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Temporal variability of benthic-pelagic coupling in shallow enclosed environment: A case study with eutrophying shrimp ponds

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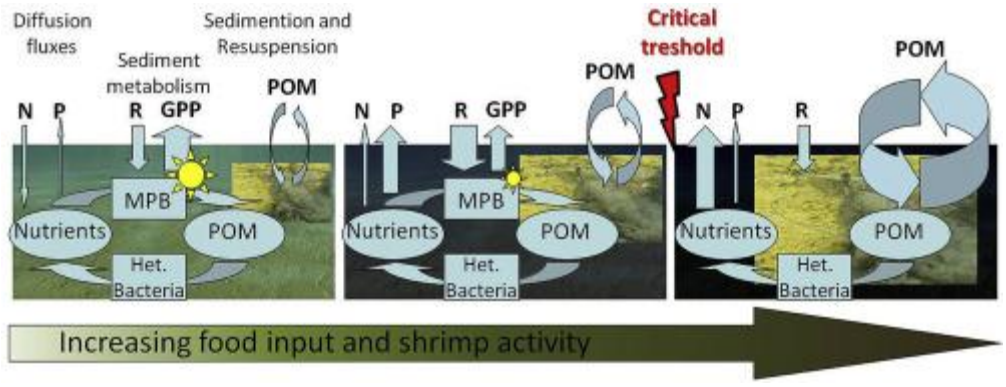
Abstract :

The evolution of benthic pelagic coupling was followed in two semi-intensive shrimp ponds in New Caledonia, with a special emphasis on the role of microphytobenthos (MPB). Three distinct periods could be identified. During the first period, MPB activity led to relative decoupling between the water column and the sediment, both compartments being autotrophic with low nutrients exchanges. During the second period, the sediment operated at the edge of a functional switch between autotrophy and heterotrophy. The amplitude of nutrient fluxes depended of the pool considered (DIN, DIP) and showed light dark variation. In the last period, sediment switched to heterotrophy with the establishment of benthic-pelagic coupling concomitantly to a massive sediment resuspension due to the shrimp activity. These findings should be considered for the management of aquaculture ponds and shallow enclosed water bodies.

Graphical abstract



Highlights

► Feed input and shrimp sediment resuspension were the main forcing function driving the benthic pelagic coupling. ► Microphytobenthos could reach a higher biomass and an equivalent areal productivity when compared to phytoplankton. ► Sediment metabolism functioned at the edge of a shift from autotrophy to heterotrophy. ► Exchanges between the sediment and water column were dominated by particulate organic matter resuspension. ► Microphytobenthos activity led to a complex dynamic of the nature and the amount of the diffusive nutrients fluxes.

Keywords : tropical aquaculture, Shrimp, Eutrophication, Benthic-pelagic coupling, 24 Microphytobenthos, Bioturbation

1. Introduction

Shrimp farming is a major aquaculture activity worldwide and a major part of the production came from tropical large species in the family Penaeidae. In New Caledonia, shrimp aquaculture is based on a semi-intensive model in shallow ponds (1 m) of 10 ha to produce *Litopenaeus stylirostris*. The rearing cycles last about 200 days and between two cycles, ponds are drained and left to dry in the sun for a period of several weeks.

From a biogeochemical standpoint, these agrosystems are the site of complex internal cycles dominated by interactions between the sediment and the water column. In fact these two compartments share the processes of organic matter and nutrient cycling. They have numerous exchange pathways, with molecular diffusion of nutrients at the interface, sedimentation, and sediment resuspension caused by shrimp activity (e.g. Burford and Longmore, 2001). These processes are impacted by rapid change from a mesotrophic to a hypertrophic state following the increase of the food input to feed animals (Lemonnier et al., 2010). Much of this feed is not retained by the species cultured but enters the pond system as particulate and dissolved nutrients (Briggs and Funge-Smith, 1994; Martin et al., 1998; Jackson et al., 2003). The standard representation is an increase of water column phytoplankton abundance and biomass with time, leading to an increase in sedimentation of organic matter. This process enhances sediment respiration and the release of nutrients back to the water column, which further sustains primary production of phytoplankton (Hargreaves, 1998; Burford and Lorenzen, 2004).

However, there are no studies that take into account benthic primary production by microphytobenthos in intensive/semi-intensive ponds, as it is assumed that this micro-organism is negligible in these turbid systems. However, this may not always be the case, because a considerable amount of light can reach the sediment at the beginning of the rearing

50 period in this shallow environment. Moreover there is evidence that microphytobenthos may
51 remain viable even if not exposed to light for long periods, and that they are capable of
52 photosynthesis at very low light levels (Sundbäck and Graneli, 1988; Sundbäck et al., 2004;
53 Zilius et al., 2012). Sediment colonized by microphytobenthos can retain nutrients via their
54 assimilation both from the water column and pore water (e.g. Sundbäck et al., 2000; Bartoli et
55 al., 2003). Moreover, they represent an input of labile organic matter fuelling sediment
56 metabolism, and photosynthetic oxygen production at the interface oxidizes surface
57 sediments, with important biogeochemical implications for phosphorus (P) retention (Carlton
58 and Wetzel, 1988) and for early diagenic processes (Welker et al., 2002; Hochard et al.,
59 2010).

60 Thus, microphytobenthos might have a significant function during the rapid eutrophication
61 events that occur in shrimp ponds. Indeed benthic primary producers are known to play a key
62 role during eutrophication processes in a shallow ecosystem. They can act as a buffer
63 (McGlathery et al., 2001; McGlathery et al., 2007; Larson and Sundbäck, 2008), but their
64 disappearance with severe eutrophication could lead to environmental shifts in these
65 ecosystems (McGlathery et al., 2007; Viaroli et al., 2008; 2010). Such shifts are characterized
66 by a change in the sediment trophic status from autotrophy to heterotrophy, thereby changing
67 its role in the system from a productive compartment retaining nutrients in the sediment to a
68 detrital compartment fuelling pelagic primary production with nutrients (e.g. Dunn et al.,
69 2012).

70 In many aspects, processes observed in shrimp ponds can be a model for the investigation of
71 eutrophication in shallow marine environments. They represent medium scale systems that are
72 semi-controlled and highly monitored. In this study, we examined the temporal variability of
73 benthic-pelagic coupling in relation with eutrophication state in two semi-intensive shrimp
74 ponds, focussing on the role of microphytobenthos.

2. Materials and methods

2.1. General approach

To analyse this temporal variability, field surveys were conducted in the same industrial farm – "La Sodacal" – during the 2012 and 2013 austral summers, in two ponds, designated U and A assuming negligible inter-annual variability due to climatic conditions. Bi-monthly samplings were carried out at two stations in each pond from the beginning of the rearing until day 120 which corresponded generally to the highest feed input in ponds. Stations were chosen in each pond from a map of their sediment organic matter content established during the previous drying period. For each pond, the first and second stations represented the low and high organic matter (OM) content, respectively. This choice was supposed to reflect areas where the variability of the processes linked to benthic - pelagic coupling was maximum.

2.2. Management of the ponds

Ponds were dried for one month before filling with sea water pumped from the Teremba bay. The ponds (U: 10.1 ha; A: 9.2 ha; around 1 m depth) were supplied with post-larvae (0.03 g) *Litopenaeus stylirostris* on 16 February 2012 (pond U) and 13 February 2013 (pond A). Each pond was stocked at the density of 16 animals.m^{-2}. Shrimp were fed on a daily basis with commercial feed containing 35-40% protein. The ponds were managed by the farmer in accordance with his usual techniques. Water was renewed daily, ranging from about 5% to 30% of the total pond volume depending on the shrimp biomass. Mechanical aeration was also used in relation to the shrimp biomass. During rearing, shrimp mortality was estimated by counting dead and moribund shrimp at the pond edges or on the filters located on the

97 effluent gates. This estimation should be viewed in this study as a qualitative method for
98 identifying periods of mortality.

