Reply to Reviewer 1 comments

SPECIFIC COMMENTS

Abstract: -

The top-down control is not demonstrated in this work, you cannot mention this so securely as a major driver, neither as a result of your work. Same remark for the discussion section.

Answer. Yes indeed, so we replaced this sentence by "*We found that "bottom-up" control accounted for a large part in the variability of phytoplankton productivity*". We also modified the discussion in this way and only suggested the top-down as a potential factor.

Introduction:

- The part related to climate change is really oversized when compared to real scientific questions addressed by this paper in its current version. Moreover, the consideration of PAH rather balance the study towards local changes because of human activities. This introduction should at least be less oriented towards climate change and present the challenge of understanding the functioning of such continental to marine environments interface in a context of growing anthropization, which seems to fit better to the design and results of the study.

Answer. We deleted the whole paragraph referenced to global change and added the challenge put forward by this remark.

- The consideration of PAH is not mentioned in the introduction. It is surprising to see it appear in the methods section without any mention in the objectives! The results and the discussion related to PAH are interesting, but you need to include it in your objectives, otherwise it is really hard to see how useful it can be to consider PAH regarding to your scientific objectives. This comment and the previous one are really connected, they should be taken into consideration together. The way PAH are mentioned in the abstract, the importance of oil-related compounds is obvious, this should be presented in this way in the introduction.

Answer. We agree with the Reviewer. We thus added two paragraphs dedicated to PAHs in the introduction:

"*Local anthropogenic inputs of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) may also affect bacterial diversity and activities (Lekunberri et al., 2010; Rodríguez-Blanco et al., 2010; Jiménez et al., 2011). Indeed, PAHs, which can comprise as much as 25–35 % of total hydrocarbon content in crude oils (Head et al., 2006), are among the most abundant and ubiquitous pollutants in the coastal environment (González-Gaya et al., 2016). These compounds are recognized by the European and US environmental agencies as priority pollutants for the aquatic medium due to their toxicity, persistence and ability to accumulate in the biota (Kennish, 1992). Hence, the presence of PAHs in the marine environment may induce an increase in the indigenous populations of marine bacteria that can break down and utilize these chemicals as carbon source, the so called "PAH-degrading bacteria" or "PAH degraders". These bacteria are generally strongly selected in oil-impacted ecosystems, where they may account for 70 to 90% of the total bacterial community (Head et al., 2006; Gutierrez et al., 2014).*"

"*Also, Terminos Lagoon is potentially impacted by PAHs, which may come from a diversity of sources including sea-based activities (spills from ships, platforms and pipelines, ballast* *water discharge, drilling…) but also rivers, surface runoffs and the atmosphere that carry various urban and industrial wastes (fuel combustion, traffic exhaust emissions...). Nevertheless, to our knowledge, little is known about the PAH content in this ecosystem. Even though Noreña-Barroso et al. (1999) have reported PAH concentrations in the American Oysters Crassostrea virginica and Rendon-Von Osten et al. (2007), PAH concentrations in surface sediments, no data are currently available about dissolved PAH concentrations in surface waters of the Terminos Lagoon.*"

In the objectives, we also added "*dissolved PAHs*" and "*including PAH-degrading bacteria*".

We removed one reference from the reference list:

Aguayo, P., Gonzalez, C., Barra, R., Becerra, J., and Martinez, M.: Herbicides induce change in metabolic and genetic diversity of bacterial community from a cold oligotrophic lake, World journal of microbiology & biotechnology, 30, 1101-1110, 2014.

And we added several references dealing with PAHs/PAH-degrading bacteria:

González-Gaya, B., Fernández-Pinos, M. C., Morales, L., Abad, E., Piña, B., Méjanelle, L., Duarte, C. M., Jiménez, B., and Dachs, J.: High atmosphere-ocean Exchange of semivolatile aromatic hydrocarbons, Nature Geoscience, 9, 438-442, 2016.

Gutierrez, T., Rhodes, G., Mishamandani, S., Berry, D., Whitman, W. B., Nichols, P. D., Semple, K. T., and Aitken, M. D.: Polycyclic Aromatic Hydrocarbon Degradation of Phytoplankton-Associated Arenibacter spp. and Description of Arenibacter algicola sp. nov., an Aromatic Hydrocarbon-Degrading Bacterium, Applied and Environmental Microbiology, 80, 618-628, 2014.

Head, I. M, Martin Jones, D., and Roling, W. F. M.: Marine microorganisms make a meal of oil, Nature, 4, 173-182, 2006.

Jiménez, N., Viñas, M., Guiu-Aragonés, C., Bayona, J. M., Albaigés, J., and Solanas, A. M.: Polyphasic approach for assessing changes in an autochthonous marine bacterial community in the presence of Prestige fuel oil and its biodegradation potential, Applied Microbiolal Biotechnology, 91, 823-834, 2011.

Lekunberri, I., Calvo-Díaz, A., Terira, E., Morán, X. A. G., Peters, F., Nieto-Cid, M., Espinoza-González, O., Teixeira, I. G., and Gasol, J.M.: Changes in bacterial activity and community composition caused by exposure to a simulated oil spill in microcosm and mesocosm experiments, Aquatic Microbial Ecology, 59, 169-183, 2010.

Noreña-Barroso, E., Gold-Bouchot, G., Zapata-Perez, O., and Sericano, J. L.: Polynuclear Aromatic Hydrocarbons in American Oysters Crassostrea virginica from the Termines Lagoon, Campeche, Mexico, Marine Pollution Bulletin, 38, 637-645, 1999.

Rendon-von Osten, J., Memije, M., Ortiz, A., and Benitez, J.: Potential sources of PAHs in sediments from Terminos lagoon, Campeche, Mexico, Toxicology Letters, 172, S162, 2007.

Material and methods:

- Section 2.6 and later all along the manuscript: flow cytometry cannot distinguish bacteria from archaea, thus most of the "bacterial" should be replaced by "prokaryotic" or "heterotrophic prokaryotes".

Answer. We have done the replacement in the whole text when appropriated.

- Section 2.9: Could you explicit the calculation of this MPN? The way such calculation is performed after two weeks of incubation sounds weird, how is the number of bacteria enumerated after two weeks related to the initial sample? Also refer to my corresponding comment in the result section.

Answer. Sorry, but the reviewer's remark is not very clear. The principle of the MPN technique is based on serial dilution on the reference sample to be analysed. Therefore, the calculation takes into account the initial number of bacteria and the dilution where resazurin changed color. It makes no sense to count the bacteria after two weeks incubation and use this count in the calculation. Here, the MPN is giving an estimation of the most probable number of bacteria able to degrade the mix of six PAHs as sole carbon source within two weeks. We reassure the reviewer that the use of classical MPN table based on symmetrical dilution series with a constant, table-specified dilution factor, as explained in the material and method section.

A reference has been added in the material and method section, in case the reader wants to have more information on this method classically used in microbiology.

Alexander, M., 1982. Most probable number method for microbial populations. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, 2nd ed. American Society of Agronomy, Madison, WI, pp. 815-820.

Results: - Lines 279-280: very consistent, but could you indicate the approximate value of the ratio in this area?

Answer. We indicated in the text the Phaeo:CHL ratio of the area (<25%)

- Lines 292-294: the correlations concerning aminopeptidase activities are very weak, it should not be presented at the same level than the ones concerning phosphatase activities, which are stronger. This probably means that there is indeed a correlation between these variables, but they are not linear and Spearman's correlation tests the linearity of the relation. You should try non parametric and non linear correlation analyses to further precise the link between these variables.

Answer. We now precised "…. to a lesser extent, between phosphatase activities…."

- Lines 296-299: Need to explain how such percentage is obtained. As it is explained in ,the current version of the manuscript, one could understand that you counted bacteria in your 2 week enrichments and divided this number by the number of bacteria in your initial samples... This does not sound correct.

Answer. See our previous response on - Section 2.9 for this remark.

- Lines 300-303: I don't understand the cause-to-consequence relationship you mention here (second part of the sentence). You should split the sentence into two very descriptive ones. Moreover, the PAH distribution should be presented at the beginning of this paragraph, then followed by PNM counts, and finally the correlation analysis. Thus the two components of the title should also be switched.

Answer. We agree with the reviewer. We modified this paragraph 3.5 accordingly:
We replaced the paragraph title "*3.5 Estimated abundance of bacterial PAH-degraders and PAH concentrations*" by "*3.5 Dissolved PAH concentrations and estimated abundance of bacterial PAH-degraders*"

And we replaced the whole concerned paragraph:

"*Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada river (estimated at 4.6 104 cells mL^{-1}, equivalent to 4.4 % of the total bacterial abundance) (Fig. 5A). Lower values were found close to the Chumpan embouchure (estimated at 4.7 103 cells mL^{-1}, equivalent to 0.2 % of the total bacterial abundance), and commonly represented less than 0.1 % of the bacterial abundance in the rest of the lagoon. Quantification by MPN counts showed significant even if low spearman rank correlation with dissolved total PAH concentrations (R=0.37, p<0.05, n=35), mainly because of PAH distribution (Fig. 5B) showing higher concentrations close to the El Carmen inlet (332 ng L^{-1}) and relatively lower concentrations close to Palizada river (187 ng L^{-1}) and to the Chumpan embouchure (166 ng L^{-1}). Correlations (p<0.05, n=35) were stronger with PP (R=0.65) and CHL (R=0.53). PAH concentrations were generally lower in the rest of the lagoon (<130 ng L^{-1}).*

By the paragraph:

"*Dissolved total PAH concentrations (Fig. 5A) were higher close to the El Carmen inlet (332 ng L^{-1}) and relatively lower close to Palizada river (187 ng L^{-1}) and to the Chumpan embouchure (166 ng L^{-1}). They were generally lower in the rest of the lagoon (<130 ng L^{-1}). Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada river (estimated at 4.6 104 cells mL^{-1}, equivalent to 4.4 % of the total bacterial abundance) (Fig. 5B). Lower values were found close to the Chumpan mouth (estimated at 4.7 103 cells mL^{-1}, equivalent to 0.2 % of the total bacterial abundance), and commonly represented less than 0.1 % of the bacterial abundance in the rest of the lagoon. Quantification by MPN counts showed significant even if low spearman rank correlation with dissolved total PAH concentrations (R=0.37, p<0.05, n=35). Correlations (p<0.05, n=35) were stronger with PP (R=0.65) and CHL (R=0.53).*"

Consequently, we inverted the order of Fig. 5A and 5B:
Figure 5: as Figure 2 for A. total dissolved PAHs (ng L-1) and B. the most-probable-number (MPN in count)

Discussion:

- Lines 346-348: The corresponding figures are puzzling because, yes, the normalized productivity per cell seems to be higher, but since the CHL concentration is also lower, one could think that the productivity of the area expressed by unit of volume could be lower. Since you have CHL concentration per liter and since the productivity is expressed is expressed by unit of CHL, I suggest that you calculate a productivity by volume of water in order to better compare the productivity of the two sites. According to your graph, you have less than a 2-fold difference in CHL concentration between the two sites but a 6- to 7-fold difference in C fixation rates. Thanks to such analysis, your conclusion will be more robust.

