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## Impact of contaminated sediment elutriate on coastal phytoplankton community (Thau lagoon, Mediterranean Sea, France)

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### Abstract :

Effects of sediment-released contaminants and nitrogen were assessed on phytoplankton communities sampled from Thau lagoon (France, Mediterranean Sea) and one close offshore marine station. Phytoplankton was exposed to sediment elutriate (seawater containing a mix of metals, organic chemicals, and nutrients) or to ammonium enrichment for four days using immersed microcosms exposed to natural conditions of light and temperature. Functional (production - respiration balance) and structural (taxonomy and cell densities) responses of the phytoplankton community were assessed. In the lagoon, both treatments stimulated phytoplankton growth, compare to controls. Conversely in the offshore station, the phytoplankton growth was stimulated only with the sediment elutriate addition. In offshore and lagoon stations, both treatments caused a shift in the taxonomic composition of the phytoplankton. Proliferation of potentially toxic diatoms and dinoflagellates resulted from the addition of elutriate. Correspondence analysis determined that phytoplankton from the offshore station was more sensitive to both treatments compared to the lagoon community. According to daily production and respiration balance, lagoon community metabolism remained heterotrophic ( $P < R$ ) for all treatments, whereas only transient shifts to net autotrophy ( $P > R$ ) were observed in the offshore community. Direct toxicity of contaminants released from sediment, if any, was therefore masked by nutrient enrichment effects, whereas indirect evidence of contaminant pressure was highlighted by changes in community composition and metabolism.

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## Highlights

► Contaminated sediment elutriates trigger changes in phytoplankton dynamics. ► Phytoplankton community structure and metabolism are affected. ► Elutriates effects are not identical to those of nutrient addition alone. ► Elutriates effects differ depending on the initial community structure.

**Keywords** : Sediment resuspension, Mediterranean lagoon, Contaminants, Nutrients, Phytoplankton community

## 44 **1. Introduction**

45 Anthropogenic contaminants in marine ecosystems are often eventually trapped by sediments,  
46 which act as a sink for pollutants. Resulting contaminated sediments affect mainly the benthic  
47 organisms but can also impede pelagic compartments when chemicals are released to the water  
48 column (Birch and O'Hea 2007; Jonas and Millward 2010; Roberts, 2012). Disturbance of  
49 contaminated sediments by anthropogenic (dredging and disposal of dredged materials, trawling,  
50 ship movements and propeller wash) and natural processes (tides, storms and bioturbation) may  
51 affect a wide range of species, eventually being incorporated into food webs (Burton and Johnston  
52 2010). Resuspension of contaminated sediments (RCS) has been shown to be an important source of  
53 dissolved chemicals in historically contaminated estuaries (Kalnejais *et al.* 2010, Roberts 2012).  
54 Dissolved polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) concentrations  
55 have been shown to increase due to dredged material disposal, even though the levels did not  
56 exceed water quality criteria (Cornelissen *et al.*, 2008). For example, in conditions of turbulent mixing  
57 in oxygenated seawater, organic (PAH and PCB) and inorganic (trace metals) contaminants were  
58 desorbed from sediments (Josefsson *et al.*, 2010; Voie *et al.*, 2002). In anthropized coastal areas,  
59 such mixing events also cause nutrients release, with consequent enrichment of the water column  
60 (Cantwell *et al.*, 2002; Kalnejais *et al.*, 2010), and ammonia may be released in greater amounts  
61 (Jones-Lee and Lee, 2005). A few studies have reported that nutrients and contaminants may have  
62 opposite results, with the beneficial effect of the nutrients overcompensating for the detrimental  
63 impact of the contaminants (Crain *et al.*, 2008). The effects of RCS on pelagic organisms are still being  
64 studied and have not yet been fully incorporated into ecological risk assessment for management of  
65 coastal ecosystems (Roberts, 2012). Several studies have shown that marine organisms (such as fish,  
66 bivalves, algae and polychaetes) are sensitive to RCS by many pathways (Edge *et al.*, 2015; Edge *et*  
67 *al.*, 2016, Hill *et al.*, 2009; Tolhurst *et al.*, 2007; Voie *et al.*, 2002).

68 Phytoplankton feeds pelagic food webs and determines aquatic ecosystem primary production and  
69 functioning at higher trophic levels (Field *et al.*, 1998). Any change in its community structure and

70 metabolism may, therefore, trigger a cascade of indirect effects throughout the plankton ecosystem  
71 (De Hoop *et al.*, 2013). Understanding how phytoplankton responds to contaminants is of primary  
72 importance for forecasting the ecological consequences of chemical contamination and for targeting  
73 priority management and restoration policies for aquatic systems. There is however no general  
74 consensus about the responses of primary producers, in direction and extent, to the simultaneous  
75 load of pollutants and nutrients. For example, Riedel *et al.* (2003) reported that phytoplankton  
76 growth was inhibited after exposure to a combination of contaminants (mix of trace metals) and  
77 nutrients, whereas Lafabrie *et al.* (2013a, 2013b) showed that RCS can stimulate growth and modify  
78 phytoplankton community structure, suggesting that the toxic effect of chemicals if any could be  
79 offset by nutrients.

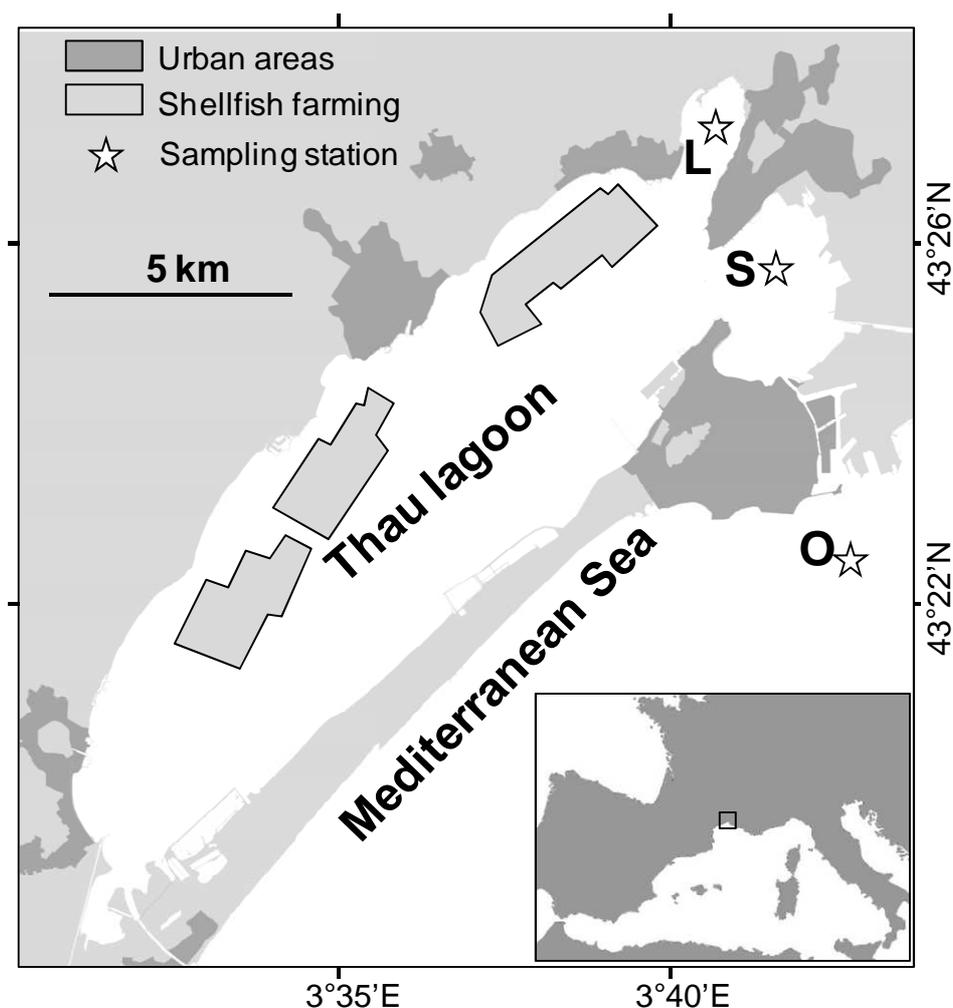
80 Within the marine environment, coastal ecosystems such as lagoons have become particularly  
81 impacted by chemical pollutants from anthropogenic inputs (agricultural, urban, and industrial)  
82 (Lafabrie *et al.*, 2013a; Levin *et al.*, 2001). This increased chemical contamination can cause a drastic  
83 alteration of the lagoon environment and eventually a threat to the services associated with lagoon  
84 environments (fisheries, aquaculture, tourism). This is the case for example of Thau lagoon (France),  
85 located on the northern shores of the Western Mediterranean Sea. The Thau Lagoon is one of the  
86 largest French Mediterranean Lagoons and is one the most important shellfish farming areas in  
87 Europe (Castro-Jimenez *et al.*, 2008). It sustains a large population of exploited fish (gilthead  
88 seabream *Sparus aurata* and European seabass *Dicentrarchus labrax*) and reared shellfish (mussels,  
89 clams and oysters) (Fouilland *et al.*, 2012). This lagoon appears to be under intense anthropogenic  
90 pressure characterized by an increase in industrial, agricultural and urban activities (La Jeunesse,  
91 2001). Recent studies in the Thau lagoon have revealed moderate to high chemical contamination in  
92 the sediment (Rigollet *et al.*, 2004), and high nutrient enrichment of the water (Anschutz *et al.*, 2007;  
93 Fouilland *et al.*, 2012) whereas the combined effects of pollutants and nutrients on the planktonic  
94 organisms of the lagoon have retained lesser attention. The Thau lagoon like all shallow marine  
95 ecosystems is subject to sediment resuspension that may have important consequences for the

96 ecosystem functioning, and impact aquaculture activities (changes in the structure and contribution  
97 of potentially toxic phytoplankton for example). Among factors that can trigger resuspension  
98 (reviewed in Roberts, 2012) in the area, flash floods and windstorms are documented (Fouilland *et*  
99 *al.* 2012), together with dredging activities in the nearby waterways and harbor. This study aimed to  
100 assess the effects of elutriates prepared from RCS on the structure and the functioning of the Thau  
101 Lagoon phytoplankton community incubated under natural outdoor conditions of temperature and  
102 irradiance, compared to near coastal community, as well as on the dynamics and metabolism of  
103 autotrophic and heterotrophic compartments. The present work focused on the soluble fraction  
104 resulting from sediment resuspension considered as directly bioavailable for plankton communities,  
105 taking in mind that suspended sediment itself is prone to have different effects even through physical  
106 damage (Edge *et al.*, 2015; Edge *et al.*, 2016). The two stations were chosen to compare the  
107 responses of a pre-exposed community from a closed lagoon, subjected to local inputs of  
108 contaminants to an a priori unexposed community from offshore station, considered as a reference.

