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Impacts of chemical contamination on bacterio-phytoplankton coupling

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Abstract :

Phytoplankton and bacterioplankton are the key components of the organic matter cycle in aquatic ecosystems, and their interactions can impact the transfer of carbon and ecosystem functioning. The aim of this work was to assess the consequences of chemical contamination on the coupling between phytoplankton and bacterioplankton in two contrasting marine coastal ecosystems: lagoon waters and offshore waters. Bacterial carbon demand was sustained by primary carbon production in the offshore situation, suggesting a tight coupling between both compartments. In contrast, in lagoon waters, due to a higher nutrient and organic matter availability, bacteria could rely on allochthonous carbon sources to sustain their carbon requirements, decreasing so the coupling between both compartments. Exposure to chemical contaminants, pesticides and metal trace elements, resulted in a significant inhibition of the metabolic activities (primary production and bacterial carbon demand) involved in the carbon cycle, especially in offshore waters during spring and fall, inducing a significant decrease of the coupling between primary producers and heterotrophs. This coupling loss was even more evident upon sediment resuspension for both ecosystems due to the important release of nutrients and organic matter. Resulting enrichment alleviated the toxic effects of contaminants as indicated by the stimulation of phytoplankton biomass and carbon production, and modified the composition of the phytoplankton community, impacting so the interactions between phytoplankton and bacterioplankton.

Highlights

► Phytoplankton and bacterioplankton were strongly coupled in offshore waters. ► Coupling between both compartments was less evident in lagoon waters. ► Sediment resuspension and chemical contamination differently impacted lagoon and offshore waters. ► Chemical contamination loosened the coupling between both compartments. ► Nutrients and organic matter released during sediment resuspension strongly decreased the bacterio–phytoplankton coupling.

Keywords : Pesticides, Trace metallic elements, Carbon cycle, Primary production, Bacterial carbon demand

1 Introduction

Bacterioplankton and phytoplankton are the key players in the carbon cycle of aquatic systems. The two microbial communities are involved in the de novo production of organic matter by phytoplankton, as well as in its mineralization by bacterioplankton. Since they represent the first levels of the pelagic marine food web (Berglund et al., 2007), it is imperative to understand their functioning and their interactions within the environment to better assess the consequences of natural or anthropic perturbations for a given aquatic ecosystem. In coastal zone and shallow-water environments, water column chemistry is closely related to the sediment interface dynamics (Hochard et al., 2010), thus, the functioning of the bacterioplankton and phytoplankton can be strongly affected during storm events resulting in the release of nutrients, microorganisms and contaminants into the water column (Roberts, 2012).

The impact of chemical contamination on the first levels of the trophic chain can be observed from a structural point of view, with changes observed in the microbial community, through the selection of species considered tolerant to contaminants (Dorigo et al., 2010; Lekunberri et al., 2010; Pesce et al., 2009). The impact can also be observed from a functional point of view, with modifications of the carbon cycle such as the stimulation of heterotrophy versus autotrophy upon metal spiking (Rochelle-Newall et al., 2008). Although the extent of the threat of contamination is large and has been increasing due to demographic patterns and resulting anthropic pressure on the coastal zone (Schwarzenbach et al., 2006; Small and Nicholls, 2003), pollution remains one of the least-studied stressors in ecology to date (Johnston et al., 2015). As a general rule, chemical contaminants are often considered as stressors due to their toxic potential. In this context, chemical contaminants were demonstrated to induce marine ecosystem functioning alterations, mainly by decreasing productivity (primary production) and increasing respiration (Johnston et al., 2015). These changes in ecosystem functioning by chemical contamination can be concomitant

with modification of the biodiversity (alpha and beta) and/or diversity losses (Johnston and Roberts, 2009), with consequences on interactions between bacterioplankton and phytoplankton (Goni-Urriza et al., 2018) that may, in turn, impact the higher trophic levels (Hansson et al., 2013; Hulot et al., 2000).

The existence of phytoplankton–bacterioplankton coupling is supported by a significant correlation between primary production and heterotrophic bacterial production (Bouvy et al., 1998; Cole et al., 1982), but revisited more recently using a larger dataset comparing dissolved primary production and bacterial carbon demand in different aquatic ecosystems (Fouilland and Mostajir, 2011, 2010). In coastal areas, bacteria production may or not strongly depends on phytoplankton exudates according to the availability of other sources of carbon such recycled trophic carbon sources (Fouilland et al., 2014) or external terrestrial sources (Fouilland et al., 2018; Morán et al., 2002). In these latter cases, the bacterial carbon demand can largely exceed phytoplankton production (Morán et al., 2002)The coupling between phytoplankton and bacterioplankton mediates the carbon transfer to the trophic web and consequently controls the ecosystem functioning. Anthropogenic perturbations like nutrient loading (Prieto et al., 2015), global warming (Hoppe et al., 2008) or acidification (Hornick et al., 2017) can severely alter the coupling between primary producers and heterotrophic bacteria. Nevertheless, the availability of labile carbon sources is often linked with chemical molecules whose effects are difficult to discern on the interactions between both compartments. We suggest that the release of contaminants by sediments in coastal waters might affect the degree of coupling between phytoplankton and bacteria through two ways: i) the inhibition of phytoplankton communities linked to the toxicity of chemical compounds, and ii) the addition of external source of carbon for heterotrophic bacteria.

In this context, the goal of this study was to assess the impact of chemical contamination on the coupling between phytoplankton and bacterioplankton in two

contrasting ecosystems, marine offshore waters and lagoon waters. The two contrasting ecosystems were sampled to test the hypothesis that the bacterial and phytoplankton response to chemical contamination would be different in the two sampling sites, with possible consequences for the coupling between both compartments, considering the possible selective pressure and the adaptation that can be triggered by the chemical environment according to the concept of pollution-induced community tolerance (Blanck, 2002). For that purpose, water incubations in 9L microcosms were performed in different seasons, with additions of a sediment elutriate or an artificial mixture of contaminants mimicking the main contaminants present in sediment elutriate (pesticides and metal trace elements). The chemical changes, along with the experiments with both contaminant exposures, were previously analyzed (Bancon-Montigny et al., 2019), and the present study focused on the changes observed for phytoplankton and bacterioplankton activities and the consequences for the coupling between both compartments.

2 Materials and Methods

2.1 Study sites and sampling

The study was conducted in southwestern Mediterranean ecosystems, the lagoon and the bay of Bizerte (Fig. S1 supplementary materials) during four distinct seasons spring (April 2014), winter (February 2015), fall (November 2015) and summer (July 2016). Like most Mediterranean coastal lagoons (Arvanitidis et al., 2009), the Bizerte lagoon (lagoon station) in the north of Tunisia is a polluted coastal ecosystem subject to intense agriculture, urbanization and industrialization pressures, as well as pressures from naval and commercial shipping harbors (Barhoumi et al., 2014a, 2014b; Yoshida et al., 2002). Consequently, sediments are strongly contaminated by a wide range of pollutants, including organic contaminants (polycyclic aromatic hydrocarbons, pesticides and herbicides) and metals (Barhoumi et al., 2014a, 2014b; Yoshida et al., 2002). The Bizerte bay is less contaminated than the lagoon, although

local polycyclic aromatic hydrocarbons (PAH) contamination has been recorded in the effluent from the oil refinery located on the shore of the bay (Mhadhbi et al., 2019; Zrafi-Nouira et al., 2009, 2008). The sampling was carried out in an offshore station (station O, 37° 16' 46.46'' N 9° 53' 50.98'' E) and a lagoon station (station L, 37° 12' 43.96'' N 9° 50' 79.78'' E), as shown in Fig. S1 (supplementary materials). Water samples were collected from a 2 m depth; more details of the sampling procedure can be found in Pringault et al. (2016). Contaminated water was obtained from the resuspension of polluted sediment following the protocol described by Bonnet et al. (2000); this protocol, recommended by the US Environmental Protection Agency (US EPA), has been successfully used to characterize the contamination level and toxicity of sediments using nonadapted species (Bonnet et al., 2000; Carr and Chapman, 1995). Polluted sediment was sampled in front of a cement factory in the lagoon channel (station CH, 37° 15' 40.22'' N 9° 51' 30.49'' E) as shown in Fig. S1, using a Van Veen grab. On the same day of sediment sampling, water was also sampled in the same station CH (see Pringault et al., 2016); this channel zone between lagoon and marine bay is among the most polluted sites in the Bizerte lagoon (Ben Said et al., 2010; Yoshida et al., 2002). Just after sampling, the sediment was sieved (2 mm mesh) to remove large particles and stored in a cool box until its return to the laboratory. In the laboratory, sediment was mixed with channel water (1:4 w/v ratio), and sediment was gently stirred for 8 h. After a 12 h settling period, the overlying solution, called thereafter as "elutriate", was smoothly siphoned off and stored in the dark at 4 °C until use in microcosm incubations.

2.2 Incubation procedure

During the four studied seasons, seawater was incubated for four days in 9 L glass microcosms (22.5 cm diameter and 23 cm height), covered with a quartz lid to allow full penetration of the natural sunlight, including UV radiation, according to the protocol described in Pringault et al. (2016) and briefly detailed below. A series of three microcosms per treatment was filled with 6 L of sample water (L and O

stations; control microcosms, C). Another series of three microcosms was filled with 4.5 L of sample water (L and O stations) completed with 1.5 L of elutriate to get a final dilution of 25% (contaminated water microcosms; CW). The third series of three microcosms (artificial contaminated water microcosms; ACW) was filled with 6 L (L and O stations) and spiked an artificial solution of pesticides and some metabolites (acetochlore, alachlore, DCPU 1-(3,4-dichlorophenyl)urea, DIA deisopropylatrazine, diuron, linuron and simazine) and metals (Ni, Cu, Zn, Cd, As, Pb). Final concentration of pesticides and metals were adjusted to mimic the concentrations observed in elutriate as wall as possible (Table S1, supplementary materials) (see Bancon-Montigny et al., 2019, for more details). All microcosms, performed in triplicate, were incubated outside under natural sunlight in a 3 m^3 pool where seawater was circulating (open system) to maintain *in situ* water temperature. Two light sensors (Skye, England), measuring incident photosynthetically active radiation (PAR; 400–700 nm, quantum SKP 215) and ultraviolet A and B radiation (315–380 nm, SKU 420 and 280–315 nm, SKU 430, respectively), monitored the light received at the surface of microcosms.

2.3 Chemical analysis

Chemical analysis of the nutrients, Chlorophyll *a* (Chl*a)* dissolved organic carbon (DOC), organic contaminants (pesticides and polycyclic aromatic hydrocarbons) and metal trace elements (MTE) were performed using the analytical protocols described in Bancon-Montigny et al. (2019) and Pringault et al. (2016).

