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 #1: Anonymous

General comments:

The manuscript describes changes in nutrients and microbial communities in a laboratory-based jellyfish decomposition experiment. The manuscript is well written, the subject area is of interest and particularly the biodiversity aspect is novel.

[**R1.1**] The authors need to take more account of the incubation system used for the presentation and discussion of the data. Firstly, there is an evolution of processes during decomposition resulting from colonisation of the biomass, microbial growth dynamics and the sequential nature of the decomposition of particulate organic matter.

Our study was aimed at measuring fluxes at the sediment-water interface following jellyfish (specifically *R. nomadica*) decomposition. To do that correctly, we had to use the core incubation technique, which limited the temporal resolution of our study. We define this aim and acknowledge the method limitations in lines 77-80: "Nutrient fluxes were measured using the whole core incubation technique previously described by Denis et al. (2001). Although restricting this study for testing short term responses, this method follows the best practices for measuring oxygen and nutrient fluxes and dynamics at the sediment-water interface (Glud, 2008; Hammond et al., 2004; Pratihary et al., 2014; Skoog and Arias-Esquivel, 2009)".

[**R1.2**] Secondly, in the discussion the limitations of the incubation method which resulted in large changes in conditions and in particular oxygen concentrations needs to be acknowledged and put into context of the smaller changes that would occur in situ.

We accept the reviewer's recommendation. In the revised manuscript, we added the following text to the Discussion (lines 256-257): "Here we found that the decomposition of the invasive jellyfish *Rhopilema nomadica* triggered deoxygenation of the seawater overlying the sediment to hypoxic and eventually anoxic levels, although the complete dissipation of oxygen is likely due to the experimental conditions". Nevertheless, we have recently performed a large-scale experiment (in a climate-change context), in a flow-through mesocosm system with high flux rate using realistic concentrations of *R. nomadica* carcasses, and measured low oxygen (hypoxic) levels in the water column in the first 24 hours of exposure. These results will be shown in a different separated publication focused on ocean warming.

Specific comments:

[**R1.3**] In the abstract, impacts on phytoplankton are mentioned, but there is no discussion of possible links between bloom decomposition and phytoplankton community structure and production in the introduction 33-45.

In the revised manuscript, we have added to the Introduction the following text (lines 38-41): "Both in the water column and on the sediment, jelly-falls undergo bacterial decomposition, directly affecting nutrient cycling (Qu et al., 2015; West et al., 2008), potentially altering plankton community composition (Xiao et al., 2019) and stimulating algal blooms (Møller and Riisgård, 2007)".

The link between decomposition and phytoplankton community structure is further discussed in lines 308-321.

[**R1.4**] As well as providing a food source to scavenging fauna, the presence of jellyfish carcasses on the sediment surface also simultaneously blocks oxygen transfer to the underlying sediment and stimulate anaerobic respiration processes, resulting in sediment reduction and accumulation of toxic sulphides (See cited Chelsky et al paper). These changes in sediment conditions result in migration or mortality of infauna, which are in turn a major influence on nutrient cycling (See for example Welsh 2000 Chemistry & Ecology 19, 321-342; Stief 2013 Biogeosciences 10, 2829-46 for reviews). These potential negative effects on benthic fauna and the indirect effect this has on nutrient cycling deserve a mention here, especially since they are again mentioned in the abstract.

Following the reviewer's suggestion, we have added the following text to the Introduction (lines 38-41): "Both in the water column and on the sediment, jelly-falls undergo bacterial decomposition, directly affecting nutrient cycling (Qu et al., 2015; West et al., 2008). Changes in the sediment conditions may result in migration or mortality of infauna (Chelsky et al., 2016), which in turn affect indirectly nutrient cycling (Stief, 2013; Welsh, 2003)".

Additionally, the potential negative effects of jellyfish decomposition on benthic fauna are mentioned throughout the Discussion: deoxygenation and acidification (line 274-279), ammonium toxicity effect (lines 279-280), and dissolved sulfides (lines 308-309).

[**R1.5**] L65. This biomass addition is equivalent to approx. 3.5 kg per square metre. How realistic is this for a natural bloom collapse in the study area?