## 109 **2. Materials and Methods**

### 110 **2.1. Study area**

111 The Thau lagoon, one of the largest French Mediterranean Lagoons (43°24'N-3°36'E; Fig. 1) with an  
112 area of 70 km<sup>2</sup>, is an important shellfishing area. Climate is characterized by autumnal storm events,  
113 and a single flood can contribute a quarter of the annual nitrogen supply from watershed (Tournoud  
114 *et al.*, 2006). Consequently, dissolved ammonium, especially in the oyster farming areas, can reach a  
115 significant concentration (Fouilland *et al.*, 2012; Gilbert *et al.*, 1997). Thau lagoon has also been  
116 strongly affected by industrial activities and an intense urbanization since the mid 20<sup>th</sup> century, which  
117 have resulted in a significant concentration of contaminants in sediments including polycyclic  
118 aromatic hydrocarbons PAH (440 - 7700 µg Kg<sup>-1</sup>), polychlorobiphenyls PCB (600 - 30,000 ng Kg<sup>-1</sup>),  
119 trace metals (8.9-6098 ng g<sup>-1</sup>) and pesticides (2921 ng Kg<sup>-1</sup>) as reported in several studies (Kawakami  
120 *et al.*, 2008; Léauté, 2008; Rigollet *et al.*, 2004).



121  
 122 **Figure 1.** Situation of the Thau Lagoon in Southern France, and location of sampling stations:  
 123 sediment and water for elutriate preparation(S), lagoon (L) and offshore (O) water inoculum.

## 124 2.2. Sediment elutriate preparation and chemical analyses

125 Water and sediment were sampled in June 2011 in the “Eaux Blanches” bay (Station S, Fig. 1). The  
 126 sediments in this area have previously been characterized as highly contaminated by trace metals,  
 127 PAH and PCB (Kawakami *et al.*, 2008; Léauté, 2008). The sediment was sampled using a Van Veen  
 128 grab and screened directly through a 2 mm mesh stainless steel sieve to remove stones, macrofauna  
 129 and plant fragments. Water for elutriate preparation was collected from the surface in a bucket and  
 130 immediately filtered through a 80 µm mesh to remove the largest organisms and debris. The  
 131 sediment elutriate was prepared as described by Bonnet *et al.* (2000). Sediment was stirred in water  
 132 (ratio 1:4 v:v) for 8 h to mimic resuspension. After settling for 8 h, the supernatant was collected and

133 then filtered through 1 and 0.2  $\mu\text{m}$  membranes to remove any native microorganisms and leave only  
134 dissolved chemical contaminants. The elutriate was used as contaminated water (CW) for the  
135 microcosm experiment.

136 Ammonium concentration in the elutriate was measured spectrophotometrically using indophenol  
137 blue complexation in basic medium (Koroleff, 1969), with a detection limit of 0.04  $\mu\text{M}$ .

138 Sub-samples of sediment and elutriates were preserved for further determination of 16 PAHs  
139 (naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene,  
140 benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene,  
141 dibenzo(a,h)anthracene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene and acenaphthylene) and 7 PCB  
142 congeners (180, 28, 52, 101, 118, 138, and 153) by GC-MS method after liquid-liquid extraction for  
143 the CW and soxhlet extraction for the sediment. Trace metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, U, V, Mn,  
144 Fe, Co and Mo) concentrations in elutriate were measured by ICP-MS, and the  $\text{NH}_4^+$  concentration in  
145 the CW was determined using fluorometry (Holmes *et al.*, 1999). Analytical methods involved the use  
146 of spiked deuterated standards and Canadian National Research Council standard seawater, for PAH  
147 and metal analysis respectively as described in Pringault *et al.* (2016).

148 Contaminant concentrations were compared when available to published quality guidelines and  
149 effects evaluation such as Effect Range Low and Effect Range Median (ERL and ERM, Long *et al.*,  
150 1995), and harmful concentrations for 5% of tested species (HC5, Ben Othman *et al.*, 2012, Wang *et*  
151 *al.*, 2014, Wheeler *et al.*, 2002) for sediment and water respectively.

### 152 **2.3. Outdoor microcosm experiment**

153 Seawater was sampled at station L ("*Lagoon*"), a highly-contaminated lagoon area characterized by  
154 regular occurrences of toxic dinoflagellate blooms (Laabir *et al.*, 2011) and station O ("*Offshore*")  
155 chosen as an offshore reference station (Fig. 1). Water samples (50 L), collected from 0 – 2 m depth,  
156 was immediately filtered through an 80  $\mu\text{m}$  mesh to remove large grazers and particles, and then  
157 stored in the dark in coolers at ambient temperature before processing. Eighty  $\mu\text{m}$ -filtered water

158 (FW) was used as plankton inoculum in microcosms, submitted to three different treatments:  
159 sediment elutriate addition (E: 3L of FW + 1L of CW), N-nutrient addition (N: 4L of FW + 20  $\mu\text{M}$  of  
160  $\text{NH}_4^+$ , final concentration, close to the actually measured ammonium concentration in elutriate) and  
161 control with no addition (C: 4L of FW). All treatments were performed in triplicate using 5L glass  
162 bottles (Schott-Duran) which contained 1L of air to allow gas exchange (de la Broise and Palenik,  
163 2007). The E treatment (for elutriate) allowed the effect of both released pollutants and nutrients on  
164 the phytoplankton community to be determined, whereas N treatment investigated the impact of  
165  $\text{NH}_4^+$  enrichment alone. Ammonium was chosen because it is among the most significant N nutrients  
166 in the Thau lagoon prone to have direct effects on planktonic communities (Fouilland *et al.* 2012;  
167 Trottet *et al.* 2016).

168 All microcosms were incubated for 96 h in an outdoor tank exposed to natural sunlight and filled with  
169 seawater, which was renewed directly from the lagoon by a pumping-draining system allowing  
170 microcosms to support the same temperature as nearby seawater. This simulated the natural  
171 conditions of light and temperature of the Thau lagoon surface waters. Each microcosm was sampled  
172 at the beginning and at 24, 48, 72 and 96 h of incubation to measure chl *a* and plankton production  
173 and respiration. Sub-samples were also taken to determine the initial (at 0 h) and final (at 96 h) cell  
174 abundances and taxonomic composition of the phytoplankton communities.

#### 175 **2.4. Chlorophyll *a* analysis**

176 Sub-samples (200 ml) for chl *a* were filtered onto glass fiber filters (GF/F, Whatman), and  
177 immediately frozen at  $-80^\circ\text{C}$  until analysis. Chlorophyll *a* was extracted using 90% acetone for 24 h in  
178 the dark at  $4^\circ\text{C}$ , followed by ultrasonic grinding in an ice bath. After centrifugation of the extract  
179 (3500 rpm for 15 min), pigment concentration in the supernatant was measured by  
180 spectrofluorimetry (Neveux and Lantoiné, 1993).

#### 181 **2.5. Phytoplankton identification and count**

182 To identify and count the phytoplankton, 250 ml sub-samples were fixed with formaldehyde (4% final  
183 concentration). The phytoplankton cells were counted on a settled volume of 100 ml (Utermöhl,

184 1938), using an upright microscope (Zeiss AX10) with a Sony XCD- U100CR digital camera, or an  
185 inverted microscope (Olympus IX70) with a Moticam MoticamPro digital camera. At least 500 cells in  
186 each sample were counted to achieve reliable estimates.

## 187 **2.6. Production and respiration measurement**

188 The net primary production (NPP) and dark respiration ( $R_{\text{dark}}$ ) rates were measured using short time  
189 incubations (< 6 h) in gas-tight 30 mL vessels, according to the oxygen light-dark incubation method  
190 (Pringault *et al.*, 2007) with a Clark-type oxygen microsensor (Unisense, Denmark). Samples were  
191 kept at ambient field temperature and light irradiance during the incubation period. Oxygen  
192 concentrations were measured in each flask at one hour intervals, corrected for temperature, salinity  
193 and barometric pressure, and oxygen evolution rates calculated from linearized data providing hourly  
194 net primary production rates (NPP,  $O_2$  evolution in the light) and dark respiration rates ( $R_{\text{dark}}$ ,  $O_2$   
195 evolution in the dark). The gross primary production (GPP) was then calculated from the NPP and  
196  $R_{\text{light}}$  values ( $GPP = |R_{\text{light}}| + NPP$ ) considering that  $R_{\text{light}}$  represented three times  $R_{\text{dark}}$  following the  
197 procedure of Pringault *et al.* (2007). The primary production to community respiration ratios (P:R)  
198 were considered from the GPP and R daily rates that were calculated from the GPP  $R_{\text{dark}}$  and  $R_{\text{light}}$   
199 hourly rates, respectively. A light period of 12 h was considered for integration of daily GPP rates,  
200 and daily respiration was calculated as the sum of hourly rates of  $R_{\text{light}}$  (\*12 hours) and hourly rates of  
201  $R_{\text{dark}}$  (\*12 hours).

## 202 **2.7. Statistical analyses**

203 Differences in variables between treatments (fixed factor, 3 levels) and sampling times (fixed factor,  
204 5 levels) were tested by two-way factorial analysis of variance (ANOVA). When ANOVA revealed  
205 significant differences, Tukey HSD post-hoc comparison test was performed to identify the significant  
206 differences. The conditions of distribution normality (Kolmogorov–Smirnov test) and variance  
207 homogeneity (Bartlett-Box test) were respected. These analyses were done using SPSS 14.0 statistical  
208 software for Windows. The Shannon-Wiener index  $H$  was calculated using PAST freeware (Hammer  
209 and Harper, 2001) to analyze changes in phytoplankton diversity for each treatment.

210 Correspondence analysis was performed using PAST from the absolute species abundances to detect  
 211 any phytoplankton composition change and highlight taxa most responsible for community  
 212 structuring.