2.4 Phytoplankton counts

Water samples were collected from each microcosm (245 mL) at the beginning and the end of the microcosm incubation, then immediately fixed with buffered formaldehyde at 2% final concentration. Species identification and counts were realized with a BX60 Olympus microscope coupled with a Progress C3–cooled Jenoptik camera. Each species was identified to the highest possible taxonomic level (class, genus or species) according to Halse et al. (1997), Hoppenrath et al. (2009),

Kraberg et al. (2010), Viličić (2014) and the WoRMS database (http://www.marinespecies.org). Counts were realized according to the Utermöhl technique (1958) and the AFNOR standards (2006). Each taxon was counted individually, except *Cyanophyceae* spp., *Chaetoceros* spp., *Pseudonitzschia* spp., *Prymnesiophyceae* spp., *Euglenoidea* spp. and many flagellates, which were grouped into general taxa because of species identification difficulties. Two groups of flagellates were distinguished by size class, <10 μm and 10–15 μm, without distinction of the taxonomic class.

2.5 Metabolic activities

Community respiration (CR) and primary production (PP) was measured using an oxygen microelectrode (Unisense, Denmark) following the protocols described by Briand et al. (2004) for CR, and by Pringault et al. (2007) for PP. Water samples (1 mL) were placed in gas-tight glass microchambers and immersed in a water bath with controlled temperature and light (*in situ* temperature and *in situ* light). Dissolved O_2 concentration was measured a minimum of four times during the 8–12 h of incubation in the vessels. Community respiration and net production (NP) were deduced from the linear regression established on measurement points in the dark for CR and in the light for NP. Primary production was then computed as the sum CR + NP. Bacterial respiration (BR) was expressed in μg C L^{-1} h^{-1} using a respiratory quotient of 1 (del Giorgio and Cole, 1998) and assuming that BR represented 30% of the total community respiration (Aranguren-Gassis et al., 2012). Bacterial respiration was used to estimate bacterial carbon demand (BCD), which represents the sum BP + BR, BP representing bacterial production measured by ^{3}H-thymidine incorporation (Smith and Azam, 1992). A detailed protocol can be found in Pringault et al. (2016). Thymidine incorporation rates were converted into carbon production using the conversion factors of 2.10^{18} cells produced per mole of thymidine incorporated and 20 fg C per cell (Lee and Bong, 2008).

2.6 Statistical analysis

Coupling between phytoplankton and bacterioplankton was estimated using the approach proposed by Moran et al. (2002) by calculating the linear regression between PP and BCD with a regression significance when $p < 0.05$. Analysis of variance (ANOVA) were performed to test the significance of the differences observed. Prior to analysis of variance, normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were checked. When these conditions were not met, differences were evaluated using the nonparametric Kruskall–Wallis ANOVA test. Posteriori paired multiple-comparisons were then performed using the Tukey HSD (honestly significant difference) test. ANOVA tests and Tukey's HSD tests were carried out with the level of significance set at $p < 0.05$ (Statgraphics Centurion XV software).

3 Results

3.1 In situ conditions

The environmental conditions (Table 1) were significantly different depending on the periods (seasons) and sampling sites (L and O stations). Chl*a*, nutrients and DOC were significantly ($p < 0.05$) higher in lagoon waters. Metabolic activities exhibited also differences according to season and sites. The highest rate of primary production (PP) were observed during summer for offshore waters whereas community respiration (CR) was maximal during fall for the lagoon system (Table 1). In the lagoon ecosystem, CR was always higher than PP (PP:CR ≤0.3), while PP largely exceeded CR (PP:CR = 1.5–1.6) in winter and summer in offshore waters. Seasonal variations for environmental conditions were observed for both sites; minimum values for phytoplankton biomass were observed during winter, whereas maximum Chl*a* were observed during spring for offshore and fall for lagoon waters. Metabolic activities also showed significant variations ($p < 0.05$) according to the season, with maximum rates during summer and fall and minimum values during winter for both

sites. As observed for environmental conditions, the phytoplankton community structure (expressed as relative abundance of the main phytoplankton groups) also exhibited significant variations according to the season and sampling site (two factors ANOVA p <0.05) (Fig. 1). Lagoon and offshore waters were dominated by Ochrophyta in summer, whereas dinoflagellates were the dominant group in spring for lagoon, and in fall for offshore waters. Winter was characterized by the dominance of Haptophyta in offshore waters and flagellates in lagoon waters.

Data regarding the chemical contamination of both sites by pesticides and metal trace elements (Table S1, supplementary materials) are given in more details in Bancon-Montigny et al. (2019).

3.2 Chlorophyll a, phytoplankton groups and metabolic activities during microcosm incubation

Incubation in microcosms during 96 h provoked significant changes for biomass (Fig. 2 and 3) and metabolic activities (Fig. 4 and 5) in all treatments, whatever the season and the sampling site. As a general rule, incubation with elutriate (CW) provoked a strong increase in Chl*a* and bacterial biomass (Fig. 2 and 3) especially in offshore waters, with values up to 5 times the concentration (Chl*a*) observed in controls (C) at the end of the incubation period. It worth noticing that sediment elutriate (CW) provoked a significant increase of bacterial biomass relative to control (Student Test, p<0.05), noticed at the beginning of the incubation (Fig. 3). The changes in Chl*a* concentrations were also accompanied by modifications of the phytoplankton community composition (relative abundance of the main phytoplankton groups) with significant differences among treatments (one factor ANOVA p<0.05) (Fig. S2 Supplementary materials).. Marked changes were noticed, especially during winter and fall, in both ecosystems. As observed for Chl*a*, the main significant modifications of phytoplankton composition relative to C microcosm were observed with elutriate incubation (CW). During summer, offshore and lagoon waters were dominated by Ochrophyta, representing up to 80% of the phytoplankton relative abundance for L

station; this dominance was not modified by the presence of sediment elutriate (CW). In contrast, in the fall, when the dinoflagellates and flagellates (lagoon and offshore sites) were the dominant phytoplankton groups, the impact of the elutriate and artificial contaminant cocktail on phytoplankton structure were more pronounced with significant (p <0.05) modifications of the relative abundance of the main phytoplankton groups, relative to the phytoplankton composition observed in the controls. A similar pattern was also observed during winter when the flagellates (lagoon) and the Haptophyta (offshore) were the dominant phytoplankton groups. Upon sediment elutriate, Cryptophyta became the most abundant group in lagoon waters (in fall and winter), whereas Ochrophyta (winter) and flagellates (winter and fall) dominated in offshore waters. The large increases in Chl*a* concentrations in CW treatments were concomitant with an important stimulation of primary production, especially in offshore waters, well superior to those observed in lagoon waters (Fig. 4 and 5). The most pronounced effects of elutriate (CW) on Chl*a* and metabolic activities were observed during spring and summer for offshore waters and during spring for lagoon. As a general rule, changes were less marked in ACW microcosms, where the artificial spiking with a cocktail of pesticides and metals provoked minor changes regarding phytoplankton biomass as well as in the phytoplankton composition. The most pronounced significant (p <0.05) inhibition effects were observed in offshore waters, with final phytoplankton biomass representing less than 50% and 30% of the control values for fall and winter, respectively. For lagoon waters, inhibition of phytoplankton biomass by ACW treatment was even less pronounced, with a significant (p <0.05) inhibition (around 50%) only observed during winter. Surprisingly, during the fall Chl*a* concentrations were higher in ACW microcosms relative to control conditions.

Regarding the metabolic activities (PP and BCD), spiking with the artificial contaminant cocktail provoked significant inhibition of PP and to a less extent of BCD in offshore waters, with values representing on some occasions less than 50% of the control values. As observed for Chl*a* concentrations, the impact of artificial

277 contamination (ACW) was significantly less pronounced in lagoon waters for both
278 PP and BCD (two factors ANOVA $p<0.05$). The most significant ($p<0.05$) effects on
279 both metabolic activities were observed during summer with up to 50% for PP and
280 30% for BCD of inhibition relative to control conditions.

281 *3.3 Bacterio–Phytoplankton coupling*

282 The metabolic activities (PP and BCD) measured during microcosm incubation were
283 used to estimate the strength of the phytoplankton–bacterioplankton coupling
284 depending on the treatments. Data measured at each season were pooled, and the
285 linear relationship between PP and BCD was estimated according to the
286 experimental conditions imposed (Fig. 6 and 7). In offshore waters, for the control
287 conditions, PP and BCD were strongly positively correlated ($r = 0.806$, $p<0.0001$) with
288 a slope very close to 1 (1.05 ± 0.10). In contrast, for the C treatment in lagoon waters,
289 the linear relationship between PP and BCD was weaker ($r = 0.504$) with a significant
290 decrease of the slope, 0.35 ± 0.08, relative to the value measured in offshore waters
291 for the same conditions. Incubation with elutriate (CW) and with an artificial cocktail
292 of pesticides and MTE (ACW) both provoked similar effects in offshore and lagoon
293 waters; a significant reduction of the slope and of the correlation coefficient, relative
294 to the respective values measured in C treatment (Student test, $p<0.05$). The most
295 pronounced differences were measured for offshore waters in CW microcosms, with
296 a slope of 0.27 ± 0.07 ($r = 0.472$, $p = 0.0007$), relative to 1.05 ± 0.10 measured in C
297 microcosms. In lagoon waters, incubation with elutriate (CW) also resulted in a
298 significant reduction of the slope with a value of 0.21 ± 0.06 relative to 0.37 ± 0.08
299 measured in C microcosms. Changes were less marked in ACW microcosm for both
300 ecosystems, nevertheless, the decrease of the slope relative to the control value
301 remained significant (Student test, $p<0.05$).

4 Discussion

The aim of the present study was to assess the consequences of chemical contamination on the coupling between phytoplankton and bacterioplankton in two contrasting ecosystems, an open oceanic ecosystem versus a semi-enclosed lagoon ecosystem. The contaminated sediment used for sediment resuspension was sampled in the lagoon channel, and according to the water circulation and the wind regime (Harzallah, 2003), contaminants released by sediment resuspension following strong gusts or stormy events can affect the pelagic communities in both studied ecosystems. The two studied Mediterranean ecosystems exhibited contrasting environmental conditions during the four seasons (Table 1) that were concomitant to marked differences in chemical contamination (Table S1, supplementary materials); offshore waters were significantly less contaminated than the lagoon (Bancon-Montigny et al., 2019). These two contrasted environments allowed testing of the hypothesis that the bacterial and phytoplankton response to chemical contamination would be different in the two sampling sites, with possible consequences for the coupling between both compartments, considering the possible selective pressure and the adaptation that can be triggered by the chemical environment, according to the concept of pollution-induced community tolerance (Blanck, 2002).