Answer. Thanks for this remark. In fact, we have actually about 2-fold difference in CHL concentration between the two sites but about the same ratio and not 6- to 7-fold difference in C fixation rates. So, for similar amount of nutrients (and DOM and DOP also…) we have similar production for half chlorophyll… Here is the table for mean values of the 2 groups (I included 4 stations in each group…)
I also added a sentence in the text concerning this comparison.

	CHL	P^b_m	PP max	NO3	PO4	NH4
	$mg.m^{-3}$	$mgC.mgCHL^{-1}.h^{-1}$	$mgC.m^{-3}.h^{-1}$	µM	µM	µM

mean G palizada	6.34	4.34	27.5	0.02	0.08	0.05
mean G inlet	3.95	7.65	30.2	0.03	0.08	0.06

- Lines 352-353: Could you provide references to support such hypothesis since you did not measure it?

Answer. We now modified this paragraph to modulate the affirmation.

- Lines 359-361: This sentence should be rewritten in a more prudent way since it is only speculation, you have no clue for the top down control and even though you have PAHs concentrations, the direct link with phytoplankton productivity remains to be demonstrated.

Answer. We now modified the sentence as follow *"Finally, it is clear that bottom-up (nutrients and humic substances) drove the differential responses of phytoplankton productivity in the eastern and western part of the lagoon, certainly in conjunction with grazing activity (top-down control").*

- Lines 378-380: sounds weird to justify a recent study by an old one... The old reference could be removed without making the sentence meaningless or doubtful.

Answer. We deleted the reference.

- Lines 383-386: These Redfield ratios were not presented in the results sections. They are meaningful and should be presented extensively!

Answer. We added the map for the distribution of this ratio in the lagoon.

- Lines 410-413: That could appear contradictive. If you suppose that higher concentrations of DOC but lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria, you should clearly state it.

Answer. We now explained that higher DOC concentrations associated with lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria. The high aminopeptidase activity in the Palizada River plume confirmed the presence of recalcitrant organic matter from terrestrial origin, as opposed to minimum activities in Puerto Real marine waters or in Candelaria mouths, where DOC concentrations were maximal.

- Lines 425-427: So vicinity of Palizada river = phosphatase activity but P-depleted zone, meaning very low P-availability for phytoplanktonic growth, which seems consistent with le smaller C fixation rates observed in this zone, am I right? If yes, such a link between nutrients and biological productivity could be added to this discussion, this would greatly strengthen the end of the first sub-section which was up to now very speculative. Moreover, this would also strengthen the links between the geochemical and the biological sides of this paper which appear very few connected for the moment.

Answer. Thanks for this pertinent remark that we included in the text.

- End of section 4.3: A small discussion could be added about distance-based effects and community turnover: according to your data, one could think that strong local selective pressures led to strong shift in the community composition, instead of having progressive gradients and/or fast dispersion that would rather just promote a turnover within the active fraction. Such thinking points out the existence of strong driving pressures that you did not measured in your study, since you do not have such strong correlation

Answer. As describe above, we changed the sentence into: “These results indicated that most of the free-living bacterial community detected by molecular fingerprinting (DNA-based) were active (RNA-based) among the lagoon, with the exception of the local transition zones between the lagoon waters and the coastal (El Carmen inlet) or rivers (Palizada and Candelaria).”
This sentence points out that some transitions zones strongly affect bacterial diversity and activities. We agree with the reviewer’s comment but it is difficult to state on either a strong local selective pressure or progressive gradient driving the strong shift of the community observed in this study. Because our dataset aimed to give an overview of the entire lagoon rather than focusing on the several transition zones of the lagoon, we decided to be cautious and not over interpreting our results.

- Lines 460-466: These two sentences should rather be placed at the beginning of the previous paragraph, before discussing about community structure.

Answer. We moved these sentences at the beginning of the paragraph 4.3 as recommended

- Lines 475-477: Adding a short discussion about "distance-based" similarities in present and active communities, as proposed in a previous comment would perfectly fit with this discussion.

Answer. We added the short discussion suggested

- The link between the severe drought and the potential predictive aspect of this study is not mentioned all along the discussion section, whereas it represents most of your introduction. You need either to remove this “climate-change” part in the intro, or discuss it in the discussion.

Answer. We modified the introduction and discussion in consequence

Conclusion
- Lines 512-514: You cannot say that as it was unambiguous, you did not even measure any top-down parameter.

Answer. We modulated our affirmation, in indicating that we supposed a possible shift. In fact we have some signals that confirm this hypothesis; it is the reason why we chose to keep it in the text.

- Lines 514-517: This sentence does not sound correct since you measured C fixation, thus phytoplankton activity. To measure growth, you need repeated measures in time, that you do not have. So you can definitely not state that there is no growth. I understand it is probably not what you wanted to say, but your sentence could be misinterpreted

Answer. We replaced growth by C-fixation

- Lines 528-530: again, you are over confident in your hypothesis: "seems to support", you do not have any temporal data to support this hypothesis.

Answer. done

TECHNICAL CORRECTIONS

Answer. All the 15 remarks have been taken into account and fixed

Reply to Reviewer 2 comments

SPECIFIC COMMENTS

Abstract:

- The abstract states that the study will help to “understand how the severe drought period influenced biogeochemical cycling and phyto- and bacterio-plankton communities”, nevertheless the study does not compare the data obtained during this period to any other records (maybe due to the fact that they do not exist) obtained during in more “classic” situation. This statement needs to be nuanced.

Answer. Effectively, we have not such data to compare, so we modulated this statement in the text, and added " *… under such conditions*" to be clearer.

- “Coupling between top-down and bottom-up controls accounted for the diverse responses in phytoplankton productivity” : I didn’t see any demonstration of top-down control in the study.

Answer. Yes indeed, so we replaced this sentence by "*We found that "bottom-up" control accounted for a large part in the variability of phytoplankton productivity*".
In the discussion, we re written the paragraph 4.1 in order to modulate the top-down control, and considered it as an hypothesis, and not as an affirmation

- The PAH-relative measurements need to be better integrated in the global aim of the study.

Answer. We modified the abstract, introduction and discussion to better integrate our PAH results. In the abstract, we replaced the part “*A large set of biogeochemical (nutrients, dissolved and particulate organic matter), phytoplanktonic (biomass and photosynthetic activity) and bacterial (bacterial diversity and ectoenzymatic activities) parameters….*”
By the part: “*A large set of biogeochemical [nutrients, dissolved and particulate organic matter, dissolved polycyclic aromatic hydrocarbons (PAHs)], phytoplanktonic (biomass and photosynthetic activity) and bacterial (bacterial diversity, including PAH-degrading bacteria, and ectoenzymatic activities) parameters…*”
We also added the following sentence: “*The highest dissolved total PAH concentrations were measured in El Carmen inlet, suggesting an anthropogenic pollution of the zone probably related to the oil platform exploitation activities in the shallow waters of the South of the Gulf of Mexico.*”

Introduction

- Overall the introduction is too much climate change orientated in regards to the results presented in the manuscript. Introduction needs to be more focused on the local dynamic of this lagoon and on the importance of considering PAH in this specific area. This study is more focused on the dynamic of land/sea transitional coastal areas under combined climatic and chemical pressures than on climate change effects.

Answer. We deleted the whole paragraph referenced to global change and focused the introduction on the challenge to understand the functioning of continental to marine environments interface in a context of growing anthropization.

- The importance of PAH in the studying area is not mentioned anywhere in the introduction or in the objectives. However, PAH concentrations and PAH-degraders appeared as a substantial part of the results and discussion sections. If PAH is an important factor to consider in the lagoon, this should be clearly presented in the objectives of the study.

Answer. We agree with the Reviewer. We thus added two paragraphs dedicated to PAHs in the introduction:

"*Local anthropogenic inputs of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) may also affect bacterial diversity and activities (Lekunberri et al., 2010; Rodríguez-Blanco et al., 2010; Jiménez et al., 2011). Indeed, PAHs, which can comprise as much as 25–35 % of total hydrocarbon content in crude oils (Head et al., 2006), are among the most abundant and ubiquitous pollutants in the coastal environment (González-Gaya et al., 2016). These compounds are recognized by the European and US environmental agencies as priority pollutants for the aquatic medium due to their toxicity, persistence and ability to accumulate in the biota (Kennish, 1992). Hence, the presence of PAHs in the marine environment may induce an increase in the indigenous populations of marine bacteria that can break down and utilize these chemicals as carbon source, the so called "PAH-degrading bacteria" or "PAH degraders". These bacteria are generally strongly selected in oil-impacted ecosystems, where they may account for 70 to 90% of the total bacterial community (Head et al., 2006; Gutierrez et al., 2014).*"

"*Also, Terminos Lagoon is potentially impacted by PAHs, which may come from a diversity of sources including sea-based activities (spills from ships, platforms and pipelines, ballast water discharge, drilling…) but also rivers, surface runoffs and the atmosphere that carry various urban and industrial wastes (fuel combustion, traffic exhaust emissions...). Nevertheless, to our knowledge, little is known about the PAH content in this ecosystem. Even though Noreña-Barroso et al. (1999) have reported PAH concentrations in the American Oysters Crassostrea virginica and Rendon-Von Osten et al. (2007), PAH concentrations in surface sediments, no data are currently available about dissolved PAH concentrations in surface waters of the Terminos Lagoon.*"

In the objectives, we also added "*dissolved PAHs*" and "*including PAH-degrading bacteria*".

We removed one reference from the reference list:

Aguayo, P., Gonzalez, C., Barra, R., Becerra, J., and Martinez, M.: Herbicides induce change in metabolic and genetic diversity of bacterial community from a cold oligotrophic lake, World journal of microbiology & biotechnology, 30, 1101-1110, 2014.

And we added several references dealing with PAHs/PAH-degrading bacteria:

González-Gaya, B., Fernández-Pinos, M. C., Morales, L., Abad, E., Piña, B., Méjanelle, L., Duarte, C. M., Jiménez, B., and Dachs, J.: High atmosphere-ocean Exchange of semivolatile aromatic hydrocarbons, Nature Geoscience, 9, 438-442, 2016.

Gutierrez, T., Rhodes, G., Mishamandani, S., Berry, D., Whitman, W. B., Nichols, P. D., Semple, K. T., and Aitken, M. D.: Polycyclic Aromatic Hydrocarbon Degradation of Phytoplankton-Associated Arenibacter spp. and Description of Arenibacter algicola sp. nov., an Aromatic Hydrocarbon-Degrading Bacterium, Applied and Environmental Microbiology, 80, 618-628, 2014.

Head, I. M, Martin Jones, D., and Roling, W. F. M.: Marine microorganisms make a meal of oil, Nature, 4, 173-182, 2006.

Jiménez, N., Viñas, M., Guiu-Aragonés, C., Bayona, J. M., Albaigés, J., and Solanas, A. M.: Polyphasic approach for assessing changes in an autochthonous marine bacterial community in the presence of Prestige fuel oil and its biodegradation potential, Applied Microbiolal Biotechnology, 91, 823-834, 2011.