### 213 3. Results

#### 214 3.1. Contaminant and nutrient levels

215 Low levels of PCBs ranging from 0.002 to 0.017  $\mu\text{g g}^{-1}$  were measured in the sediment, whereas  
 216 greater amounts of PAHs were found (Table 1). The most abundant PAHs in sediment were  
 217 fluoranthene (1.7  $\mu\text{g g}^{-1}$ ), pyrene (1.24  $\mu\text{g g}^{-1}$ ), benzo(a)anthracene (0.78  $\mu\text{g g}^{-1}$ ) and benzo(a)pyrene  
 218 (0.87  $\mu\text{g g}^{-1}$ ).

219 The contaminated water (CW) prepared from these sediment contained high metal levels at  
 220 concentrations ranging from 0.03 (for Co) to 217.1  $\mu\text{g l}^{-1}$  (for Mo) (Table 1). Apart from these  
 221 potentially toxic contaminants, a significant concentration of  $\text{NH}_4^+$  (21.8  $\mu\text{M}$ ) was also measured in  
 222 CW. Elutriate did not contained any detectable dissolved PAH or PCB.

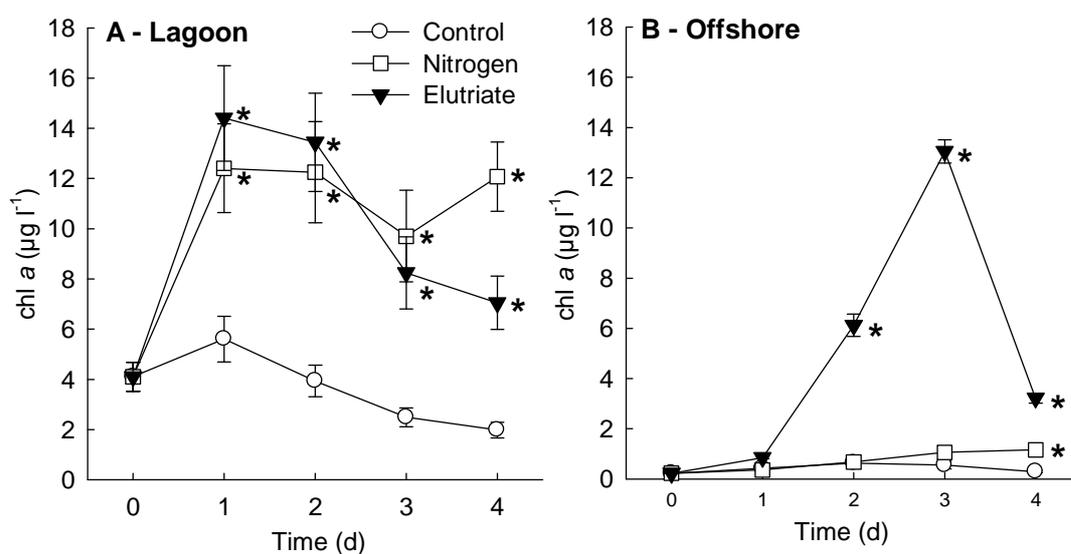
	Sediment		CW			Sediment		CW			Sediment		CW	
PAH					PCB					TME				
Anthracene	0.22	bdl	PCB 101	0.002	bdl	Mo	-	217.1						
Benzo(a)anthracene	0.78	bdl	PCB 118	0.012	bdl	Cd	-	0.021						
Benzo(a)pyrene	0.87	bdl	PCB 138	0.005	bdl	Pb	-	0.088						
Benzo(b)fluoranthene	0.67	bdl	PCB 135	0.007	bdl	U	-	33.3						
Benzo(g,h,i)perylene	0.68	bdl	PCB 180	0.017	bdl	V	-	3.91						
Benzo(k)fluoranthene	0.5	bdl	PCB 28	0.01	bdl	Cr	-	0.47						
Chrysene	0.72	bdl	PCB 52	0.006	bdl	Mn	-	22.67						
Fluoranthene	1.7	bdl				Fe	-	1.53						
Indenopyrene	0.55	bdl				Co	-	0.03						
Naphthalene	0.03	bdl				Ni	-	0.62						
Phenanthrene	0.74	bdl				Cu	-	2.49						
Pyrene	1.24	bdl				Zn	-	1.75						
						As	-	9.56						

223 **Table 1.** Contaminant levels in the Thau lagoon sediment ( $\mu\text{g g}^{-1}$  dry weight) and in the prepared  
 224 elutriate (CW,  $\mu\text{g l}^{-1}$ ) (bdl: below detection limit of 0.01  $\mu\text{g l}^{-1}$ ; -: not determined). PAH: polycyclic  
 225 aromatic hydrocarbons; PCB: polychlorobiphenyl congeners; TME: trace metal elements.

#### 226 3.2. Effect of the treatments on chlorophyll *a* concentrations

227 In lagoon station L, the concentration of chl *a* was 4.1  $\mu\text{g l}^{-1}$  in the inoculum, and after 24 h, it  
 228 increased 2 and 3 times compared to the control (5.6  $\mu\text{g l}^{-1}$ ) in microcosms E (added CW) and N

229 (added  $\text{NH}_4^+$ ), respectively (Fig. 2A). Levels remained significantly higher ( $p < 0.05$ ) in E (average of  
 230  $11.24 \mu\text{g l}^{-1}$ ) and N (average of  $12.25 \mu\text{g l}^{-1}$ ) than in the control (average  $3.50 \mu\text{g l}^{-1}$ ) until the end of  
 231 incubation (Fig. 2A) and no significant difference was observed between E and N treatment. By  
 232 contrast, the concentration of chl *a* was  $0.225 \mu\text{g l}^{-1}$  in the inoculum from the offshore station O.  
 233 After 48h, it increased 6 and 13 times compared to the control in microcosms E (48h:  $6.123 \mu\text{g l}^{-1}$  in E  
 234 and  $0.63 \mu\text{g l}^{-1}$  in C; 72h:  $13.21 \mu\text{g l}^{-1}$  in E and  $0.7 \mu\text{g l}^{-1}$  in C) but at the end of the incubation the  
 235 biomass decreased in microcosms E ( $p < 0.05$ ) ( $3.21 \mu\text{g l}^{-1}$ ). In general, no significant difference was  
 236 observed between C and N treatments ( $p < 0.05$ ) for the offshore inoculum all along the experiment,  
 237 levels remained closely equivalent in C ( $0.50 \mu\text{g l}^{-1}$ ) and N microcosms ( $0.70 \mu\text{g l}^{-1}$ ) (Fig. 2B).



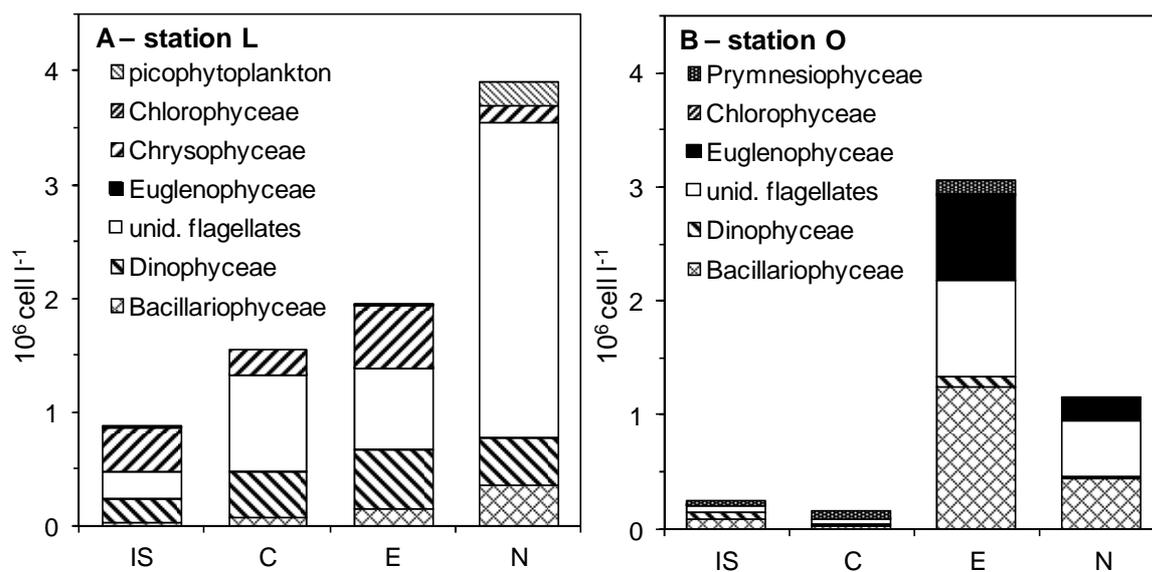
238  
 239 **Figure 2.** Evolution of chlorophyll *a* concentrations in the microcosms for Lagoon (A) and Offshore  
 240 experiments (B). Mean  $\pm$  SE,  $n = 3$ .

### 241 3.3. Effects of the treatments on phytoplankton abundance and whole community 242 composition

243 In inoculums, the species diversity of the phytoplankton communities expressed as *H* index was  
 244 significantly lower in lagoon ( $1.25 \pm 0.05 \text{ bits ind}^{-1}$ ) than in offshore station ( $1.45 \pm 0.006 \text{ bits ind}^{-1}$ ).

#### 245 3.3.1. Lagoon phytoplankton community (station L)

246 In station L, the phytoplankton inoculum ( $0.85 \times 10^6 \text{ cells l}^{-1}$ ) was dominated by chrysophyceae ( $0.38$   
 247  $10^6 \text{ cells l}^{-1}$ ), the most abundant species being the loricate chromulinale *Ollicola vangoorii* (known as  
 248 mixotrophic in Mediterranean waters, Novarino *et al.* 2002) and an unidentified chrysophyceae (50%  
 249 of total cell counts). The phytoplankton community contained similar proportions (~25%) of  
 250 dinoflagellates ( $0.21 \times 10^6 \text{ cells l}^{-1}$ ) and small unidentified flagellates ( $0.23 \times 10^6 \text{ cells l}^{-1}$ ) (Fig. 3A).



251  
 252 **Figure 3.** Contribution of the various taxonomic groups to the total phytoplankton abundances in the  
 253 initial sample community (IS) and after four days in the control (C), sediment elutriate treatment (E),  
 254 and nitrogen enrichment treatment (N) for Lagoon (Fig. 3A) and Offshore (Fig. 3B) experiments.