4.1 In situ biomass and metabolic activities

The two Mediterranean ecosystems, lagoon and offshore, were sampled during the four seasons. The metabolic rates (CR: community respiration and PP: primary production) and the phytoplankton biomass measured in the present study were in agreement with previous studies in this region (Meddeb et al., 2018; Sakka Hlaili et al., 2008) and were comparable to reports from similar ecosystems in the Mediterranean basin (Ciavatta et al., 2008; Pérez-Ruzafa et al., 2005; Sfriso et al., 2003), with seasonal variations for both marine and lagoon waters (Salhi et al., 2018). As a general rule, phytoplankton biomass and metabolic activities involved in the C cycle (PP, CR and BCD) were significantly higher in lagoon waters relative to

offshore waters except for PP in summer were higher rates were observed in offshore waters relative to lagoon. Similarly, significant differences were observed in the phytoplankton composition between the two ecosystems, confirming the particular trophic status played by semi-enclosed ecosystems relative to their counterpart marine adjacent waters (Newton et al., 2014). Despite a large open channel to coastal sea, the watershed influence and the very long water residence time (> 200 days) observed in the Bizerte lagoon (Bejaoui et al., 2017) relative to the offshore adjacent waters (<20 days; (Sayol et al., 2013), allows accumulation of nutrients, DOC and chemical contaminants within the lagoon. In addition, temperature and salinity variations showed distinct patterns with lower temperature and salinity during winter and spring in lagoon waters (compared to the offshore situation), whereas an opposite situation was observed during the summer, where temperature and salinity values were maximal in the lagoon system (Béjaoui et al., 2016). These distinct environmental conditions favored the development of different planktonic species in the lagoon environment relative to the marine adjacent waters, as confirmed by the phytoplankton composition (Fig. 1) but also for the bacterioplankton structure (Goni-Urriza et al., 2018).

Seasonal variations were observed for the phytoplankton diversity and the metabolic activities involved in the C cycle, suggesting a strong impact on the organic matter cycle with cascading consequences for the carbon transfer within the trophic web. Using a modeling approach to estimate the flux and exchanges between the different components of the pelagic marine trophic web, it was possible to assess the functioning and stability of the trophic web (Sakka Hlaili et al., 2014). Meddeb et al. (2019, 2018) reported food-web structure differences between the marine and lagoon ecosystems in the Bizerte area, with herbivorous and multivorous dominant carbon pathways in the marine and the lagoon, respectively. The ecological indices, like the total system throughput (Niquil et al., 2012), used as proxies of the ecosystem functioning, suggests a more efficient carbon transfer in the open marine ecosystem, whereas the lagoon environment was more active, but more stressed and less

organized (Meddeb et al., 2018). According to the ratio between primary production and community respiration (PP:CR; Table 1), a strong autotrophic activity occurred in offshore waters during winter and summer seasons, while the lagoon ecosystem seemed to be mainly driven by heterotrophic activity (CR>>PP); this supports the assumption that an intense recycling of organic matter occurred in the lagoon waters through the bacterial use of organic matter derived from the sediments and the watershed depending on the bioavailability of OM which strongly affect its mineralization by bacteria (Blanchet et al., 2017; Decho and Gutierrez, 2017).

4.2 Impact of contamination on diversity and function

Microbial communities from open (offshore) and lagoon waters were exposed to sediment elutriate and to a cocktail of chemical contaminants (MTE and pesticides). The contaminants cocktail was designed from the main compounds identified and analysed in sediment elutriate (Table S1, supplementary materials) in order to assess the toxic effects of these contaminants without confounding chemical factors, nutrients and/or dissolved organic matter that are released during sediment resuspension (Pringault et al., 2016). Nutrients and organic matter are known to strongly mitigate the toxicity of pesticides and TME against microorganisms, mainly attributed to a decrease of contaminant bioavailability by adsorption on dissolved organic matter (Boyd et al., 2005; Coquillé et al., 2018; Lorenzo et al., 2002) and changes in the nutrient status, toxicity increasing under nutrient limitation (Chia et al., 2016; Miao and Wang, 2006). For offshore waters, spiking with pesticides and MTE resulted in an inhibition of phytoplankton biomass as well as a reduction of metabolic activities involved in the carbon cycle (PP and BCD). Previous studies have observed that environmentally relevant concentrations of pesticides, within the range of ng $L^{-1,}$ similar to those observed in the artificial contamination (ACW), can severely affect primary production of microalgae with a strong reduction of photosynthetic efficiency up to 50% (Devilla et al., 2005). Similarly, MTE can also strongly reduce phytoplankton biomass and primary production of natural phytoplankton

communities (Pérez et al., 2006) at μg L^{-1} concentrations, in the same order of magnitude than those used in ACW microcosm for the artificial spiking. As a general rule, when assessing the impact of pollution on the marine carbon cycle and ecosystem functioning, primary production is more affected by chemical contamination than respiration, especially upon herbicide or metal trace contamination, with a strong reduction of O_2 production or CO_2 fixation, whereas respiration remains less impacted (Johnston et al., 2015). Nevertheless, the inhibition of metabolic processes or biomass production observed when pelagic communities are exposed to a single molecule can be alleviated when contaminants are used in mixture (Johnston et al., 2015), due to the antagonist effects than pollutant can exert between them (Franklin et al., 2002; Sharma et al., 1999). This hypothesis can be advanced to explain the absence of clear toxic effects on phytoplankton biomass and metabolic rates of the artificial mixture from pesticides and metal trace elements (ACW) for the lagoon waters. In addition, as mentioned before, the lagoon ecosystem is characterized by a very long water residence time (>200 days), which favors the accumulation of pollutants and nutrients. This long period can promote the development of a community resistant to chemical pollution according to the concept of pollution induced community tolerance (Blanck, 2002) and observed for meiobentic communities (Boufahja and Semprucci, 2015). Furthermore, the accumulation of pollutants and nutrients in lagoon waters results in weak enrichment when exposed to the artificial cocktail of contaminants (Bancon-Montigny et al., 2019), reducing the potential toxicity of the added contaminants for the microbial lagoon communities.

It is worth noting that the toxic effects observed in offshore waters upon artificial spiking (ACW) were alleviated when the contaminants present in the sediment elutriate were accompanied by nutrients and dissolved organic matter (CW). When offshore waters were exposed to elutriate (CW), phytoplankton biomass and bacterial abundances strongly increased as well as metabolic activities (primary production and bacterial carbon demand). The strong stimulation of BCD in CW

treatment (Figs. 4) can partly be explained by the external supply of bacteria by sediment resuspension, as indicated by the significant increase in bacterial abudance observed at the onset of microcosm incubation in CW relative to control (Fig. 3). Nevertheless, previous study showed that this increase in bacterial biomass upon sediment resuspension provoked minor changes on bacterial diversity and community structure (Goni-Urriza et al., 2018). Stimulation of biomass and carbon production was most obvious during spring and fall when flagellates and dinoflagellates dominated the phytoplankton community. In anthropogenic coastal environment, sediment resuspension results in the release in the water column of a trail of contaminants, nutrients and dissolved organic matter as well as benthic microorganisms (Roberts, 2012), leading to enhancement of primary production and phytoplankton biomass, directly by increasing the number of microalgae cells and indirectly by stimulating, through nutrients supply, phytoplankton growth (Guallar and Flos, 2017). In the present study, sediment elutriate was artificially mixed (1/4 final volume) with offshore or lagoon waters. This mixing resulted in important enrichment in nutrients (especially in dissolved ammonium and silicates), dissolved organic matter, metal trace elements (Mn, Fe, Mo, Zn and Ni) and pesticides (simazine, alachlor, deisopropylatrazine and 1-(3,4-dichlorophenyl)urea) (Bancon-Montigny et al., 2019) and exogenous bacteria by sediment resuspension (Goni-Urriza et al., 2018). Enrichments in nutrients, DOM and chemical contaminants were more pronounced in offshore waters relative to lagoon waters, explained by higher initial concentrations of compounds in the lagoon; thus the addition of sediment elutriate with their compounds does not change the chemical status of the lagoon significantly (Bancon-Montigny et al., 2019). For offshore waters, the metabolic and biomass changes upon elutriate were more pronounced during spring and fall with Chl*a* concentration and primary production up to five times more than observed in the control. Spring and fall were characterized by important enrichment in nutrients, iron and manganese upon elutriate exposure (CW) whereas chemical contamination by Ni, Zn and pesticides were relatively low (Bancon-Montigny et al., 2019). In

445 contrast, during winter, the impact of elutriate was reduced for both phytoplankton
446 biomass and metabolic activities. This period was characterized by a strong
447 enrichment in ammonium (33µM relative to *in situ* 0.4 µM before mixing)
448 concomitant with enrichment in pesticides and Ni, with twice the *in situ*
449 concentration (Bancon-Montigny et al., 2019). It is very likely that this strong
450 enrichment in ammonium and chemical contaminants did not promote favorable
451 conditions for phytoplankton growth, as indicated by the low Chl*a* concentration and
452 PP rates achieved after exposure relative to control values.

453 *4.3 Bacterio–phytoplankton coupling and chemical contamination*

454 Primary production (PP) and bacterial carbon demand were strongly correlated (r =
455 0.806, p <0.0001) in control treatments for offshore waters with a slope close to 1
456 (1.046) suggesting a tight coupling between phytoplankton and bacterioplankton; the
457 carbon demand of the heterotrophic compartment could be totally sustained by
458 primary production (Morán et al., 2002). For lagoon waters, despite a significant
459 positive linear relationship, the slope was strongly lower (0.35) as well as the
460 correlation coefficient of the slope. This clearly indicated a weaker interdependency
461 between both compartments, especially during fall and winter. In these two seasons,
462 lagoon BCD strongly exceeded primary production, consequently, bacterioplankton
463 must rely on allochthonous organic carbon to sustain its carbon requirements. These
464 differences between lagoon and offshore situations in the bacterio–phytoplankton
465 coupling are in agreement with the main observations reported in marine
466 ecosystems, with tighter coupling and interdependency between bacterioplankton
467 and phytoplankton increasing from the coast to the open ocean (Fouilland et al.,
468 2018; Morán et al., 2002).

469 When lagoon or offshore waters were incubated with a mixture of contaminants
470 (pesticides and MTE, ACW), the linear relationships between both compartments
471 slightly changed, with, in both cases, a significant decrease of the slope and the
472 coefficient correlation, suggesting a decrease in the carbon dependency with respect

to primary production to sustain the heterotrophic metabolism. These changes on the carbon dependency were more pronounced during winter and fall in offshore waters with a strong reduction of primary reduction when Haptophyta or dinoflagellates dominated the phytoplankton community. Inhibition of primary production were concomitant to significant changes of the relative abundance of the main phytoplankton groups. Interactions between phytoplankton and bacteria are species-dependent (Seymour et al., 2017) and a shift in phytoplankton community structure can modify bacterial production, bacterial abundance and bacterial community structure (Camarena-Gómez et al., 2018) with consequently incidences on the bacterio-phytoplankton coupling. In addition, under chemical contamination, phytoplankton can release dissolved organic matter as extracellular polymeric substances (EPS), binding out of the cell, the toxic compounds like metal trace elements, or pesticides (Decho and Gutierrez, 2017; Naveed et al., 2019). Such defense mechanisms were reported in phytoplankton cultures (Decho and Gutierrez, 2017; Herzi et al., 2013a, 2013b) as well as in natural phytoplankton communities (Rochelle-Newall et al., 2008, 2011) or peryphyton biofilm (Ivorra et al., 2000). The binding of chemical compounds on the phytoplankton-derived dissolved organic matter can modify its composition and its physicochemical properties (Naveed et al., 2019) with incidences on its bioavailability and lability (Enya et al., 2020; Wada and Suzuki, 2011), decreasing so the potential utilization by the heterotrophic compartment (Seto et al., 2013), eventually loosening the coupling between both compartments.