Lekunberri, I., Calvo-Díaz, A., Terira, E., Morán, X. A. G., Peters, F., Nieto-Cid, M., Espinoza-González, O., Teixeira, I. G., and Gasol, J.M.: Changes in bacterial activity and community composition caused by exposure

to a simulated oil spill in microcosm and mesocosm experiments, Aquatic Microbial Ecology, 59, 169-183, 2010.
Noreña-Barroso, E., Gold-Bouchot, G., Zapata-Perez, O., and Sericano, J. L.: Polynuclear Aromatic Hydrocarbons in American Oysters Crassostrea virginica from the Termines Lagoon, Campeche, Mexico, Marine Pollution Bulletin, 38, 637-645, 1999.
Rendon-von Osten, J., Memije, M., Ortiz, A., and Benitez, J.: Potential sources of PAHs in sediments from Terminos lagoon, Campeche, Mexico, Toxicology Letters, 172, S162, 2007.

Material and Methods

- Section 2.1 (last paragraph): I suggest including the sub-sampling information in the relative subsequent technical sections Is there any available data concerning water circulation in this lagoon ? This information could be relevant for your study

Answer. As suggested, we moved the subsampling informations in each relative subsequent section (Nutrients and POM; see file for details).
Concerning the circulation, we added a specific paragraph containing recent reference to describe the sampling zone (section 2.1)=

"Recent results on tidal current modeling (Contreras Ruiz Esparza et al., 2014) revealed a dynamic inshore current entering the lagoon through Carmen passage, flowing through the southern half of the lagoon and coming out through Puerto Real and a much slower inverse water current flooding the northern central part of the lagoon. That tidally induced hydrodynamic trend generated a counter clock wise circulation gyre located in the center of the lagoon leeward from Carmen Island."

- Section 2.6: Only free bacteria can be measured according to this protocol not "total bacteria" as mentioned in the text (attached bacteria are not measured)

Answer. Effectively, we agree with reviewer's comment. "Total bacteria" has been changed into "Free-living heterotrophic prokaryote" all through the text to also be in agreement with reviewer 1 remark.

- Section 2.7: In turbid waters, such prefiltration step may lead to the retention of attached bacteria on the filter and induce a bias in bacterial diversity assessment. This method is adequate to collect the bacteria present in aquatic sample but this bias need to be mentioned in the discussion section.

Answer. As recommended by the reviewer, we pointed this aspect in the discussion section: "In the present study, we focused on the free-living bacteria and disregarded the particle-attached fraction by pre-filtrating the water by 3 µm, which allowed eliminating the problem of DNA eukaryotic chloroplasts that may have biased our results in the context of gradients of productive zones"

- Section 2.9: The authors used a mixture of 6 PAHs for MPN but there is no explanation of this choice. In addition, what is the ratio of each PAH in the mixture? Why did they use a 10 µg/mL final concentration (is this concentration realistic when dealing with environmental samples)? Additional information is required in this section.

Answer. This section has been modified as follow:
A mixture of 6 PAHs from 2 to 5 rings (naphthalene, fluorene, phenanthrene, fluoranthrene, pyrene and benzo[a]pyrene) prepared in dichloromethane in equimolar concentration was introduced into each well at a final concentration of 10 µg mL-1, as previously described by Sauret et al. (2016). It corresponds to very high concentration of PAH in nature, i.e. 50 times higher than the values found in the harbour of Leghorn (Cincinelli et al., 2001).

Sauret C, Tedetti M, Guigue C, Dumas C, Lami R, Pujo-Pay M, Conan P, Goutx M, Ghiglione JF (2016) Influence of PAHs among other coastal environmental variables on total and PAH-degrading bacterial communities. Environmental Science and Pollution Research, 23: 4242-4256
Cincinelli, A., Stortini, A.M., Perugini, M., Checcini, L., Lepri, L., 2001. Organic pollutants in sea-surface microlayer and aerosol in the coastal environment of Leghorn (Tyrrhenian Sea). Mar. Chem. 76, 77–98.

Note that all paragraphs referencing to PAH have been changed in the revised version.

SPECIFIC COMMENTS
Results
- PAH concentrations should be included in section 3.2

Answer. We agree that PAH concentrations could appear in the 3.2 section, but it is also logical to deal with concentrations and abundance of bacterial PAH-degraders together as we did in 3.5. So, we preferred to not modify.

- Section 3.4: Biomass is the total amount of living material in a given habitat, population, or sample. Specific measures of biomass are expressed in dry weight per unit area of land or unit volume of water. In this section, you report bacterial abundance (cells/ml) and not bacterial biomass.

Answer. It has been fixed in the revised version of the text.

- The standard deviation values for MUF-P and MUF-Lip are higher than the measured mean values. The use of a range of values would be more suitable. In addition the values presented in the text do not fit with the values presented in the legend of Figure 4. This needs to be corrected

Answer. We apologize for the values presented in the legend of Figure 4. We thank the reviewer who realized that these values were wrong and it has now been fixed. Concerning the scale given by means and standard deviations, in fact, we compared the higher values found in Palizada and Chumpan rivers embouchures northward towards El Carmen Island to the rest of the lagoon by giving these mean values. We agree that some of the standard deviation values for MUF-P and MUF-Lip in the rest of the lagoon were higher than the measured mean values, but this is informative of the variability of the data. Giving range of values would not be informative on this aspect.

- Section 3.5: As previously mentioned, flow cytometry does not consider attached bacteria. The protocol used for MPN determination allows the growth of free and attached PAH-degraders. As a consequence, the measured percentage is not accurate.

Answer. As recommended, we modified the whole section and gave precisions concerning the calculation method. A reference has been added in the material and method section, in case the reader wants to have more information.

Alexander, M., 1982. Most probable number method for microbial populations. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, 2nd ed. American Society of Agronomy, Madison, WI, pp. 815-820.

Discussion

- Lines 352-361: No grazing activity was measured in this study, so this statement need to be presented as a other way to explain the data and not as an affirmation.

Answer. We re-written the paragraph 4.1 in order to modulate the top-down control, and considered it as a hypothesis, and not as an affirmation

- Section 4.3: Please explain the last sentence of the section : " These results indicated that most of the communities detected by molecular fingerprinting were active, with no specific distinction through the lagoon."

Answer. We apologize for being unclear in this sentence, which has been changed to: Here, the combination of DNA and RNA showed similar tendencies within the total and active communities presenting eastern, middle and western distribution among the lagoon. These results indicated that most of the free-living bacterial communities detected by molecular fingerprinting (DNA-based) were active (RNA-based) among the lagoon, with the exception of the local transition zones between the lagoon waters and the coastal (El Carmen inlet) or rivers (Palizada and Candelaria).

Conclusion

- No demonstration of any top-down control in this study

Answer. It has been modified everywhere in the text as previously detailed

- The conclusion gives a nice overview of the main results obtained in the lagoon but does not respond to the climate change questions mentioned in the introduction. This needs clarification or rephrasing of the introduction.

Answer. It has been modified as suggested.

TECHNICAL AND TYPOS CORRECTIONS

Answer. All the 9 remarks have been taken into account and fixed

NOTE = Some minor comments have been accepted for clarity and therefore do not appear in correction mode, but all that is indicated in response to reviewer's comments has been modified as reported

Title:

Biogeochemical cycling and phyto- and bacterio-plankton communities in a large and shallow tropical lagoon (Terminos Lagoon, Mexico) under 2009-2010 El Niño Modoki drought conditions

Authors:

Pascal Conan[1], Mireille Pujo-Pay[1], Marina Agab[1], Laura Calva-Benítez[2], Sandrine Chifflet[3], Pascal Douillet[3], Claire Dussud[1], Renaud Fichez[3], Christian Grenz[3], Francisco Gutierrez Mendieta[2], Montserrat Origel-Moreno[2,3], Arturo Rodríguez-Blanco[1], Caroline Sauret[1], Tatiana Severin[1], Marc Tedetti[3], Rocío Torres Alvarado[2], Jean-François Ghiglione[1]

(1) Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique, F-66650, Banyuls/mer, France

(2) Universidad Autonoma Metropolitana, Departamento de Hidrobiología, México D.F., Mexico

(3) Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO) UM 110, 13288, Marseille, France

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Correspondence to: Pascal Conan (pascal.conan@obs-banyuls.fr)

Abstract

A large set of biogeochemical [nutrients, dissolved and particulate organic matter, dissolved polycyclic aromatic hydrocarbons (PAHs)], phytoplanktonic (biomass and photosynthetic activity) and bacterial (diversity and abundance, including PAH-degrading bacteria, and ectoenzymatic activities)A large set of biogeochemical (nutrients, dissolved and particulate organic matter), phytoplanktonic (biomass and photosynthetic activity) and bacterial (bacterial diversity and ectoenzymatic activities) parameters were determined to understand how the severe drought period relative to the 2009-2010 El Niño Modoki episode influenced biogeochemical cycling and phyto- and bacterio-plankton communities in Terminos Lagoon (Mexico) under such conditions, potentially prefiguring future environmental conditions due to expected trends in climate change. During the study period, the water column of Terminos Lagoon functioned globally as a sink of carbon, and especially as a "nitrogen assimilator", because of high production of particulate and dissolved organic matter although exportation of autochthonous matter to the Gulf of Mexico was weak. Coupling between top-down andWe found that "bottom-up" controls accounted for a large part in the variability of the diverse responses in phytoplankton productivity. Nitrogen and phosphorus stoichiometry mostly accounted for the heterogeneity in phytoplankton and free-living prokaryotesbacteria distribution in the lagoon. In the Eastern part, we found a clear decoupling between areas enriched in dissolved inorganic nitrogen in the North close to Puerto real coastal inlet and areas enriched in phosphate (PO_4) in the South close to the Candelaria estuary. Such a decoupling limited the potential for primary production resulting in an accumulation of dissolved organic carbon and nitrogen (DOC and DON, respectively) close to the river mouths. In the Western part of the lagoon, maximal phytoplankton development resulted from the coupling between Palizada river inputs of nitrate (NO_3) and particulate organic phosphorus -PP- (but depleted in PO_4) and bacterial activity transforming PP and dissolved organic phosphorus (DOP) to available PO_4. The Chumpan River only marginally contributed to PO_4 inputs due to its very low contribution to overall river inputs. The highest dissolved total PAH concentrations were measured in El Carmen inlet, suggesting an anthropogenic pollution of the zone probably related to the oil platform exploitation activities in the shallow waters of the South of the Gulf of Mexico. We also found that a complex array of biogeochemical and phytoplanktonic parameters were the driving force behind the geographical distribution of bacterial community structure and activities. Finally, we showed that nutrients brought by the Palizada River supported an abundant bacterial community of PAH-degraders, which are of significance in this important oil production zone.We also found that a complex array of biogeochemical and phytoplanktonic parameters were the driving force behind the geographical distribution of bacterial community structure and activities. Finally, we showed that nutrients brought by the Palizada River supported an abundant bacterial community of polycyclic aromatic hydrocarbon (PAH)-degraders, which are of significance in this important oil production zone.