Our biomass estimation is realistic based on the published data on the density of *R. nomadica* in blooms in the EMS, as well as personal observations (please see an example below). The jellyfish densities are discussed in lines 302-307: "Reported densities of *R. nomadica* aggregations from the EMS are $1.6 \cdot 10^5$ km^{-2} in the Israeli coast (Lotan et al., 1992; Lotan et al., 1994), $1 \cdot 10^6$ km^{-2} in the Lebanese coast (Lakkis and Zeidane, 1991), and $9 \cdot 10^5$ km^{-2} in the Mediterranean Egyptian coast (Madkour et al., 2019). The average wet weight of *R. nomadica* changes seasonally, 1340 ±953 g ind^{-1} during summer and 2450 ±1854 g ind^{-1} during winter (N=40, T.G.-H. unpublished data), yielding ca. 1.3 kt km^{-2}". Accordingly, the densities of *R. nomadica* blooms in the EMS are 0.2-1 ind m^{-2}. Therefore, our biomass estimation (25 g 78.5 cm^{-2} = 3.2 kg m^{-2}) falls at the high limits within the realistic range. In particularly, jelly-falls of

of 1-5 ind m^{-2} on the sediment, depending on substrate topography (see below photos), thus our biomass concentration in the experiment may have been an underestimation. We plan to present *in-situ* measurements of such jelly-falls in a future publication.

Jelly-falls of *Rhopilema nomadica* and *Rhizostoma pulmo* in Dor, Israel (15-m depth). Photos: courtesy of H. Nativ (University of Haifa).

[**R1.6**] L70-80. Were the cores incubated under light or dark conditions i.e. are there any effects of photoautotrophic activity on oxygen and nutrient concentrations.

The cores were incubated under PAR= 100 µmol photons·m^{-2}·s^{-1} with a photoperiodicity of 14:10 (L:D). This information was added to the Methods section (lines 83-84).

[**R1.7**] L90-95. There are several issues with using this equation to calculate average fluxes over the entire incubation period as done in the results. Firstly, the equation assumes that the change in concentration is linear (consistent flux rate), but as the figure shows this is not true and fluxes rates evolve over time, as would be expected during decomposition (see cited decomposition studies), and in some cases reverse direction. At least in some cases, this impact could be minimised by calculating between time points, when conc changes would be closer to linear and changes between periods would show the evolution of flux rates over time.

We thank the reviewer for this important comment. In the revised manuscript, we address the non-linearity of fluxes by analyzing them over time, i.e., applying several linear phases (lines 175-199, 288-291). We also address the changes in the direction of the fluxes, which were evident in NO_x and PO_4. To calculate the diel fluxes (Tables 1,2), we have integrated the changes over time and indicated the time span used for calculation.

[**R1.8**] Secondly, fluxes are largely due to diffusion and diffusion rates depend on the concentration gradient between the sediment porewater and the overlying water. Therefore, in a closed system like the one used here, the changes in water column solute concentrations caused by

the fluxes inhibit the rate of the flux that creates them by decreasing the concentration gradient between the sediment and water. This is especially true for oxygen where the water column conc falls to zero i.e. there is no oxygen consumption at the end of the experiment because there is no oxygen demand, but because there is no oxygen to supply the oxygen demand.

In our experiment, the controls (N=3) showed no change in nutrient concentrations (Fig. 4). Therefore, although we have not measured pore water chemistry, it can be reasonably assumed that diffusive flux is negligible. The higher oxygen flux rate at the end of the experiment (under low oxygen concentration) is not due to diffusive flux but rather due to heterotrophic microbial activity.

[**R1.9**] Thirdly, as the extremely large change in water column oxygen concentration and therefore fluxes, aerobic processes become increasingly inhibited over time causing a shift to anaerobic processes, which would impact both nutrient dynamics and microbial community composition.

We acknowledge that over time (in our experiment, after >26 hrs) oxygen levels were reduced to hypoxic levels (<4 mg L^{-1}), impacting both nutrient dynamics and microbial community composition. Nevertheless, nutrient flux and bacterial abundance and production within the first 24 hours of exposure show large changes which we focus on in the discussion.

[**R1.10**] L121-127. Presumably the 1.7 mL incubated refers to the seawater in the cores. However, it would be expected that the bulk of bacterial production would occur associated with the jellyfish tissues and the sediment in contact with these.

The 1.7 ml water samples for bacterial productivity incubations as well as the 1.6 ml samples for flow cytometry were drawn at each time point from above the jellyfish/sediment (see Fig. 2). Both bacterial abundance and production were significantly higher in the jellyfish treatments than in the controls (at respective depth), suggesting that not only the jellyfish tissue and the below sediment but also the overlying waters are affected by increased bacterial abundance and production. Studying the jellyfish epi-biome microbial dynamics is out of the scope of the present study.

[**R1.11**] L129-144. As above, this is not measuring the overall changes in populations, just those in the water column.

See the response to R1.10. The samples were taken from the overlying waters and represent the communities at the sediment-water interface.

[**R1.12**] L145-160. What statistical analyses were performed on the oxygen and nutrient data?