When the natural community from offshore and lagoon waters were incubated with elutriate (CW), the slope and the correlation coefficient of the linear relationships between BCD and PP strongly decreased relative to their corresponding control value, especially for offshore waters, with a slope of 0.27 ($r = 0.472$) relative to 1.05 ($r = 0.806$) in the control. For lagoon waters, the slope was even lower (0.18) relative to 0.35 in the control treatment. These very low values, clearly indicated that the dependency between both compartments was weak when exposed to sediment

elutriate. Sediment resuspension released a significant amount of organic matter, nutrients and bacteria, which resulted in a strong enrichment in DOM, nutrients and bacterial abundance in offshore and lagoon waters, knowing the low concentrations of DOM and nutrients measured *in situ* (Bancon-Montigny et al., 2019). The release of DOM and nutrients consecutive to sediment resuspension is known to stimulate both phytoplankton and bacterioplankton metabolisms (Roberts, 2012; Uchimiya et al., 2016) with serious consequences on the biogeochemical cycles (Cotner, 2000) depending on the bioavailability of OM that strongly affects its recycling (Blanchet et al., 2017; Decho and Gutierrez, 2017). These conditions favor a loosening of the coupling between phytoplankton and bacterioplankton; the heterotrophic compartment may have access to external carbon sources, decreasing its potential dependency on the phytoplankton exudates. In addition, nutrients released by sediment can also modify the phytoplankton composition by alleviating nutrient competition within the community and allowing the growth of microalgae taxa previously nutrient-limited. A previous study has shown that sediment resuspension may alter the synchrony between bacterial and phytoplanktonic compartments (Goni-Urriza et al., 2018). Consequently, the tight coupling observed in offshore waters between phytoplankton and bacterioplankton can be loosened upon sediment resuspension by several non exclusive factors i) a modification of the phytoplankton composition through the release of sediment nutrients, altering so interactions with the heterotrophic bacteria, ii) the supply of an external source of labile carbon that can be preferentially used by heterotrophs, iii) the supply of exogenous bacteria that can enhance the overall bacterial carbon demand.

5 Conclusion

The result of the present study clearly showed that phytoplankton and bacterioplankton were strongly coupled in offshore waters while the coupling was less obvious in the lagoon ecosystem. We clearly showed that the presence of contaminants altered the interactions between phytoplankton and bacterioplankton,

with a loosening of the coupling observed in offshore waters. This fact was even more marked upon sediment resuspension due to the strong supplies of nutrients, dissolved organic carbon and exogenous bacteria. Considering the climate scenarios in the Mediterranean basin that predict an increase of storms and paroxystic events (Cramer et al., 2018), with consequently higher occurrences of sediment resuspension, the functioning of coastal ecosystems will be severely impacted by strong alterations of the interactions between bacterioplankton and phytoplankton with non-negligible consequences for the carbon cycle and the CO_2 emissions.

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7 References cited

AFNOR, AFNOR, 2006. Norme guide pour le dénombrement du phytoplankton par microscopie inverse (méthode Utermöhl). , Comité Technique CEN/TC 230 "Analyse de l'eau."

Aranguren-Gassis, M., Teira, E., Serret, P., Martinez-Garcia, S., Fernandez, E., 2012. Potential overestimation of bacterial respiration rates in oligotrophic plankton communities. Mar. Ecol. Prog. Ser. 453, 1–10. https://doi.org/10.3354/meps09707

Arvanitidis, C., Somerfield, P.J., Chatzigeorgiou, G., Reizopoulou, S., Kevrekidis, T., Eleftheriou, A., 2009. Do multivariate analyses incorporating changes in pattern across taxonomic levels reveal anthropogenic stress in Mediterranean lagoons? J. Exp. Mar. Bio. Ecol. 369, 100–109. https://doi.org/10.1016/j.jembe.2008.10.032

Bancon-Montigny, C., Gonzalez, C., Delpoux, S., Avenzac, M., Spinelli, S., Mhadhbi, T., Mejri, K., Hlaili, A.S., Pringault, O., 2019. Seasonal changes of chemical contamination in coastal waters during sediment resuspension. Chemosphere

235, 651–661. https://doi.org/10.1016/j.chemosphere.2019.06.213

Barhoumi, B., LeMenach, K., Devier, M.H., Ben Ameur, W., Etcheber, H., Budzinski, H., Cachot, J., Driss, M.R., 2014a. Polycyclic aromatic hydrocarbons (PAHs) in surface sediments from the Bizerte Lagoon, Tunisia: levels, sources, and toxicological significance. Environ. Monit. Assess. 186, 2653–2669. https://doi.org/10.1007/s10661-013-3569-5

Barhoumi, B., LeMenach, K., Devier, M.H., El Megdiche, Y., Hammami, B., Ben Ameur, W., Ben Hassine, S., Cachot, J., Budzinski, H., Driss, M.R., 2014b. Distribution and ecological risk of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in surface sediments from the Bizerte lagoon, Tunisia. Environ. Sci. Pollut. Res. 21, 6290–6302. https://doi.org/10.1007/s11356-013-1709-7

Béjaoui, B., Armi, Z., Ottaviani, E., Barelli, E., Gargouri-Ellouz, E., Chérif, R., Turki, S., Solidoro, C., Aleya, L., 2016. Random Forest model and TRIX used in combination to assess and diagnose the trophic status of Bizerte Lagoon, southern Mediterranean. Ecol. Indic. 71, 293–301. https://doi.org/10.1016/j.ecolind.2016.07.010

Bejaoui, B., Solidoro, C., Harzallah, A., Chevalier, C., Chapelle, A., Zaaboub, N., Aleya, L., 2017. 3D modeling of phytoplankton seasonal variation and nutrient budget in a southern Mediterranean Lagoon. Mar. Pollut. Bull. 114, 962–976. https://doi.org/10.1016/j.marpolbul.2016.11.001

Ben Said, O., Goni-Urriza, M., El Bour, M., Aissa, P., Duran, R., 2010. Bacterial Community Structure of Sediments of the Bizerte Lagoon (Tunisia), a Southern Mediterranean Coastal Anthropized Lagoon. Microb. Ecol. 59, 445–456.

Berglund, J., Müren, U., Båmstedt, U., Andersson, A., 2007. Efficiency of a phytoplankton-based and a bacteria-based food web in a pelagic marine system. Limnol. Oceanogr. 52, 121–131. https://doi.org/10.4319/lo.2007.52.1.0121

Blanchet, M., Pringault, O., Panagiotopoulos, C., Lefèvre, D., Charrière, B., Ghiglione, J.-F., Fernandez, C., Aparicio, F.L., Marrasé, C., Catala, P., Oriol, L., Caparros, J.,

586 Joux, F., 2017. When riverine dissolved organic matter (DOM) meets labile DOM
587 in coastal waters: changes in bacterial community activity and composition.
588 Aquat. Sci. 79, 27–43. https://doi.org/10.1007/s00027-016-0477-0
589 Blanck, H., 2002. A critical review of procedures and approaches used for assessing
590 pollution-induced community tolerance (PICT) in biotic communities. Hum.
591 Ecol. Risk Assess. 8, 1003–1034.
592 Bonnet, C., Babut, M., Ferard, J.F., Martel, L., Garric, J., 2000. Assessing the potential
593 toxicity of resuspended sediment. Environ. Toxicol. Chem. 19, 1290–1296.
594 Boufahja, F., Semprucci, F., 2015. Stress-induced selection of a single species from an
595 entire meiobenthic nematode assemblage: is this possible using iron enrichment
596 and does pre-exposure affect the ease of the process? Environ. Sci. Pollut. Res.
597 22, 1979–1998. https://doi.org/10.1007/s11356-014-3479-2
598 Bouvy, M., Arfi, R., Cecchi, P., Corbin, D., Pagano, M., Saint-Jean, L., Thomas, S.,
599 1998. Trophic coupling between bacterial and phytoplanktonic compartments in
600 shallow tropical reservoirs (Ivory Coast, West Africa). Aquat. Microb. Ecol. 15,
601 25–37. https://doi.org/10.3354/ame015025
602 Boyd, T.J., Wolgast, D.M., Rivera-Duarte, I., Holm-Hansen, O., Hewes, C.D., Zirino,
603 A., Chadwick, D.B., 2005. Effects of dissolved and complexed copper on
604 heterotrophic bacterial production in San Diego Bay. Microb. Ecol. 49, 353–366.
605 https://doi.org/10.1007/s00248-003-1065-0
606 Briand, E., Pringault, O., Jacquet, S., Torréton, J.P., 2004. The use of oxygen
607 microprobes to measure bacterial respiration for determining bacterioplankton
608 growth efficiency. Limnol. Oceanogr. Methods 2.
609 https://doi.org/10.4319/lom.2004.2.406
610 Camarena-Gómez, M.T., Lipsewers, T., Piiparinen, J., Eronen-Rasimus, E., Perez-
611 Quemaliños, D., Hoikkala, L., Sobrino, C., Spilling, K., 2018. Shifts in
612 phytoplankton community structure modify bacterial production, abundance
613 and community composition. Aquat. Microb. Ecol.
614 https://doi.org/10.3354/ame01868

615 Carr, R.S., Chapman, D.C., 1995. Comparison of Methods for Conducting Marine and
616 Estuarine Sediment Porewater Toxicity Tests - Extraction, Storage, and Handling
617 Techniques. Arch. Environ. Contam. Toxicol. 28, 69–77.
618 Chia, M.A., Dauda, S., Jibril, T.Z., 2016. Toxicity of atrazine to Scenedesmus
619 quadricauda under different nitrogen concentrations. Environ. Earth Sci. 75, 960.
620 https://doi.org/10.1007/s12665-016-5766-3
621 Ciavatta, S., Pastres, R., Badetti, C., Ferrari, G., Beck, M.B., 2008. Estimation of
622 phytoplanktonic production and system respiration from data collected by a
623 real-time monitoring network in the Lagoon of Venice. Ecol. Modell. 212, 28–36.
624 https://doi.org/10.1016/j.ecolmodel.2007.10.025
625 Cole, J.J., Likens, G.E., Strayer, D.L., 1982. Photosynthetically produced dissolved
626 organic carbon: An important carbon source for planktonic bacteria. Limnol.
627 Oceanogr. 27, 1080–1090. https://doi.org/10.4319/lo.1982.27.6.1080
628 Coquillé, N., Ménard, D., Rouxel, J., Dupraz, V., Éon, M., Pardon, P., Budzinski, H.,
629 Morin, S., Parlanti, É., Stachowski-Haberkorn, S., 2018. The influence of natural
630 dissolved organic matter on herbicide toxicity to marine microalgae is species-
631 dependent. Aquat. Toxicol. 198, 103–117.
632 https://doi.org/10.1016/j.aquatox.2018.02.019
633 Cotner, J.B., 2000. Intense winter heterotrophic production stimulated by benthic
634 resuspension. Limnol. Oceanogr. 45, 1672–1676.
635 Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J.P., Iglesias, A., Lange, M.A.,
636 Lionello, P., Llasat, M.C., Paz, S., Peñuelas, J., Snoussi, M., Toreti, A., Tsimplis,
637 M.N., Xoplaki, E., 2018. Climate change and interconnected risks to sustainable
638 development in the Mediterranean. Nat. Clim. Chang.
639 https://doi.org/10.1038/s41558-018-0299-2
640 Decho, A.W., Gutierrez, T., 2017. Microbial extracellular polymeric substances (EPSs)
641 in ocean systems. Front. Microbiol. 8, 922.
642 https://doi.org/10.3389/fmicb.2017.00922
643 del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic

644 systems. Annu. Rev. Ecol. Syst. 29, 503–541.
645 Devilla, R.A., Brown, M.T., Donkin, M., Tarran, G.A., Aiken, J., Readman, J.W., 2005.
646 Impact of antifouling booster biocides on single microalgal species and on a
647 natural marine phytoplankton community. Mar. Ecol. Prog. Ser. 286, 1–12.
648 https://doi.org/10.3354/meps286001
649 Dorigo, U., Berard, A., Rimet, F., Bouchez, A., Montuelle, B., 2010. In situ assessment
650 of periphyton recovery in a river contaminated by pesticides. Aquat. Toxicol. 98,
651 396–406. https://doi.org/10.1016/j.aquatox.2010.03.011
652 Enya, O., Heaney, N., Iniama, G., Lin, C., 2020. Effects of heavy metals on organic
653 matter decomposition in inundated soils: Microcosm experiment and field
654 examination. Sci. Total Environ. 724, 138223.
655 https://doi.org/10.1016/j.scitotenv.2020.138223
656 Fouilland, E., Le Floc'h, E., Brennan, D., Bell, E.M., Lordsmith, S.L., Mcneill, S.,
657 Mitchell, E., Brand, T.D., Elena García-Martín, E., Leakey, R.J.G.G., Floc'h, E. Le,
658 Brennan, D., Bell, E.M., Lordsmith, S.L., Mcneill, S., Mitchell, E., Brand, T.D.,
659 García-Martín, E.E., Leakey, R.J.G.G., 2018. Assessment of bacterial dependence
660 on marine primary production along a northern latitudinal gradient. FEMS
661 Microbiol. Ecol. 94, 150. https://doi.org/10.1093/femsec/fiy150
662 Fouilland, E., Mostajir, B., 2011. Complementary support for the new ecological
663 concept of "bacterial independence on contemporary phytoplankton
664 production" in oceanic waters. FEMS Microbiol. Ecol.
665 https://doi.org/10.1111/j.1574-6941.2011.01170.x
666 Fouilland, E., Mostajir, B., 2010. Revisited phytoplanktonic carbon dependency of
667 heterotrophic bacteria in freshwaters, transitional, coastal and oceanic waters.
668 FEMS Microbiol. Ecol. 73, 419–429. https://doi.org/10.1111/j.1574-
669 6941.2010.00896.x
670 Fouilland, E., Tolosa, I., Bonnet, D., Bouvier, C., Bouvier, T., Bouvy, M., Got, P., Le
671 Floc'h, E., Mostajir, B., Roques, C., Sempéré, R., Sime-Ngando, T., Vidussi, F.,
672 2014. Bacterial carbon dependence on freshly produced phytoplankton exudates

673 under different nutrient availability and grazing pressure conditions in coastal
674 marine waters. FEMS Microbiol. Ecol. 87, 757–769. https://doi.org/10.1111/1574-
675 6941.12262
676 Franklin, N.M., Stauber, J.L., Lim, R.P., Petocz, P., 2002. Toxicity of metal mixtures to
677 a tropical freshwater alga (Chlorella sp): The effect of interactions between
678 copper, cadmium, and zinc on metal cell binding and uptake. Environ. Toxicol.
679 Chem. 21, 2412–2422. https://doi.org/10.1897/1551-
680 5028(2002)021<2412:tommta>2.0.co;2
681 Goni-Urriza, M., Moussard, H., Lafabrie, C., Carre, C., Bouvy, M., Sakka Hlaili, A.,
682 Pringault, O., 2018. Consequences of contamination on the interactions between
683 phytoplankton and bacterioplankton. Chemosphere 195.
684 https://doi.org/10.1016/j.chemosphere.2017.12.053
685 Guallar, C., Flos, J., 2017. Enhanced primary production in summer and winter-
686 spring seasons in a populated NW Mediterranean coastal ecosystem. J. Sea Res.
687 120, 1–12. https://doi.org/10.1016/j.seares.2016.10.009
688 Halse, G.R., Syvertsen, E.E., Steidinger, K.A., Tangen, K., Throndsen, J., Heimdal,
689 B.R., 1997. Identifying marine phytoplankton. Academic Press.
690 Hansson, L.A., Nicolle, A., Graneli, W., Hallgren, P., Kritzberg, E., Persson, A., Bjork,
691 J., Nilsson, P.A., Bronmark, C., 2013. Food-chain length alters community
692 responses to global change in aquatic systems. Nat. Clim. Chang. 3, 228–233.
693 https://doi.org/10.1038/nclimate1689
694 Harzallah, A., 2003. Transport de polluants dans la lagune de Bizerte simulé par un
695 modèle de circulation de l'eau. Bull. l'Institut Natl. des Sci. Technol. la Mer,
696 Salambôo 1–19.
697 Herzi, F., Jean, N., Zhao, H.Y., Mounier, S., Mabrouk, H.H., Sakka Hlaili, A., 2013a.
698 Copper and cadmium effects on growth and extracellular exudation of the
699 marine toxic dinoflagellate Alexandrium catenella: 3D-fluorescence spectroscopy
700 approach. Chemosphere 93, 1230–1239.
701 https://doi.org/https://doi.org/10.1016/j.chemosphere.2013.06.084

Herzi, F., Sakka Hlaili, A., Le Poupon, C., Mabrouk, H.H., Mounier, S., 2013b. Characterization of exudates released by the marine diatom Skeletonema costatum exposed to copper stress: a 3D-fluorescence spectroscopy approach. Biometals 26, 773–781. https://doi.org/10.1007/s10534-013-9655-x

Hochard, S., Pinazo, C., Grenz, C., Evans, J.L.B., Pringault, O., 2010. Impact of microphytobenthos on the sediment biogeochemical cycles: A modeling approach. Ecol. Modell. 221. https://doi.org/10.1016/j.ecolmodel.2010.04.002

Hoppe, H.G., Breithaupt, P., Walther, K., Koppe, R., Bleck, S., Sommer, U., Jürgens, K., 2008. Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: A mesocosm study. Aquat. Microb. Ecol. 51, 105–115. https://doi.org/10.3354/ame01198

Hoppenrath, M., Elbrachter, M., Drebes, G., 2009. Selected Microphytoplankton Species from the North Sea Around Helgoland and Sylt. Kleine Senckenberg-Reihe 49, Stuttgart, Germany.

Hornick, T., Bach, L.T., Crawfurd, K.J., Spilling, K., Achterberg, E.P., Woodhouse, J.N., Schulz, K.G., Brussaard, C.P.D., Riebesell, U., Grossart, H.P., 2017. Ocean acidification impacts bacteria-phytoplankton coupling at low-nutrient conditions. Biogeosciences 14, 1–15. https://doi.org/10.5194/bg-14-1-2017

Hulot, F.D., Lacroix, G., Lescher-Moutoue, F.O., Loreau, M., 2000. Functional diversity governs ecosystem response to nutrient enrichment. Nature 405, 340–344. https://doi.org/10.1038/35012591

Ivorra, N., Bremer, S., Guasch, H., Kraak, M.H.S., Admiraal, W., 2000. Differences in the sensitivity of benthic microalgae to Zn and Cd regarding biofilm development and exposure history. Environ. Toxicol. Chem. 19, 1332–1339.

Johnston, E.L., Mayer-Pinto, M., Crowe, T.P., 2015. Chemical contaminant effects on marine ecosystem functioning. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.12355

Johnston, E.L., Roberts, D.A., 2009. Contaminants reduce the richness and evenness of marine communities: A review and meta-analysis. Environ. Pollut.

https://doi.org/10.1016/j.envpol.2009.02.017

Kraberg, A., Baumann, M., Dürselen, C.-D., 2010. Coastal phytoplankton. Photo guide for Northern European Seas. Verlag, Munchen Germany.

Lee, C.W., Bong, C.W., 2008. Bacterial abundance and production, and their relation to primary production in tropical coastal waters of Peninsular Malaysia. Mar. Freshw. Res. 59, 10–21. https://doi.org/10.1071/MF07099

Lekunberri, I., Calvo-Diaz, A., Teira, E., Moran, X.A.G., Peters, F., Nieto-Cid, M., Espinoza-Gonzalez, O., Teixeira, I.G., Gasol, J.M., 2010. Changes in bacterial activity and community composition caused by exposure to a simulated oil spill in microcosm and mesocosm experiments. Aquat. Microb. Ecol. 59, 169–183.

Lorenzo, J.I., Nieto, O., Beiras, R., 2002. Effect of humic acids on speciation and toxicity of copper to Paracentrotus lividus larvae in seawater. Aquat. Toxicol. 58, 27–41. https://doi.org/10.1016/s0166-445x(01)00219-3

Meddeb, M., Grami, B., Chaalali, A., Haraldsson, M., Niquil, N., Pringault, O., Sakka Hlaili, A., 2018. Plankton food-web functioning in anthropogenically impacted coastal waters (SW Mediterranean Sea): An ecological network analysis. Prog. Oceanogr. 162. https://doi.org/10.1016/j.pocean.2018.02.013

Meddeb, M., Niquil, N., Grami, B., Mejri, K., Haraldsson, M., Chaalali, A., Pringault, O., Hlaili, A.S., 2019. A new type of plankton food web functioning in coastal waters revealed by coupling Monte Carlo Markov chain linear inverse method and ecological network analysis. Ecol. Indic. 104. https://doi.org/10.1016/j.ecolind.2019.04.077

Mhadhbi, T., Pringault, O., Nouri, H., Spinelli, S., Beyrem, H., Gonzalez, C., 2019. Evaluating polar pesticide pollution with a combined approach: a survey of agricultural practices and POCIS passive samplers in a Tunisian lagoon watershed. Environ. Sci. Pollut. Res. 26. https://doi.org/10.1007/s11356-018-3552-3

Miao, A.J., Wang, W.X., 2006. Cadmium toxicity to two marine phytoplankton under different nutrient conditions. Aquat. Toxicol. 78, 114–126.