Keywords: biogeochemistry in coastal lagoon, microbial ecology and ecotoxicology, El Niño, lagoon pollution, Gulf of Mexico, Terminos Lagoon

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1. **Introduction**

Coastal lagoons are complex environments, combining features of shallow inland water bodies wholly or partly sealed off from the adjacent coastal oceans, influenced by tide, river input, precipitation *versus* evaporation balance and surface heat balance. Interactions between freshwater and marine sources generate strong gradients of salinity, light and nutrient availability (Hauenstein and Ramírez, 1986). Biological diversity is generally high in these environments (Milessi et al., 2010). Located in the Southern Gulf of Mexico near Campeche sound, Terminos Lagoon is one of the largest tropical coastal lagoon worldwide and its recognised environmental importance and protected status ~~is~~ are potentially threatened by petroleum-related industrial activities inshore and offshore (García-Ríos et al., 2013). A first tentative budget of salt and nutrient concluded that Terminos Lagoon was slightly autotrophic on a yearly basis (David, 1999), but this assessment was clearly based on scarce environmental data. Chlorophyll-*a* (CHL) concentration and phytoplankton net production have been reported to respectively range from 1 to 17 $\mu g\ L^{-1}$ and from 20 to 300 $gC\ m^{-2}\ a^{-1}$ (Day et al., 1982), suggesting potential shift from oligotrophic to eutrophic conditions.

In aquatic ecosystems, bacteria utilize a large fraction (up to 90 %) of primary production, since algal carbon exudates might be the principal source for bacterial production (Cole et al., 1988; Conan et al., 1999). Beside the utilization of a considerable part of the available organic matter, bacterioplankton communities also absorb inorganic nutrients thus competing with phytoplankton communities (Conan et al., 2007; Hobbie, 1988). The bulk of organic matter is a highly heterogeneous matrix among which are labile substrates such as proteins or peptides, oligosaccharides, and fatty acids, but most of it is accounted for by complex and refractory substrates (Hoppe et al., 2002). Extracellular enzymes hence are essential to aquatic microorganisms as they allow for the partitioning of complex organic substrates, including high molecular weight compounds which cannot pass through the cell membrane (Arnosti and Steen, 2013). As a function of genetic diversity, the capacity to produce extracellular enzymes is differently distributed in the bacterial community, directly impacting the range of substrates metabolized (Zimmerman et al., 2013). This phenomenon has global-scale implications, since several meta-analysis clearly evidenced differences in the metabolic capacities of microorganisms from temperate, tropical or high latitude waters (Amado et al., 2013; Arnosti et al., 2011). At a local scale, alteration of the evaporation/precipitation balance due to climate change can be challenging especially in the case of coastal lagoon, as it is well known that changes in salinity may alter bacterial diversity and activities (Pedrós-Alió et al., 2000). Local anthropogenic inputs of organic pollutants such as polycyclic aromatic hydrocarbons ~~or herbicides~~(PAHs) may also ~~be detrimental to~~affect bacterial diversity and activities

((Aguayo et al., 2014; Jiménez et al., 2011; Rodríguez-Blanco et al., 2010). Indeed, PAHs, which can comprise as much as 25–35 % of total hydrocarbon content in crude oils (Head et al., 2006), are among the most abundant and ubiquitous pollutants in the coastal environment (González-Gaya et al., 2016). These compounds are recognized by the European and US environmental agencies as priority pollutants for the aquatic medium due to their toxicity, persistence and ability to accumulate in the biota (Kennish, 1992). Hence, the presence of PAHs in the marine environment may induce an increase in the indigenous populations of marine bacteria that can break down and utilize these chemicals as carbon source, the so called "PAH-degrading bacteria" or "PAH degraders". These bacteria are generally strongly selected in oil-impacted ecosystems, where they may account for 70 to 90% of the total bacterial community (Head et al., 2006;(Gutierrez et al., 2014; Head et al., 2006).

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Despite their importance, few studies consider the bacterial community in tropical inland aquatic ecosystems (Roland et al., 2010) -or coastal lagoons (Abreu et al., 1992; Hsieh et al., 2012; MacCord et al., 2013; They et al., 2013) and almost none dealt with tropical coastal lagoons (Scofield et al., 2015). Though, the present challenge is to really understand the functioning of such continental to marine environments interface in a context of growing anthropization. In such a background lacking context, the identified challenge was to foresee how phytoplankton and bacterial abundance, diversity and activity could be affected by incoming climate change in large and shallow tropical lagoons. According to Global Change scenarios, Centro America is a region where most significant climate change will occur in the second half of the century (Giorgi 2006) mostly leading to significant decrease in rainfall (Hidalgo et al., 2013). We therefore took the opportunity of the strong drought conditions related to the 2009-2010 El Niño Modoki episode to sample water in Terminos lagoonLagoon, a large and shallow tropical lagoon from the Gulf of Mexico, as a way to assess how expected from long term climate change might affect the structure and functioning of plankton communities.

In comparison to the numerous coastal lagoons fringing the Gulf of Mexico, Terminos Lagoon received moderate scientific attention (Grenz et al., in rev). Among the existing studies, very few have been conducted on bacterial communities and most of the latter have been based on culture-dependent methods (Lizárraga-Partida et al., 1987; Lizárraga-Partida et al., 1986). However, cultivable bacteria represent a very small fraction of total present bacteria (<0.1 %; Ferguson et al., 1984) and culture-independent methods are requested to more accurately assess the diversity and activity of whole bacterial communities in such a vast and understudied system. Also, Terminos Lagoon is potentially impacted by PAHs, which may come from a diversity of sources including sea-based activities (spills from ships, platforms and pipelines, ballast water discharge, drilling…) but also rivers, surface runoffs and the atmosphere that carry various urban and industrial wastes (fuel combustion, traffic exhaust emissions...). Nevertheless, to our knowledge, little is known about the PAH content in this ecosystem. Even though Noreña-Barroso et al. (1999) have reported PAH concentrations in the American Oysters *Crassostrea virginica* and Rendon-Von Osten et al. (2007), PAH concentrations in surface sediments, no data are currently available about dissolved PAH concentrations in surface waters of the Terminos Lagoon.

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~~Our study aimed at evaluating the links between : i) biogeochemical (nutrients, dissolved and particulate organic matter, dissolved PAHs), ii) phytoplanktonic (biomass and photosynthetic activity) and iii) bacterial (bacterial diversity, including PAH-degrading bacteria, and ectoenzymatic activities) parameters in Terminos Lagoon (Mexico) after a sustained period of minimum river discharge relative to the 2009-2010 El Niño Modoki episode (Fichez et al., 2016).~~

Our study aims at evaluating the links between: i) biogeochemical (nutrients, dissolved and particulate organic matter), ii) phytoplanktonic (biomass and photosynthetic activity) and iii) Free-living prokaryotes (diversity, including PAH-degrading bacteria, and ectoenzymatic activities) parameters in the water column of Terminos Lagoon (Mexico) after a sustained period of minimum river discharge relative to the 2009-2010 El Niño Modoki episode. After having identified the main sources of nutrients in the lagoon (focused on nitrogen and phosphorus), we propose a geographical organization of the ecosystem to explain the distribution of the microbial pelagic communities across the lagoon.

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2. Materials and methods

2.1 Study site and sampling

Terminos Lagoon is a large (1,936 km^2, volume 4.65 km^3) and shallow (average depth 2.4 m) coastal lagoon located in the Mexican state of Campeche (Fig. 1), 18°20' to 19°00' N and 91°10' to 92°00' W. Temperature shows low seasonal variation (27 to 33 ºC), but salinity oscillates from brackish to marine waters due to high variability in river runoff (Fichez et al., 2016; Gullian-Klanian et al., 2008). River discharge, precipitation, and groundwater seepage account for 95.44, 4.53 and 0.03 %, respectively~~, –~~. The Chumpan, Candelaria/Mamantel (hereafter Candelaria), and Palizada estuaries account for 5, 19, and 76 %, respectively of freshwater delivered yearly (~12 10^9 m^3 a^{-1}; i.e. about 2.6 times the lagoon volume) to the lagoon~~and of the averaged ~12 10^9 m^3 a^{-1} of freshwater delivered yearly to the lagoon (i.e. about 2.6 times the lagoon volume) the Chumpan, Candelaria/Mamantel (hereafter Candelaria), and Palizada estuaries account for 5, 19, and 76 %, respectively~~ (Fichez et al., 2016). The lagoon is connected to the coastal Sea by 2 inlets: El Carmen on the North Western side (4 km long) and Puerto Real on the North Eastern side (3.3 km). About half of the water volume is renewed every 9 days, mostly as a result of tidal exchange. The tide is mainly diurnal, with a mean range of 0.3 m (David and Kjerfve, 1998).

Recent results on tidal current modeling (Contreras Ruiz Esparza et al., 2014) revealed a dynamic inshore current entering the lagoon through Carmen ~~passage~~inlet, flowing through the southern half of the lagoon and coming out through Puerto Real and a much slower inverse water current flooding the northern central part of the lagoon. That tidally induced hydrodynamic trend generated a counter clock wise circulation gyre located in the ~~center~~ centre of the lagoon leeward from Carmen Island.

Samples were collected at 0.2 m depth at 35 stations distributed over the whole lagoon (Fig. 1) from the 21st to the 27th of October, 2009. In 2009, a yearly cumulated discharge of 4.83 ± 1.71 10^9 m^3 broke a historical deficit record over the 1992-2011 period for the Palizada River (average yearly cumulative discharge of 7.19 ± 4.22 10^9 m^3 s^{-1}) (Fichez et al., 2016). That exceptional drought period impacted the whole Mesoamerican region during the 2009-2010 El Niño Modoki episode, and resulted in a salinity positive anomaly in Terminos Lagoon that most strongly developed during the post wet season period (Fichez et al., 2016), at the time of our sampling.

A vertical profile of temperature, salinity and fluorescence was carried out at each of the 35 stations with a SeaBird CTD probe (SBE 19) with a precision of 0.01°C for temperature and 0.001 for salinity. Once the profile completed, water was sampled using a 5L Niskin bottle maintained horizontally at 0.2 m below the surface.

2.2 Nutrients and Dissolved organic matter

As soon as the sampling Niskin was retrieved on board, a 40 mL Schott® glass vial previously acid washed was rinsed with sampled water, filled, immediately injected with the fluorometric detection reagent for ammonia determination (as described in Holmes et al., 1999), sealed, and stored in the dark for later analysis at the laboratory. Then, two 30 mL and one 150 mL plastic acid washed vials were then rinsed with sampled water, filled, stored in a specifically dedicated and refrigerated ice cooler, to be later deep-frozen at the laboratory awaiting analysis of dissolved inorganic and organic nutrients as follow.