255 In station L, after 96 h of incubation, the phytoplankton density increased in all microcosms, reaching  
 256  $1.54$ ,  $1.93$  and  $3.90 \times 10^6 \text{ cells l}^{-1}$  in the C, E and N microcosms, respectively (Fig. 3A). Diatoms ( $0.06 \times 10^6$   
 257  $\text{cells l}^{-1}$  in C,  $0.13 \times 10^6 \text{ cells l}^{-1}$  in S), chrysophyceae (*Ollicola vangoorii* and the unidentified  
 258 chrysophyceae) ( $0.22 \times 10^6 \text{ cells l}^{-1}$ , in C and  $0.54 \times 10^6 \text{ cells l}^{-1}$  in E), and dinoflagellates ( $0.40 \times 10^6$  in C and  
 259  $0.51 \times 10^6 \text{ cells l}^{-1}$  in E) were stimulated by the addition of elutriate. In the N microcosms, the  
 260 flagellates density increased significantly (74.9%) followed by the diatoms (9%) and cyanobacteria  
 261 (5%), the community being dominated by the unidentified flagellates (71%). By comparison, the  
 262 community in the E microcosms comprised mainly dinoflagellates (11%), chrysophyceae (3.9%) and  
 263 diatoms (9%). The contribution of diatoms in the N microcosms (9%) seemed to be higher than in the

264 E microcosms (7.1%). As well as changes in the contributions from the various taxonomic groups to  
 265 the phytoplankton community, there were changes in the species composition inside taxonomic  
 266 groups. Phytoplankton diversity increased after 96 h of incubation in all three microcosms compared  
 267 to the initial samples. The Shannon index  $H$  was  $1.7 \pm 0.03$ ;  $1.55 \pm 0.02$  and  $1.6 \pm 0.01$  bits ind<sup>-1</sup>,  
 268 respectively in C, E and N microcosms and there was no significant difference between treatments ( $p$   
 269  $> 0.05$ ).

### 270 **3.3.2. Offshore phytoplankton community (station O)**

271 In station O, the initial community of phytoplankton ( $0.24 \cdot 10^6$  cells l<sup>-1</sup>) was dominated by diatoms  
 272 ( $0.085 \cdot 10^6$  cells l<sup>-1</sup>) and dinophyceae ( $0.053 \cdot 10^6$  cells l<sup>-1</sup>). After 96 h of incubation, the algal density  
 273 increased in N and E microcosms, respectively  $1.25$  and  $3.04 \cdot 10^6$  cells l<sup>-1</sup>, compared to the control  
 274 ( $0.15 \cdot 10^6$  cells l<sup>-1</sup>). Diatoms ( $1.24 \cdot 10^6$  cells l<sup>-1</sup> in E,  $0.014 \cdot 10^6$  cells l<sup>-1</sup> in C), unidentified flagellates ( $0.03$   
 275  $\cdot 10^6$  cells l<sup>-1</sup> in C and  $0.85 \cdot 10^6$  cells l<sup>-1</sup> in E) and chrysophyceae (0 in C and  $0.73 \cdot 10^6$  cells l<sup>-1</sup> in E) were  
 276 stimulated by the addition of elutriate (Fig. 3B). In the N and E microcosms, the diatoms (40.8 % in E  
 277 and 37.5 % in N) and chrysophyceae (24.3 % in E and 16.1 % in N) were significantly increased  
 278 compared to the control (Diatoms: 9.9 %; Chrysophyceae: 0 %). By contrast, dinoflagellates showed a  
 279 sharp decrease in E (2.8 %) and N (1.4 %) compared to the control (14.1 % of total cell densities).  
 280 Unidentified flagellates were stimulated in E microcosms (42.6 %) compared to the control and N  
 281 microcosms (23.7 and 28 % respectively). In the offshore sample experiment, the diversities in C  
 282 microcosms ( $1.41 \pm 0.04$  bits ind<sup>-1</sup>) were significantly higher after 96h than in E ( $1.32 \pm 0.002$  bits ind<sup>-1</sup>)  
 283 and N microcosms ( $1.19 \pm 0.03$  bits ind<sup>-1</sup>).

### 284 **3.4. Effect of the treatments on diatom community composition**

285 In station L, the initial diatom community was dominated by three pennate taxa, the potentially toxic  
 286 *Pseudo-nitzschia* sp. (43%) and *Nitzschia longissima* (43%), followed by *Nitzschia* sp. (14%) (Table 2).  
 287 After 4 days of incubation, the diatom diversity remained similar in the C and N microcosms, with a  
 288 large contribution of *Pseudo-nitzschia* sp. (80-90%) and a small contribution of *Nitzschia longissima*

289 (5%) (Table 2). Until the end of incubation time, *Pseudo-nitzschia* spp. were the only diatoms  
 290 remaining under elutriate addition (E microcosms).

	Lagoon				Offshore			
	IS	C	E	N	IS	C	E	N
<i>Asterionellopsis glacialis</i>					31.4			
<i>Chaetoceros</i> spp.					25.6	11.8	3.5	62.8
<i>Leptocylindrus</i> sp.					4.1			
<i>Nitzschia longissima</i>	42.9	4.8		12.5	3.3		2.7	3.3
<i>Nitzschia</i> sp.	14.3							
<i>Pseudo-nitzschia</i> sp.	42.9	90.5	100	87.5	35.5	76.5	90.3	29.5
<i>Thalassiosira</i> sp.						11.8	3.5	4.4
Unidentified diatoms		4.8						

291 **Table 2.** Diatom species composition (expressed as % of total cell counts in the group) in initial  
 292 sample community (IS) and after four days in the control (C), sediment elutriate treatment (E), and  
 293 nitrogen enrichment treatment (N) for lagoon and offshore experiments. Empty cells: taxa not found  
 294 in the sample.

295 In the station O, the initial community of diatoms (Table 2) was dominated by potentially toxic  
 296 *Pseudo-nitzschia* spp. (35.5 %), *Asterionellopsis glacialis* (31.4 %) and *Chaetoceros* spp. (25.6 %). After  
 297 96h exposure, the two species *Asterionellopsis glacialis* and *Leptocylindrus* sp. disappeared in the  
 298 three treatments. Conversely, the centric diatom *Thalassiosira* sp. appeared in large numbers in C  
 299 (11.8 %), N (4.4 %) and E (3.5 %) microcosms. The potentially toxic *Pseudo-nitzschia* spp. were the  
 300 dominant diatom species in C and E microcosms (76.5 % and 90.3 %, respectively), accounting for a  
 301 smaller contribution in N microcosms (29.5 %) compared to the control (Table 2).

### 302 3.5. Effect of the treatments on dinoflagellate community composition

303 In station L, at the beginning of the experiment, the dinophyceae were dominated by *Alexandrium*  
 304 sp., which contributed up to 72% to total dinoflagellate community. The contributions of other  
 305 species varied from 0.9% (*Gonyaulax polygramma*, *Gonyaulax spinifera*, *Gyrodinium* sp., *Oblea* sp.,  
 306 *Prorocentrum lima* and *Protoperidinium* sp.) to 5.2% (*Prorocentrum gracile* and *Gymnodinium* sp.)  
 307 (Table 3).

308 At the end of incubation, the genus *Alexandrium* was still the dominant one in all three microcosms  
 309 (54.7 % in C, 56.0% in E and 74.2 % in N). There was also a significant development of *Prorocentrum*

310 species (*P. gracilis*, *P. micans* and *P. minimum*) in the N microcosm. *Katodinium* sp., which was  
 311 present in the control, disappeared from the E and N microcosms after the incubation.

	Lagoon				Offshore			
	IS	C	E	N	IS	C	E	N
<i>Alexandrium</i> sp.	72.4	54.7	56.1	74.2				
<i>Gonyaulax polygramma</i>	0.9		0.6	2.2				
<i>Gonyaulax spinifera</i>	0.9				1.9			
<i>Gymnodinium</i> sp.	5.2	13.1	19.5	5.6	52.8	51.6	57.1	16.7
<i>Gyrodinium</i> sp.	0.9	2.2	2.4		17.0			
<i>Katodinium</i> sp.		2.2						
<i>Neoceratium fusus</i>					1.9			
<i>Oblea</i> sp.	0.9	0.7	0.6					
<i>Peridinium quinquecorne</i>	2.6		0.6					
<i>Prorocentrum gracile</i>	5.2	4.4	3.7	6.7				16.7
<i>Prorocentrum lima</i>	0.9							
<i>Prorocentrum micans</i>	3.4		4.3	1.1	3.8	9.7	14.3	
<i>Prorocentrum minimum</i>	2.6	1.5	1.2	5.6		3.2		
<i>Prorocentrum</i> sp.						4.8		
<i>Protoperidinium diabolus</i>		1.5						
<i>Protoperidinium</i> sp.	0.9	0.7	1.2	3.4				
<i>Scrippsiella</i> sp.	2.6	2.9	1.2	1.1	1.9	6.5		
Unidentified dinoflagellates	0.9	16.1	8.5		20.8	24.2	28.6	66.7

312 **Table 3.** Dinoflagellates species composition (expressed as % of total cell counts in the group) in  
 313 initial sample community (IS) and after four days in the control (C), sediment elutriate treatment (E),  
 314 and nitrogen enrichment treatment (N) for lagoon and offshore experiments. Empty cells: taxa not  
 315 found in the sample.

316 In station O, the initial community of dinoflagellates was dominated by *Gymnodinium* spp. with  
 317 52.83% of relative contribution (Table 3), followed by unidentified dinoflagellates (20.8 %) and  
 318 *Gyrodinium* sp. (17 %). After 96h of incubation, *Gymnodinium* spp. were dominant in the control C  
 319 (51.6 %) and E treatment (57.1 %), followed by unidentified dinoflagellates (C: 24.2 %; E: 28.6 %). By  
 320 contrast, unidentified dinoflagellates were most abundant in the N microcosms (66.7 %) compared to  
 321 *Gymnodinium* spp. and *Gyrodinium* sp. (16.7 % for both).

### 322 3.6. Effect of the treatments on the other phytoplankton community composition

323 In station L, various flagellates belonging to several classes were present in the inoculum sample  
 324 dominated by *Ollicola vangoorii* and colored flagellates, together with few cyanobacteria,  
 325 chrysophyceae, unidentified chrysophyceae and chlorophyceae (Table 4). The chrysophyceae *O.*  
 326 *vangoorii* was the dominant species (59.3 %). At the end of incubation, the contribution of *O.*  
 327 *vangoorii* was lower in microcosms C (20.1 %) and N (4.7 %) relative to microcosms E (42.6 %). There

328 was a higher contribution of other unidentified flagellates in the three microcosm conditions (78.8 %  
 329 in C, 56.4 % in E and 88.5 % in N) than in the inoculum.