99 2.3. Design of the study

100 During these surveys, bi-monthly samplings were carried out at each station to measure in
101 duplicate the net primary production (NPP), the respiration (R), the particulate and nutrient
102 fluxes at the water-sediment interface. Water was collected in polycarbonate flasks (2-l) at the
103 surface and near the bottom at each station to measure turbidity and chlorophyll *a* content (chl
104 *a*). One undisturbed core was manually collected at each station by a diver to analyse, total
105 organic matter content, chlorophyll *a* and nutrients in pore water. To assess the temporal
106 evolution of the pond environmental conditions, daily temperature, dissolved oxygen and
107 fluorescence (DO) were taken from the farm's record books. Salinity and Secchi measured
108 about twice a week were also furnished by the farmer. Daily rainfall data were obtained from
109 the weather Forecast Meteorological data Services. Daily photosynthetically active radiation
110 (PAR; 400-700 nm) was measured near the farm using a LI-COR Li1400 data logger.

111 2.4. Metabolism

112 2.4.1. Water

113 Oxygen fluxes in the water column were measured in light and dark bottles (Strickland and
114 Parsons, 1972). These bottles (300 ml), made of borosilicate glass, had been soaked in dilute
115 HCI (1% vol/vol) for several days before the measurements. The bottles were rinsed and filled
116 with pond water taken at each incubation location. Two pairs of light and dark bottles were
117 incubated at each station, one in the water column at 10 cm below water surface and the
118 others10 cm above the sediment surface.

119 2.4.2. Benthos

120 To determine oxygen fluxes from sediment at each station, four acrylic chambers (two clear
121 and two dark) were fastened to 0.075 m^2 PVC cores pushed about 10 cm into the substrate by
122 a scuba diver, thus ensuring minimum sediment disturbance while avoiding the presence of
123 shrimp in chambers (Clavier et al., 2008). Enclosed water (7.8 l) was homogenized with
124 adjustable submersible pumps (~ 0.25 $l.min^{-1}$). The oxygen sensor spot method was used to
125 follow this parameter in chambers, but also in bottles as described in Warkentin et al. (2007).
126 SP-PST3-PSUP-YOP-D5 oxygen sensor spots (Presens GmbH), and a fibre-optic oxygen
127 meter (Fibox 3: Presens GmbH) were used for this study. Oxygen fluxes were assessed from
128 the time course of oxygen. The incubation started between 9-11 am in order to have
129 favourable O_2 condition and the time steps varied form 30 min to 1 hour. The time step and
130 incubation duration were adjusted depending on the initial oxygen concentration in order to
131 avoid hypoxic conditions and to respect a maximum change of oxygen of 20% in the benthic
132 chambers (Dalsgaard et al. 2000). Oxygen flux (dDO/dt in $\mu mo1.l^{-1}.h^{-l}$) was expressed as the
133 slope of the linear regression of oxygen content in the incubation (Boucher et al., 1994).
134 Sediment metabolism ($\mu mol.m^{-2}.h^{-1}$) was calculated by correcting oxygen flux in the benthic
135 chamber (dDO_{bc}/dt) with bottom water oxygen flux (dDO_{bw}/dt) and was expressed in $\mu mol.m^{-2}.h^{-1}$:
136
137 Sediment metabolism ($\mu mol.m^{-2}.h^{-1}$) = (dDO_{bc}/dt- dDO_{bw}/dt) x (V/S)

138 V: volume of the benthic chamber (l); S: surface area of the benthic chamber (m^2).

139 Total Respiration (*R*) and Net Primary Production (NPP) rates were deduced from the oxygen
140 fluxes in the dark and light bottles and chambers respectively (Bender et al., 1987). Gross
141 primary production (GPP) represented the sum of the rate of R and NPP. Daily NPP and GPP
142 were calculated by multiplying the hourly values by the number of daylight hours (12). Daily
143 R equalled the hourly oxygen flux in the dark times 24. Daily GPP and R were used to

144 calculate the P/R ratio and assess the benthic trophic status of the sediment as in Rizzo et al.
145 (1996). Water column integrated metabolism ($\mu mo1.m^{-2}.h^{-1}$) represented the mean between
146 surface and bottom incubation integrated over 1m depth. Phytoplankton nutrient demand was
147 calculated from integrated water column daily GPP and the Redfield ratio as in Lemonnier et
148 al. (2017).

149 2.5. Particulate matter fluxes

150 One plate plastic boxe used as sediment traps (30 cm long, 23 cm wide and 8 cm high) was
151 deployed on the pond bottom for 48 h at each station in pond U. Measures were conducted in
152 duplicate in pond A. The top of the boxes was protected with a honeycomb-style baffle,
153 trapping all the sediment material (net sedimentation + local resuspension) (Kassila and
154 Hussenot, 2006). The area of exchange for trapping the sediment was 0.0173 m^2 per box. The
155 purpose of the baffle was to decrease the size of the eddies and to avoid shrimp entering the
156 trap access. The trapped material was collected, dried at 60°C and weighed to measure the
157 total flux mass. Flux was expressed in $g.m^{-2}.h^{-1}$. To distinguish net sedimentation from
158 resuspension in the trapped material, Fe and Al were used as tracers, on the assumption that
159 particles originating from the water column are mainly organic, while particles resuspended
160 from the pond bottom are mainly mineral and contain high concentrations of Fe and Al
161 (Avnimelech et al., 1999). Fe and Al were analysed in sediment (0 - 2 cm) and in trapped
162 material by ICP-OES Varian 730-ES after standard digestion. One hundred milligrams of
163 sample was fused at 1,100°C with 1 g of $Li_2B_4O_7$ for 20 min. The fusion residue was then
164 dissolved with 5% HCl.

165 An independent estimation of the C sedimentation fluxes was calculated following
166 Avnimelech et al. (1999) and Kassila and Hussenot (2006). It was hypothesized that this flux

corresponded to unconsumed food carbon and integrated water NPP, assuming 50% C in food and unconsumed food equal 30% of food input.

2.6. Nutrient fluxes

Chambers and bottles were also set up to measure nutrients fluxes from sediment. Following deployment of benthic chambers, water samples were collected in the water column with polycarbonate flasks (2 l) at the beginning of the incubation at each incubation location. Water samples were also collected within the chambers using polyethylne syringes (0.05 l). The sampling equipment (syringes, flasks) was washed with HCl (10%) and rinsed with deionised water. Around four hours later, samples were collected in each chamber and incubated bottle to determine nutrient concentrations. Water samples were filtered through a GF/F Whatman filter and sub-sampled for the different nutrient analyses. Ammonium (NH_4^+) and soluble reactive phosphorus (SRP) analyses were carried out immediately on fresh water samples, while the other nutrients were measured on frozen samples. Ammonium was analysed according to the method described by Grasshoff and Johannsen (1972). SRP were measured in accordance with the molybdenum blue reaction described by Murphy and Riley (1962) using a spectrophotometer (Shimadzu UV1700). Nitrate and nitrite [(NO_2^-+NO_3^-)-N] (NOx) were determined using standard colorimetric techniques on a Bran + Luebbe AutoAnalyser III (Raimbault et al., 1990). Dissolved organic nitrogen (DON) was analysed following oxidation procedure described by Raimbault et al. (1999). Pre-oxidation dissolved inorganic nitrogen (DIN) concentrations were subtracted from the post-oxidation total dissolved nitrogen (TDN) concentration so as to derive the DON concentrations.

Nutrient fluxes were calculated using followed formula:

$$\text{Nutrient Fluxes } (\mu mol.m^{-2}.h^{-1}) = ((C_{(Tf)} - C_{(T0)}) - (B_{(Tf)} - B_{(T0)})) / T) \times (V / S)$$

where $B_{(T0)}$ and $B_{(Tf)}$: nutrient concentrations at the beginning and at the end of the incubation in bottles located water near the bottom($\mu mol.l^{-1}$); $C_{(T0)}$ and $C_{(Tf)}$: nutrient concentrations at the beginning and at the end of the incubation in the chamber ($\mu mol.l^{-1}$); T: time (h); V: volume of the benthic chamber (l); S: surface area of the benthic chamber (m^2).

2.7. Water and Sediment state variables

2.7.1. Water

Turbidity was measured using an Aquafluor TM Handheld fluorometer (Turner Design). To analyse the chl *a* concentration, 25 to 50 ml water samples were filtered through Whatman GF/F filters and then stored frozen at -20°C until they were analysed. Chlorophyll *a* and pheophytin *a* concentrations were determined in methanol extract before and after acidification using a fluorometer (Model TD700, Turner designs) in accordance with the method described by Herbland et al. (1985).