Nitrate (NO_3 ± 0.02 µM), nitrite (NO_2 ± 0.01 µM), phosphate (PO_4 ± 0.01 µM) and silicate ($Si(OH)_4$ ± 0.05 µM) concentrations were measured on a continuous flow autoanalyzer Technicon® AutoAnalyzer II (Aminot and Kérouel, 2007) as previously described in Severin et al. (2014). Ammonium (NH_4 ± 10 nM) was

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detected at nanomolar concentrations by fluorometric detection (Holmes et al., 1999) on a Turner Design Trilogy fluorometer.

Samples for dissolved organic matter (DOM) were filtered through 2 precombusted (24h, 450°C) glass fiber filters (Whatman GF/F, 25 mm). 20 mL were collected for dissolved organic carbon (DOC), into precombusted glass tubes, acidified with orthophosphoric acid (H_3PO_4), and analyzed by high temperature catalytic oxidation (HTCO) (Cauwet, 1999) on a Shimadzu TOCV analyzer. Typical analytical precision is ± 0.1–0.5 (SD) or 0.2–1 % (CV). 20 mL of samples were collected in Teflon vials for dissolved organic nitrogen (DON) and phosphorus (DOP), and were analyzed by Persulfate wet-oxidation according to Pujo-Pay and Raimbault (1994) and Pujo-Pay et al. (1997).

2.3 Particulate organic matter, chlorophyll and phaeopigment

A 4 L plastic acid washed container was also sub-sampling from the Niskin bottle. It was rinsed with sampled water, filled, and stored in a dedicated ice cooler awaiting filtration back at the laboratory as follow. 250 mL of seawater were filtered through a precombusted (24h, 450°C) Whatman GF/F glass filters (25 mm), placed into a Teflon vial and oxidized for particulate organic nitrogen (PON) and phosphorus (PP) measurements (according to Pujo-Pay and Raimbault, 1994). ~1 L was filtered on precombusted (24 h, 450°C) glass fiber filters (Whatman GF/F, 25mm) for particulate organic carbon (POC) and PON measurements. Filters were dried in an oven at 50°C and stored, in ashed glass vial and in a dessicator until analyses when return from the cruise, on a CHN Perkin Elmer 2400.

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For chlorophyll (CHL), 250 mL were filtered on 25 mm diameter Whatman® GF/F filters and immediately stored in liquid nitrogen. CHL and phaeopigment (Phaeo) were later extracted from filters by 100 % methanol (Marker, 1972) and concentrations were determined by the fluorometric technique (Lorenzen, 1966) on a Turner Design Trilogy fluorometer.

2.4 Photosynthetic parameters

Photosynthetic-irradiance parameters (α, P^b_m and I_k) were measured using radioactive ^{14}C-tracer technique (Fitzwater et al., 1982) in a homemade incubator specifically design. 10x60mL Nunc® culture vials were cautiously filled and inoculated with $Na_2H^{14}CO_3$ (final activity of ~0.2 µCi mL^{-1}), incubated for 45 min in a 10 light levels irradiance gradient (from 0 to 1327 W m^{-2}), then filtered on Whatman GF/F 25 mm filters, rinsed with 10 % HCl, dried at 45°C for 12 h, and placed into scintillation vials. 10 ml of a liquid scintillation cocktail

(Ultima Gold uLLT) were added to the set of scintillation vials 6 h before processing in a Beckman Scintillation Counter. The photosynthetic parameters were determined by fitting each obtained curve with the 'hyperbolic tangent model without photoinhibition' proposed by Jassby and Platt (1976).

2.5 Measurements of dissolved total PAH concentrations

Dissolved total ~~polycyclic aromatic hydrocarbons (~~PAH~~s)~~ concentrations were determined by using the EnviroFlu-HC submersible UV fluorometer (TriOS Optical Sensors, Germany), a commercially available instrument dedicated to the *in situ* and real time quantification of PAHs in water. The sensor was calibrated in the laboratory before the cruises according to Tedetti et al. (2010) and Sauret et al. (2016). In this work, the mean dissolved total PAH concentrations derived from the sensor are given in ng L^{-1} with a mean coefficient of variation of 10 %.

2.6 ~~Bacterial abundance~~Abundance of prokaryotes

Free-living prokaryotes~~bacteria~~ were determined by flow cytometry (Mével et al., 2008). 2 mL seawater samples were fixed with 2 % formaldehyde for 1 h at 4°C. 1 mL sub-sample was incubated with SYBR Green I ~~(~~(Sigma Aldrich, final conc. 0.05% [v/v] of the commercial solution~~final conc. 0.05 % [v/v] of the commercial solution~~) for 15 min at 20°C in the dark and analysed with a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an air-cooled argon laser (488 nm, 15 mW). Data acquisition and analysis were done with Cell-Quest software (Becton Dickinson). The discrimination of the heterotrophs is done by the use of sybr green which induces a green fluorescence and then prokaryotes are separated using the SSC diffraction parameter.

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2.7 Total and metabolically active bacterial community structure

Nucleic acids were extracted on 0.2 µm-pore-size filter (47 mm, PC, Nucleopore) by filtration of 1 L of pre-filtered (3 µm) water. Co-extraction of DNA and RNA was performed after chemical cell lysis (Ghiglione et al., 1999) with the Qiagen Allprep DNA/RNA extraction kit using manufacturer instructions. DNA and cDNA (by M-MLV reverse transcription of 16S rRNA, Promega) were used as a template for PCR amplification of the variable V3 region of the 16S rRNA gene (*Escherichia coli* gene positions 329–533; Brosius et al., 1981). The primer w34 was fluorescently labelled at the 5'-end position with phosphoramidite (TET, Applied Biosystems).

CE-SSCP analysis was performed using the 310 Genetic Analyzer and Genescan analysis software (Applied Biosystems), as previously described (Ortega-Retuerta et al., 2012).

2.8 Extracellular enzymatic activities

Aminopeptidase, β-glucosidase and lipase were measured using a VICTOR3 spectrofluorometer (Perkin Elmer) after incubations of 2 h at *in situ* temperature with L-leucine-7amido-4-methyl coumarin (LL, 5 µM final), MUF-β-D-glucoside (β-Glc, 0.25 µM final) or MUF-palmitate (Lip, 0.25 µM final). These saturated concentrations and optimized time incubations were determined prior to the extracellular enzymatic activities measurement, as previously described (Van Wambeke et al., 2009).

2.9 Quantification of PAH-degrading bacteria by Most-Probable-Number

The quantification of PAH-degrading bacteria was performed by the most-probable-number (MPN). A total of ~~100~~ 100 ~~µL~~ µL of each sample was introduced in triplicate in a 48-well microplate with 900 µL of sterile minimum medium, as previously described (Rodríguez-Blanco et al., 2010; Sauret et al., 2016). A mixture of 6 PAHs from 2 to 5 rings (naphthalene, fluorene, phenanthrene, fluoranthrene, pyrene and benzo[a]pyrene) prepared in dichloromethane in equimolar concentration was introduced into each well at a final concentration of 10 µg mL^{-1}, as previously described by Sauret et al. (2016). It corresponds to very high concentration of PAH in nature, i.e. 50 times higher than the values found in the harbour of Leghorn (Cincinelli et al., 2001)~~A mixture of 6 PAHs with different ring numbers (naphthalene, fluorene, phenanthrene, fluoranthrene, pyrene and benzo[a]pyrene) prepared in dichloromethane in equimolar concentration was introduced into each well at a final concentration of 10 µg mL^{-1}~~. After 2 weeks of incubation, the change from blue to pink, indicating oxidation of the resazurin contained in the medium was checked and each sample was analysed by flow cytometry. Classical MPN table gave the most probable number of bacteria able to degrade the mixture of six PAHs (Alexander, 1982).

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2.10 Statistical analysis

Comparative analysis of 16S rDNA- or 16S rRNA-based CE-SSCP fingerprints was carried out with the PRIMER 6 software (PRIMER-E, Ltd., UK) using Bray-Curtis similarities. We used the similarity profile test SIMPROF (PRIMER 6) to test the null hypothesis of randomly that a specific sub-cluster can be recreated by permuting the entry ribotypes and samples, when using hierarchical agglomerative clustering. The significant branch (SIMPROF, p<0.05) was used as a prerequisite for defining bacterial clusters, and clusters were reported on non-metric multidimensional scaling (MDS) representation.

Canonical correspondence analysis (CCA) was used to investigate the variations in the CE-SSCP profiles under the constraint of our set of environmental variables, using CANOCCO software (version 5.0), as previously described in Berjeb et al. (2011). Significant variables (i.e. variables that significantly explained changes in 16S rDNA- and 16S rRNA-based fingerprintings) in our data set were chosen using a forward-selection procedure. Explanatory variables were added until further addition of variables failed to contribute significantly ($p < 0.05$) to a substantial improvement to the model's explanatory power. Environmental parameters were previously transformed according to their pairwise distributions, and ~~spearman~~Spearman's rank pairwise correlations between the transformed environmental variables were used to determine their significance with Statel v2.7.

3. Results

3.1 Distribution of physical parameters

At the studied period, Terminos Lagoon was characterized by a North West-South East positive gradient of temperature from >30 to about 27°C (Fig. 2A). Salinity was maximal at Puerto Real inlet (37.50) and along the southern limits of El Carmen Island, intermediate at Candelaria and Chumpan River ~~embouchures~~mouths, and minimal (21.57) close to the Palizada River (Fig. 2B).

3.2 Distribution of biogeochemical parameters

Nitrate and ammonium concentrations (Fig. 2C and 2D) were maximum close to the Palizada embouchure (16.6 and 0.3 µM, respectively) and to the Puerto Real inlet (2.5 µM in NO_3 and the highest NH_4 concentration of 1 µM). In the rest of the lagoon, NO_3 concentrations were quite low and homogeneous (close to the detection limit of 0.01 µM). NH_4 concentrations were more variable with minimum values on the northern side of the lagoon, and concentration in the range 0.1 to 0.3 µM on the southern inshore side.

The distribution pattern for PO_4 (Fig. 2E) significantly differed from N-nutrients. Minimum concentrations (<0.05 µM) were measured in the western part of the lagoon under the influence of the Palizada River, indicating very low PO_4 inputs from that river as opposed to nitrogen-nutrients. PO_4 concentrations were also low (<0.10 µM) in the centre of the lagoon. The highest PO_4 concentration was measured in front of the

Chumpan River (0.17 µM), whereas significant inputs in the Eastern part came from Candelaria River (0.13 µM) and Puerto Real inlet (0.12 µM).

The distributions of dissolved organic carbon (DOC; Fig. 2F), nitrogen (DON; Fig. 2G) and phosphorus (DOP; Fig. 2H) concentrations followed a pattern comparable to the one of PO_4. The highest concentrations of 400, 20 and 1 µM for DOC, DON and DOP, respectively, were measured in the South Eastern part of the lagoon, either in front of the Chumpan ~~and~~ or Candelaria estuaries. Maximal DOC and DON concentrations (82% and 95%, respectively) were measured in front of Candelaria River, whereas the maximal DOP concentrations were observed in front of the Chumpan River~~Contrary to DOC and DON, DOP concentration was maximal in front of the Chumpan river compare to Candelaria River (82, 95, and 180 % respectively)~~. Lowest concentrations <200, 5 and 0.1 µM of DOC, DON and DOP were measured in front of the Palizada ~~embouchure~~ mouth and in the case of DOC it even spread along the northern shore of Carmen Island. Significant ~~spearman~~Spearman's rank correlations (n=35, p<0.05) were found between DON and DOC (~~P=~~ρ=0.64), DOP (~~P=~~ρ =0.64) and temperature (~~P=~~ρ =-0.32).