	Lagoon				Offshore			
	IS	C	E	N	IS	C	E	N
cyanobacteria	0.6		0.5	6.6				
chlorophyceae	0.6		0.2		2.2	9.8	0.6	1.8
coccolithophorids					38.8	59.0	6.9	2.1
<i>Eutreptiella braarudii</i>		0.3	0.2					
<i>Ollicola vangoorii</i>	59.3	20.1	42.6	4.7	2.8		43.0	26.3
Unidentified chysophyceae	2.1	0.8		0.2				
Unidentified flagellates	37.4	78.8	56.4	88.5	56.2	31.1	49.6	69.8

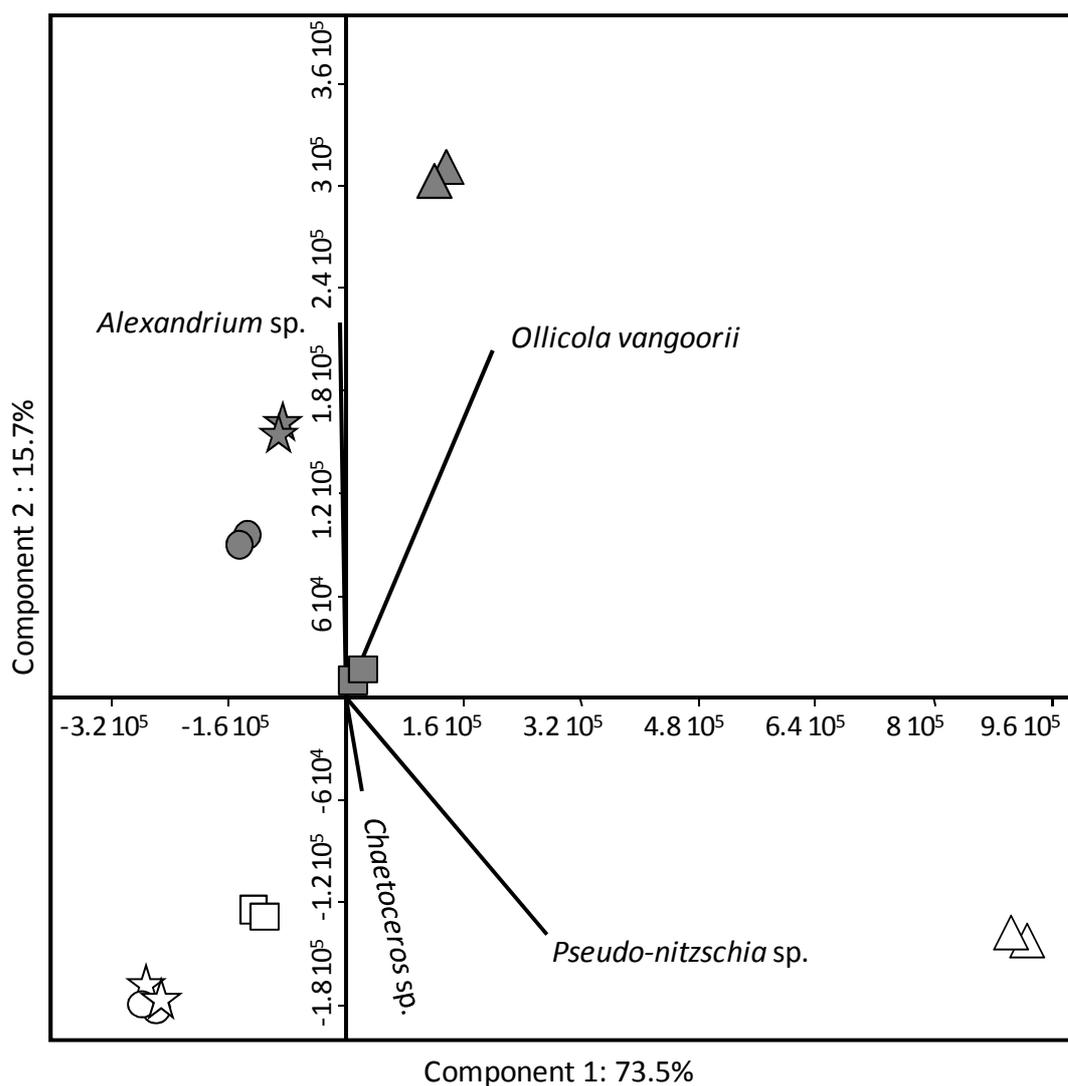
330 **Table 4.** Chlorophyceae, cyanobacteria and pigmented flagellate species composition (expressed as  
 331 % of total cell counts in the group) in initial sample community (IS) and after four days in the control  
 332 (C), sediment elutriate treatment (E), and nitrogen enrichment treatment (N) for lagoon and offshore  
 333 experiments. Empty cells: taxa not found in the sample.

334 In station O, the inoculum community was dominated by unidentified flagellates (56.2 %) and  
 335 coccolithophorids (38.8 %). The remaining part of cell density was constituted by *Ollicola vangoorii*  
 336 and unidentified chlorophyceae. After 96h, unidentified flagellates were dominant in the two  
 337 treatments E and N (E: 49.6 %; N: 69.8 %) compared to the control (31.1 %). The opposite pattern  
 338 was noticed for coccolithophorids (C: 59.0 %; N: 2.1 %; E: 6.9 %). For the chrysophyceae *Ollicola*  
 339 *vangoorii*, almost the same evolution pattern was observed as in the station L with a large increase in  
 340 cell numbers during the incubation. In fact, this species showed a higher contribution in E  
 341 microcosms (43.0 %) compared to the N (26.3 %) and C (0 %) ones (Table 4).

### 342 3.7. Correspondence analysis

343 The correspondence analysis, of which the two main axes explained 60.4 % of the observed  
 344 variances, highlighted the marked changes between phytoplankton composition at the initial and the  
 345 end of the experiments in the stations L and O (Fig. 4). In lagoon station L, the final community  
 346 composition in N and E microcosms differed from inoculum and control microcosms. The average  
 347 treatment effect was more pronounced in offshore station compared to the lagoon station. Results  
 348 demonstrated a pronounced divergence between the initial samples used as inoculum and the two  
 349 treatments N and E after incubation (Fig. 4), the control remaining close to the inoculum. The species  
 350 *Alexandrium* sp., *Chaetoceros* sp., *Pseudo-nitzschia* sp., and *Ollicola vangoorii* may have been mainly

351 responsible for these differences between the different microcosms (inoculum, C N and E). For the  
 352 lagoon station, cyanobacteria *Spirulina* sp., and *Prorocentrum* species (*P. micans*, *P. gracile* and *P.*  
 353 *minimum*) contributed to the difference between E treatment and the others (inoculum, N and C),  
 354 whereas *Asterionellopsis glacialis*, coccolithophorids, *Prorocentrum micans*, *P. gracile*, *Gyrodinium* sp.  
 355 and unidentified flagellates may have been mainly responsible for these differences between the  
 356 different samples (inoculum, C, N and E) in the station O (data not illustrated).



357  
 358 **Figure 4.** Correspondence analysis scatter plot obtained from the relative contributions of identified  
 359 phytoplankton taxa for the Lagoon (grey symbols) and Offshore (open symbols) experiments. Stars:  
 360 inoculum; circles: controls; squares: nitrogen enrichment; triangles: sediment elutriate treatment.  
 361 Species with loadings  $>0.5$  are shown.

### 3.8. Effect of the treatments on community metabolism: production and respiration

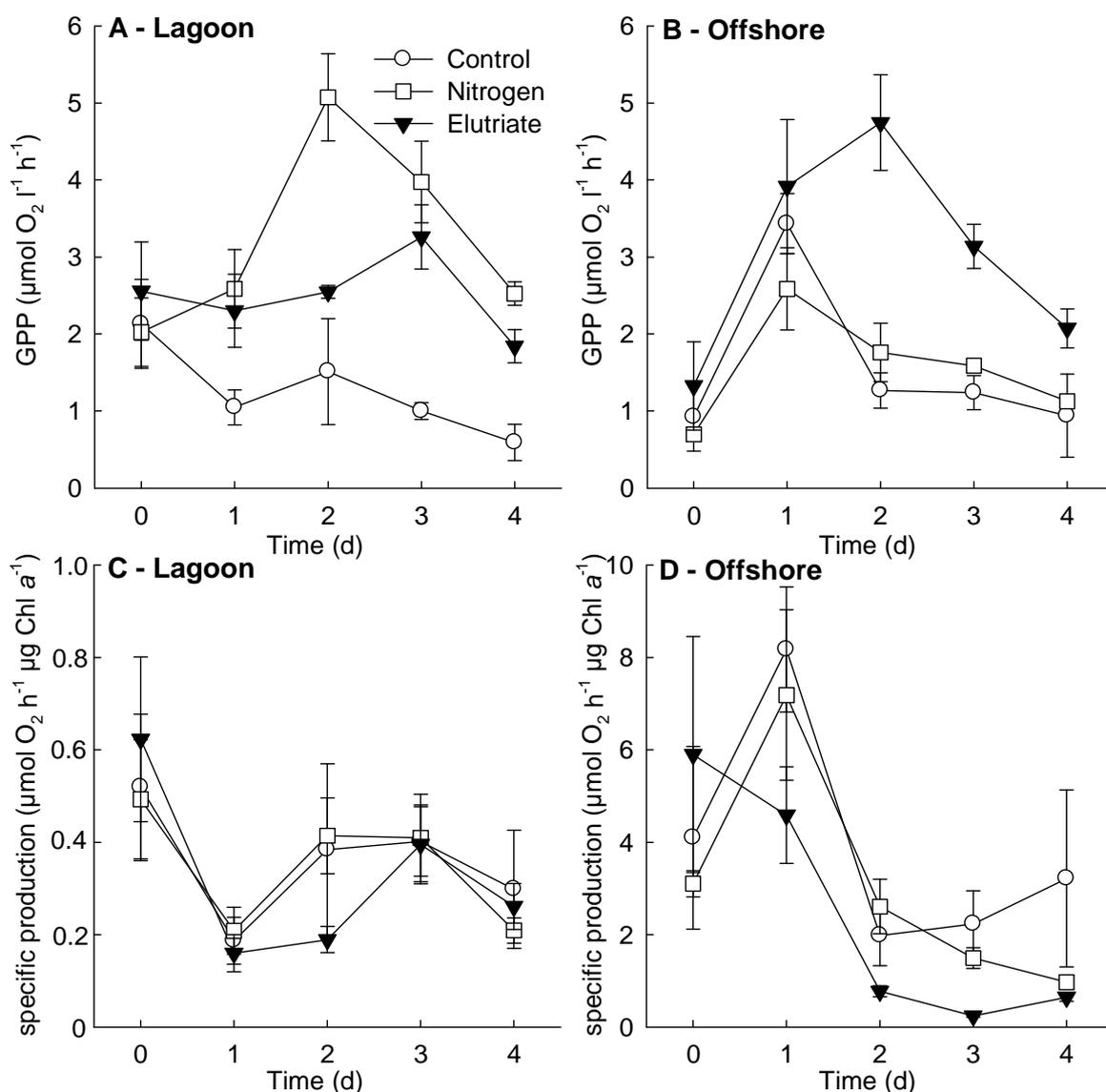
In the lagoon station L, gross primary production (GPP) rates showed a peak ( $> 5 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) in nitrogen enriched microcosm relative to control and elutriate treatments after 48h of incubation (Fig. 5A). Nevertheless, oxygen evolution rates were almost identical in N and E treatments for all the other incubation times, whereas GPP in controls was lower (below  $2 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) and decreased continuously, from start to 96 h. Both incubation time and treatment modified to metabolic pattern (Table 5). In the offshore station, GPP values were significantly stimulated under the three treatments after a short time, from 0.9 to  $3.43 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$  in C, from 0.69 to  $2.58 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$  in N and from 1.32 to  $3.91 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$  in E microcosms (Fig. 5B). Accordingly, during the later part of the incubation period, a decrease in GPP was noticed in the three microcosms. GPP rates were significantly stimulated in E microcosms compared to the C and N ones (Table 5,  $p < 0.05$ ). The average GPP rate was  $3.03 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$  in E, almost doubled compared to the C ( $1.55 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) and N microcosms ( $1.56 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) (Fig. 5B).