2.7.2. Sediment

Pore water was extracted using soil moisture rhizons characterized by a vertical resolution of 1 cm (Song et al., 2003; Seeberg-Elverfeldt et al., 2005). Nutrients (NH_4^+ and SRP) were analysed in pore waters following the methods described above for water. One layer of soil 0 – 2 cm was sampled using a 40 cm long PVC tube of 10 cm diameter. The samples then were dried at 60°C for 1 week and analysed for organic matter by loss on ignition using a muffle furnace at 350°C for 8 h (Queiroz and Boyd, 1998). The Kjeldhal method was used to determine total nitrogen (TN) (Nelson and Sommers, 1982). Total organic carbon (TOC) was measured by the Walkley–Black potassium dichromate-sulphuric acid oxidation method with external heating (Boyd, 1995). Chlorophyll *a* concentration was analysed from frozen samples (1 cm core layer) collected in triplicate at each station. Frozen sediment samples were freeze-dried for 24 h before extraction using methanol. The extract was analysed before and after

acidification using a TD-700 fluorometer (Holm-Hansen et al., 1965) in accordance with the method described by Underwood (2002). The concentration of sediment chl *a* was expressed in $mg.m^{-2}$.

2.8. Statistical analyses

The non-parametric Mann-Whitney U test was used to compare the initial organic matter content in sediment between ponds. To analyse spatial and temporal variation, samplings were grouped in three periods (P1, P2 and P3) taking into account two samplings so as to have similar periods between ponds and feed input level (fig. 1a). P1, P2 and P3 were characterized by lowest, medium and highest inputs, respectively. Variations in all variables were investigated by means of a two-way analysis of variance (ANOVA), with the station (U1, U2, A3 and A4) and sampling time as major sources of variance, assuming negligible inter-annual variability due to climatic conditions. Before analysis, data were checked for 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-transformation, squared, 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 at each period. Differences were considered significant at $p < 0.05$. T-test was used to compare among dark and light incubations. When data were not normally distributed, the non-parametric Wilcoxon-Mann-Whitney test was used as an alternative to the t-test.

3. Results

3.1. Survival, growth and feeding of shrimp

Although the two rearings studied began with very similar zootechnical conditions, they displayed some divergence during the crop. Food inputs were higher and more variable in pond U than in pond A (Fig. 1a). Growth was higher in pond U than in pond A at the beginning of the rearing period, but mean weights (20 g) were equivalent at the end of our survey (week 13) (data not shown). Significant mortality appeared in pond A at day 80 until the end of the survey. A mortality outbreak was observed around day 87 in pond U. In both cases, the beginning of the mortality corresponded to a fall in temperature to 22°C. These mortalities were linked to the pathogen *Vibrio penaeicida*, the aetiological agent of a disease known as Syndrome 93, which affects the shrimp industry at the beginning of the cold season (e.g. Mermoud et al., 1998). Estimated survival at the end of the survey (day 90) was 80% for pond U and less than 60% for pond A (day 91). Final survival at harvest for the two crops were 65% (day 147) and 41% (day 127) for ponds U and A, respectively.

3.2. Temporal evolution of state variables in the water column

For both surveys, climatic conditions corresponded to the end of the warm season and the transition to the cool season. Water temperature and PAR decreased similarly during both surveys from 27°C to 22°C (Fig. 1b) and from 650 to 400 µmol photons.m^{-2}.s^{-1}, respectively. Occasional falls in PAR (not shown) corresponded to rainfall events that occurred in week 5 (81 mm) and week 6 (26 mm) in 2012 for pond U and around week 7 (35 mm) and week 9 (54 mm) in 2013 for pond A. Salinity was lower in pond U (30) than in pond A (40) during the first part of the rearing, reflecting differences of precipitation and water exchange rates (Fig. 1c). Secchi gradually decreased from around 0.9 m to 0.5 m in both ponds. From week 5, Secchi was usually lower in pond U than in pond A (Fig. 1d). Between week 1 (not shown) and week 7, daily minimal values of DO decreased from 6-7 mg.l^{-1} to 3-4 mg.l^{-1} (Fig. 1e). The introduction of mechanical aeration during week 7 induced an increase of the concentration until the end of the rearing. Fluorescence showed different trends in the two ponds (Fig. 2f).

In pond A, fluorescence increased slightly from the beginning to the end of the survey. In pond U, there was first (until week 10) a slight increase, which was later followed by high values (up to 151 µg 1^{-1}). Table 1 shows the mean values of chl *a*, and turbidity during our samplings. The first and second sampling were characterized by lowest chl *a* (< 35 µg.l^{-1}) and turbidity (~ 10 NTU) values. Concerning the other samplings in pond U, chl *a* and turbidity reached 142 µg.l^{-1} and 39 NTU, respectively. Difference between the beginning and the end of the survey for these parameters was not as pronounced in pond A as in pond U as already observed for Secchi and fluorescence.

3.3. Temporal evolution of the sediment characteristics

Among the structural variables, the clearest and significant differences between station was for organic matter (OM) and C:N ratio (Fig. 2a,b, Table 2). Carbon and nitrogen were highly correlated with OM representing 46% (n = 23; r^2 = 0.94; p < 0.01) and 5.4% (n = 23; r^2 = 0.91; p < 0.01) of its content. The C/N ratio, varying between 7.3 and 12.1, was significantly higher in pond A than in pond U. There was a station x period interaction for Chl *a* and pheophytine % (Table 2), indicating that the difference between stations depended on the period. Period affected negatively Chl *a* in pond U after week 7, but only after week 11 in pond A (Fig. 2c). Both main factors (station and period) affected the NH_4^+ concentration of pore water. This nutrient increased with time (Fig. 2d). No significant effects at all were found for SRP. As SRP values were very low (< 5 µmol l-1), N/P ratios in the pore water were high (> 100 µmol l^{-1}).

3.4. Benthic-pelagic coupling

3.4.1. Metabolism

Water column integrated GPP varied between 116 ± 58 and 456 ± 118 mmol.m^{-2}.d^{-1} (Table 1) and was within the same range for both ponds. In pond U, the ratio between surface and

bottom GPP showed a large increase and variability between stations at the end of the survey from < 3.1 to > 7.0, reflecting the poorer light conditions. In pond A, this ratio varied from 1 to 3.1. Water column integrated R (Table 1) varied between -71 ± 33 and -285 $mmol.m^{-2}.d^{-1}$. R was quite steady in pond A, while there was an increase of respiration at the end of the survey in pond U. This was particularly pronounced in bottom water where mean respiration increased from -115 to -375 $mmol.m^{-2}.d^{-1}$ (data not shown).

Mean GPP in the sediment ranged from 7 ± 19 to 274 ± 14 $mmol.m^{-2}.d^{-1}$ (Fig. 3a). As observed for chl *a*, results of the ANOVA (Table 3) showed a significant effect of station on GPP with higher values in pond A than in pond U. The trend was similar for all stations, with an increase of GPP from the beginning of the survey to weeks 5 - 7 followed by a decrease of the values after weeks 9 - 11. Mean respiration varied between -13 ± 7 and -176 ± 18 $mmol.m^{-2}.d^{-1}$ (Fig. 3b) and showed a significant effect of the period and station (Table 3). We observed higher respiration values at week 5 and 7 in pond A than in pond. Mean P/R ratio significantly decreased during samplings at station U1, U2 and A4 (Fig. 3c). This ratio fell to a value of less than 1 from week 9 in pond U and from week 11 at station A4. Analysis of variance also revealed a significant effect of the station x period interaction on this ratio, indicating that the difference between periods depended on the station (Table 3).

3.4.2. Particulate fluxes

Total trapped material ranged from 413 and 4996 $g.m^{-2}.day^{-1}$ for pond U and between 109 ± 3 and 480 ± 9 $g.m^{-2}.day^{-1}$ for pond A (Fig. 4). While values were quite steady in pond A, they greatly increased after week 7 in pond U, rising to more than 4000 $g.m^{-2}.day^{-1}$. Effects of the period and station and the interaction station x period were significant (Table 3). Tukey's test revealed the presence of two groups, formed by U1 - U2 and A3 - A4. Considering all the samplings, mean OM content of trapped material was 6.4 ± 0.7% and 8.5 ± 1.0% for ponds U

and A, respectively. The organic carbon (C) and nitrogen (N) trapped varied between 5 - 121 $gC.m^{-2}.d^{-1}$ and 1 - 18 $gN.m^{-2}.d^{-1}$. Based on the dilution method, total solids resuspension amounted on average to 82 ± 13% of the total trapped material (Table 4). This method led to a smaller mean contribution of resuspension for C and N, with 39 ± 13% and 32 ± 11%, respectively. The mean percentage of resuspension in C particulate exchanges was significantly higher (mean 79 ± 15 %) when the independent estimation of C sedimentation (C calc) was used.