The 3 rivers were clearly the main sources of particulate organic nitrogen (PON) and phosphorus (PP) in the lagoon (Fig. 3). PON reached a maximum concentration of 9.3 µM in front of the Chumpan estuary and progressively decreased while spreading to the North (Fig. 3A). Concerning PP, the Palizada River was the main source with concentrations close to 0.9 µM, progressively decreasing to 0.6 µM while spreading along the southern shore toward the Chumpan Estuary and 0.5 µM in the north-eastern drift toward Puerto Real passage (Fig. 3B). Significant ~~spearman~~Spearman's rank correlations (n=35, p<0.05) were found between PP and PON (~~R=~~ ρ=0.73), NO_3 (ρ=~~R=~~0.57) and salinity (ρ=~~R=~~-0.56).

3.3 Photosynthetic pigment and activity

Chlorophyll (CHL) and phaeopigment (Phaeo) followed a convergent distribution pattern (Fig. 3C and 3D) with maximum concentrations close or in the vicinity to the Palizada mouth (>6 µgCHL L^{-1} and ~2 µgPhaeo L^{-1}). A range of 1-6 µgCHL L^{-1} and 1-2 µgPhaeo L^{-1} was encountered in the western part of the lagoon. Concentrations <1 µgCHL L^{-1} and 1-2 µgPhaeo L^{-1} were mostly confined to the eastern part. On a global view, Phaeo accounted for 28 ± 8 % of CHL on average, hence attesting of rather active phytoplankton communities. Significant ~~spearman~~Spearman's rank correlations (n=35, p<0.05) were found between CHL and~~,~~ Phaeo (ρ=~~R=~~0.82) ~~and~~ or P~~O~~P (~~P~~ρ=0.74).

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The maximum rate of carbon production per unit of chlorophyll at light saturation (P^b_m, Fig. 3E) was minimal (<0.5 mgC mgCHL^{-1} h^{-1}) in the Palizada plume in association with the maximum Phaeo:CHL ratio measured ~~(<~~(>44 %). Maximum P^b_m values in excess of 8.0 mgC mgCHL^{-1} h^{-1} were measured close the Chumpan estuary in an area of low Phaeo:CHL ratio (<25 %).

3.4 Bacterial~~Bacterial a~~abundance and extracellular enzymatic activities

Free-living prokaryotes~~Bacterial~~ abundance ~~–~~ranged from 1.0 to 4.8 10^6 cell mL^{-1} (mean=2.8 10^6 cell mL^{-1}, SD=0.9 10^6 cell mL^{-1}, n=35), with maximum values observed in the Puerto Real passage and close to the river ~~embouchures~~ mouths (Candelaria and Chumpan rivers), except for the Palizada river which showed the highest river-lagoon gradient from maximum to minimal values cited above (Fig. 3F).

Cell specific aminopeptidase (Leu-MCA), and phosphatase (MUF-P) activities reached maximum values close to Palizada and Chumpan rivers ~~embouchures~~ mouths (33, and 131.9 fmol L^{-1} h^{-1} cell^{-1}, respectively (Fig. 4A, and 4B). Cell specific lipase activity (MUF-Lip) was maximum (10.9 fmol L^{-1} h^{-1} cell^{-1}; Fig. 4C) from Chumpan river ~~embouchure~~ mouth northward towards El Carmen Island, crossing the lagoon approximately in its middle following the isotherms (Fig. 2A). Much lower activities were found over most of the lagoon for all the activities (mean values in fmol L^{-1} h^{-1} cell^{-1} are 12.6 ± 8.4 for Leu-MCA, 12.1 ± 24.2 for MUF-P and 2.4 ± 2.6 for MUF-Lip). Significant ~~spearman~~Spearman's rank correlations (n=35, p<0.01) were found between aminopeptidase activities and DOC (ρ=~~R=~~ -0.27), PON (ρ=~~R=~~0.33) and to a lesser extent, between phosphatase activities and PO_4 (ρ=~~R=~~ -0.46), PP (ρ=~~R=~~0.60), NO_3 (ρ=~~R=~~0.69), CHL (ρ=~~R=~~0.53).

3.5 Dissolved PAH concentrations and ~~Estimated~~ estimated abundance of bacterial PAH-degraders ~~and PAH concentrations~~

Dissolved total PAH concentrations (Fig. 5~~B~~A) were higher close to the El Carmen inlet (332 ng L^{-1}) and relatively lower close to Palizada river (187 ng L^{-1}) and to the Chumpan ~~embouchure~~mouth (166 ng L^{-1}). ~~PAH concentrations~~They were generally lower in the rest of the lagoon (<130 ng L^{-1}). Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada river (estimated at 4.6 10^4 cells mL^{-1}, equivalent to 4.4 % of free-living prokaryotes~~bacteria~~) (Fig. ~~5A~~5B). Lower values were found close to the Chumpan ~~embouchure~~ mouth (estimated at 4.7 10^3 cells mL^{-1}, equivalent to 0.2 % of free-living prokaryotes~~bacteria~~), and commonly represented less than 0.1 % of the free-living prokaryote~~bacterial~~ abundance in the rest of the lagoon. Quantification by MPN counts showed significant even if low

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~~spearman~~Spearman's rank correlation with dissolved total PAH concentrations (~~P~~ρ=0.37, $p<0.05$, n=35)~~.,~~ MPN counts c~~mainly because of PAH distribution (Fig. 5B) showing higher concentrations close to the El Carmen inlet (332 ng L^{-1}) and relatively lower concentrations close to Palizada river (187 ng L^{-1}) and to the Chumpan embouchure (166 ng L^{-1}). C~~orrelation~~s~~ ($p<0.05$, n=35) were stronger with PP (ρ~~R~~=0.65) and CHL (ρ~~R~~=0.53). ~~PAH concentrations were generally lower in the rest of the lagoon (<130 ng L^{-1}).~~

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3.6 Spatial distribution of total and metabolically active bacteria by CE-SSCP fingerprints.

Bacterial community structure defined as a function of 16S rDNA-based fingerprints from each sample singled out 3 individual stations (Palizada river, El Carmen inlet and Candelaria river) and aggregated 5 groups of stations (Fig. 6A). Three of those groups included a large number of samples: cluster I grouped 9 stations located in the North-eastern part of the lagoon close to Puerto Real inlet; cluster II grouped 9 stations positioned in the middle of the lagoon up North from Chumpan river to the Carmen Island; cluster III grouped 8 stations situated in the South western of the Carmen Island. Two other groups with fewer stations identified intermediated communities found between the El Carmen inlet and the Palizada River in the western part of the lagoon (cluster V; stations 2, 4, 6) and in the middle of the lagoon, close to the Candelaria river (cluster IV; stations 22, 24, 27).

Metabolically active bacterial communities as a function of 16S rRNA-based fingerprints singled out 2 stations (Palizada river and El Carmen inlet) and aggregated 5 groups of stations which are slightly different from the DNA-based clusters (Fig. 6B). Three of those groups included a large number of samples: cluster I formed the largest cluster with 15 stations located in the Eastern part of the lagoon; cluster II grouped 9 stations in the middle of the lagoon up North from Chumpan river to the Carmen Island; cluster III grouped 5 stations in the North Western part of the lagoon, close to El Carmen inlet. Two other groups with fewer stations showed intermediate~~d~~ communities found close to the Palizada river mouth (cluster IV; stations 6 and 8) and further east (cluster V; stations 9 and 12).

3.7 Environmental drivers of the total and active prokaryote~~bacterial~~ community structures

To analyse the main environmental factors controlling the spatial distribution of total (Fig. 7A) and active (Fig. 7B) prokaryote~~bacterial~~ communities, we performed a canonical correspondence analysis (CCA). In both DNA- and RNA- based analysis, the cumulative percentage of variance of the species-environment relationship indicated that the first and second canonical axis explained 48 % and 24 % of the total variance, respectively for DNA and 45 % and 31 % for RNA. The remaining axes accounted for less than 14 % of the total variance each, and thus were not considered as significant enough.

In the DNA-based CCA, the first canonical axis was positively correlated with NO_3^- and CHL and negatively correlated with concentration of DOC, DOP, DON and oxygen. In the RNA-based CCA, the first canonical axis was positively correlated with NO_3^- and PAHs and negatively correlated with the concentration of POC, PON, oxygen, salinity, PO_4 and CHL. The concomitant effect of those parameters explained 27 % and 40 % (ratio between the sum of all canonical eigenvalues and the sum of all eigenvalues) of the changes in bacterial community structure found in the DNA- and RNA-based fractions, respectively (Figure 7).

4. Discussion

4.1 Biogeochemical characteristics of Terminos Lagoon under low River discharge conditions

With a contribution of about 76 % to river inputs in the lagoon (Fichez et al., 2016; Jensen et al., 1989), Palizada River delivers most of the new nitrogen inputs as nitrate and ammonium. High concentrations in nitrogen were also measured in the Puerto Real inlet, suggesting a second nitrogen source from coastal seawater. These two sources have clearly different impact on primary producer development and activity as shown by the Phaeo:CHL ratio (<20 % in the vicinity of the Palizada River, but >30 % close to the Puerto Real inlet) and P^b_m values (low in the Palizada area and higher close to the inlet). So, despite greater chlorophyll degradation (indicated by high Phaeo concentrations), phytoplanktonic cells were more productive under the influence of waters from the Gulf of Mexico when compared to those under the river influence. Specifically, in terms of average values for the two zones, with similar nutrient, DOM and POM concentrations, we measured a similar potential primary production per unit volume (27.5 and 30.2 mgC m^{-3} h^{-1} for Palizada River and Puerto Real inlet respectively), but for about 2-fold lower stock of chlorophyll in the area of the inlet (6.3 and 3.9 mgCHL m^{-3} for Palizada River and Puerto Real inlet respectively). This is in apparent contradiction with what has been classically reported on the influence of rivers inputs in coastal areas that generally largely enhanced primary productivity (see for example the Rhone River in the Mediterranean Sea; Pujo-Pay et al., 2006). Decreasing turbidity along the estuarine to inlet transect is a first factor explaining the seaward offset of phytoplankton productivity. But higher grazing activity by herbivores in the coastal waters or in the vicinity of the Inlet could be an explanation to further justify the conjunction of higher Phaeo concentrations together with active phytoplankton physiology (this should be further study).