variable	Lagoon						Offshore					
	treatment	time	interaction	E-C	N-C	E-N	treatment	time	interaction	E-C	N-C	E-N
chl <i>a</i>	0.0000	0.0000	0.0049	*	*	<i>ns</i>	0.0000	0.0000	0.0000	*	*	*
GPP	0.0000	0.0000	0.0000	*	*	*	0.0000	0.0000	0.0000	*	<i>ns</i>	*
R <sub>dark</sub>	0.0000	0.0000	0.0005	*	*	<i>ns</i>	0.0000	0.0000	0.0000	*	<i>ns</i>	*
NP	0.0004	0.0000	0.0000	*	*	*	<i>0.7165</i>	0.0000	0.0496	<i>ns</i>	<i>ns</i>	<i>ns</i>
P:R	0.0000	0.0000	0.0001	<i>ns</i>	*	*	0.0038	0.0000	0.0000	*	*	<i>ns</i>

375

**Table 5.** Output of multifactorial ANOVA on chl *a* concentrations and metabolic rates for the Lagoon and Offshore experiments, depending on treatment or incubation time. GPP: gross primary production; R<sub>dark</sub>: community respiration in the dark; NP: net production; P:R: production to respiration ratio. P-values are given for each variable; non significant differences are highlighted by italics. Significant differences at 95% confidence levels for treatment comparison (elutriate-control: E-C; nitrogen-control: N-C; elutriate-control: E-C) are marked with an asterisk. *ns*: non significant.

382

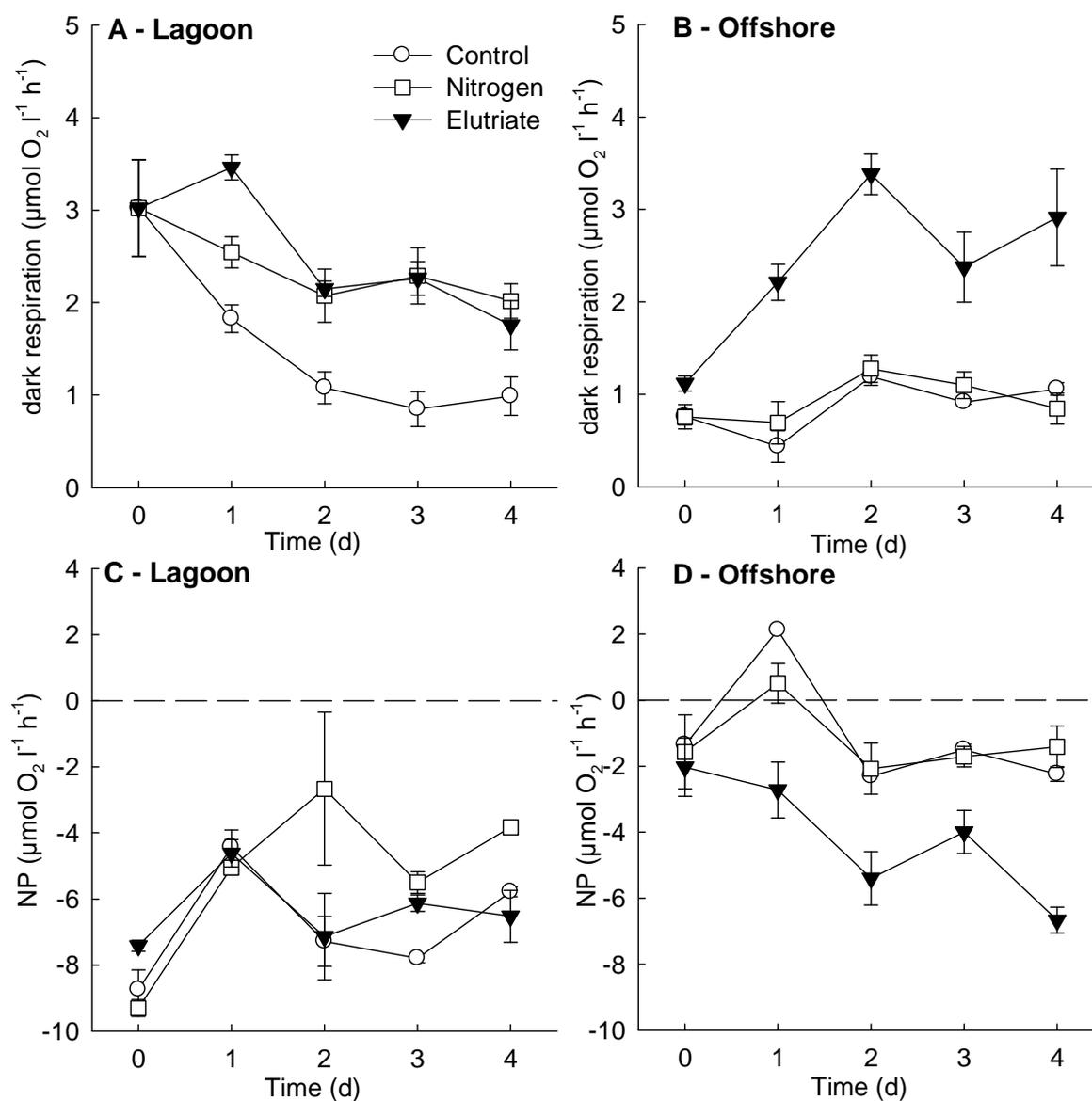


383  
 384 **Figure 5.** Gross oxygen production rates (GPP) measured in the Lagoon (Fig. 5A) and offshore (5B)  
 385 experiment during the incubation, and specific oxygen production per unit of chl *a* in the same  
 386 microcosms (Fig. 5C and 5D). Please note the different y scales for 5C and 5D graphs.

387 From GPP values and chl *a* concentrations, the specific phytoplankton activity was calculated (specific  
 388  $\text{GPP} = \text{GPP} / [\text{chl } a]$ ). A significant decrease was observed in the oxygen productivity in the lagoon  
 389 experiment over the first 24h with a fall from 0.52 to 0.18  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$  in C microcosms, 0.62  
 390 to 0.15  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$  in E microcosms and 0.49 to 0.2  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$  in N microcosms  
 391 (Fig. 5C). No significant differences arose for specific gross primary production between the three  
 392 treatments ( $p > 0.05$ ). GPP increased over the rest of the incubation period and the average specific

393 phytoplankton activity was 0.36, 0.3 and 0.34  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$  respectively for C, E and N  
394 microcosms (Fig. 5C), without any significant differences between the three treatments ( $p>0.05$ ). The  
395 specific phytoplankton activity in the offshore experiment was marked by a significant increase at  
396 24h in C (from 4.1 to 8.2  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$ ) and N (from 3.1 to 7.2  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$ )  
397 microcosms at 24h but a decrease in E (from 6 to 4.5  $\mu\text{mol O}_2 \text{ h}^{-1} \mu\text{g chl } a^{-1}$ ). For the remnant of the  
398 incubation experiment, specific activities decreased regardless of treatment (Fig. 5D). In a general  
399 way, specific activities of communities from the offshore station were one order of magnitude higher  
400 than the ones measured for lagoon communities.

401 The same patterns were observed for dark respiration measurements compare to GPP. In Lagoon  
402 experiment,  $R_{\text{dark}}$  increased significantly ( $p < 0.05$ ) in the E and N microcosms relative to controls  
403 during all the incubation period (Fig. 6A). The average respiration rates measured during the  
404 experiment were 1.55; 2.23 and 2.39  $\mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$  in the C, E and N microcosms, respectively.  
405 Throughout the experiment, the net production did not reach positive values in any of the  
406 microcosms. Compared to the lagoon station, the respiration in the station O was significantly  
407 stimulated only in E microcosms (average rate: 2.39  $\mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) compare to the C (average rate:  
408 0.87  $\mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) and N (average rate: 0.93  $\mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ ) ones (Fig. 6B, Table 5). No significant  
409 differences arose between the control and N microcosms ( $p>0.05$ ). In the offshore station, and  
410 throughout the experiment, net production was not positive in any of the microcosms, except for a  
411 transient state after 24h incubation, where net autotrophy was noticed in C and N microcosms (Fig.  
412 6C). On the contrary, in E microcosms the system appeared heterotrophic throughout the whole  
413 incubation time (Fig. 6D, Table 5).



414  
 415 **Figure 6.** Dark respiration rates measured in the Lagoon (Fig. 6A) and Offshore (6B) experiments  
 416 during the incubation, and net oxygen production (NP) in the same microcosms (Fig. 6D and 6D).  
 417 Dashed line figures the exact balance between gross production and respiration (NP = 0).

## 418 4. Discussion

### 419 4.1. Contaminants and nutrients released from the sediments

420 Concentrations of PAHs in Thau lagoon sediments found during this study were significant ( $\Sigma\text{PAH}_{16} =$   
 421  $8.7 \mu\text{g g}^{-1}$  dry weight), similar to previous reports from studies in the same area ( $\Sigma\text{PAH}_{16} = 0.44$  to  $7.7$   
 422  $\mu\text{g g}^{-1}$  dry weight, Léauté, 2008). Sediments from *Eaux Blanches* bay and *Crique de l'Angle* creek

423 (station S and station L respectively in Fig. 1) have been found to be mainly contaminated with PAHs,  
424 with total amounts of 8 and 7  $\mu\text{g g}^{-1}$  dry weight, respectively (Léauté, 2008). PAHs concentrations in  
425 the sediments of the eastern part of Thau lagoon were similar to those found in other highly  
426 contaminated ecosystems, such as in sediments from Ushuaia Bay (7.6  $\mu\text{g g}^{-1}$  dry weight; Massara  
427 Paletto *et al.*, 2008), but much higher than those found in Daya Bay (South China) (0.04 to 0.158  $\mu\text{g g}^{-1}$   
428 dry weight; Yan *et al.*, 2009), Gironde Estuary (up to 2  $\mu\text{g g}^{-1}$  dry weight; Budzinski *et al.*, 1997) and  
429 Bizerte lagoon (1.49  $\mu\text{g g}^{-1}$  dry weight; Lafabrie *et al.*, 2013b) for example. Polychlorobiphenyls (PCBs)  
430 were also found in significant levels in sediments, at higher concentrations ( $\Sigma\text{PCB7} = 0.059 \mu\text{g g}^{-1}$  dry  
431 weight) than previously reported in the same area ( $\Sigma\text{PCB7} = 0.0006$  to  $0.030 \mu\text{g g}^{-1}$  dry weight;  
432 Léauté, 2008). Despite these high levels in sediment, the concentrations of all PAHs and PCBs in the  
433 elutriate (CW filtered through 0.2  $\mu\text{m}$ ) were below detection limits (Table 1). These PAH and PCB  
434 contaminants have hydrophobic properties and a strong affinity for particles in the water column  
435 (Luellen and Shea, 2002; Tolosa *et al.*, 1995), and may have been removed by filtration together with  
436 particulate matter.

437 The shift from anoxic (sediment in place) to oxic conditions (suspended sediment) may change the  
438 partition and speciation of contaminants, particularly adsorbed trace metals prone to solubilization  
439 (Petersen *et al.*, 1996). Metals were not measured in the sediment in the present study, but previous  
440 published works reported high level of contamination in the *Eaux Blanches* bay (Kawakami *et al.*,  
441 2008). From the top five centimeters layer of a sediment core sampled in the same location (station  
442 5, Fig. 2 in Kawakami *et al.*, 2008), these authors previously found concentrations of 93  $\mu\text{g.g}^{-1}$  for  
443 lead, 2.2  $\mu\text{g.g}^{-1}$  for cadmium, 95.3  $\mu\text{g.g}^{-1}$  for copper, and 29.3  $\mu\text{g.g}^{-1}$  for nickel (sediment dry weight).  
444 This could explain the high concentrations of trace metals in the elutriate (Table 1). A similar study  
445 performed in Biguglia lagoon (Corsica) also reported low levels of PAHs in total elutriates (although  
446 not filtered through 0.2  $\mu\text{m}$ ) ranging from 0.007 to 0.011  $\mu\text{g l}^{-1}$ , but comparatively high  
447 concentrations of trace metals (ranging from 0.07 to 27  $\mu\text{g l}^{-1}$ ) (Lafabrie *et al.*, 2013a). Results

448 presented here show that the resuspension of sediments resulted mostly in the release of metal  
 449 contaminants and nutrients, especially ammonium (20  $\mu\text{M}$ ), in dissolved form in the water column.  
 450 Sediment quality guidelines and ecological safety values for contaminants in marine waters are still  
 451 debated, but several studies attempted to summarize toxicity values for individual chemicals in the  
 452 context of risk assessment. The measured concentration of chemicals and metals in the sediment  
 453 and elutriates used in the present study were compared to known concentrations with predicted  
 454 effects on biota (Table 6). Long *et al.* (1995) defined two threshold levels as Effect Range Low (ERL)  
 455 and Effect Range Median (ERM) derived from effect data distribution on sediment, namely the 10<sup>th</sup>  
 456 and 50<sup>th</sup> percentile respectively, whereas harmful concentration for 5% of the species (HC5) in sea  
 457 water were derived from species-sensitivity distribution curves (Ben Othman *et al.*, 2012, Wang *et*  
 458 *al.*, 2014, Wheeler *et al.*, 2002). For all the values but one measured in the present study,  
 459 contaminant concentrations in sediment were between ERL and ERM, whereas contaminated water  
 460 values were always below documented HC5. Only naphthalene concentration in sediment was below  
 461 ERL (Table 6).

	in sediment (ppb = $\mu\text{g kg}^{-1}$ dry weight)			in contaminated water ( $\mu\text{g l}^{-1}$ )	
	measured*	ERL <sup>§</sup>	ERM <sup>§</sup>	measured*	HC5
organics					
Anthracene	220	85.3	1100	bdl	
Benzo(a)anthracene	780	261	1600	bdl	
Benzo(a)pyrene	870	430	1600	bdl	0.011 <sup>&amp;</sup>
Chrysene	720	384	2800	bdl	
Fluoranthene	1700	600	5100	bdl	21 <sup>!</sup>
Naphthalene	30	160	2100	bdl	
Phenanthrene	740	240	1500	bdl	2.33 <sup>&amp;</sup>
Pyrene	1240	665	2600	bdl	1.09 <sup>&amp;</sup>
$\Sigma_{\text{PCB congeners}}$	59	22.7	180	bdl	
	(ppm = $\mu\text{g g}^{-1}$ dry weight)			(ppm = $\mu\text{g l}^{-1}$ )	
metals	measured <sup>#</sup>	ERL <sup>§</sup>	ERM <sup>§</sup>	measured*	HC5 <sup>£</sup>
Lead	93	46.3	218	0.088	238.2
Cadmium	2.2	1.2	9.6	0.021	8.22
Copper	95.3	34	270	2.49	22.7
Nickel	29.3	20.9	51.6	0.62	597.7

462 **Table 6.** Comparison of sediment and elutriate contamination levels (\*: this study) to documented  
 463 sediment quality guidelines (ERL and ERM) and predicted ecological effects (HC5) for marine waters.  
 464 §: data from Long *et al.*, 1995; #: data from Kawakami *et al.*, 2008; &: data from Wang *et al.*, 2014; !:  
 465 data from Ben Othman *et al.*, 2012; £: data from Wheeler *et al.*, 2002. bdl: below detection limit.

#### 466 **4.2. Effect of contaminated sediment elutriate on phytoplankton growth**

467 Both E and N treatments increased phytoplankton growth in lagoon station, resulting in final chl *a*  
468 concentrations that were two and three times higher in the E and N microcosms, respectively, than  
469 in the control microcosms. By comparison, in the offshore station, supplementation by ammonium  
470 (N microcosms) did not induce increase ( $p > 0.05$ ) in chlorophyll *a* compared to the control (Fig. 2B),  
471 whereas E treatment resulted in a ten times increase compare to C and N treatments. These results  
472 suggest that the nutrients counteract the possible harmful effects of contaminants on phytoplankton  
473 and that nitrogen in the form of  $\text{NH}_4^+$  was likely to be the most limiting element for the  
474 phytoplankton in the lagoon station. In such a situation, nitrogen alone limits the growth of  
475 phytoplankton and elutriate addition can release this limitation. In the offshore community, nitrogen  
476 addition alone did not trigger phytoplankton growth, whereas an unknown limiting factor was made  
477 available by elutriate addition.

478 Such phytoplankton growth stimulation was also observed for example after a wind-mixing event in a  
479 shallow subtropical bay in Florida (Lawrence *et al.*, 2004) and the increases in  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$   
480 concentrations in the water column were identified as the main causes of this effect. Lafabrie *et al.*  
481 (2013b) showed that both elutriate-contaminated and nutrient enriched water increased  
482 phytoplankton growth compared to the control in an experiment performed in the Bizerte Lagoon  
483 (Tunisia). Other experiments by the same research group in the Biguglia lagoon (Corsica) suggest that  
484 brief sediment resuspension events strongly stimulate growth of phytoplankton communities  
485 (Lafabrie *et al.*, 2013a), even when sediments are contaminated by toxic chemicals. By contrast,  
486 other studies have reported a decrease in the total phytoplankton biomass after exposure to a  
487 combination of contaminants (mix of trace elements) and nutrients (*e.g.*, Riedel *et al.*, 2003). The  
488 responses of phytoplankton to a combination of contaminants and nutrients may, therefore, depend  
489 on threshold levels above which the contaminants become toxic.

490 There may be other mechanisms that stimulate phytoplankton growth in the present study. The E  
491 microcosms were slightly enriched with metallic micronutrients, such as nickel and zinc (Table 1)

492 which are known to be essential for cell metabolism. These metals are involved in the stabilization of  
493 protein structure and also facilitate transfer reactions and/or catalysis of the enzymatic reactions  
494 (Torres *et al.*, 2008). For example, Zn plays a key role in the photosynthetic electron transport  
495 between the thylakoids (Raven *et al.*, 1999). Molybdenum (which was the most abundant trace  
496 metal in the E microcosms) Mn and Fe are involved as enzyme cofactors in respiration,  
497 photosynthesis and nitrogen metabolisms and may be limiting for natural phytoplankton populations  
498 *in situ* (Boyd *et al.*, 1996; Howarth and Cole 1985). Essential metal-micronutrients (Zn, Mo, Mn and  
499 Fe) which were abundant in the elutriate could therefore favor algal growth provided that their  
500 concentrations do not exceed the tolerance thresholds of organisms. It has been shown that some  
501 dissolved metals (Co, Ni and Zn) are able to increase the chlorophyll biomass, but they may also be  
502 harmful at higher concentrations (Chakraborty *et al.*, 2010).

#### 503 **4.3. Effect of contaminated sediment elutriate on phytoplankton structure**

504 The results of the present study in lagoon station showed that diatoms were stimulated in the N  
505 microcosms compared to other treatments. This appeared contradictory with Lafabrie *et al.* (2013a)  
506 who showed that the high  $\text{NH}_4^+$  levels (100 $\mu\text{M}$ ) in their experiment resulted in a decrease in diatom  
507 abundance. Actually, this concentration was 5 times higher than the  $\text{NH}_4^+$  levels (20 $\mu\text{M}$ ) used in the  
508 present study, and can be explained by a potential toxicity of ammonium at high concentrations. In  
509 the offshore station, diatoms were stimulated in the E microcosms compare to other treatments.  
510 Chikhaoui *et al.* (2008) highlighted the fact that dissolved silicate can be a limiting factor and an  
511 essential element to the growth and metabolism of diatoms communities, even in coastal, nutrient-  
512 rich waters. Diatoms in the E microcosms were represented exclusively by potentially toxic *Pseudo-*  
513 *nitzschia* species in both experiments (inoculum from lagoon and offshore stations). This genus has  
514 been shown to produce domoic acid, an amnesic shellfish toxin, which is able to chelate various  
515 metals (such as Fe and Cu) and, therefore, can counteract the toxicity of these pollutants (Lelong *et*  
516 *al.*, 2012; Rue and Bruland, 2001). It has also been reported that some *Pseudo-nitzschia* species (*P.*  
517 *australis* and *P. multiseriis*) withstand toxic concentrations of Cu by increasing domoic acid

518 production (Ladizinsky, 2003; Maldonado *et al.*, 2002). This could suggest that the dominance of  
519 *Pseudo-nitzschia* in the E microcosms in the two stations, enriched in trace metals from sediment,  
520 may be related to their ability to produce domoic acid. This makes them more resistant to metal  
521 toxicity than other species that do not have this ability, such as *Nitzschia longissima* in lagoon station,  
522 which disappeared from the E microcosms, and the decrease of the contribution of *Chaetoceros* spp.  
523 in offshore station, suggesting that these species were sensitive to dissolved trace metals.

524 An increase in the cyanobacteria biomass was observed in lagoon station in the ammonium enriched  
525 microcosms (N microcosms). Such a pattern was also reported by Fouilland *et al.* (2012), concluding  
526 that inorganic nitrogen limited the growth of prokaryotic picophytoplankton in Thau lagoon. More  
527 generally, cyanobacteria are often considered as more tolerant to chemicals, such as reported for  
528 example in the study of Stachowski-Haberkorn *et al.* (2009), after exposing marine phytoplankton to  
529 a fungicide (Opus at  $100\mu\text{g l}^{-1}$ ) which resulted in an increase in *Synechococcus* population density.

530 Regarding the dinoflagellates group, the persistence of *Alexandrium* sp. and relatively high  
531 abundance in the E microcosms (with high level of heavy metals) in the lagoon experiment suggests a  
532 relative tolerance of this species to metals. The close relative *Alexandrium catenella* was shown to be  
533 able to resist to Cu toxicity by increasing dissolved organic carbon (DOC) release (Herzi *et al.*, 2013).  
534 For dinoflagellates from offshore station, many species in E and N microcosms were depauperate  
535 compare to the control microcosms. These species were *Scrippsiella* sp.; *Prorocentrum* sp. and  
536 *Prorocentrum minimum*, appearing therefore not tolerant to the sediment elutriate.

537 Correspondence analysis highlighted the fact that treatments effects were more pronounced in  
538 offshore station compared to lagoon station (Fig. 5), and demonstrated a wide divergence between  
539 the initial sample and the three treatments C, N and E microcosms in offshore station. This suggests  
540 that phytoplankton sampled from offshore station were more sensitive to the sediment elutriate  
541 effects and to the added ammonia compared to the phytoplankton from lagoon station. This can be  
542 supported by the observed changes in Shannon index  $H$  values. At the end of incubations, the  
543 phytoplankton diversity in lagoon station did not differ in the E and N treatments compared to the

544 control ( $H$  index:  $1.7 \pm 0.03$ ;  $1.55 \pm 0.02$  and  $1.6 \pm 0.01$  bits  $\text{ind}^{-1}$ , respectively in C, E and N  
545 microcosms) whereas in offshore station the diversity in C microcosms ( $1.41 \pm 0.04$  bits  $\text{ind}^{-1}$ ) was  
546 significantly higher than in E ( $1.32 \pm 0.002$  bits  $\text{ind}^{-1}$ ) and N microcosms ( $1.19 \pm 0.03$  bits  $\text{ind}^{-1}$ ).

#### 547 **4.4. Effect of contaminated sediment elutriate on community production and respiration**

548 In lagoon experiment, community respiration followed the same trend as phytoplankton biomass.  
549 Actually, the  $R_{\text{dark}}$  increased 3 times in N and E microcosms compared to the control ( $P < 0.05$ ). For the  
550 offshore experiment, only E treatment resulted in an up to 3-fold increase in dark respiration  
551 compared to control microcosms. In this last experiment,  $R_{\text{dark}}$  did not change compared to the  
552 control ( $P > 0.05$ ) under N treatment. This suggests that the respiration in lagoon station was limited  
553 by nitrogen, whereas this element was not a limiting factor for respiratory activity at offshore  
554 station.

555 The specific phytoplankton activity (GPP:chl  $a$ ) decreased significantly in the control and both  
556 treatments (N and E microcosms) during all the incubation time in lagoon station. Almost the same  
557 pattern was observed in the three microcosms treatments in offshore station but, only after 24h, a  
558 transient increase of GPP:chl  $a$  was noticed only in C and N treatments compared to E ones. The  
559 studied phytoplankton communities in N and C microcosms appeared physiologically more efficient  
560 than in E microcosms after 24h incubation in offshore station. Increase in the specific phytoplankton  
561 activity, together with slight phytoplankton community structure changes, have been previously  
562 reported in a mesocosm experiment for communities exposed to a mix of nutrients with an organic  
563 contaminant (pyrene; Hjorth *et al.*, 2008).

564 The net production gives information on the “trophic state” of a system, currently linked to the  
565 production:respiration ratio;  $P:R > 1$  indicates a net autotrophy state, whereas  $P:R < 1$  indicates a net  
566 heterotrophy state (Dodds and Cole, 2007). In lagoon station, net production was always negative,  
567 highlighting the fact that the three microcosms C, N and E were net heterotrophic during all the  
568 incubation time. The systems were also heterotrophic in the offshore station in E, N and C  
569 microcosms but for this experiment a transient state appeared for only C and N microcosms, during

570 which the plankton communities were net autotrophic. Previous field-based studies also highlighted  
571 the drift of planktonic communities' metabolism towards heterotrophy shortly after sediment  
572 resuspension events (Cotner *et al.*, 2000; Lawrence *et al.*, 2004). Similarly, laboratory experiments  
573 showed a reduction of the *P:R* ratio after exposure to trace metal, reduction of the *P:R* ratio that  
574 resulted in a bacterially dominated heterotrophic system (Nayar *et al.*, 2004; Rochelle-Newall *et al.*,  
575 2008).

## 576 **5. Conclusion**

577 This ecotoxicological experiment, conducted on natural phytoplankton sampled from the Thau  
578 lagoon and Mediterranean offshore waters, showed that elutriate prepared from sediments  
579 stimulated phytoplankton growth despite significant contamination by potentially toxic chemicals.  
580 Sediments involved in exposure experiment were considered as significantly contaminated by metals  
581 and organics based on quality assessment thresholds. For both phytoplankton communities,  
582 significant increases in phytoplankton biomass and cell abundance were observed after elutriate  
583 addition. For the lagoon station, the stimulatory effect of elutriate was similar to that of adding  $\text{NH}_4^+$   
584 alone, without any toxic chemical. It is, therefore, likely that the nutrient present in elutriate  
585 (especially nitrogen) caused the proliferation of primary producers. On the other hand, the  
586 phytoplankton community from the offshore reference station was clearly beneficial from elutriate  
587 addition based on observed growth, since nitrogen alone did not promote community proliferation.  
588 This may mask the possible harmful effects of contaminants, making it difficult to detect them. On  
589 the contrary, the community in the reference station was not limited by the  $\text{NH}_4^+$  addition.  
590 The elutriate used in the present study was particularly contaminated with dissolved metals, some of  
591 these known to be toxic to phytoplankton (Cd, Pb), while others (Zn, Mo, Mn, Fe) may be essential  
592 for cellular metabolism at low concentrations. As a consequence, the presence of essential trace  
593 metals in sediment elutriate could be a possible cause of increased phytoplankton growth, provided  
594 that their levels remain below the tolerance threshold of these organisms.

595 Contamination also changed the community structure of Thau and offshore phytoplankton. It  
596 appears that adding sediment elutriate was most beneficial to species that were potentially toxic.  
597 The most significant change concerned the pronounced proliferation of potentially toxic diatoms  
598 (*Pseudo-nitzschia* sp.) and dinoflagellates (*Alexandrium* sp. in lagoon station and *Gymnodinium* sp. in  
599 offshore station) after the addition of sediment elutriate. Hence, it could be suggested that these  
600 microalgae have defense mechanisms (synthesis of toxin excretion of dissolved organic matter, for  
601 example) making them more resistant to chemical contamination (especially metallic in the present  
602 study). For the phytoplankton in offshore, and in the very short term, the system shifted to  
603 autotrophy after sediment elutriate addition compared to the control. By contrast, the lagoon  
604 community metabolic balance remained net heterotrophic during all the experiments and for all  
605 treatments. Apart from changes in community composition, such a transient shift in the global  
606 metabolic balance of plankton community should be considered when addressing the ecological  
607 impact of contaminated sediment in coastal marine ecosystems. The present results showed also  
608 that the treatments effects were more pronounced in offshore station compared to the lagoon  
609 station, suggesting that phytoplankton in offshore station were more sensitive to both elutriate and  
610 added ammonia compared to the phytoplankton in lagoon station. Future progress in the risk  
611 assessment of contaminated sediment for planktonic organisms will benefit from enhanced  
612 knowledge on toxicity of pure chemicals to marine microorganisms (e.g. Ben Othman *et al.*, 2012)  
613 together with community level evaluation of toxic chemical cocktails taking into consideration the  
614 stimulating effects of co-released nutrients (Pringault *et al.*, 2016). These approaches will help in  
615 discriminating true toxic effects, linked to toxic contamination, from nutrient enrichment effects that  
616 accompany sediment resuspension events.

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