for normality using log, square root or arcsine transforms. If data did not meet the test criteria after appropriate transformations, comparisons were made using the non-parametric Kruskal–Wallis test. Differences were considered significant at $p < 0.05$. For analysis, data were grouped within 2 periods according to climatic conditions: Days 8 to 18 (period A) and Days 29 to 43 (period B); mean values for each response variable in each mesocosm were calculated over each period. Mean data were transformed for normality and submitted to a repeated-measures ANOVA model where period was the 2-level repeated-measures variable and the treatments density and access to sediment were the 2 main factors. This procedure was used to explore major trends in the phytoplankton compartment over the entire course of the experiment.

RESULTS

Survival and growth of shrimp

Survival rates of shrimp at the end of the experiment ranged between 81 and 95% and were not significantly different between treatments (2-way ANOVA; see Table S1 in the Supplement). Inversely, the final mass varied between treatments at 11.2 ± 0.2, 12.3 ± 1.1, 14.5 ± 0.4 and 16.1 ± 0.5 g in $D12S^-$, $D4S^-$, D12S+ and $D4S^+$, respectively. The growth was significantly higher in tanks with low shrimp density (D4) and with access to sediment (S^+).

Environmental conditions and physico-chemical parameters

The experiment was marked by 2 significant periods (Fig. 1). The first period (A) was a dry and cool period with mean water temperature around 27°C and mean daily incident PAR around 650 µmol photons m^{-2} s^{-1}. The second period (B) was rainy and hot, with a decrease in temperature at the end of the experiment. Rain fell almost continuously, with 3 peaks centered on Days 29, 34 and 39. This period brought 161 mm of rain in 2 wk, representing a mean dilution rate of less than 2% of the water column per tank per day. Precipitation events were associated with mean daily PAR decreases down to 268 µmol m^{-2} s^{-1}. The salinity in tanks increased from 34.5 to 36.5 during period A and decreased thereafter, with a minimum level (33.0) reached at the end of the experiment. Mean DO data are presented in Fig. 2a. The minimum mean value was 1.4 mg l^{-1}. The results from the daily 2-way ANOVA show a significant effect of density ($p < 0.05$) from Day 4 to the end of the experiment, with lower concentrations in treatment D12 (Fig. 2a). Significant changes in DO were also found in relation to sediment access, but only from Day 22 to the end of the experiment. The S^+ treatment showed higher values of DO than the S^- treatment. pH (see Fig. S2 in the Supplement) varied from 7.8 to 8.5 with a decrease from Day 11 to Day 22, followed by stabilization and then an increase during the last few days. This trend was similar in all treatments, but the amplitude of variation changed with density and sediment access, especially from Day 15 to the end of the experiment (not significant at Days 36 and 39). At each sampling date (except at Day 1), turbidity was significantly different (Kruskal–Wallis test; $p < 0.05$) between treatments. Mean values ranged from 6.2 to 12.9 NTU and from 10.9 to 46.5 NTU for the $D4S^+$ and $D12S^+$ treatments, respectively (Fig. 2b). Turbidity in the other treatments did not exceed 3.4 NTU. Note that in the $D12S^+$ treatment, turbidity increased quickly after shrimp stocking, with a maximum (32–46 NTU) at Day 8, and decreased during the rainy period (B) to a minimum (11 NTU) at Day 36.

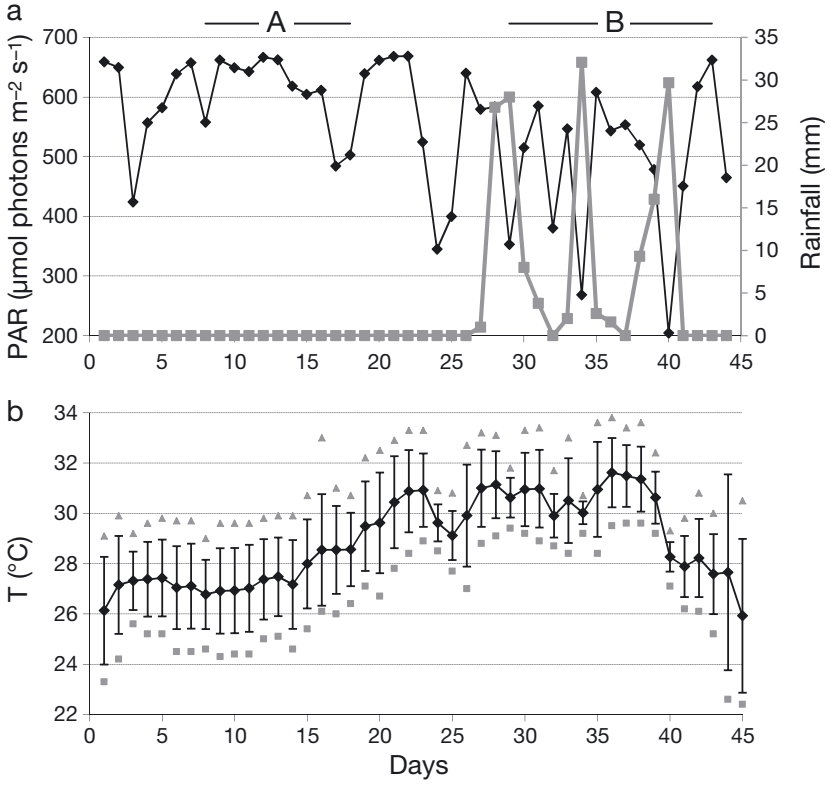

Fig. 1. (a) Temporal variations in daily rainfall (squares) and mean daily photosynthetic active radiations PAR (diamonds). A and B refer to dry and rainy periods, respectively. (b) Temporal variations in daily mean temperature (N = 24; diamonds). Squares and triangles represent the daily minimum and maximum values, respectively. Error bars are SD

Nutrients (NH_4^+, NO_x, SRP) and DON

Ammonium concentration in tanks ranged from 0 to 16.8 µmol l^{-1}. The daily 2-way ANOVA showed a significant effect of sediment access on NH_4^+ from Day 22 to the end of the experiment (except at Days 29 and 39), with lower concentrations in S^+ than in S^- treatments (Fig. 2c). The effect of density

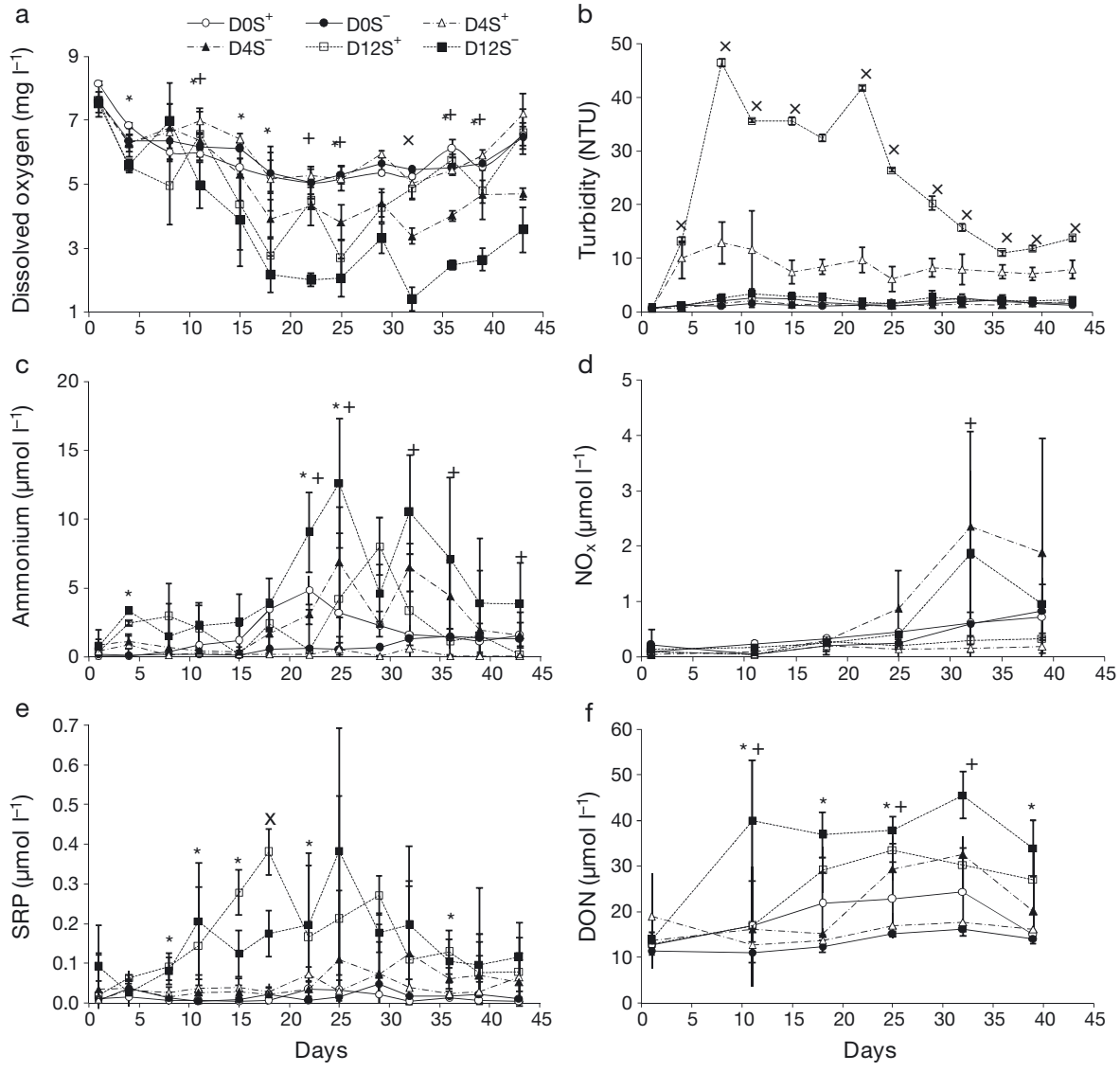

Fig. 2. Temporal variations of (a) dissolved oxygen, (b) turbidity, (c) ammonium, (d) nitrates and nitrites (NO_x), (e) soluble reactive phosphorus (SRP) and (f) dissolved organic nitrogen (DON) in the different treatments. Treatment combinations are as follows: (1) high shrimp density + access to the sediment ($D12S^+$), (2) high density − access ($D12S^-$), (3) low density + access ($D4S^+$), (4) low density − access ($D4S^-$), (5) no shrimp, without enclosure ($D0S^+$) and (6) no shrimp, with enclosure ($D0S^-$). Values are means (±SD) of 3 ($D4S^-$; $D4S^+$; $D12S^-$ and $D12S^+$) or 2 ($D0S^-$ and $D0S^+$) replicate tanks per sampling time in each treatment. * and +: significant effect of density and access to sediment, respectively ($p < 0.05$; 2-way ANOVA); x: significant difference between daily values ($p < 0.05$; Kruskal–Wallis test)

was less evident and only occasionally significant, at Days 4, 22 and 25. NO_x concentrations remained very low (<0.3 µmol l^{-1}) except at the end of the experiment (Days 32 and 39) in S^- tanks ($D4S^-$ and $D12S^-$), with concentrations up to 1 µmol l^{-1} (Fig. 2d). Mean SRP concentrations (Fig. 2e) were below 0.1 µmol l^{-1} except in treatments with high stocking density ($D12S^+$ and $D12S^-$). These treatments showed values ranging from 0.02 to 0.38 µmol l^{-1} with maxima observed between periods A and B. Results showed a significant positive effect of density on SRP concentrations from Day 8 to 22 and at Day 36 (Fig. 2e). Conversely, access to the sediment did not significantly change the SRP concentration in the water column. Silicate concentrations ranged from 0 to 35 µmol l^{-1}. No significant effect of sediment access or density on Si concentrations was shown. The daily repeated-measures ANOVA showed a significant effect of period for NH_4^+ and NO_x but not for SRP (Table 1). The N/P ratio (mole/mole) increased from around 20

Table 1. *F*-values from repeated-measures ANOVA for nutrients. Significant results are highlighted by asterisks ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)

Treatment	NH_4^+	NO_x	SRP	N/P	NH_4^+/NO_x
Density (1)	6.769*	0.001	99.383***	14.212***	4.425*
Sediment access (2)	5.383*	11.525***	0.532	1.333	0.190
Period (3)	9.204**	48.545***	0.334	1.392	20.676***
1 × 2	5.467*	5.482*	1.777	13.597***	0.316
1 × 3	0.076	0.206	2.390	1.725	0.775
2 × 3	7.863*	16.746***	2.177	0.246	0.117
1 × 2 × 3	4.406*	4.539**	0.403	9.449***	0.356

Table 2. Temporal variability of N/P (mole/mole) and NH_4^+/NO_x ratios in the different treatments. Treatment combinations are as follows: (1) high shrimp density + access to the sediment ($D12S^+$), (2) high density – access ($D12S^-$), (3) low density + access ($D4S^+$), (4) low density – access ($D4S^-$), (5) no shrimp, without enclosure ($D0S^+$) and (6) no shrimp, with enclosure ($D0S^-$). N refers to ammonium + NO_x. A and B refer to dry and rainy periods, respectively

		—Period A—			—Period B—		
	Treatment	Day 1	Day 11	Day 18	Day 25	Day 32	Day 39
N/P	$D0S^-$	22	16	34	>50	>50	>50
	$D0S^+$	19	>50	>50	>50	>50	>50
	$D4S^-$	>50	19	>50	>50	>50	>50
	$D12S^-$	16	16	23	>50	>50	44
	$D4S^+$	>50	9	16	23	17	13
	$D12S^+$	35	12	7	26	47	28
NH_4^+/NO_x	$D0S^-$	10	17	2	2	2	1
	$D0S^+$	8	4	11	7	3	1
	$D4S^-$	18	5	6	9	3	1
	$D12S^-$	6	15	17	30	10	3
	$D4S^+$	5	8	1	2	3	0
	$D12S^+$	6	40	6	18	9	3

to values higher than 50 in treatments without shrimp and treatments without access to sediment ($D0S^+$, $D0S^-$, $D4S^-$ and $D12S^-$), especially during period B (Table 2). In the other treatments, N/P values were generally <50, averaging 24 ± 21 and 26 ± 15 in the $D4S^+$ and $D12S^+$ treatments, respectively. The NH_4^+/NO_x ratio ranged from 0.2 to 40 (Table 2). The daily repeated-measures ANOVA showed a significant effect of period for the NH_4^+/NO_x ratio but not for the N/P ratio (Table 1).