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In fact, a higher activity of grazing by herbivores in the coastal waters could explain the higher Phaeo concentrations. Moreover, Day *et al.* (1982) have already founddemonstrated that small additions of filtered mangrove water had a stimulatory effect on pelagic primary production in Terminos Lagoon. This observation was later confirmed by Rivera-Monroy *et al.* (1998), b the latterwho also evidencing evidenced a large temporal variability in stimulating effect, and a rapid inhibition due to variable humic substance concentrations. The relative decrease of productivity close to the Palizada plume could be due to humic matter as we also found relatively high concentrations in dissolved PAHs (see hereafter §4.4). Finally, it is clear that bottom-up (nutrients and humic substances) drove the differential responses of phytoplankton productivity in the eastern and western part of the lagoon, certainly in conjunction with grazing activity (top-down control).Finally, a combined top-down (grazing) and bottom-up (humic substances) drove the differential responses of phytoplankton productivity.

At the time of our study, the Palizada River and the Puerto Real inlet were major sources of nitrogen to the lagoon. Sediments are generally considered to be a significant internal source of nutrients in shallow coastal ecosystems but they may also be a net sink of dissolved nitrogen at least during certain times of the year At the time of our study, the Palizada River and the Puerto Real inlet were major sources of nitrogen to the lagoon. Sediments are generally considered to be a significant internal source of nutrients in shallow coastal ecosystems but they may also be a net sink of dissolved nitrogen at least during certain times of the year (Sundbäck et al., 2000; Tyler et al., 2003). Rivera-Monroy et al. (1995a) measured nitrogen fluxes between Estero Pargo (an unpolluted tidal creek), and a fringe mangrove forest in Terminos Lagoon. They reported that mangrove sediments were a sink of NO_3 and NH_4 throughout the year. Denitrification, the dissimilatory reduction of NO_3 to produce N_2O and N_2, was considered as the main process that contributed to NO_3 loss. However, direct measurements of denitrification rates in the fringe and basin mangroves of Terminos Lagoon indicated low sink of NO_3 (Rivera-Monroy et al., 1995b) on the contrary to what has been evidenced in other mangrove forests (i.e. Twilley, 2013). This was confirmed later by Rivera-Monroy *et al.* (Rivera-Monroy et al., 2007) who hypothesised that most of the inorganic nitrogen was retained in the sediments and not lost via denitrification. They also measured a decoupling between sources of nitrogen and phosphorus and because P is a limiting nutrient, they assumed that the dominant source was tidal flooding as opposed to remineralization from organic matter in the soil. During our study, Origel Moreno (2015) found that benthic carbon mineralization consumed a large proportion (between 67 and 86 %) of the pelagic carbon production. These values are in the higher part of the range calculated for sub-tropical lagoons (Grenz et al., 2010; Machado and Knoppers, 1988) and indicate

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Mis en forme : Police :10 pt, Anglais (États-Unis) high biological activity in the sediments. Futhermore, Origel Moreno (2015) estimated that 50 to 95 % of nitrogen was mineralized in the sediment through various N-consuming processes but also that nitrogen was more efficiently mineralized than phosphorus.During our study, Origel Moreno (2015) found that benthic carbon mineralization consumed a large proportion (between 67 and 86 %) of the pelagic carbon production. These values are in the higher part of the range calculated for sub-tropical lagoons (Grenz et al., 2010; Machado and Knoppers, 1988) and indicate high biological activity in the sediments. Futhermore, Origel Moreno (2015) estimated that 50 to 95 % of nitrogen was mineralized in the sediment through various N-consuming processes but also that nitrogen was more efficiently mineralized than phosphorus.

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Our large scale study considering the whole lagoon brings some information about the potential origin of phosphorus in the water column. It is clear from our measurements that phosphate distribution in the lagoon was disconnected from nitrogen. This impacted the stoichiometry of particulate organic matter (N:P ratio) through the whole lagoon as shown by the surprising and relative low values of PON:PP ratio (<13) at all stations (indicating a particulate nitrogen deficit), except for those located in the southwest part of the lagoon where a canonical Redfied ratio of 16 was measured (Fig. 8). To sustain their growth requirement, primary producers have the ability to decouple their consumption of phosphorus and nitrogen in respect to a variable metabolic plasticity (Conan et al., 2007). In comparison to the previously discussed 2 main sources of NO_3 and NH_4 (Palizada River and Puerto Real inlet) located in the West and North part of the lagoon, we identified two distinct main sources of PO_4 in Terminos Lagoon during the sampled period: (i) river inputs from Candelaria and Chumpan in the South part, even though their contribution to the overall river discharge is low, and (ii) mineralization of organic phosphorus (PP and DOP) by prokaryotes bacterial activity (coherent with ectoenzymatic activities; see hereafter §4.2). Note that the major source of PP in the lagoon was the Palizada River, whereas accumulation of DOP was measured between the Palizada and Chumpan Rivers in the South West of the lagoon. In this area, distribution of dissolved oxygen was minimal compared to the rest of the lagoon, which was coherent with high rates of organic matter mineralization in the water column. Finally during our study, the dominant source of PO_4 was not tidal flooding as hypothesized by Rivera-Monroy *et al.* (2007), but the mineralization of organic matter by free-living prokaryotesbacteria in the water column. If that conclusion appears valid in the context of weak river discharges, further studies will be necessary to test its potential extension to other environmental conditions (rainy periods, river flood, tidal amplitude...).

4.2 Relationship between biogeochemical conditions and prokaryoticbacterial activities

Our analysis of biogeochemical trends in Terminos Lagoon has been combined with the study of the spatial distribution of prokaryotic extracellular activity. Bacterial aminopeptidase and lipase extracellular activities play a key function in the transformation of biopolymer into small monomers, since a large part of organic matter is in the form of large size molecules but only small molecules (<600 Da) are directly assimilable by bacteria (Weiss et al., 1991). The expression of aminopeptidase activity indicates the absence of direct bacterial assimilation of dissolved organic matter and their ability to actively release enzymes outside the cells (Van Wambeke et al., 2009). Moderate but significant negative correlations were found between aminopeptidase activity per cells and DOC concentration in Terminos Lagoon (~~P~~ρ=0.27, n=35, p<0.01). Higher DOC concentrations associated with lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria. The high aminopeptidase activity in the Palizada River plume confirmed the presence of recalcitrant organic matter from terrestrial origin, as opposed to minimum activities in Puerto Real marine waters or in Candelaria ~~embouchures~~mouths, where DOC concentrations were maximal. Lipase activities showed different trends, with higher activities found in the middle of the lagoon up North from Chumpan River to Carmen Island. We previously published that ambient quantity and quality of hydrolysable acyl-lipids clearly coupled with the measurement of their *in situ* hydrolysis rates (Bourguet et al., 2009). The differences between spatial distributions of ectoenzymatic aminopeptidase and lipase activities suggest that organic matter from different composition resided in the central zone of Terminos Lagoon, a result in strong agreement with a recent study on hydrodynamics that identified a large circulation cell in the same central area (Contreras Ruiz et al., 2014). Unfortunately, the contribution of the protein or lipid pool to total organic matter was not measured at the time of the study, which may have strengthen our hypothesis on the role of the composition of organic matter in the spatial distribution of extracellular enzymes activities. This lack of information may explain the very low or absence of correlation found between extracellular activities and measured biogeochemical parameters.

Phosphatase activity is well known to be controlled by the availability of soluble reactive phosphorus (Van Wambeke et al., 2009). This activity was essentially observed in the vicinity of Palizada River, and not in Puerto real inlet, the two P-depleted zones which indirectly influence the stoichiometry of particulate organic matter, as discussed above (Fig. 8). Thus, a clear phosphatase activity but P-depleted zone means very low P-availability for phytoplanktonic growth. This observation is consistent with the low phytoplankton productivity indicating a weak C-fixation rates in this zone, strengthens our bottom-up control hypothesis. Extracellular phosphatase activity was significantly (p<0.05, n=35) negatively correlated with PO_4 (~~P~~ρ=-0.46) and positively with PP (~~P~~ρ=0.60). Our data therefore converge with the model previously proposed by Robadue *et al.* (2004)

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predicting a different behaviour between the Eastern and Western sides of the lagoon in terms of water budget as well as ecosystem functioning, this distinction being mostly driven by the respective influences of the Palizada River discharge in the West and the Puerto Real marine water inputs in the North East.

4.3 Prokaryotic~~Bacterial~~ community structure and ectoenzyme activities

Molecular fingerprinting (such as CE-SSCP) and next-generation sequencing technologies generally yielded converging results (Ghiglione et al., 2005; Ghiglione and Murray, 2012; Ortega-Retuerta et al., 2012; Sauret et al., 2015) evidencing clear shifts in bacterial community structure as a function of changes in biogeochemical characteristics (Ghiglione et al., 2005). Numerous factors can regulate microorganism population dynamics, often simultaneously, and several evidences found in the literature (Berdjeb et al., 2011; Fuhrman et al., 2013; Ghiglione et al., 2008) underlined the importance of relevant statistical analysis to investigate the relative importance of environmental factors in predicting the bacterial community dynamics.

It is generally recognized that the expression of ectoenzyme activities could result from species selection and population dynamics (Martinez et al., 1996) and the zonation of prokaryotic~~bacterial~~ community structure in the Eastern, middle and Western parts of the lagoon agreed with such a paradigm. The community composition in the Eastern part could be divided into two sub-clusters corresponding to the respective influences of Palizada River mouth and El Carmen inlet. Both DNA- and RNA-based fingerprinting showed that Palizada River and El Carmen inlet hosted distinct prokaryotic~~bacterial~~ communities, as previously observed in transition zones such as river (Ortega-Retuerta et al., 2012) or lagoon mouth (Rappé et al., 2000). The relation between community composition and ectoenzyme activities was particularly evident when considering the lipase and aminopeptidase rates. Lipase activity was magnified in the middle of the lagoon with a South to North increasing gradient from Chumpan River to Carmen Island that coincided with specific communities (cluster II in both DNA- and RNA-based fingerprinting). Other communities were found in the Western part under the influence of the Palizada River, where higher aminopeptidase activity was measured.