760 Morán, X.A.G., Estrada, M., Gasol, J.M., Pedros-Alio, C., 2002. Dissolved primary
761 production and the strength of phytoplankton bacterioplankton coupling in
762 contrasting marine regions. Microb. Ecol. 44, 217–223.
763 Naveed, S., Li, C., Lu, X., Chen, S., Yin, B., Zhang, C., Ge, Y., 2019. Microalgal
764 extracellular polymeric substances and their interactions with metal(loid)s: A
765 review. Crit. Rev. Environ. Sci. Technol. 49, 1769–1802.
766 https://doi.org/10.1080/10643389.2019.1583052
767 Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A.C., Colijn, F., Riva, S.D., Gertz,
768 F., Hansen, J.W., Holmer, M., Ivanova, K., Leppäkoski, E., Canu, D.M., Mocenni,
769 C., Mudge, S., Murray, N., Pejrup, M., Razinkovas, A., Reizopoulou, S., Pérez-
770 Ruzafa, A., Schernewski, G., Schubert, H., Carr, L., Solidoro, C., PierluigiViaroli,
771 Zaldívar, J.-M.M., Viaroli, P., Zaldívar, J.-M.M., 2014. An overview of ecological
772 status, vulnerability and future perspectives of European large shallow, semi-
773 enclosed coastal systems, lagoons and transitional waters. Estuar. Coast. Shelf
774 Sci. 140, 95–122. https://doi.org/10.1016/j.ecss.2013.05.023
775 Niquil, N., Chaumillon, E., Johnson, G.A., Bertin, X., Grami, B., David, V., Bacher, C.,
776 Asmus, H., Baird, D., Asmus, R., 2012. The effect of physical drivers on
777 ecosystem indices derived from ecological network analysis: Comparison across
778 estuarine ecosystems. Estuar. Coast. Shelf Sci. 108, 132–143.
779 https://doi.org/10.1016/j.ecss.2011.12.031
780 Pérez-Ruzafa, A., Fernández, A.I., Marcos, C., Gilabert, J., Quispe, J.I., García-
781 Charton, J.A., 2005. Spatial and temporal variations of hydrological conditions,
782 nutrients and chlorophyll a in a Mediterranean coastal lagoon (Mar Menor,
783 Spain), in: Hydrobiologia. pp. 11–27. https://doi.org/10.1007/s10750-005-4356-2
784 Pérez, P., Estévez-Blanco, P., Beiras, R., Fernández, E., 2006. EFFECT OF COPPER
785 ON THE PHOTOCHEMICAL EFFICIENCY, GROWTH, AND CHLOROPHYLL
786 A BIOMASS OF NATURAL PHYTOPLANKTON ASSEMBLAGES. Environ.
787 Toxicol. Chem. 25, 137. https://doi.org/10.1897/04-392R1.1
788 Pesce, S., Batisson, I., Bardot, C., Fajon, C., Portelli, C., Montuelle, B., Bohatier, J.,

2009. Response of spring and summer riverine microbial communities following glyphosate exposure. Ecotoxicol. Environ. Saf. 72, 1905–1912.

Prieto, A., Barber-Lluch, E., Hernández-Ruiz, M., Martínez-García, S., Fernández, E., Teira, E., 2015. Assessing the role of phytoplankton-bacterioplankton coupling in the response of microbial plankton to nutrient additions. J. Plankton Res. 38, 55–63. https://doi.org/10.1093/plankt/fbv101

Pringault, O., Lafabrie, C., Avezac, M., Bancon-Montigny, C., Carre, C., Chalghaf, M., Delpoux, S., Duvivier, A., Elbaz-Poulichet, F., Gonzalez, C., Got, P., Leboulanger, C., Spinelli, S., Sakka Hlaili, A., Bouvy, M., 2016. Consequences of contaminant mixture on the dynamics and functional diversity of bacterioplankton in a southwestern Mediterranean coastal ecosystem. Chemosphere 144, 1060–1073. https://doi.org/10.1016/j.chemosphere.2015.09.093

Pringault, O., Tassas, V., Rochelle-Newall, E., 2007. Consequences of respiration in the light on the determination of production in pelagic systems. Biogeosciences 4. https://doi.org/10.5194/bg-4-105-2007

Roberts, D.A., 2012. Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments. Environ. Int. 40, 230–243.

Rochelle-Newall, E., Mari, X., Rouchon, C., Torreton, J.P., Pringault, O., 2008. Zinc induces shifts in microbial carbon flux. Aquat. Microb. Ecol. 52, 57–68.

Rochelle-Newall, E.J., Chu, V.T., Pringault, O., Amouroux, D., Arfi, R., Bettarel, Y., Bouvier, T., Bouvier, C., Got, P., Nguyen, T.M.H., Mari, X., Navarro, P., Duong, T.N., Cao, T.T.T., Pham, T.T., Ouillon, S., Torréton, J.-P., 2011. Phytoplankton distribution and productivity in a highly turbid, tropical coastal system (Bach Dang Estuary, Vietnam). Mar. Pollut. Bull. 62. https://doi.org/10.1016/j.marpolbul.2011.08.044

Sakka Hlaili, A., Grami, B., Niquil, N., Gosselin, M., Hamel, D., Troussellier, M., Mabrouk, H.H., 2008. The planktonic food web of the Bizerte lagoon (south-western Mediterranean) during summer: I. Spatial distribution under different anthropogenic pressures. Estuar. Coast. Shelf Sci. 78, 61–77.

https://doi.org/10.1016/j.ecss.2007.11.010

Sakka Hlaili, A., Niquil, N., Legendre, L., 2014. Planktonic food webs revisited: Reanalysis of results from the linear inverse approach. Prog. Oceanogr. 120, 216–229. https://doi.org/http://dx.doi.org/10.1016/j.pocean.2013.09.003

Salhi, N., Triki, H.Z., Molinero, J.C., Laabir, M., Sehli, E., Bellaaj-Zouari, A., Yahia, N.D., Yahia, O.K.-D., 2018. Seasonal variability of picophytoplankton under contrasting environments in northern Tunisian coasts, southwestern Mediterranean Sea. Mar. Pollut. Bull. 129, 866–874. https://doi.org/10.1016/j.marpolbul.2017.10.029

Sayol, J.M., Orfila, A., Simarro, G., Lõpez, C., Renault, L., Galán, A., Conti, D., 2013. Sea surface transport in the Western mediterranean sea: A lagrangian perspective. J. Geophys. Res. Ocean. 118, 6371–6384. https://doi.org/10.1002/2013JC009243

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. Science (80-.). 313, 1072–1077.

Seto, M., Wada, S., Suzuki, S., 2013. The effect of zinc on aquatic microbial ecosystems and the degradation of dissolved organic matter. Chemosphere 90, 1091–1102. https://doi.org/10.1016/j.chemosphere.2012.09.014

Seymour, J.R., Amin, S.A., Raina, J.-B., Stocker, R., 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. Nat. Microbiol. 2, 17065. https://doi.org/10.1038/nmicrobiol.2017.65

Sfriso, A., Facca, C., Ghetti, P.F., 2003. Temporal and spatial changes of macroalgae and phytoplankton in a Mediterranean coastal area: The Venice lagoon as a case study. Mar. Environ. Res. 56, 617–636. https://doi.org/10.1016/S0141-1136(03)00046-1

Sharma, S.S., Schat, H., Vooijs, R., Van Heerwaarden, L.M., 1999. Combination toxicology of copper, zinc, and cadmium in binary mixtures: Concentration-dependent antagonistic, nonadditive, and synergistic effects on root growth in

Silene vulgaris. Environ. Toxicol. Chem. 18, 348–355.

Small, C., Nicholls, R.J., 2003. A global analysis of human settlement in coastal zones. J. Coast. Res. 19, 584–599.

Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using [3H]-leucine. Mar. Microb. Food Webs 6, 107–114.

Uchimiya, M., Motegi, C., Nishino, S., Kawaguchi, Y., Inoue, J., Ogawa, H., Nagata, T., 2016. Coupled response of bacterial production to a wind-induced fall phytoplankton bloom and sediment resuspension in the chukchi sea shelf, Western Arctic Ocean. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00231

Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Method. Int. Ver. Theor. Angew. Limnol. 9, 1–39.

Viličić, D., 2014. Ecology and Composition of Phytoplankton in the Adriatic Sea. Koeltz Scientific Books.

Wada, S., Suzuki, S., 2011. Inhibitory effect of zinc on the remineralisation of dissolved organic matter in the coastal environment. Aquat. Microb. Ecol. 63, 47–59. https://doi.org/10.3354/ame01481

Yoshida, M., Hamdi, H., Nasser, I.A., Jedidi, N., 2002. Contamination of Potentially Toxic Elements (PTEs) in Bizerte lagoon bottom sediments, surface sediments and sediement repository, in: Ghrabi, A., Yoshida, M. (Eds.), Study on Environmental Pollution of Bizerte Lagoon. INRST-JICA, p. 139.

Zrafi-Nouira, I., Khedir-Ghenim, Z., Bahri, R., Cheraeif, I., Rouabhia, M., Saidane-Mosbahi, D., 2009. Hydrocarbons in Seawater and Water Extract of Jarzouna-Bizerte Coastal of Tunisia (Mediterranean Sea): Petroleum Origin Investigation Around Refinery Rejection Place. Water Air Soil Pollut. 202, 19–31.

Zrafi-Nouira, I., Khedir-Ghenim, Z., Zrafi, F., Bahri, R., Cheraeif, I., Rouabhia, M., Saidane-Mosbahi, D., 2008. Hydrocarbon pollution in the sediment from the Jarzouna-Bizerte coastal area of Tunisia (Mediterranean Sea). Bull. Environ.

876 Contam. Toxicol. 80, 566–572.

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Figure legends

Figure 1: Main phytoplankton groups observed in Offshore and Lagoon waters as a function of the season.

Figure 2: Chlorophyll a concentration during microcosm incubation of Offshore and Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 3: Bacterial abundance during microcosm incubation of Offshore and Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 4: Temporal variations of primary production and bacterial carbon demand observed during the microcosm incubation of Offshore waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 5: Temporal variations of primary production and bacterial carbon demand observed during the microcosm incubation of Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

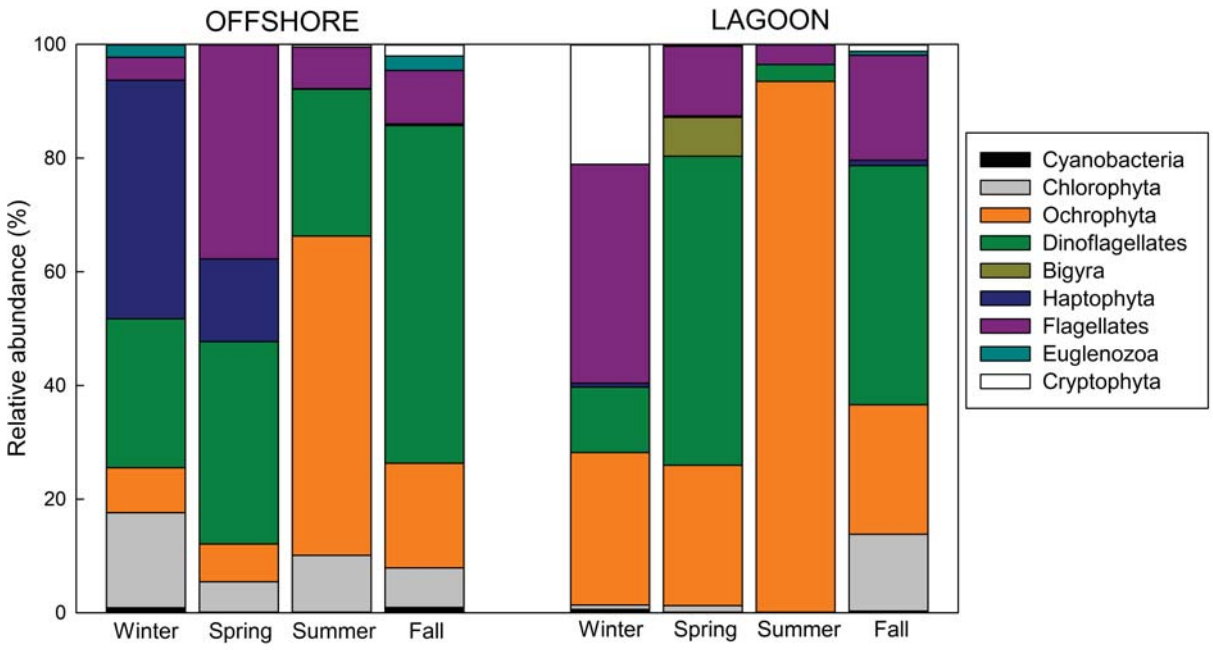
Figure 6: Scatter plot of bacterial carbon demand vs primary production in Offshore waters for the four seasons as a function of treatments. The fitted solid lines represent the linear regression. The dotted line indicates the 1:1 relationship between the two metabolic processes.