Oxygen and nutrient data were correlated with bacterial abundance and bacterial production using Pearson correlation using R package Hmisc (Harrell, 2004). See line 163 and Table B1.

[**R1.13**] L160-190. As above the effects of decomposition processes evolve over time due to colonisation processes, the sequential nature of decomposition e.g. PON decomposed to DON and DON to ammonium, shifting conditions and ultimately depletion of the biomass. This is shown by

the non-linearity of the concentration changes that show that the production/consumption processes causing the fluxes are changing with in some cases the flux changing direction. Therefore, data need to be analysed in a manner that shows these shifting rates and the changing nutrient ratios they produce. It would also be useful to indicate what fraction of the C, N & P in the added biomass were actually mineralised over the course of the experiment. Especially as the data in the figure indicate that the decomposition rate had not even peaked by the end of the experiment, as ammonium production rates were still increasing at the end of the experiment. Indeed the highest rate of oxygen demand was at the end of the experiment, despite low water column concentration present at this time.

We thank the reviewer for this important comment. Indeed the non-linearity of the concentration changes indicate a sequential nature of decomposition, likely due colonization and POM breakdown. In the revised manuscript (lines 175-199, 288-291), we have addressed the non-linearity of fluxes by analyzing them over time, i.e., applying several linear phases. We also address the changes in the direction of the fluxes, which were evident in NO_x and PO_4. To calculate the diel fluxes (Tables 1,2), we have integrated the changes over time and indicated the time span used for calculation.

[**R1.14**] There is no description of the sediment analyses in the methods section.

This information was already included in the initial manuscript in the Methods section (lines 135-137): “250 mg from 0-1 and 1-2 cm sediment sections were transferred into the extraction tube. DNA was extracted from water and sediment using the DNeasy PowerSoil Kit (Qiagen, California, USA), using the manufacturer's protocol that included a FastPrep-24™ (MPBIO, Ohio, USA) bead-beating step (2x40 sec at 5.5 m/s, with a 5 min interval)”.

[**R1.15**] 4.1. This section would be much improved by reanalysing the oxygen and nutrient flux with time. This would show how these evolved over time and how the composition of the TDN and TDP fluxes shifted over time. This would allow discussion of the decomposition process e.g. leaching versus decomposition, sequential mineralisation etc. Also some data on the proportion of particularly the N and P present in the biomass that was actually mineralised during the experiment would be useful, as it appears the decomposition process was only partially completed, so overall effects would be greater over longer time periods. Finally, some context needs to be given when making comparisons to the natural system e.g. how does the biomass density compare? How does a closed system with a 40 cm water column compare to in situ conditions with a large water column, which can be resupplied by water movements such as currents and exchange with the atmosphere i.e. potential in situ effects would be very, very much lower than those measured.

This is a summary of former comments made by the reviewer. See responses to R1.1-R1.14.

[**R1.16**] L275-278. This N:P ratio is incorrect. It is not a %:% (weight:weight) ratio, it is an atom:atom (Mol:Mol) ratio. Therefore, the weights of N and P need to be divided by the atomic masses of N & P and the ratio of these compared.

We thank the reviewer for this comment. The study N:P ratios throughout the manuscript are presented correctly as mol:mol ratios. However, the N:P ratio derived from Lucas et al. (2011) was incorrectly calculated from %:%. In the revised manuscript, this ratio was corrected to mol:mol (lines 296-297): “Elemental body composition of scyphozoan jellyfish, in general, is 2.48 N %DW (dry weight) and 0.22 P %DW, hence an N:P ratio of 25:1 (Lucas et al., 2011)”.

[**R1.17**] L317-324. Growth efficiency also depends on the type of respiration and decreases in the order of aerobic Approx. 0.5) > nitrate reduction > metal reductions > sulfate reduction (.0.2). Therefore, fixed production does not equal fixed rate of respiration as the type of respiration, which is taking place shifts with oxygen conditions. Such changes would be even greater in jellyfish associated biofilms and in the surface sediments (See cited paper by Chelsky et al. 2016, which shows a shift to iron and sulfate reduction in the sediment in situ). The shift in your nitrate data from production (net nitrification) to consumption (net nitrate reduction), demonstrate this shift in dominance from aerobic to anaerobic processes in the benthos. Whereas, the water column effect in situ is likely very, very different from the changes that occurred in your cores.

We agree. Following the reviewer's suggestion, we have added to the revised manuscript (lines 291-294): "The shift from nitrate production to nitrate consumption 36 hours from the onset of the experiment likely reflects the shift from aerobic to anaerobic processes due to the low, hypoxic (and eventually anoxic) levels and may be regarded as an experimental artefact, although such changes were previously showed in surface sediments (Chelsky et al., 2016)".