Interactive comment on "**The effects of decomposing invasive jellyfish on biogeochemical fluxes and microbial dynamics in an ultraoligotrophic sea**" by Tamar Guy-Haim et al.

Reviewer comments are in italics, answers follow within a text box in a dark blue font. The line numbers are compatible with the revised manuscript.

REVIEWER #2: Anonymous

General comments:

The paper of Guy-Haim et al. provides new information on the impact that the decomposition of jellyfish's carcasses can have on nutrients dynamics and on the bacteria living in sediments and in surrounding waters. The study focuses in particular on the jellyfish *Rhopilema nomadica*, a non-indigenous species that has established in recent decades in some regions of the eastern Mediterranean, where swarms of this species are regularly reported with detrimental effects for different activities of high economical relevance. An experimental set-up is built to allow measuring nutrients and dissolved oxygen as well as assessing bacteria abundance, productivity and composition, throughout different phases of the carcasses' decomposition process. Results show that jellyfish degradation determines significant changes in nutrients supply, oxygen concentration/pH and in the composition and abundance of bacteria living in the sediments and in the above water.

Overall, the study addresses a highly relevant scientific question, providing a significant contribution towards a better understanding of the impact of jellyfish blooms on biogeochemical fluxes. Research outcomes here presented can be used to improve current ecosystem models, implementing the effects of jellyfish blooms, more specifically blooms of *R. nomadica*, on biogeochemical fluxes and on the first levels of the trophic web (i.e. bacterial communities).

[**R2.1**] The paper is quite comprehensive, though needs some revisions in the description of the methods and possibly in the presentation of some results. In particular, session 2.6 should include more details on the numerical methods here adopted, as the reader is not necessarily familiar with the R routines indicated in the text and need to understand what has been done with the data.

Following the reviewer's suggestion, in the revised manuscript we have detailed all abbreviations used for statistical methods (lines 156-165). The full details of the statistical and bioinformatics methods can be found in the cited references, and are customary in studies of microbial community diversity using amplicon sequences (reviewed in Knight et al., 2018, Prodan et al., 2020).

Knight, R., Vrbanac, A., Taylor, B.C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.I., McDonald, D. and Melnik, A.V., 2018. Best practices for analysing microbiomes. *Nature Reviews Microbiology*, *16*(7), pp.410-422.

Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A.H., Nieuwdorp, M. and Levin, E., 2020. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *Plos one*, *15*(1), p.e0227434.

[**R2.2**] For instance, it should be mentioned on which data set (supposedly 30 + 30 groups shown in Fig. 7 and fig. C1?) the diversity indices have been calculated and possibly why these three specific diversity indices (Chao, Shannon and Simpson) have been selected.

The dataset on which Fig. 7 and Fig. C1 are based on is the 16S amplicon sequences obtained by Illumina high-throughput sequencing (see Materials and Methods section 2.5), which was analyzed according to the described pipeline (see Materials and Methods section 2.6). The dataset was deposited in NCBI GenBank, in the Sequence Read Archive (SRA): BioProject PRJNA626084 (see section Data Availability).

The alpha diversity indices used in our study (Chao1, Shannon and Simpson) are the most common indices used in microbial diversity research to compare the diversity among samples and between treatments with controls. Chao1 is an abundance-based estimator of species richness. Simpson Index is an estimator of species richness and species evenness, with more weight on species evenness; whereas Shannon Index is estimator of species richness and species evenness, with more weight on species richness. See the following review:

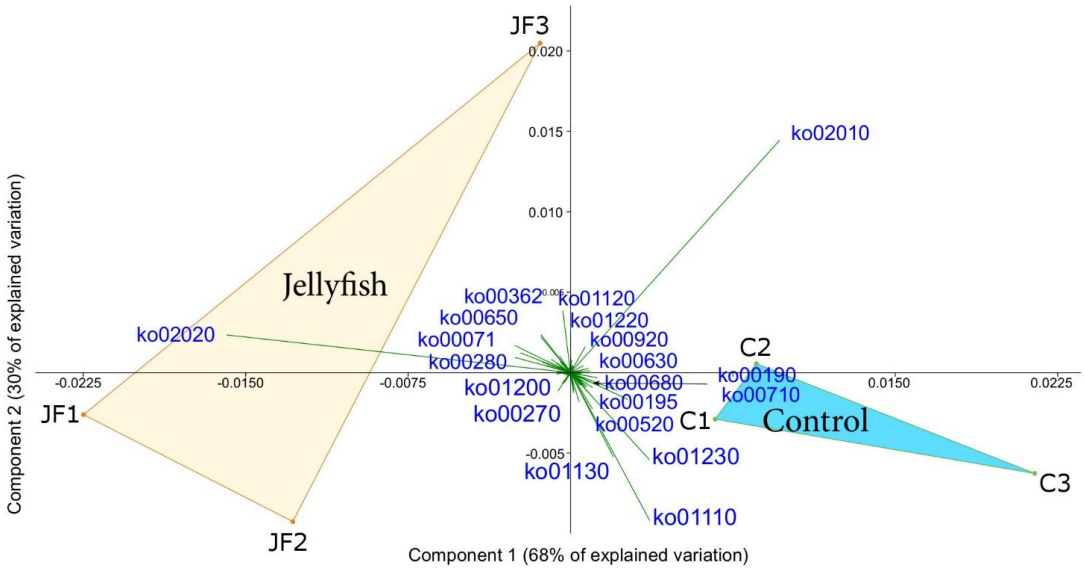
Kim, B.R., Shin, J., Guevarra, R., Lee, J.H., Kim, D.W., Seol, K.H., Lee, J.H., Kim, H.B. and Isaacson, R.E., 2017. Deciphering diversity indices for a better understanding of microbial communities. *J Microbiol Biotechnol*, *27*(12), pp.2089-2093.