3.4.3. Nutrient fluxes

Daily mean DIN fluxes stayed low in pond A with values between -0,44 and 0.64 $mmol.m^{-2}.d^{-1}$ (Fig. 5a). Pond U showed comparably low mean values for the beginning of the survey but a large increase in fluxes occurred after week 11 regardless of the station, with a mean value of 8.6 ± 1.5 $mmol.m^{-2}.d^{-1}$. While during low flux periods NO_x could account for a substantial component of DIN fluxes, high fluxes were largely driven by NH_4^+ (> 97% for DIN fluxes) (Online Resource 1a, 1b, 1c and 1d). No significant light-dark effects were found for the NH_4^+ and NOx fluxes ($P > 0.05$ Wilcoxon-Mann-Whitney test). Results of the Kruskal-Wallis test showed a significant spatial variability of NH_4^+ fluxes for periods 2 and 3 in dark conditions (Table 5).

Daily mean DON fluxes ranged from -4.5 to 2.1 $mmol.m^{-2}.d^{-1}$ during the survey of pond U and did not reveal any clear trend. Pond A showed large negative DON fluxes for the first sampling date (-6.7 and -14.5 $mmol.m^{-2}.d^{-1}$ for A3 and A4). Thereafter mean fluxes increased, with a maximum value of 4.0 $mmol.m^{-2}.d^{-1}$ (Fig. 5b). T-test showed no significant differences ($P < 0.05$) between dark and light incubations (Online Resource 1e, 1f). However, results showed that the period significantly changed values in light incubations (Table 3). The period and station did not have any effect on fluxes measured in dark conditions.

Daily SRP fluxes ranged from -0.03 to 0.99 mmol.m^{-2}.d^{-1} and from -0.11 and 1.07 mmol.m^{-2}.d^{-1} for ponds U and A, respectively (Fig. 5c). Mean fluxes were low at the beginning and at the end of the surveys for both ponds, with mean maximum fluxes occurring at week 5 for pond A and at week 7 for pond U. A T-test showed significant differences ($P < 0.01$) between light and dark incubation. Indeed mean fluxes were less than 0.7 mmol.m^{-2}.d^{-1} in light incubations, while they reached 1.6 mmol.m^{-2}.d^{-1} in dark incubations (Online Resource 1g and 1h).

4. Discussion

4.1. Microphytobenthic biomass and sediment metabolism

Our survey clearly exhibited a rapid eutrophication trend characterized by the development of algae in the water column and at the water-sediment interface. The MPB biomass can be very high in this system and was in the upper range of what is usually recorded in subtidal environments (1 - 560 mg chl *a* m^{-2}) (Barranguet et al., 1993; McIntyre, 1996). This was confirmed by sediment metabolism, which showed that this pool was photosynthetically active, with a GPP that could be as high as 270 mmolO_2.m^{-2}.d^{-1}. These observations clearly demonstrate that MPB is potentially an important primary producer in this system. Indeed, this pool is often considered to be negligible in aquaculture ponds (Burford and Longmore, 2001), but this appears not to be the case for semi-intensive systems and possibly for intensive systems at the beginning of rearing.

During the first 7 weeks of both rearings in the present study, MPB biomass doubled in response to eutrophication processes. This is likely due to the combination of an increase of nutrient availability and sufficient light regime, as reported in works on natural gradient and experimental fertilization (eg. Bourgeois et al., 2010). This increase in biomass coincided with a proportional increase in benthic metabolism for both primary production and

359 respiration. During this period, the benthic trophic status was clearly autotrophic for both
360 surveys and MPB primary production was likely to fuel part of the heterotrophic pathways
361 (Middelburg et al., 2000; Cook et al., 2007). Nevertheless, the relationship between MPB and
362 eutrophication is not always straightforward. Benthic systems are known to respond in a non-
363 linear way to eutrophication processes, with threshold effects leading to environmental shifts
364 (McGlathery et al., 2007; Viaroli et al., 2008). These shifts are triggered by the growth of
365 phytoplankton as observed in our study, which lead both to a reduction of light availability at
366 the sediment surface and to an increase in organic matter sedimentation fuelling heterotrophic
367 processes (McGlathery et al., 2007; Viaroli et al., 2008). We observed a clear divergence is
368 the response of MPB biomass and benthic metabolism between the two surveys after week 7.
369 Pond A showed the relative persistence of benthic processes with high MPB biomass and
370 primary production, while pond U underwent a shift in sediment functioning. On week 9 in
371 pond U, the sediment showed a large decrease in GPP values and an increase in its
372 respiration. This situation led to a shift of P/R toward heterotrophy, as might expected from
373 the conceptual model published by Viaroli et al. (2008). Finally in pond U, GPP collapsed to
374 very low value and the sediment metabolism was almost totally heterotrophic.
375 The reason for such a difference between pond A and U is unclear, as pond depth and
376 zootechnical conditions were comparable. One of the reasons might be a more active and
377 stable MPB biofilm in pond A. This is supported by the fact that pond A displayed respiration
378 and production rates that were twice as high as in pond U. Two factors might explain this
379 trend. The first one was the presence of more labile organic sediment pool as suggested by
380 higher ammonium pore water concentrations in pond A. The second was more favourable
381 light condition in this pond as supported by water column turbidity and Secchi values. Shrimp
382 bioturbation should also be considered as a major forcing function of benthic metabolism

intensity and stability. Indeed, the recorded shrimp resuspension rate was at least twice lower in pond A, which means less sediment disturbance.

4.2. Exchanges of particulate matter at the water-sediment interface

Shrimp bioturbation appeared to be a major exchange pathway between the sediment and the water column in terms of mineral and organic particulate matter. Thus sediment resuspension, accounted for between 50 and 95% of the total solid fluxes between the sediment and the water column. Direct comparison with other studies is difficult because this process depends on the species reared, with regard to their weight, biomass (Avnimelech et al., 1999) and behaviour, and the nature of the pond bottom (Jimenez-Montealegre et al., 2002). Indeed, even though shrimp biomass was comparable between the ponds, sediment resuspension was far higher in pond U than in pond A. Moreover this process did not show any linear evolution for either pond, as might be expected from the study by Rivto et al. (1997). In pond U, resuspension rate showed a sudden increase after week 7 up until the end of the survey, which suggest that a threshold value existed above which shrimp activity led to massive sediment resuspension rather than a constant increase. In their study, Walker and Grant (2009) showed how a microbial mat can initially biostabilize sediment against erosion through 'armouring' of the sediment, but that the same sediment can experience abrupt erosion once these mats fail above a critical shear velocity. This mat which was higher in pond A than in pond U could explained the low sediment resuspension measured in pond A.

These features are essential as they impact the light regime of the pond and also the exchange of OM. Indeed the total flux of organic carbon (C-org) and nitrogen (N tot) trapped varied (1 - 141 $gC.m^{-2}.d^{-1}$), which was in agreement with the studies by Avnimelech et al. (1999), Jimenez-Montealegre et al. (2002), and Kassila and Hussenot (2006). The application of the dilution method to the trapped pools of C-org and N tot led to a much higher contribution of

sedimentation compared to total solids, as in Avnimelech et al. (1999). This likely reflects the higher C and N content of particulate matter in the water column compared to the sediment. Nevertheless, C-org and N tot sedimentation fluxes seemed overestimated by this method, as stated by Kassila and Hussenot (2006). An independent estimation of C sedimentation fluxes, based on the pond's primary production and feeding rate (Avnimelech et al., 1999; Kassila and Hussenot, 2006), lead to much lower values (1,6 - 5,2 $gC.m^{-2}.d^{-1}$) and to a percentage of sedimentation similar to that of total solids. In all cases, OM resuspension appeared to actively contribute to the water column metabolism and nutrient cycling, as suggested by the increase of water column respiration (not shown) after the large resuspension event in pond U. Those results are consistent with our earlier study Lemonnier et al. (2017), which showed an increase in the organic mineralization rate in the water column as a result of sediment resuspension by bioturbation.

