

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