[**R2.3**] Also, it should be indicated the dimension of the matrix (N metabolic functions/pathways X P observations) analysed by PCA, which should not include "rare" metabolic functions, i.e. lines with too many zeros, to prevent bias in the results of the analysis.

The PCA matrix included 324 KEGG orthologs (KOs) across all samples (N=3 jellyfish-treatments and N=3 controls), after reducing rare KOs (appearing in only one replicate), to avoid zero-inflated dimensionality. This information was added to the revised manuscript (lines 241-244, Fig. 8 caption).

[**R2.4**] Finally, Figure 8 should be redone using symbols and labels that would allow reading at least the key variables discussed in the text.

We have modified Figure 8 to include larger labels (detailed in caption) for a better readability.



[**R2.5**] line 166: Table 2 should be cited instead of Table 1.

Table 1 details the diel oxygen and nutrient fluxes standardized per jellyfish biomass (µmol·g $WW^{-1}·d^{-1}$) whereas Table 2 presents the fluxes at the sediment-water interface as measured in the experimental chambers standardized to square meter (mmol m^{-2} d^{-1}). In line 166 (lines 171-172 in the revised text), we discuss the fluxes at the sediment-water interface as measured in the jellyfish treatment chambers versus the controls. Thereby, Table 1 is referred.

[**R2.6**] line 173: here it should be indicated that the NO_3 concentration in JF2 is different from the other stations and possibly the reason for it should be discussed.

The following text was added (lines 183-185): "One of the jellyfish treatments (JF2) showed higher (2-fold) concentrations of NO_3 throughout the experiment, likely due to a different initial NO_3 content derived from the mixture of jellyfish tissue, as some parts have shown to include higher concentrations of dissolved nitrogen (MacKenzie et al., 2017). Nevertheless, this has not affected the overall nutrient fluxes nor triggered different responses to the microbial communities (thus, the same direction and strength of responses were observed in all jellyfish addition treatments)".

[**R2.7**] Lines 302-307: this sentence is unclear and should be further revised. In particular, it is not clear whether the chlorophyll maximum in late-spring summer is a recurrent event that does usually follow records of jellyfish blooms. Unless the two events can be chronologically connected, the sentence here drafted should be changed or deleted.

In this section, we describe the discrepancy between the high Chl-*a* concentrations in the water column of the EMS coastal waters during winter, to the chlorophyll maximum in the sediment in late-spring summer, which was previously explained by spring bloom of benthic producers. We suggest that the summer decomposition of *R. nomadica* blooms may also contribute to the high summer concentrations measured in the sediment throughout the leaching of limiting nutrients to phytoplankton (namely N and P). For better clarification, in the revised manuscript we have emphasized the differences between the water column and sediment (lines 317-319).

[**R2.8**] Line 314: in the first and second parentheses Synechococcus and Prochlorococcus should be respectively indicated (in other words, the two parentheses have been inverted).

Corrected: "Autotrophic cyanobacteria, on the other hand, decreased (*Synechococcus*), or increased to a lower level than the unamended control (*Prochlorococcus*)" likely due to deoxygenation (Bagby and Chisholm, 2015) or out-competition…" (lines 327-329).

[**R2.9**] Lines 324-325: I suggest to revise the text along the following lines: "In the shallow waters of the EMS the peak of bacterial production observed in summer is possibly associated with the swarms of *R. nomadica*, which are frequently (regularly?) observed in this season"

The swarms of *R. nomadica* in the EMS are observed semi-annually, during both winter and summer, coinciding with the bacterial production peaks in the EMS shallow waters. However, we would like to be more careful with our statement, and therefore we prefer using "potentially contributing" than "associated with" that may suggest causality.

[**R2.10**] Lines 363-365: this sentence needs further revision, as the study does not really measure decomposition dynamics in the Mediterranean, which would imply measurements done in situ. The study does rather measure nutrients and dissolved oxygen released by remineralisation of *R. nomadica* carcasses and the potential impact of this on the bacterial community.

We revised the sentence following the reviewer's suggestion: "Our study examined, for the first time, the decomposition effects of the bloom-forming invasive jellyfish *R. nomadica* on the oxygen and nutrient fluxes and microbial communities at the sediment-water interface" (lines 377-378).

Using this experimental setup is the best practice for the study of fluxes at the sediment-water interface (Denis et al., 2001; Glud, 2008; Hammond et al., 2004; Pratihary et al., 2014; Skoog and Arias-Esquivel, 2009). Yet, we agree that *in-situ* measurements are necessary for assessing post-bloom dynamics. In the Conclusions section, we included a paragraph (lines 387-391) on necessary future research. Indeed, we are currently running thermal large-scale mesocosms and *in-situ* research that we aim at summarizing in future publications.