4.3. Nutrient exchanges at the water-sediment interface

While sediment resuspension is acknowledged to be responsible for a large transfer of particulate material, it is considered to be smaller for interstitial water (Sloth et al., 1996; Tenberg et al., 2003). Thus dissolved exchanges were likely to be dominated by diffusion. In euphotic sediment colonized by MPB, the benthic trophic status is acknowledged to predict whether sediments are net sources or sinks of inorganic nutrient (e.g. Engelsen et al., 2008). Hence, heterotrophic sediments are considered as a source and autotrophic sediment as a sink of dissolved inorganic nutrients.

Ammonium fluxes represented almost all the DIN exchanges. As observed in various studies (e.g. Engelsen et al., 2008, Hochard et al., 2012), ammonium was retained in the sediment when it was autotrophic. The principal process responsible for this retention is considered to be MPB nitrogen assimilation associated with its photoautotroph (Sundbäck and McGlathery,

2005; Hochard et al., 2010). While ammonium fluxes were low and quite conservative in pond A, large ammonium effluxes were observed when the sediment shifted toward heterotrophy in pond U. The decrease of photosynthetic activity is likely to have lowered the MPB N demand and thus allowed ammonium diffusion from the deeper layer of the sediment to overcome the MPB assimilation capacity (Tobias et al., 2003). This transition was visible at week 7 in pond U, where ammonium fluxes began to display ammonium effluxes during the dark and finally showed strong effluxes in both dark and light conditions in agreement with the values reported by Burford and Longmore (2001) in heterotrophic sediment from intensive shrimp ponds.

Nitrates only represented a small part of the DIN fluxes. The absence of inward fluxes is in accordance with the low nitrate concentration in the water column. The absence of fluxes directed toward the water column suggests the absence of nitrification and/or the assimilation of nitrate by MPB (Risgaard-Petersen, 2003; Hochard et al., 2010). Burford and Longmore (2001) reported similar low NO_3 sediment fluxes and related processes in shrimp ponds even in the absence of active MPB.

Phosphate fluxes showed a similar pattern for both surveys, with significant effluxes in the middle of the survey periods, while they remained low at the beginning and at the end of the surveys. MPB seemed to play a role in phosphate fluxes, as these displayed a day/night variation. Carlton and Wetzel (1988) demonstrated how MPB photosynthesis mediated release of phosphorus from sediments via daily formation and breakdown of an oxidized microzone. Indeed, when the sediment surface is oxidized, Fe(III) hydroxides can bind phosphate, thereby reducing its availability, while during the night the attenuation of the oxygenated layer permits its release. This is in agreement with the fact that maximum phosphate fluxes were recorded during periods of maximum benthic metabolism (primary production and respiration), which are likely to favour anoxic conditions at the sediment

458 surface during the night. The study by Kraal et al (2013) also reported that P fluxes could be
459 decoupled from water column oxygen concentration because of the strong interaction of
460 sediment P availability and sediment internal Fe and S redox cycling. In conclusion, the
461 observed P fluxes appeared to be the result of both MPB activity and sediment geochemical
462 cycles.
463 DON fluxes were of the same order of magnitude as DIN or even higher and thus represented
464 a significant part of the dissolved exchanges. This agrees with previous observations in
465 shallow-water sediments, where DON fluxes have been recognized as important (Tyler et al.,
466 2003; Sundbäck et al., 2004). Those fluxes are considered to reflect the hydrolysis of fresh
467 organic matter at the sediment surface (e.g. Blackburn and Blackburn, 1993;) as well as the
468 active uptake of DON by benthic microbes (e.g. Linares and Sundback, 2006). Nevertheless
469 as in our study, they are often reported as being much more erratic and variable than DIN
470 fluxes (Tyler et al., 2003; Sundbäck et al., 2004). Overall the sediment acted as a sink in
471 DON, which could be the result of the combination of the large pool present in the water
472 column and active microbial uptake at the sediment interface.
473
474 *4.4. Whole system functioning*
475 In semi-intensive shrimp ponds, sediment should not be considered only as a detritic
476 compartment. MPB was a major component of the functioning of the pond ecosystem, as it
477 could support up to 50% of total pond production, thus contributing significantly to the carbon
478 flow and the oxygen budget of the pond. MPB activity also led to a complex evolution of the
479 nature and the amount of the diffusive nutrient fluxes. This resulted in strong variation of the
480 stochiometry of the input of N and P from the sediment to the water column, which could
481 have consequences on the phytoplankton dynamic and diversity (Lemonnier et al., 2017). Fig.

6 presents a simplified model of the response of this system to eutrophication, that can be schematised by the 3 following periods:

- (1) MPB activity leads to relative decoupling between the water column and the sediment, both compartments being autotrophic with low nutrients exchanges.
- (2) The sediment operates at the edge of a functional switch between autotrophy and heterotrophy. Heterotrophic processes are reinforced by the increasing OM input while sediment primary production suffers from light limitation due to phytoplankton development and shrimp bioturbation. Nutrient fluxes depend of the pool considered and showed light dark variation.
- (3) Sediment switches to heterotrophy with the establishment of benthic-pelagic coupling. The sediment metabolism is mainly fuelled by water column OM sedimentation and regenerated nutrient diffuse back to the water column. This period is also characterized by massive sediment resuspension which created major OM exchanges.

While pond A did not showed a clear shift toward heterotrophy, this trend was confirmed by our previous study (Luong et al., 2016), which showed that the trophic status of the sediment was heterotrophic when food exceeded 7 $g.m^{-2}.d^{-1}$. It is also consistent with the study conducted by Burford and Longmore (2001) that did not reveal any benthic primary production in intensively farmed shrimp ponds. However, specific experiments should be conducted to investigate the combined effect of organic enrichment and bioturbation on benthic system stability. The study of these highly monitored and controlled systems can yield quantitative thresholds that could prove very useful for the management of eutrophying shallow enclosed water bodies.

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Table 1 Mean water column characteristics in ponds at each sampling (N = 4). Water column metabolism (GPP, R) represents the mean value between the surface and bottom incubation and was integrated over 1m depth to obtain a surface value. GPPs/ GPPf represent the ratio between surface and bottom primary production.

Pond	Period	Week	Chl *a* ($\mu g.l^{-1}$)	Turbidity (NTU)	GPP ($mmol.m^{-2}.d^{-1}$)	R ($mmol.m^{-2}.d^{-1}$)	GPPs/ GPPf
U	1	3	33.6 ± 5.5	8.5 ± 4.6	177 ± 50	-117 ± 30	1.6 ± 0.5
U	1	5	28.7 ± 5.3	10.7 ± 1.7	116 ± 58	-71 ± 33	3.1 ± ND
U	2	7	35.1 ± 1.4	11.2 ± 1.1	456 ± 118	-148 ± 22	1.5 ± 0.1
U	2	9	62.5 ± 11.1	24.6 ± 15.8	270 ± 113	-119 ± 20	2.2 ± 1.0
U	3	11	130.6 ± 8.5	34.1 ± 17.4	373 ± 326	-285 ± 125	24.0 ± 29.5
U	3	13	54.0 ± 8.2	39.3 ± 22.2	241 ± 218	-192 ± 61	7.4 ± 2.2
A	1	3	20.4 ± 3.4	13.6 ± 8.1	273 ± 110	-172 ± 43	2.1 ± 0.1
A	1	5	14.3 ± 1.4	6.9 ± 2.9	227 ± 63	-132 ± 61	1.4 ± 0.4
A	2	7	27.7 ± 9.4	7.1 ± 4.1	385 ± 82	-194 ± 50	1.0 ± 0.3
A	2	9	47.3 ± 3.8	25.3 ± 17.2	315 ± 176	-186 ± 40	3.1 ± 1.4
A	3	11	38.0 ± 2.2	10.0 ± 3.6	377 ± 147	-153 ± 16	2.0 ± 0.4
A	3	13	37.5 ± 4.9	6.2 ± 0.9	348 ± 123	-148 ± 58	1.9 ± 0.7

ND: not determined

Table 2 Results of the univariate analysis of variance testing for spatial-temporal variability in structural variables measured in the sediment of ponds U and A. DF = degrees of freedom; MS = mean square; F = Fratio. Bold values are significant.

Variable	Source	DF	MS	F	P
OM	Station	3	0.00	83.80	< **0.001**
	Period	2	0.00	1.50	0.27
	Station + Period	6	0.00	1.68	0.22
C/N	Station	3	15.72	35.61	< **0.001**
	Period	2	1.59	3.60	0.06
	Station + Period	6	0.50	1.13	0.40
NH_4^+	Station	3	209552.94	13.24	< **0.01**
	Period	2	182523.56	11.53	< **0.01**
	Station + Period	6	19094.81	1.21	0.37
SRP	Station	3	0.15	0.76	0.54
	Period	2	0.15	0.78	0.48
	Station + Period	6	0.14	0.75	0.62
Chl *a* in sediment	Station	3	92.14	3.76	**0.02**
	Period	2	174.73	7.13	< **0.001**
	Station + Period	6	56.69	2.31	< **0.05**
Pheophytine %	Station	3	0.03	1.22	0.06
	Period	2	0.03	2.57	0.07
	Station + Period	6	0.04	2.86	< **0.01**

Table 3 Results of the univariate analysis of variance testing for spatial-temporal variability in functional variables measured at the water-sediment interface of ponds U and A. DF = degrees of freedom; MS = mean square; F = Fratio. Bold values are significant.

Variable	Source	DF	MS	F	P
R	Station	3	11236486.24	0.84	**0.02**
	Period	2	17572077.13	3.87	< **0.01**
	Station + Period	6	1731626.59	6.06	0.73
GPP	Station	3	6619.72	0.60	< **0.01**
	Period	2	2945.49	9.27	**0.04**
	Station + Period	6	548.28	4.13	0.61
P/R	Station	3	0.41	0.77	**0.01**
	Period	2	0.50	5.79	< **0.01**
	Station + Period	6	0.11	7.04	**0.02**
Trapped sediment	Station	3	1.03	43.69	< **0.001**
	Period	2	0.80	33.93	< **0.001**
	Station + Period	6	0.10	4.25	**0.02**
DON fluxes	Station	3	104240.99	2.72	0.06
(Light)	Period	2	202295.88	5.29	< **0.001**
	Station + Period	6	139240.50	3.64	< **0.001**
DON fluxes	Station	3	116161.35	2.29	0.10
(Black)	Period	2	16547.05	0.33	0.72
	Station + Period	6	79334.84	1.57	0.19
SRP fluxes	Station	3	0.93	0.53	0.67
(Light)	Period	2	1.10	0.63	0.54
	Station + Period	6	0.88	0.50	0.81
SRP fluxes	Station	3	3.15	1.22	0.32
(Black)	Period	2	33.87	13.06	< **0.001**
	Station + Period	6	4.76	1.84	0.12

Table 4 Resuspension and sedimentation fluxes of total solids, particulate organic carbon (C), particulate organic nitrogen (N) and fluxes of particulate organic carbon estimated from water column primary production and food input (C calc.). The numbers in brackets represent the percentage of resuspension in total trapped material.

Pond	Week	Resuspension				Sedimentation			
		Total solids $g.m^{-2}.day^{-1}$	C $gC.m^{-2}.day^{-1}$	N $gN.m^{-2}.day^{-1}$	C calc. $gC.m^{-2}.day^{-1}$	Total solids $g.m^{-2}.day^{-1}$	C $gC.m^{-2}.day^{-1}$	N $gN.m^{-2}.day^{-1}$	C calc. $gC.m^{-2}.day^{-1}$
U	3	556 (90)	7.0 (44)	0.8 (42)	14.4 (90)	60	9.0	1.0	1.6
U	5	402 (90)	3.9 (35)	0.5 (37)	9.5 (86)	47	7.2	0.8	1.6
U	7	552 (87)	7.3 (29)	0.9 (27)	20.2 (79)	77	18.1	2.4	5.3
U	9	1231 (91)	24.9 (46)	3.1 (43)	50.5 (94)	128	28.9	4.2	3.3
U	11	3907 (93)	57.7 (62)	7.3 (48)	88.7 (96)	295	35.0	7.8	4.0
U	13	4672 (95)	81.4 (67)	8.7 (47)	118.2 (98)	235	39.5	9.8	2.7
A	3	90 (50)	1.6 (34)	0.2 (26)	2.4 (50)	91	3.2	0.5	2.38
A	5	98 (71)	1.8 (26)	0.2 (16)	4.7 (68)	40	5.2	1.1	2.25
A	7	288 (80)	5.5 (32)	0.7 (20)	13 (77)	70	11.7	2.6	3.89
A	9	224 (84)	3.9 (31)	0.5 (20)	9.5 (74)	43	8.8	1.8	3.28
A	11	167 (70)	3.2 (30)	0.4 (21)	6.3 (59)	70	7.7	1.5	4.36
A	13	345 (77)	6.1 (34)	0.8 (31)	14.6 (81)	103	12.0	1.8	3.52

Table 5 Results of the F-ratio for Krustal-Wallis test for spatial variability in DIN fluxes (NH_4^+ and NOx fluxes) in the sediment of ponds U and A. Bold values are significant.

	Period 1	Period 2	Period 3
NH_4^+ fluxes (Light)	0.53	0.24	0.18
NH_4^+ fluxes (black)	0.56	**0.02**	**< 0.01**
NOx fluxes (Light)	0.59	0.30	0.91
NOx fluxes (dark)	0.75	0.90	0.06

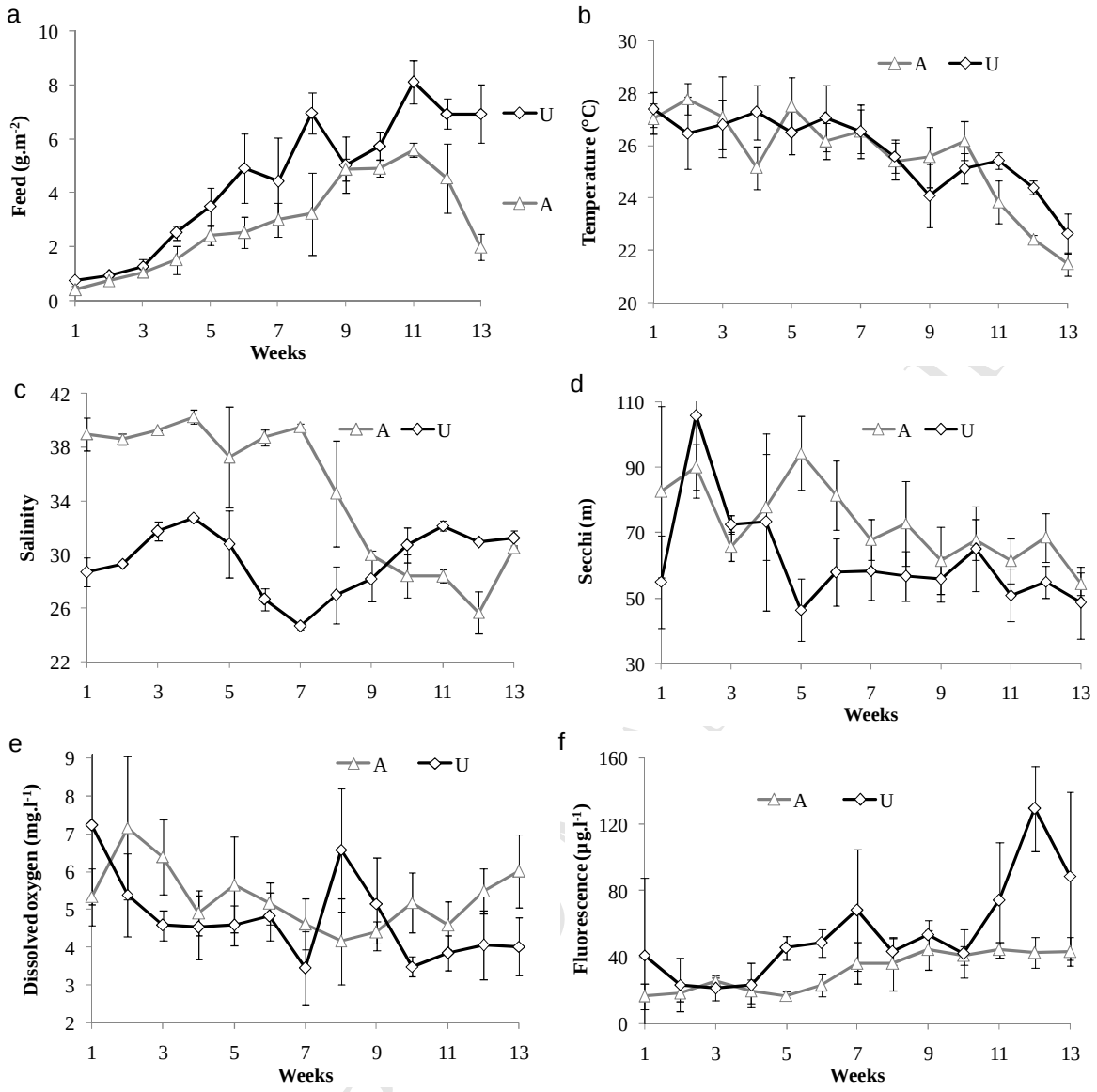

Fig. 1. Temporal variability of mean weakly (a) feed input (b) water temperature, (c) salinity, (d) Secchi, (e) dissolved oxygen, and (f) fluorescence for the surveys conducted in 2012 (pond U) and 2013 (pond A) (data source: La Sodacal). Errors bars correspond to the standard deviation.

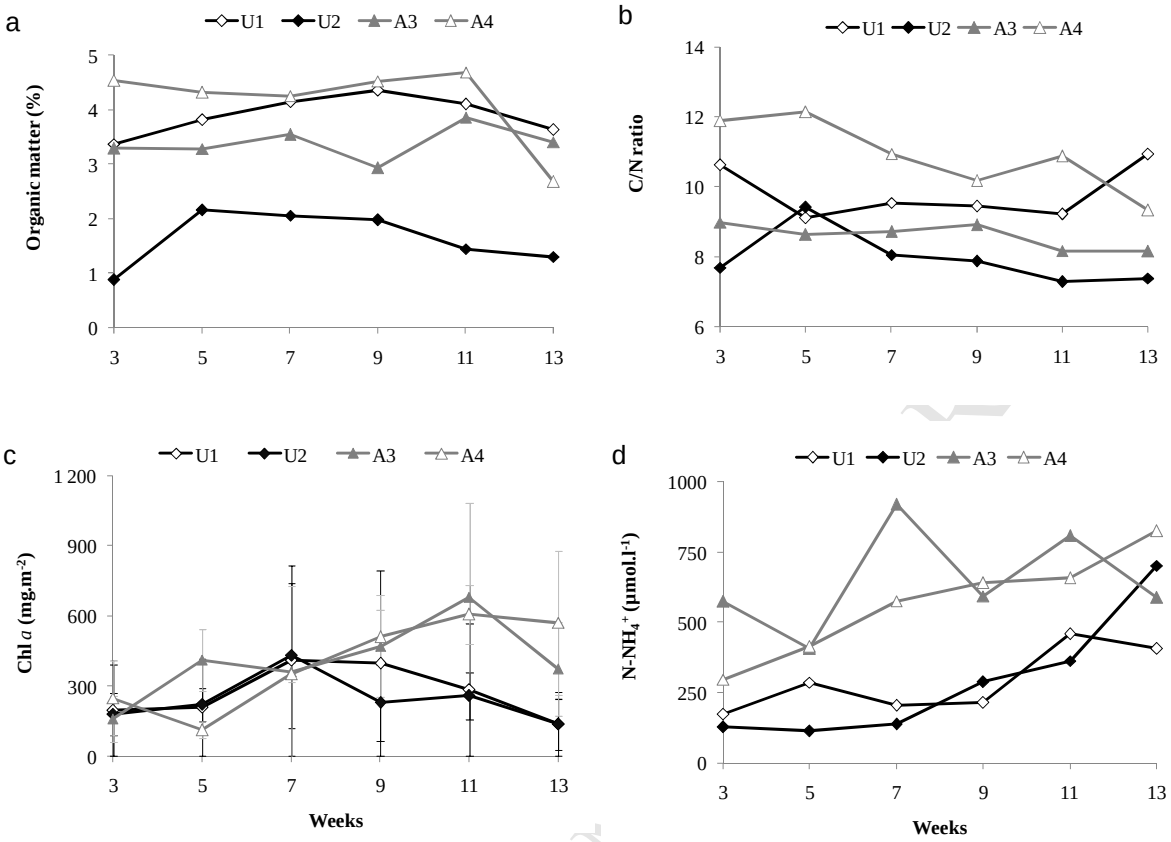

Fig. 2. Temporal variability of (a) organic matter, (b) C/N ratio (c) Chl *a* content in the sediment and d) ammonium in pore water. Chlorophyll a concentrations is presented as the mean of 3 samples collected at each station and at each sampling time.

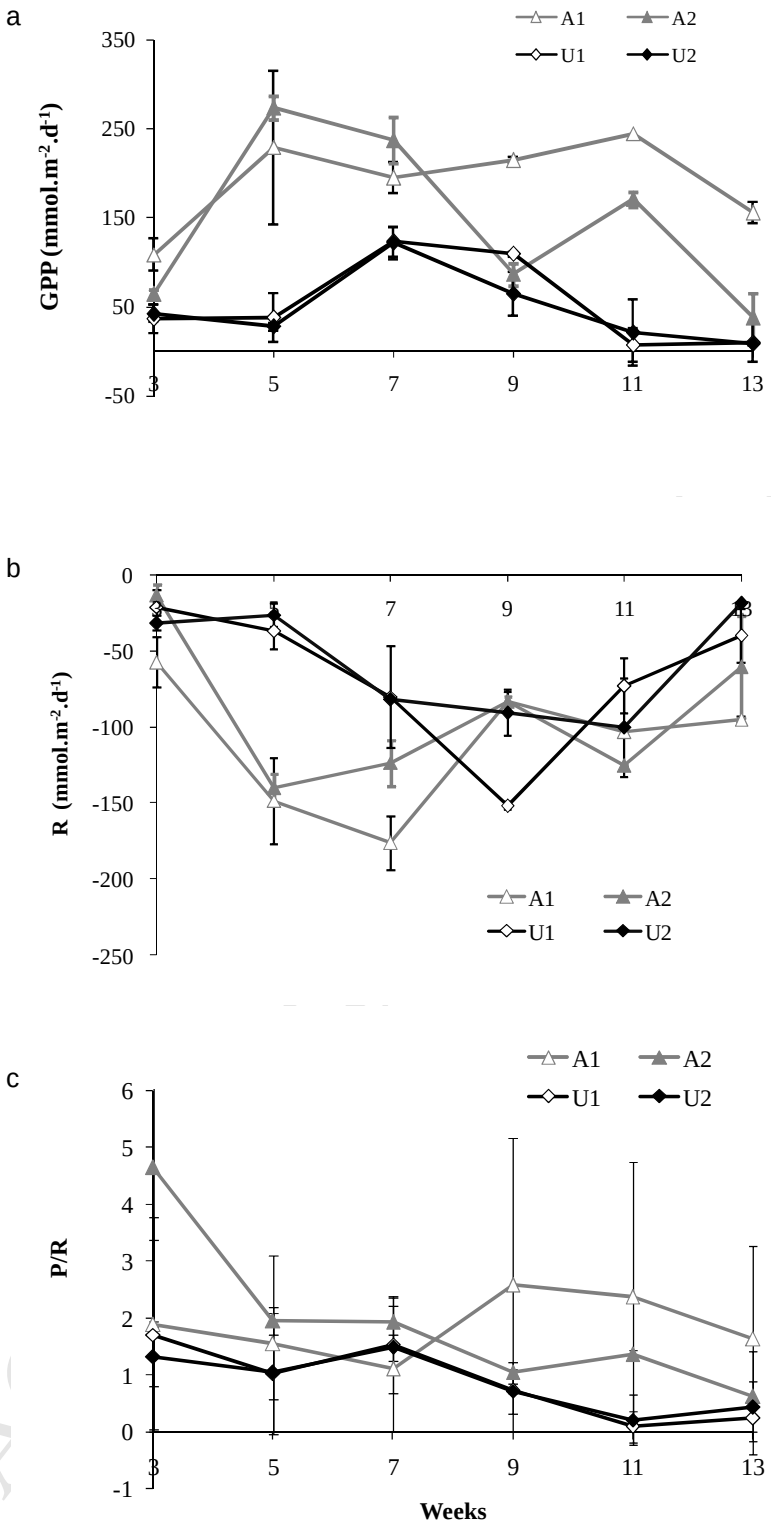

Fig. 3. Temporal variability of (a) sediment gross primary production (GPP), (b) respiration (R), and (c) the benthic trophic ratio (P/R). Measurements were conducted in duplicate at each sampling. Errors bars correspond to the standard deviation.

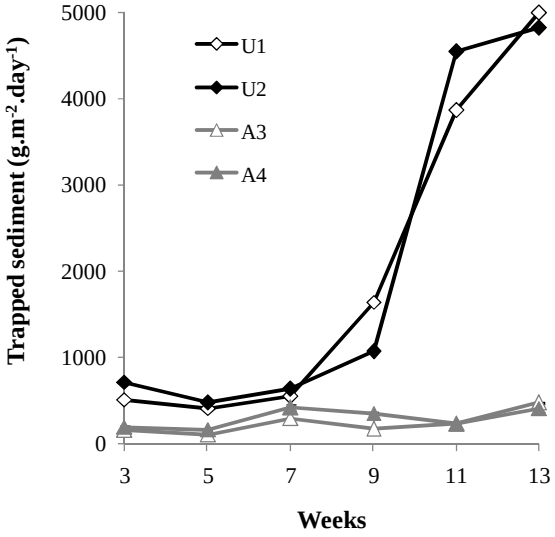

Fig. 4. Temporal variability of trapped material. Values are presented as the mean of 2 samples in pond A.

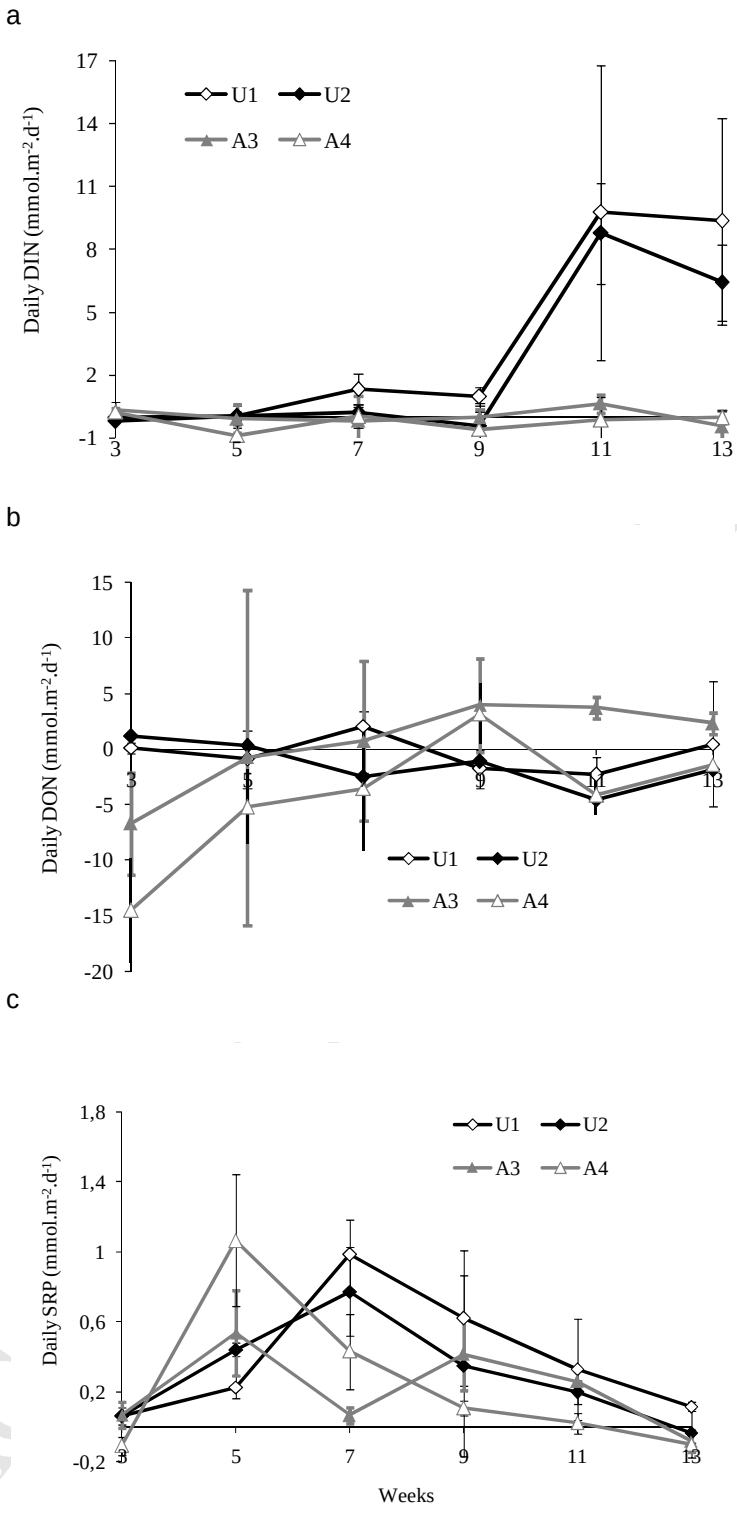

Fig. 5. Temporal variation of (a) the daily fluxes of dissolved inorganic nitrogen,(b) the daily fluxes of dissolved organic nitrogen and (a) the daily fluxes of phosphates (SRP). Measurements were conducted in duplicate at each sampling. Errors bars correspond to the standard deviation.

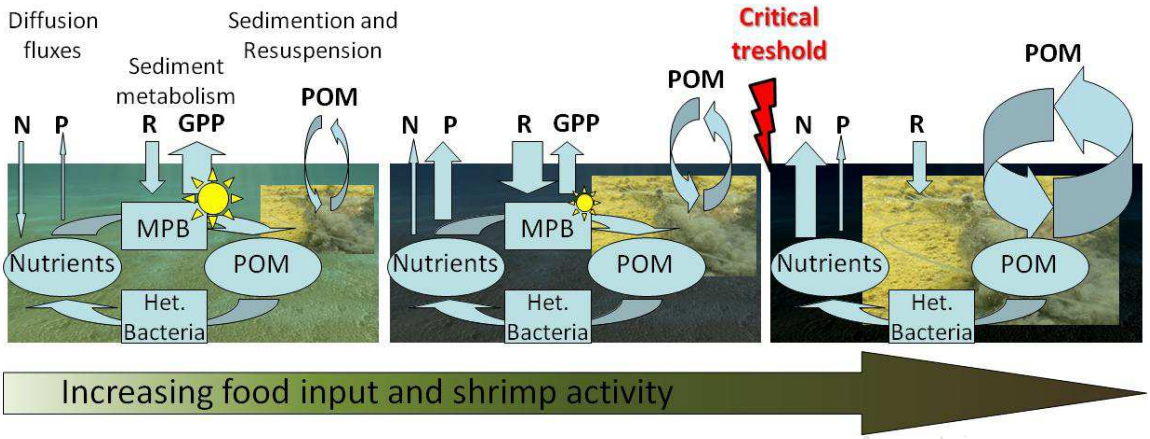

Fig. 6: Conceptual model of the temporal evolution of the sediment metabolism and exchanges. R and GPP represent sediment respiration and gross primary production, respectively. N and P are the nitrogen (DIN+DON) and phosphates exchanges, and POM the particulate organic matter sedimentation and resuspension. MPB represents the processes associated with microphytobenthos and Het. Bacteria, the processes associated with heterotrophic bacteria.

- Feed input and shrimp sediment resuspension were the main forcing function driving the benthic pelagic coupling.
- Microphytobenthos could reach a higher biomass and an equivalent areal productivity when compared to phytoplankton.
- Sediment metabolism functioned at the edge of a shift from autotrophy to heterotrophy.
- Exchanges between the sediment and water column were dominated by particulate organic matter resuspension.
- Microphytobenthos activity led to a complex dynamic of the nature and the amount of the diffusive nutrients fluxes.