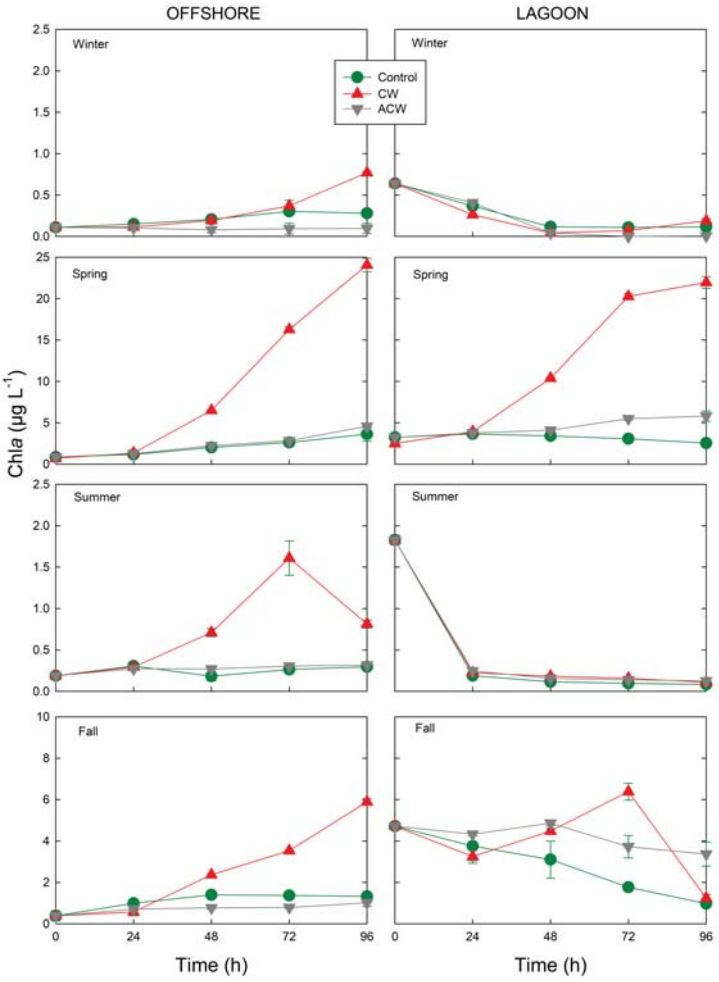
Figure 7: Scatter plot of bacterial carbon demand vs primary production in Lagoon waters for the four seasons as a function of treatments. The fitted solid lines

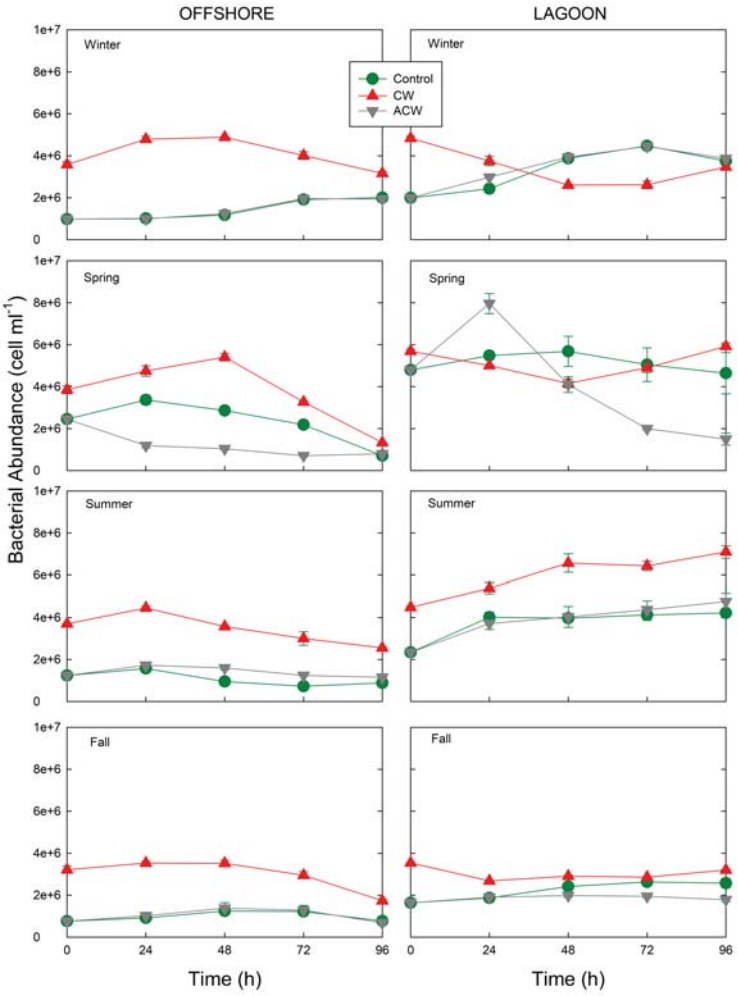
represent the linear regression. The dotted line indicates the 1:1 relationship between the two metabolic processes

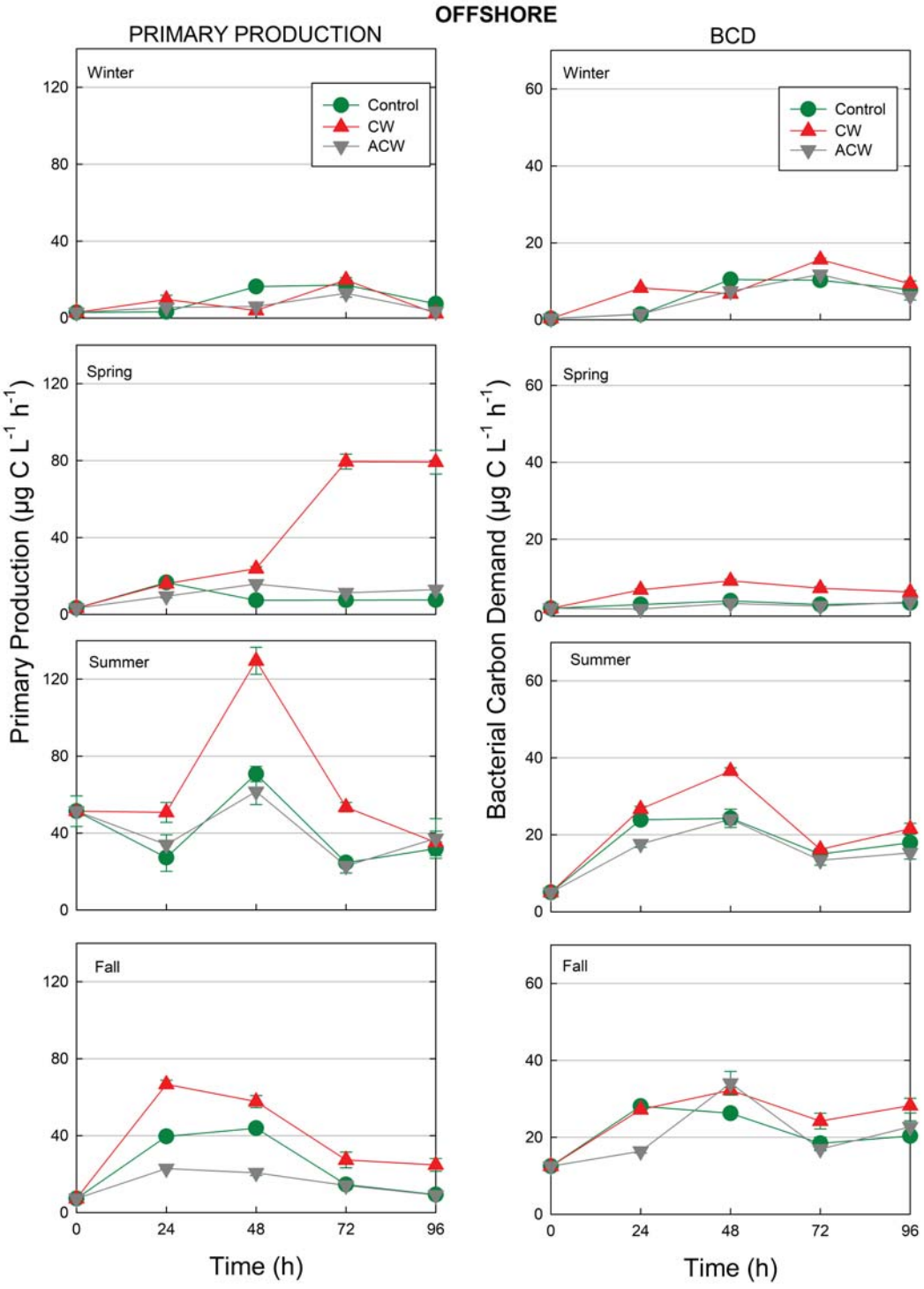
Table 1: In situ conditions observed in the two sampling stations for the four seasons. DOC: Dissolved Organic Carbon, PP: Primary Production, CR: Community Respiration. Average ± stdv (n=3)