The combination of DNA and RNA strengthen our observations as DNA-based analysis alone would have failed to distinguish between active, dormant, senescent or dead cells hence preventing to assess the level of activity of each detected bacterial population (Rodríguez-Blanco et al., 2010). Even though the abundance of bacteria in the sea is high, only a small fraction is considered to be metabolically active (Del Giorgio and Bouvier, 2002). Bacterial growth rate has been shown to correlate with cellular rRNA content (Kemp et al., 1993); therefore, information on cellular activity may be obtained by tracking reverse-transcribed 16S rRNA

(Lami et al., 2009). In the present study, we focused on the free-living- prokaryotes~~bacteria~~ and disregarded the particle-attached fraction by pre-filtrating the water by 3 µm, which allowed eliminating the problem of DNA eukaryotic chloroplasts that may have biased our results in the context of gradients of productive zones. The combination of DNA and RNA results in Terminos Lagoon showed similar trends with total and active communities presenting a strong zonation between the eastern, middle and western parts of the lagoon, to which could be added smaller transition zones located around major sources of coastal (El Carmen inlet) and river inputs (Palizada and Candelaria). Here, the combination of DNA and RNA showed similar tendencies within the total and active communities presenting eastern, middle and western distribution among the lagoon. These results indicated that most of the free-living bacterial communities detected by molecular fingerprinting (DNA-based) were active (RNA-based) among the lagoon, with the exception of the local transition zones between the lagoon waters and the coastal (El Carmen inlet) or rivers (Palizada and Candelaria). ~~These results indicated that most of the communities detected by molecular fingerprinting were active, with no specific distinction through the lagoon.~~

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4.4 Biogeochemical parameters and ~~pollutants~~ PAHs are driving the prokaryotic~~bacterial~~ community structure

~~Molecular fingerprinting (such as CE-SSCP) and next-generation sequencing technologies generally yielded converging results (Ghiglione et al., 2005; Ghiglione and Murray, 2012; Ortega-Retuerta et al., 2012; Sauret et al., 2015) evidencing clear shifts in bacterial community structure as a function of changes in biogeochemical characteristics (Ghiglione et al., 2005). Numerous factors can regulate microorganism population dynamics, often simultaneously, and several evidences found in the literature (Berdjeb et al., 2011; Fuhrman et al., 2013; Ghiglione et al., 2008) underlined the importance of relevant statistical analysis to investigate the relative importance of environmental factors in predicting the bacterial community dynamics.~~

Through the use of direct gradient multivariate ordination analyses, we demonstrated that a complex array of biogeochemical parameters was the driving force behind- prokaryotic~~bacterial~~ community structure shifts in Terminos Lagoon. Physico-chemical parameters such as nitrate, oxygen, dissolved organic matter (DOC, DON, DOP) and chlorophyll *a* acted in synergy to explain bacterial assemblage changes on rDNA level. Some differences were observed to explain the geographical patterns of the metabolically active bacterial communities (rRNA level), ~~where~~ which salinity, particulate organic matter (PON, PP) and phosphate were needed in addition to nitrate, oxygen and CHL parameters already outlined on rDNA level. The variance explained by the environmental variables selected by the statistical model only represented 27 % and 40 % of the variability at the DNA and RNA level, respectively. So, further studies will be needed to elucidate the unexplained variance of the model, due to other parameters not taken into account in our study, such as ecological relationships between bacterial community themselves or top-down control by predation and viral lysis (Ghiglione et al., 2016).

The concentration of dissolved total PAHs was also a significant explanatory variable of the metabolically active bacterial community structure. PAHs are considered the most toxic component of crude oil to marine life and are ubiquitous pollutants in the coastal environment (Kennish, 1992). Our study was performed just before the 2010 Deepwater Horizon (DWH) blowout in the Gulf of Mexico, but several offshore oil platforms exist in the shallow waters of Campeche Bank in the southern part of the Gulf of Mexico, such as the one from the Campeche field (Cheek-1) which is only 60 km North from Terminos Lagoon (Warr et al., 2013). The coast of Campeche itself has been also impacted by the 1979 oil spill of the *Ixtoc I* platform, just about 100 km Northwest of Terminos Lagoon (Warr et al., 2013). PAHs concentrations in Terminos Lagoon indicated an input into the lagoon from El Carmen inlet (maximal concentration of 332 ng L^{-1}) that mostly impacted the Eastern part, with concentration <130 ng L^{-1} in the rest of the lagoon. We observed a high enrichment of PAH-degraders in the South eastern part of the lagoon, with low but significant correlation with PAH concentrations (Pρ=0.37, $p<0.05$, n=35). This enrichment was particularly high (estimated at 4.6 10^4 cell mL^{-1}, equivalent to 4.4 % of the free-living prokaryotesbacterial abundance) in the Palizada River mouth. Nitrogen fertilization from allochthonous inputs from the Palizada River may be crucial for PAHs degradation potential in Terminos Lagoon. Indeed, it is well accepted that bacterial degradation of hydrocarbon (carbon source for bacteria) is dependent on the nutrients to re-equilibrate the C:N:P ratio (Sauret et al., 2015; Sauret et al., 2016). Some halotolerant bacteria may have the capability to degrade PAHs and survive in river, lagoon and seawater, such as *Marinobacter hydrocarbonoclasticus* sp. 17 (Grimaud et al., 2012). Further studies using PAH-stable isotopes coupled with pyrosequencing (Dombrowski et al., 2016; Sauret et al., 2016) are necessary to identify the dynamic of these functional communities in Terminos Lagoon. Using similar approaches, previous reports showed that the pollutant content and PAHs, in particular, were responsible for the dynamic of bacterial community structure in the sediment of Bizerte lagoon, Tunisia (Ben Said et al., 2010). Such massive impact of pollutants was not observed here, possibly because of the difference in the degree of pollution between the two areas (moderately contaminated in Terminos Lagoon *versus* highly contaminated in Bizerte). Metabolically active bacterial community structure in the Terminos Lagoon was significantly impacted by PAH-

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pollution even though it did not exceed the effect of other environmental parameters and their specificity at each geographical location.

5. Conclusions

This study provides a new original set of biogeochemical characteristics for one of the largest shallow tropical coastal lagoon and due to the 2009-2010 El Niño Modoki episode, climatic conditions in Terminos Lagoon region were exceptionally dry at the time of our sampling, hence potentially indicative of future environmental conditions resulting from the predicted trends in climate change in the Centro American region. We evidenced a clear distinction in ecosystem functioning between the east and west parts of the lagoon. Most of the oceanic water entering through the inlets spread toward the south-east where dissolved organic matter accumulated. This area did not support significant phytoplankton development. In the West, we hypothesized a balance shift between a top-down and a bottom-up control to explain~~ed~~ the different responses in terms of phytoplankton productivity. The decoupling between nitrogen inputs respectively brought by oceanic waters and the Palizada River, and phosphate inputs from the Chumpan River did not allow for phytoplankton ~~growth~~C-fixation. Most of the phytoplankton biomass was aggregated around the Palizada River mouth (which brought most of the freshwater in the lagoon), in a P-depleted area (low phosphate concentration and high bacterial phosphatase activity). Bacterial ectoenzyme activities were mainly observed in the middle of the lagoon, along a south to north cross section stretching from the Chumpan River up to Carmen Island. Maximum mineralization activities were found in this area, which coincided with high extracellular lipase and aminopeptidase activities and low DOC and O_2 concentrations. The lagoon produced significant quantities of particulate and dissolved organic matter thanks to nutrients inputs from the rivers, to uncoupling between nitrogen and phosphate, and to prokaryotic~~bacterial~~ activities, but in the end most of it was internally processed or stored and only a few of this autochthonous matter was exported to the Gulf of Mexico coastal waters. Hence during our study, the water colimn of Terminos Lagoon functioned globally as a sink, and especially as a "nitrogen assimilator". Highest PAH concentrations were measured in El Carmen inlet, suggesting an anthropogenic pollution of the zone probably related to the oil platform exploitation activities in the shallow waters of the South of the Gulf of Mexico and, more locally, to the efflux from El Carmen harbour that serves as a logistical support to the oil extraction industry. We also evidenced the importance of nitrogen fertilization from the Palizada River, which seems to support an abundant prokaryotic~~bacterial~~ community of PAH-degraders.

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Another significant outcome from our study was (i) to link the spatial distribution of ectoenzymatic activities with changes in prokaryotic~~bacterial~~ community structure and (ii) to show that a combination of a complex set of physical and biogeochemical parameters was necessary to explain the changes in prokaryotic ~~bacterial~~ community structure. This study also emphasizes the use of direct multivariate statistical analysis to keep the influence of pollutants in perspective, without denying the role of other physico-chemical variables to explain the dynamic of prokaryotic~~bacterial~~ community structure in polluted areas.

Our study provided an extensive dataset efficiently mixing biogeochemical status ~~information~~ with information on phytoplankton and prokaryotic~~bacterial~~ structure and dynamics, which has never been measured before in Terminos Lagoon and its outcomes offers a strong base of information and reflexion for future studies on this essential coastal system and the potential environmental conditions that might prevail as a consequence of incoming climate change. Further studies will be needed to compare our dataset with high river input regime conditions and asses how it might affect the observed uncoupling between nitrogen and phosphate as well as the dominant source of phosphorus and its consequence on the primary production and prokaryotic activities. Also, the role of the top-down control should also be investigated in order to better understand the variability of the observed responses.~~The~~

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Figure legends

Figure 1: Study site location and distribution of the 35 sampled stations in the lagoon.

Figure 2: Maps distribution of the physico-chemical parameters measured in the Terminos lagoon in October 2009 for **A.** Temperature (°C); **B.** Salinity; **C.** nitrate concentrations (NO_3 in µM); **D.** ammonium concentrations (NH_4 in µM); **E.** phosphate concentrations (PO_4 in µM); **F.** dissolved organic carbon concentrations (DOC in µM); **G.** dissolved organic nitrogen concentrations (DON in µM); and **H.** dissolved organic phosphorus concentrations (DOP in µM).

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Figure 3: as Figure 2 for **A.** particulate organic nitrogen concentrations (PON in µM); **B.** particulate organic phosphorus concentrations (PP in µM); **C.** Total chlorophyll concentrations (CHL in $mg.m^{-3}$); **D.** phaeopigments (Phaeo in $mg.m^{-3}$); **E.** maximum photosynthetic rate normalized to chlorophyll (P^b_m in $mgC.mgCHL^{-1}.h^{-1}$); **F.** Free-living prokaryotes~~bacterial~~ abundance (10^6 $cell.mL^{-1}$)

Figure 4: as Figure 2 for **A.** aminopeptidase activities ($fmol.L^{-1}.h^{-1}.cell^{-1}$); **B.** phosphatase activities ($fmol.L^{-1}.h^{-1}.cell^{-1}$); and **C.** Lipase activities ($fmol.L^{-1}.h^{-1}.cell^{-1}$)

Figure 5: as Figure 2 for **A.** -Total dissolved PAHs ($ng.L^{-1}$)~~the most-probable-number (MPN in count)~~; and **B.** the most-probable-number (MPN in count)~~Total dissolved PAHs (ng.L⁻¹)~~

Mis en forme : Police :Gras

Figure 6: Multidimensional scaling (MDS) plot of the total (**A**) and metabolically active (**B**) prokaryotic~~bacterial~~ community structures as determined from CE-SSCP profiles based on Bray–Curtis similarity index. Clusters were determined according to similarity profile test SIMPROF ($p<0.05$).

Figure 7: Canonical correspondence analysis of total (A) and active (B) bacterioplankton community structure from the 35 samples using physico-chemical parameters. Arrows point in the direction of increasing values of each variable. The length of the arrows indicates the degree of correlation with the represented axes. The position of samples relative to arrows is interpreted by projecting the points on the arrow and indicates the extent to which a sample prokaryotic ~~bacterial~~ community composition is influenced by the environmental parameter represented by that arrow. The variance explained by the environmental variables selected by the model represent 27 % and 40 % of the variability at the DNA and RNA level, respectively.

Figure 8: as Figure 2 for NOP:PP ratio

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