After shrimp introduction, DON concentrations increased regardless of treatment (Fig. 2f). A significant effect of density on daily mean concentrations was shown from Day 11. Concentrations were lower in S^+ than in S^- treatments, but the difference was statistically significant only at Days 11, 25 and 32. The highest DON values were found in treatment $D12S^-$ (>35 µmol l^{-1}).

Phytoplankton biomass

Tchl *a* exhibited the same patterns in all treatments containing shrimp, with an increase from Day 4 to Day 8 (Fig. 3). Moreover, it appeared that density and sediment access both had a positive and significant impact on the Tchl *a* concentrations even though the impact of sediment access was visible 14 d after that of density. Tchl *a* maxima were recorded in the $D12S^+$ treatment (>15 µg l^{-1}) and minima (~5 µg l^{-1}) in the $D4S^-$ treatment, whereas maximum values in $D4S^+$ and $D12S^-$ were similar at around 10 µg l^{-1}. Comparatively, concentrations in control tanks (without shrimp) remained low (<2 µg l^{-1}) and close to the initial concentrations (0.63 ± 0.68 µmol l^{-1}). Concentrations were not significantly different between periods A and B regardless of treatment (Table 3). Phaeophytin *a* represented 20% to 57% of Tchl *a*. This ratio decreased during algal blooming at the beginning of the experiment (Day 8 to Day 15), and then increased toward the end. The increase in the ratio was higher in S^- than in S^+ treatments, but the effect of sediment access was only significant at Day 32 (not shown).

Phytoplankton abundance and diversity

Pico- and nanophytoplankton abundance by flow cytometry

Mean abundances of picocyanobacteria ranged between 5.5×10^2 and 4.7×10^6 cells ml^{-1} (Fig. 4a). S^+ tanks showed higher abundances than S^- tanks from Day 11. However, due to the high data variability, differences were significant ($p < 0.05$) only at Days 8, 39 and 43. A shrimp density effect on picocyanobacteria abundance was observable only for the last sampling date.

Among the eukaryotes, the smallest were assigned to picoeukaryotes and the largest to cryptophytes (Courties et al. 2005, Lucas et al. 2010). To make the

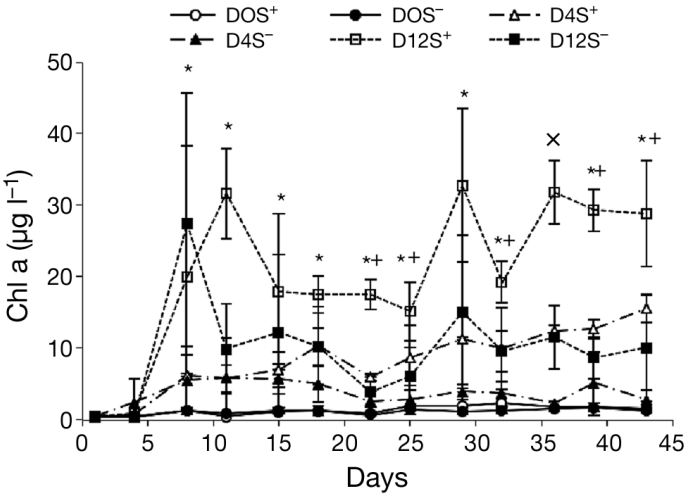

Fig. 3. Temporal mean (±SD) variations of chlorophyll *a* (chl *a*) concentrations in the different treatments (see Fig. 2 legend for treatment definitions). * and +: significant effect of density and access to sediment, respectively ($p < 0.05$; 2-way ANOVA); ×: significant difference between daily values ($p < 0.05$; Kruskal–Wallis test)

presentation of results easier, the 3 other eukaryote populations were pooled into a single group named nanophytoplankton. Mean abundances of picoeukaryoted ranged from 0 to 1.0×10^6 cells ml^{-1}. They increased from Day 11 in S$^+$ tanks (Fig. 4b) as did picocyanobacteria, but due to the high data variability, the effect of sediment access was not statistically demonstrated. Likewise, no density effect was observed. Nanophytoplankton was amongst the less abundant groups, with abundances ranging between 0.3×10^3 and 3.0×10^5 cells ml^{-1} (Fig. 4c). Maximum abundance was recorded in the D12S$^+$ treatment. Sediment access did not show any significant effect except at Day 22, with a positive effect. Density had a significant effect on abundances at Days 11, 22 and 39. Cryptophytes occurred in 85% of the samples, but their abundances (data not shown) were always lower than 70×10^3 cells ml^{-1}. Density showed a significant positive effect at Days 4, 15 and sediment access a negative and positive effect at Days 18 and 39.

Pigment-based phytoplankton composition

Chl (c_1+c_2) quickly appeared in the water column just after shrimp stocking, with concentrations ranging from 0.07 to 5.08 µg l^{-1} (Fig. 5a). They were the main accessory pigments in all the treatments during period A. However, the steady decrease of the chl (c_1+c_2)/Tchl *a* ratio from 0.2 to 0.1 (data not shown) showed that the amount of these red-brown algae fell throughout the experiment, to the benefit of green algae and picocyanobacteria, regardless of the treatment. Density regularly showed a significant positive effect on chl (c_1+c_2) concentrations. Values were generally higher in S$^+$ than in S$^-$ treatments (Fig. 5a), and a significant positive effect was observed at Days 22 and 32. Note that silicates disappeared with the increase of chl (c_1+c_2) concentrations (Fig. 6), suggesting a high presence of diatoms in this group.

Chl c_3, which is present in prymnesiophytes, chrysophytes and pelagophytes in addition to chl (c_1+c_2), ranged from 0 to 0.52 µg l^{-1} (Fig. 5b). The daily 2-way ANOVA regularly showed a positive and significant effect of density. Concentrations were generally significantly higher in S$^+$ than in S$^-$ tanks. The chl c_3/Tchl *a* ratio was generally low regardless of the treatment (<0.5). This ratio dropped throughout the experiment, indicating a progressive decrease in the proportion of these taxa within the phytoplankton community. No significant effect of sediment access or density on this ratio (except at Day 43) could be detected during the experiment. Chl *b* can be used as a biomarker of green eukaryotes (prasinophytes, chlorophytes and euglenophytes). Its concentration increased from Day 15 and reached 8 µg l^{-1} at Day 29 in the D12S$^-$ treatment (Fig. 5c). It was significantly

Table 3. *F*-values from repeated-measures ANOVA for concentration of total chl *a* (Tchl *a*) and contribution of each algal group to chl *a*. Significant results are highlighted by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

Treatment	Tchl *a*	Diatoms + dinoflagellates	Chlorophytes	Picocyanobacteria	Haptophytes	Cryptophytes
Density (1)	148.871***	0.502	1.943	4.150*	9.701***	2.046
Sediment access (2)	16.910**	0.007	3.420	9.181*	0.020	0.642
Period (3)	1.229	72.576***	115.796***	16.926***	1.306	8.847***
1 × 2	4.525*	2.351	4.429*	2.579	1.385	3.554
1 × 3	0.516	3.092	3.255	3.913*	0.472	0.466
2 × 3	4.139	0.469	7.276*	5.637*	0.035	0.114
1 × 2 × 3	1.236	3.890*	5.024**	4.156*	0.219	1.597

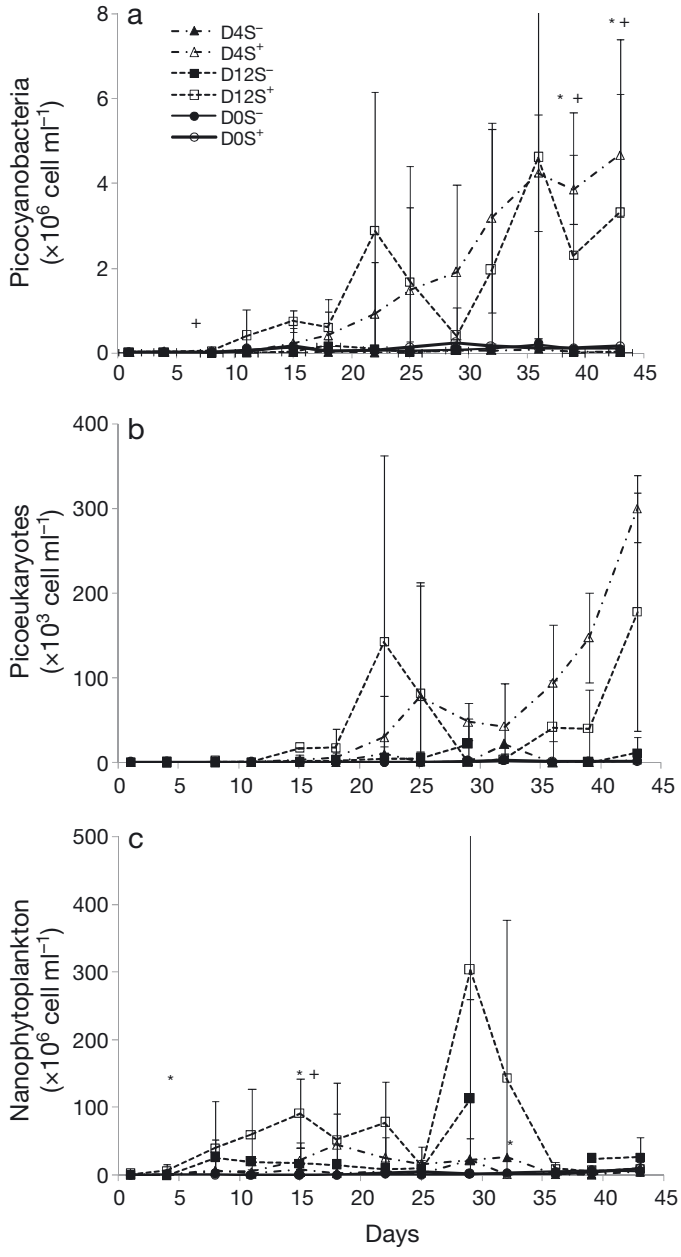

Fig. 4. Temporal mean (±SD) variations of abundance of (a) picocyanobacteria, (b) picoeukaryotes and (c) nanophytoplankton in the different treatments (see Fig. 2 legend for treatment definitions). Abundances are presented in thousand or million cells per milliliter. * and +: significant effect of density and access to sediment, respectively ($p < 0.05$; 2-way ANOVA)

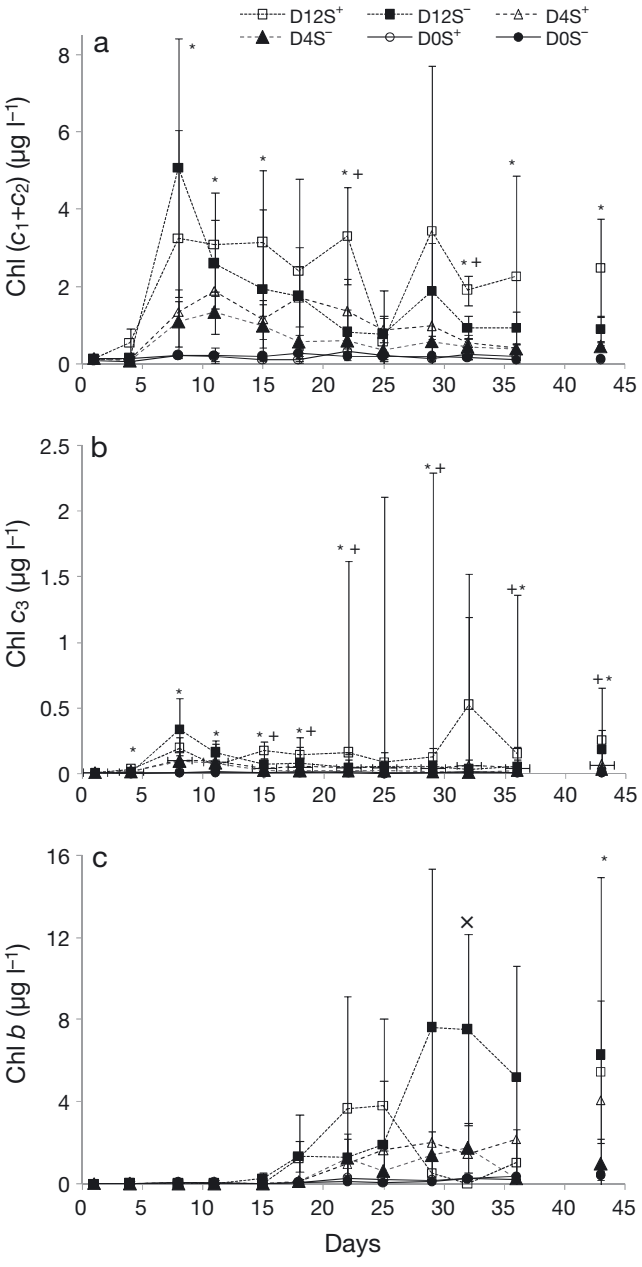

Fig. 5. Temporal mean (±SD) variations of (a) chl (c_1+c_2), (b) chl c_3 and (c) chl *b* concentrations as analyzed by spectrofluorometry. See Fig. 2 legend for treatment definitions. * and +: significant effect of density and access to sediment, respectively ($p < 0.05$; 2-way ANOVA); ×: significant difference between daily values ($p < 0.05$; Kruskal–Wallis test)

and positively affected by stocking density during the second part of the experiment. There was no significant effect of sediment access. The chl *b*/Tchl *a* ratio increased from 0 to around 0.4 during the course of the experiment (data not shown), suggesting an increase of the proportion of green algae in the phytoplankton community.

Contribution of each taxon to Tchl *a*

Based on these estimates, Fig. 7 shows the temporal variability of each group in terms of chl *a* in the different tanks. The chl *a* linked to cryptophytes was less than 1.2% of Tchl *a*, with 96% of the values <0.5%. Its contribution increased signif-

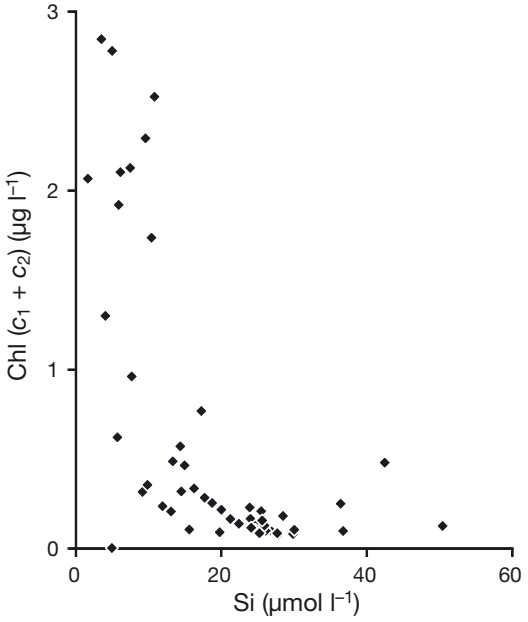

Fig. 6. Relationship between silicate concentrations and chl (c_1+c_2) in the samples

icantly between periods A and B (Table 3). Haptophytes were also a minor component of the phytoplankton biomass, representing less than 15% of Tchl *a*, but they were always detected in the samples. The contribution of picocyanobacteria to Tchl *a* ranged from 0 to 31%. With treatments D4S$^-$ and D12S$^-$, values never exceeded 6%. The daily repeated-measures ANOVA showed a significant effect of period, density and sediment access on the contribution of picocyanobacteria to Tchl *a* (Table 3). Proportions of chl *a* associated with the diatoms + dinoflagellates group (chl $a_{diat+dino}$) decreased significantly between periods A and B regardless of the treatment (Table 3), from 80% of Tchl *a* at the beginning to values ranging from 18 to 52% at the end. Green algae became the predominant contributor to Tchl *a* in the second part of the experiment in all tanks (Table 3) and represented between 40 and 65% of Tchl *a*. The shift from diatoms + dinoflagellates to green algae coincided with the occurrence of the rainy period (Fig. 1A). When generating the chl *b* versus chl a_{chloro} scatterplot diagram, 3 distinct and strong correlations were observed (Fig. S3 in the Supplement), leading to a mean chl *b*/chl a_{chloro} ratio of 0.61 (assemblage 1), 0.86 (assemblage 2) and 0.38 (assemblage 3). The 3 linear regressions were significantly different (ANCOVA; F = 261, ddl = 3, $p < 0.0001$). Table 4 shows the distribution of these assemblages according to treatment. Assemblage 1 was observed in all treatments, assemblage 2 in the treatments with shrimp and without sediment access, and assemblage 3 mainly in the treatments with shrimp and with sediment access, suggesting an effect of density and sediment access on the composition of the green algae community.

Metabolism and N budget

Daily mean GPP ranged from 5.1 ± 3.3 (D4S$^-$) to 21.2 ± 9.6 µmol l^{-1} h^{-1} (D12S$^+$) (Table 5). The daily 2-way ANOVA showed a significant positive effect of

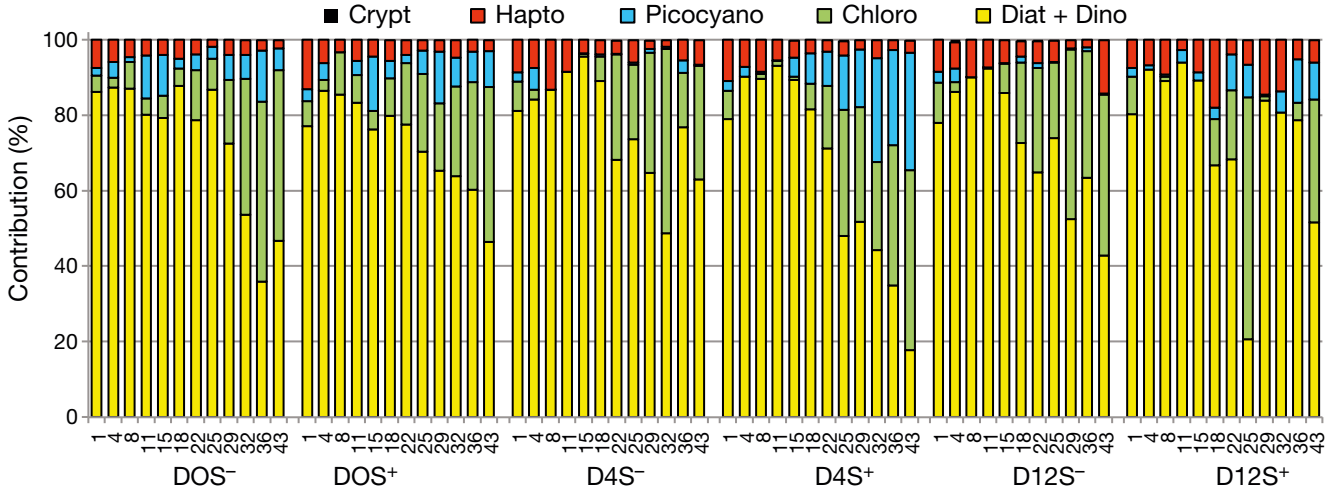

Fig. 7. Temporal (days) mean variations in the contribution of each algal group to total chlorophyll *a* (Tchl *a*) as revealed by spectrofluorometry, according to treatment (see Fig. 2 legend for treatment definitions). Crypt: cryptophytes; hapto: haptophytes; picocyano: picocyanobacteria; chloro: chlorophytes; diat + dino: diatoms + dinoflagellates

Table 4. Green algae assemblages identified in each tank at each sampling date and distinguished by treatment (see Table 2 for treatment definitions). Data indicate chlorophyll *b* (chl *b*) concentrations in µg l^{-1}. Assemblages 1, 2 and 3 are indicated by dark grey, light grey and black shading, respectively; green algae assemblages in samples containing <0.4 µg l^{-1} of chl *b* were not able to be identified; empty cells: no chl *b*

	Treatment/tank number															
	D0S⁻		D0S⁺		D4S⁻			D12S⁻			D4S⁺			D12S⁺		
Day	1606	1612	1614	1616	1601	1604	1611	1607	1608	1609	1603	1610	1615	1602	1605	1613
1	0.03		0.01	0.04	0.04	0.04	0.04	0.05	0.04	0.02	0.01	0.03	0.04	0.05	0.03	0.06
4	0.01		0.02		0.02	0.01	0.01	0.01	0.01							
8	0.11		0.11	0.08								0.09		0.11		
11	0.03		0.06	0.05					0.08				0.24			
15	0.07	0.01	0.03	0.03	0.04			0.58		0.23		0.07				
18	0.10		0.08	0.03	0.17		0.23	1.79	0.53	1.76		0.39		3.71		
22	0.18	0.04	0.10	0.41	2.44	0.02	1.33	0.28	1.8	1.9	0.14	2.79		10.01	0.94	
25	0.09	0.05	0.14	0.36	0.03	0.07	1.82	0.17	5.49	0.14	1.00	3.97		8.4	3.13	
29	0.08	0.18	0.13	0.18	0.13	1.59	2.46	4.28	16.52	2.05	1.57	4.44		1.62		
32	0.36	0.21	0.19	0.37	0.91	3.14	1.25	5.34	4.29	12.87	3.16	0.79	0.5			
36	0.44	0.35	0.22	0.25	0.28	0.12	0.41	0.95	3.35	11.35	3.66	1.11	1.82	2.89	0.01	0.32
43	0.29	0.55	0.25	0.60	0.15	0.44	2.39	16.32	0.94	1.6	4.01	4.74	3.57	8.16	1.56	6.68

density on GPP at the end of the experiment and of sediment access at Days 22, 25 and 43 (data not shown). Daily mean respiration rates ranged from 1.5 ± 1.2 to 5.7 ± 1.9 µmol l^{-1} h^{-1} for D4S⁻ and D12S⁺, respectively. Significant positive effects of density and sediment access on respiration were also regularly shown from Day 15 to the end of the experiment (data not shown).

For the D4S⁻ treatment, phytoplankton N demand was more or less equal to the nutrient input by shrimp N excretion and N remineralization (Table 5). For D4S⁺, N demand of phytoplankton increased from around 10 to 25 µmol l^{-1} d^{-1}. The N budget decreased from 0.6 ± 5.0 µmol l^{-1} d^{-1} in D4S⁻ to −14.7 ± 7.2 µmol l^{-1} d^{-1} in D4S⁺, indicating a higher capacity of the bioturbated system to recycle mineral N. Results also showed that increasing densities were associated with an increase in potential N remineralization.

Table 5. Metabolic rates and daily N budget (µmol l^{-1} h^{-1}) in the different treatments (see Table 2 for treatment definitions). Data are means ± SD (range) (n = 13). GPP: gross primary production

	D4S⁻	D4S⁺	D12S⁻	D12S⁺
GPP	5.1 ± 3.3 (2.0–13.0)	12.4 ± 5.1 (3.1–20.2)	10.2 ± 5.5 (1.4–19.0)	21.2 ± 9.6 (6.1–38.6)
Respiration rates	1.5 ± 1.2 (0.3–4.3)	2.4 ± 0.9 (0.5–3.3)	2.8 ± 1.3 (0.2–4.8)	5.7 ± 1.9 (3.1–8.4)
Phytoplankton N demand	−9.8 ± 6.7	−25.7 ± 10.1	−20.0 ± 11.1	−38.4 ± 20.4
Potential N mineralization	3.9 ± 3.4	4.9 ± 2.0	7.2 ± 4.0	12.9 ± 7.8
Shrimp N excretion	6.5 ± 0.4	6.5 ± 0.6	19.5 ± 1.3	19.5 ± 1.3
Daily N budget	0.6 ± 5.0	−14.2 ± 7.2	6.6 ± 8.8	−6.0 ± 19.5

DISCUSSION

Effects of stocking densities and bioturbation on phytoplankton biomass

Both stocking densities and bioturbation increased the phytoplankton biomass and microbial metabolism in our experiment. This result reveals the importance of these 2 factors in sustaining phytoplankton primary production in shrimp farm ponds with a soft bottom of sediment. Nevertheless, the pathways involved for each were not similar. Stocking density determined the amount of nutrients entering into the system, directly enlarging the nutrient pool available for primary production, as already reported by several authors (e.g. Martin et al. 1998). This resulted in an increase in autotrophic plankton biomass in the D12S⁻ treatment, reaching values 2.8-fold greater than in low shrimp density tanks (D4S⁻) and 9.1-fold higher than in the tank without shrimp (D0S⁻). Likewise, bioturbation by shrimp increased phytoplankton biomass, with values 2.3-fold greater in S⁺ than in S⁻ tanks, as well as productivity for the same amount of nutrient entering into the system. Thus, the maximum biomass was recorded in D12S⁺ treatments with Tchl*a* concentrations averaging 30 µg l^{-1}, a situation that is close to the biomass generally measured in earthen semi-intensive shrimp ponds in New Caledonia (Thomas

et al. 2010, Pusceddu et al. 2011). The increase of water column respiration rates and the decrease of DON in S^+ compared to S^- treatments suggest an increase in the organic matter mineralization rate in the water column as a consequence of sediment resuspension by bioturbation. This outcome could be explained by an intensification of bacterial production (BP) in the water column due to the increase of heterotrophic bacteria attached to sediment particles (e.g. Arfi & Bouvy 1995). In shrimp ponds in New Caledonia, BP associated with particles (>3 µm) accounts for more than 80% of the total BP in the water column (S. Hochard & H. Lemonnier unpubl. data). The increase in mineralization rate theoretically implies that more nutrients are available for primary producers, which may partly explain the significant and positive effect of bioturbation on primary production and phytoplankton biomasses. Resuspension of sediment was already reported to increase the mineralization rate by a factor between 2 and 5 in coastal sediments (Stahlberg et al. 2006). Thus, bioturbation appeared to be a key process for internal nutrient recycling in shrimp ponds. Another factor should be taken into account to explain the increase of Tchl *a* in the water column in treatments with sediment access: the effect of bioturbation on the microphythobenthos (MPB) resuspension. During this experiment, concentrations of Tchl *a* in the first centimeter of the sediment ranged between 86 and 151 mg m^{-2} for $D12S^+$ and did not show any significant change with time (data not shown). These biomasses represented more than 80% of the primary producer biomass (phytoplankton and MPB) in the system. It is therefore assumed that MPB resuspension by shrimp could have a significant impact on Tchl *a* measurements, as also suggested for shallow systems (Brito et al. 2012, Garstecki et al. 2002). The increase of turbidity in $D12S^+$ tanks between Day 1 (before shrimp stocking) and Day 4 (2 d after stocking) due to the resuspension of sediment by shrimp coincided with a small increase of phytoplankton biomass from 0.4 to 0.9 µg l^{-1}. This preliminary result suggests a relatively limited effect of MPB on water column Tchl *a* enrichment. However, further studies in shrimp pond ecosystems are needed to clarify this point.

Limiting factors for phytoplankton growth

Several limiting factors should be taken into account to understand phytoplankton dynamics in the different treatments. In the case of high density (D12) with and without sediment access, the high turbidity due to bioturbation and/or high phytoplankton cell abundances led to a limited increase of GPP compared to biomass. This finding suggests that under these conditions, photo-limitation including self-shading by phytoplankton could limit the upper levels of GPP (Giovannini & Piedrahita 1994). In a previous experiment conducted in the same tanks and under similar zootechnical conditions, we showed a significant increase in photo-limitation of around 50% between surface and bottom. Because of this light limitation, it is likely that nutrient products from organic matter mineralization (SRP, NH_4^+, NO_x) were accumulated in the water column, as they are not efficiently utilized by phytoplankton. This situation mainly occurred in the second part of the experiment, when PAR values decreased due to cloudy/rainy conditions. In treatment $D12S^+$, the negative effect of bioturbation on productivity of the system due to light limitation is probably mitigated by an increase of turbulent mixing. This process might favor the vertical displacement of phytoplankton to the surface and therefore its nutrient uptake capacity (MacIntyre 1993). This possibility could explain why NH_4^+ accumulation was significantly lower in $D12S^+$ than in $D12S^-$, except during bad weather conditions (Day 29) when the light was too weak for primary productivity. Another limiting factor identified during this experiment was phosphorus. In the $D4S^-$ and D0 treatments, the N:P atomic ratio was far above the Redfield ratio of 16:1, which is required for optimal phytoplankton growth. Phosphorus depletion could limit GPP in the water column in these treatments assuming that nutrients were not in excess in the water column (Table 2). In $D4S^+$, the N:P ratio was generally close to the Redfield ratio, suggesting that phytoplankton growth may not be theoretically limited by either P or N.

To conclude, light, which depends on weather, turbidity, cell abundance, depth and vertical mixing, should be taken into account to understand phytoplankton dynamics in the shrimp pond ecosystem. A second factor is nutrient availability linked to feed input, which depends on the N/P ratio and the organic matter mineralization rate. With an increase of shrimp biomass and/or density, P becomes less limiting, while light becomes more and more limiting, implying a trend towards nutrient (NH_4^+ and NO_x) accumulation in the water column.

Phytoplankton community dynamics and structure

Shrimp stocking with the corresponding daily feed input triggered the onset of a phytoplankton bloom

after 2 d. Growth dynamics of phytoplankton were typical, with an exponential increase, a short peak after 7 d followed by a decline in biomass (Day 13) until the start of a new bloom. Apart from the change in biomass, a shift in the phytoplankton assemblage was significant in all treatments. During period A, the increase of chl (c_1+c_2) in all tanks suggests that the group diatoms + dinoflagellates was dominant regardless of treatment. Note that the abundance of this group was positively correlated with the increase of shrimp stocking density, suggesting that diatoms and dinoflagellates were strongly favored by eutrophication. The spectrofluorometry data failed to discriminate dinoflagellates from diatoms. In shrimp ponds, diatoms are often reported as a major component of phytoplankton biomass even though several studies, based on microscopic observations, suggest that dinoflagellates could become episodically dominant (Casé et al. 2008, Burford 1997, Yusoff et al. 2002). In this environment characterized by high organic matter content, dinoflagellates are mostly heterotrophic (Garcés et al. 2006, Zapata et al. 2012). The organic matter content and the depletion of SRP in the water column in treatments characterized by a high N/P ratio (Table 2) could be the factors promoting the dinoflagellates against diatoms during this first period (Yamamoto 2003, Collos et al. 2009). Further confirmation with microscopic observations and/or molecular approaches is needed to understand the adaptive response of diatoms and dinoflagellates to eutrophication and to determine the dinoflagellates' trophic regime in the pond ecosystem.

During period B, the proportion of chl (c_1+c_2) decreased steadily and green algae (chlorophytes and prasinophytes) became dominant. Proliferation of tiny Prasinophyceae belonging to the picoplankton and small nanophytoplankton class sizes has already been observed in shrimp ponds in New Caledonia (Lemonnier et al. 2016), where they accounted for 10 to 53% of Tchl *a* in the present study. Shifts from diatoms to chlorophytes in the coastal environment, which have been particularly subjected to eutrophication processes, are often described as the consequence of nitrogen pool change (e.g. Donald et al. 2011, Collos & Harrison 2014, Glibert et al. 2016). The effects of change in the proportion of NH_4^+ and NO_x on assemblages were recently well illustrated and discussed by Glibert et al. (2016). When NH_4^+ is the dominant (reduced) form, flagellates, cyanobacteria and chlorophytes may proliferate while diatoms are more likely to dominate under NO_3 enrichment conditions. Chlorophytes are not only physiologically better adapted to use NH_4^+ but are also more tolerant to toxic values than diatoms. In the present study, a significant effect of period on the NH_4^+/NO_x ratio was shown in the different treatments, suggesting that N pool change could lead to a shift from diatoms to chlorophytes. Several authors have reported that diatom populations generally decrease in ponds due to silica depletion in the environment (Yusoff et al. 2002, Casé et al. 2008). However, this depletion was not observed in the present study in treatments without shrimp ($D0S^+$ and $D0S^-$), whereas the community shift did occur.

Besides the nutrient changes, temperature and light changes between period A and period B due to adverse weather conditions could also be inferred from the shift from diatoms to chlorophytes observed in tanks. With an increase of 4°C, temperature could enhance the effect of nutrients on phytoplankton community composition, as reported in the literature (e.g. Deng et al. 2014). The temperature increase could also increase the regenerated production and therefore modify the N pool, the production of NH_4^+ favoring indirectly the observed shift. Besides temperature, a possible effect of light on the shift could also be suspected. Indeed, in our case, the change in phytoplankton populations appeared during the rainy period, which was characterized by low radiation, as also reported by Leruste et al. (2016) in hypertrophic shallow lagoons. The availability and quality of light are known to drive fluctuation in phytoplankton species (Huisman et al. 1999). Diatom abundance was increased by light fluctuations, while cyanobacteria and green algae dominated under low and high constant conditions, respectively (Litchman 1998). However, the community shift was similar in all treatments characterized by different turbidity levels.

Concerning the green algae community blooming mainly during period B, 3 assemblages were distinguished according to their chl *b*/chl a_{chloro} ratios. Interestingly, the chl *b*/chl a_{chloro} ratios calculated for each assemblage (0.86, 0.61 and 0.38) from spectrofluorometry data were close to those measured in a shrimp pond from HPLC data using CHEMTAX software, i.e. 0.81, 0.65 and 0.35 (Lemonnier et al. 2016). According to the authors, the ratio 0.81 was associated with prasinophytes type 3 (with prasinoxanthin) and the ratio 0.65 with prasinophytes type 1–2 (without prasinoxanthin) (Latasa et al. 2004). The ratio 0.35 identified as chlorophytes by HPLC was slightly lower than the value found in the present study (0.38). Because chlorophytes (assemblage 3) were more frequently observed in S^+ tanks than prasinophytes (assemblages 1 and 2), we consider that this

assemblage could be more efficient in an environment characterized by high nutrient recycling rates and high ammonium concentrations.

From Day 11 to the end of the experiment, the phytoplankton assemblage underwent another modification, with a major growth of picocyanobacteria in S^+ treatments. These treatments were characterized by an increase in nutrient recycling by the microbial loop, enriched with NH_4^+ and urea from shrimp excretion and protein-rich feed given to cultured species (Burford & Williams 2001). This finding was reported in various environments and by large-scale manipulation experiments (e.g. Donald et al. 2011). In their review on this topic, Glibert et al. (2016) reported that picocyanobacteria as well as chlorophytes (see above) may be physiologically better adapted to use a reduced form of N, mainly NH_4^+, than diatoms, even if diatoms have a high nitrogen affinity, especially small-sized species (Litchman et al. 2009). Indeed, these fast-growing prokaryotic algae, like other small cells, are known to be more efficient than larger cells in nutrient uptake (Agawin et al. 2004, Furnas et al. 2005). Because of their high surface to volume ratios, picocyanobacteria are able to use resources more efficiently than larger cells (Raven, 1998). The rising temperature from period A to period B could have enhanced the effect of ammonium on cyanobacterial biomass and dominance in the second period (O'Neil et al. 2012). As suggested by results from the S^+ treatments (Table 2), a low N:P molar ratio may also be a major factor favoring cyanobacteria dominance in aquaculture ponds (Paerl & Tucker 1995). Several authors reported that abiotic factors, such as salinity or turbidity, explained part of the variability in the diversity of marine *Synechococcus* populations in coastal waters (Jing et al. 2009, Liu et al. 2014). The next research step should be to investigate the diversity of picocyanobacteria and, more generally, other taxonomic groups in shrimp ponds in order to better understand how environmental conditions (nutrients, temperature and/or light) might affect species-specific differences of these taxa.

To conclude on the phytoplankton community dynamics and structure, the differences between treatments remained limited regardless of the period. This finding suggests a high resilience of the phytoplankton community to feed input and bioturbation, and, consequently, to nutrient enrichment, the N:P ratio and turbidity in the shrimp pond ecosystem. Even if the N pool was probably the main factor, the data do not allow us to definitely conclude whether the shift from diatoms to chlorophytes observed in all treatments resulted from the modification of the N pool or from an external common factor (temperature, light). Further specific experiments are needed to define precisely the effect of each factor.

Importance of phytoplankton in maintaining the pond water quality

DON is a major (30–40%) component of the N waste produced during rearing, and most of it is leached from the feed given to the reared shrimp (Burford & Williams 2001). In this experiment, the accumulation of DON in the water column was higher in $D12S^-$ than in $D4S^-$, suggesting that DON production was higher than the recycling capacity of the system in the highest feed input treatments. Consequently, the dissolved versus particulate ratio increased in the water column with the increase in feed input. This organic matter enrichment directly impacted the stability of the system, in terms of daily pH and DO fluctuations. Indeed, the pH decrease observed in the water column in relation to feed input was likely linked to the increase in CO_2 production through bacterial metabolism. This result suggests a substantial reduction in the acid–base buffering capacity of the system with increasing shrimp density and feed inputs. Conversely, supplied organic matter in the water column also stimulated respiration, which in turn caused depleted O_2 in the morning. Based on the N daily budget calculations in the different tanks, it may also be shown that increasing stocking densities entailed an increase of phytoplankton N demand coupled with an increase of both potential N mineralization and shrimp N excretion (Table 4). As a result, the turnover of N increased. As seen above, bioturbation by shrimp may favor organic matter mineralization by heterotrophic bacteria and then rapid nutrient uptake by phytoplankton, which limits the increase in the dissolved/particulate ratio. Because it improved the recycling of organic matter in the system by the microbial loop, bioturbation mitigated the accumulation of products (ammonia and nitrites) and the drop of DO and pH, which are stressors for shrimp (e.g. Lemonnier et al. 2004, Mugnier et al. 2008). The buffering capacity of the system to recycle N waste is, however, constrained by a threshold for the turnover of N. Indeed, as presented in Fig. 8, NH_4^+ accumulated in the water column when the N turnover rate exceeded -10 µmol l^{-1} d^{-1}, while no accumulation was observed for an N turnover rate below this value. By limiting the primary production and, consequently, the uptake of NH_4^+, light is assumed to be the main

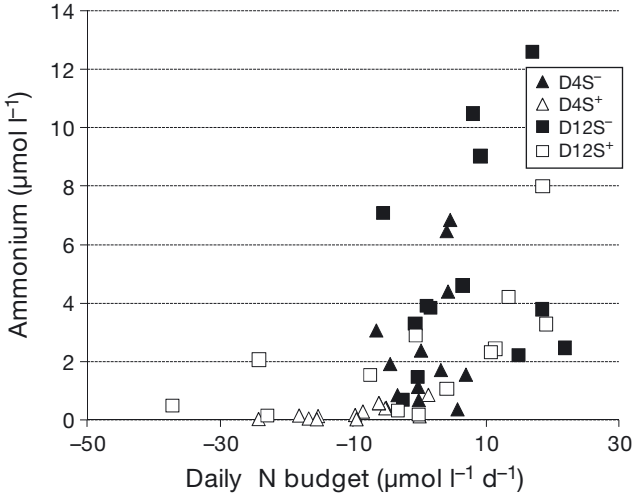

Fig. 8. Relationship between daily N budget and ammonium concentration in the water column for the different treatments (see Fig. 2 legend for treatment definitions)

factor limiting this recycling rate (see 'Phytoplankton community dynamics and structure', above). To conclude on the importance of phytoplankton in maintaining the pond water quality, this compartment counterbalances 'negative' effects of organic matter mineralization. However, this mitigation effect is governed by light availability in the system.

Consequences for water column management

A better understanding of the relative importance of food input and shrimp bioturbation in phytoplankton community dynamics could be useful for shrimp pond management. At low biomass, in our experiment, the system had strong resilience due to efficient nutrient recycling and the robustness of primary production to the changes in climatic condition linked to moderate turbidity. At higher densities, the system became more unstable due to the greater sensitivity of primary production to light availability. By using fertilizers in shrimp ponds, we can reasonably hypothesize that increasing phytoplankton biomass would thus be inefficient in stabilizing the system and might even lead to a more unstable conditions. In that case, stability of the water column might be more efficiently achieved through carbon input, in order to stabilize the nutrient pool through heterotrophic assimilation and thus buffer system oscillations (Hari et al. 2004). This result partly explains the success of the Biofloc system sometimes used to stabilize the water column at high stocking densities (Crab et al. 2012, Martínez-Córdova et al. 2015).

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