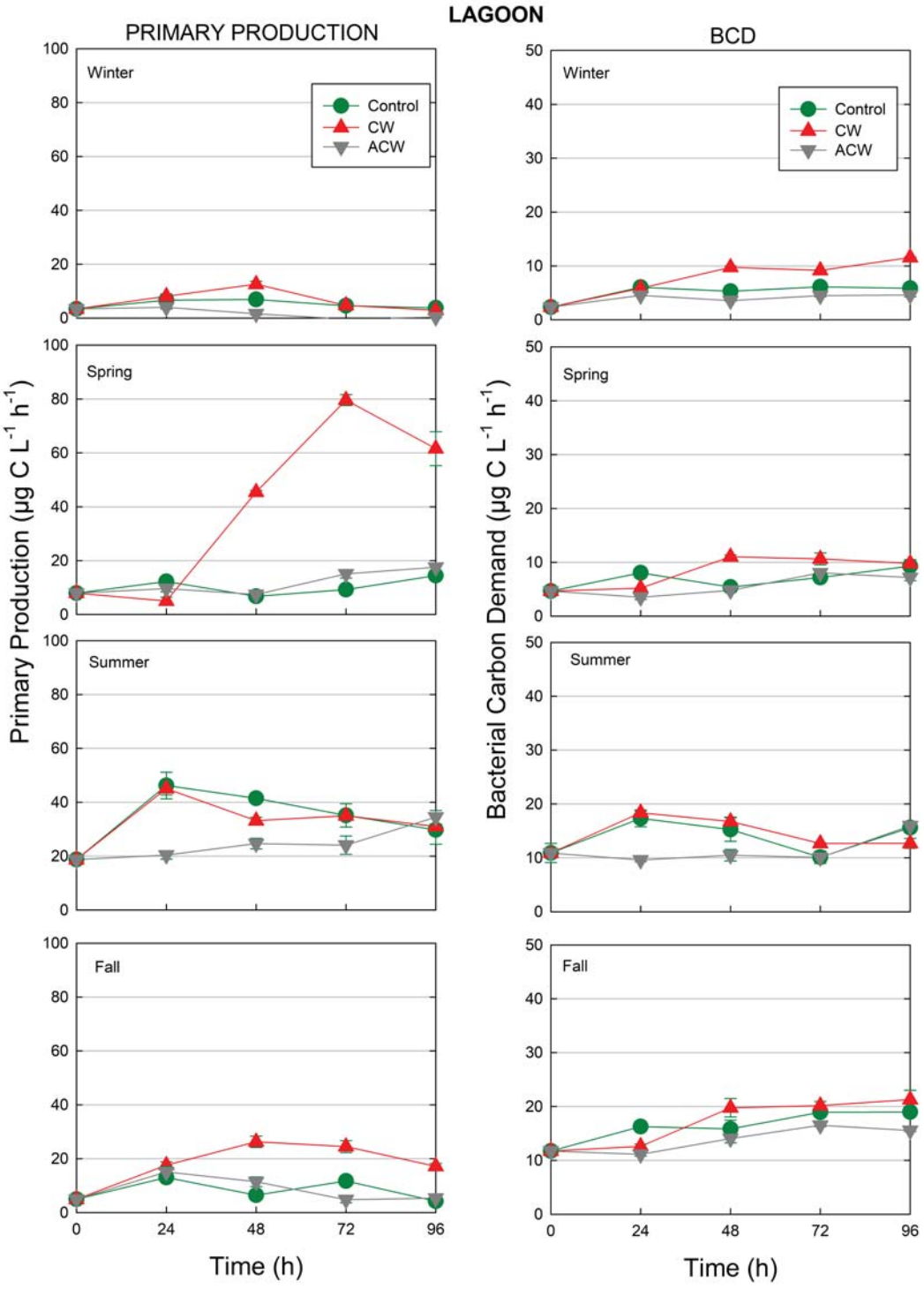
	OFFSHORE				LAGOON			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
DOC (mg C L^{-1})	1.23 ± 0.16	1.10 ± 0.02	1.08 ± 0.11	1.39 ± 0.31	2.52 ± 0.92	2.26 ± 0.18	1.93 ± 0.08	1.90 ± 0.24
$SiOH_4$ (µM)	3.13 ± 0.02	0.82 ± 0.07	1.60 ± 0.03	1.60 ± 0.04	5.83 ± 0.03	0.82 ± 0.07	1.79 ± 0.06	9.28 ± 0.66
NH_4 (µM)	0.48 ± 0.10	0.48 ± 0.11	0.22 ± 0.01	0.32 ± 0.17	0.70 ± 0.23	0.54 ± 0.11	0.81 ± 0.25	2.00 ± 0.36
PO_4 (µM)	0.07 ± 0.01	0.05 ± 0.04	0.22 ± 0.01	0.27 ± 0.02	0.08 ± 0.06	0.05 ± 0.04	0.16 ± 0.01	0.33 ± 0.03
NO_2 (µM)	0.23 ± 0.03	0.04 ± 0.05	0.05 ± 0.02	0.10 ± 0.02	0.18 ± 0.03	0.04 ± 0.01	0.20 ± 0.02	0.27 ± 0.04
NO_3 (µM)	1.50 ± 0.61	0.05 ± 0.05	0.62 ± 0.14	0.97 ± 0.15	2.94 ± 0.55	0.10 ± 0.05	1.36 ± 0.26	2.19 ± 0.23
Chl*a* (µg L^{-1})	0.11 ± 0.01	0.86 ± 0.24	0.19 ± 0.02	0.38 ± 0.15	0.64 ± 0.05	3.25 ± 0.11	1.83 ± 0.06	4.71 ± 0.13
PP (µg C L^{-1} h^{-1})	2.94 ± 0.49	3.32 ± 0.42	51.30 ± 7.95	7.26 ± 1.42	3.40 ± 0.27	7.92 ± 0.65	18.70 ± 0.11	4.92 ± 1.12
CR (µg C L^{-1} h^{-1})	1.04 ± 0.31	6.13 ± 0.49	16.28 ± 1.88	41.45 ± 0.67	7.49 ± 0.90	13.07 ± 0.93	34.09 ± 5.86	38.21 ± 1.08
P:R	1.54 ± 0.3	0.27 ± 0.02	1.57 ± 0.11	0.09 ± 0.01	0.23 ± 0.02	0.31 ± 0.04	0.29 ± 0.05	0.06 ± 0.02

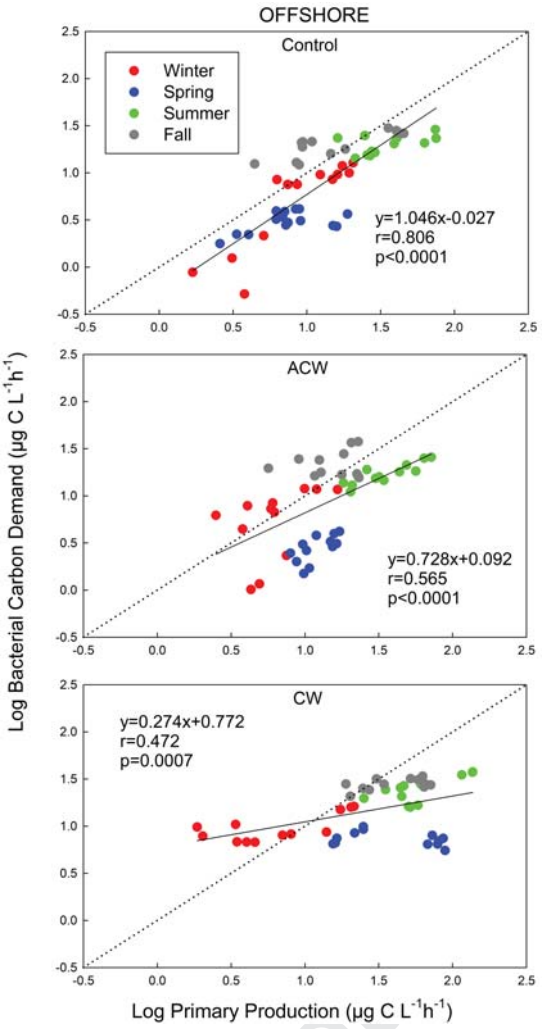


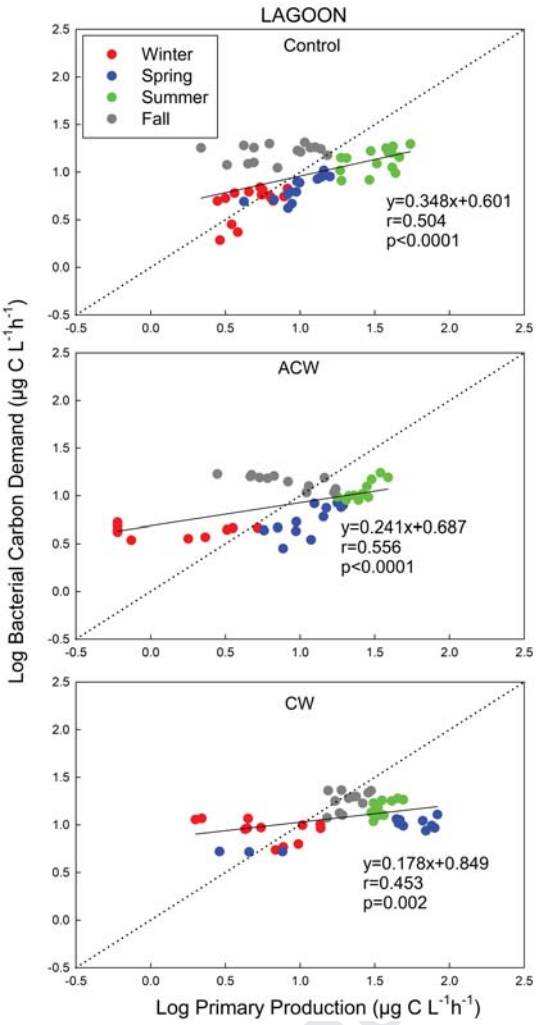

OFFSHORE
LAGOON
Winter
Spring
Summer
Fall
Control
CW
ACW
Chla (µg L-1)
Time (h)
0
24
48
72
96


OFFSHORE
LAGOON
Winter
Spring
Summer
Fall
Control
CW
ACW
Bacterial Abundance (cell ml-1)
Time (h)
0
2e+6
4e+6
6e+6
8e+6
1e+7
0
24
48
72
96


OFFSHORE
PRIMARY PRODUCTION
BCD
Winter
Spring
Summer
Fall
Control
CW
ACW
Primary Production (µg C L-1 h-1)
Bacterial Carbon Demand (µg C L-1 h-1)
Time (h)
0
24
48
72
96


LAGOON
PRIMARY PRODUCTION
BCD
Winter
Spring
Summer
Fall
Control
CW
ACW
Primary Production (µg C L-1 h-1)
Bacterial Carbon Demand (µg C L-1 h-1)
Time (h)
0
24
48
72
96


OFFSHORE
Control
Winter
Spring
Summer
Fall
y=1.046x-0.027
r=0.806
p<0.0001
ACW
y=0.728x+0.092
r=0.565
p<0.0001
CW
y=0.274x+0.772
r=0.472
p=0.0007
Log Bacterial Carbon Demand (µg C L-1h-1)
Log Primary Production (µg C L-1h-1)


LAGOON
Control
Winter
Spring
Summer
Fall
y=0.348x+0.601
r=0.504
p<0.0001
ACW
y=0.241x+0.687
r=0.556
p<0.0001
CW
y=0.178x+0.849
r=0.453
p=0.002
Log Bacterial Carbon Demand (µg C L-1h-1)
Log Primary Production (µg C L-1h-1)

Highlights

- Phytoplankton and bacterioplankton were strongly coupled in offshore waters.
- Coupling between both compartments was less evident in lagoon waters.
- Sediment resuspension and chemical contamination differently impacted lagoon and offshore waters.
- Chemical contamination loosened the coupling between both compartments.
- Nutrients and dissolved organic matter released during sediment resuspension strongly decreased the bacterio–phytoplankton coupling.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: