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25-August-2020

To: *Biogeosciences* Editorial Office

Dear Dr. Ciavatta,

We are pleased to submit for publication the revised version of BG-2020-226: "**The effects of decomposing invasive jellyfish on biogeochemical fluxes and microbial dynamics in an ultraoligotrophic sea**". We appreciate the highly constructive review of our manuscript, and are glad that the reviewers found it important, interesting and suitable for *Biogeosciences* readership.

We have addressed all of the comments made by the reviewers and the revised version was reformulated accordingly. Detailed point-by-point answers to the reviewer comments are following. The line numbers are compatible with the final display of the manuscript.

We hope that you will find our response satisfactory, and we look forward to hearing from you.

Sincerely,

Tamar Guy-Haim, PhD

National Institute of Oceanography
Zooplankton Ecology Lab, IOLR
Haifa, Israel

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

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)".

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:

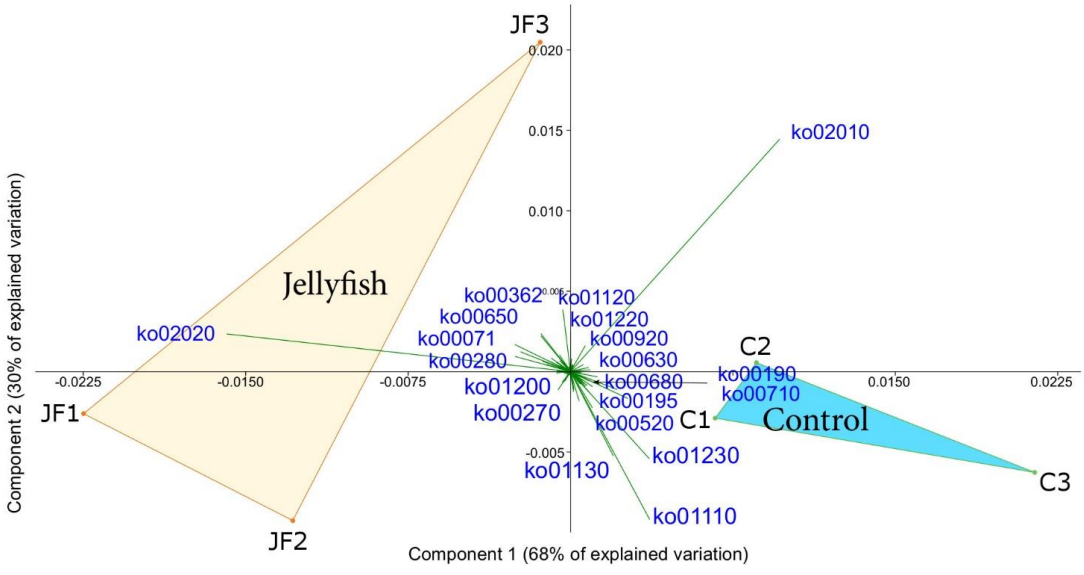
Kim, B.R., Shin, J., Guevarra, R., Lee, J.H., Kim, D.W., Seol, K.H., Lee, J.H., Kim, H.B. and Isaacson, R.E., 2017. Deciphering diversity indices for a better understanding of microbial communities. *J Microbiol Biotechnol*, *27*(12), pp.2089-2093.

[**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.

The effects of decomposing invasive jellyfish on biogeochemical fluxes and microbial dynamics in an ultraoligotrophic sea

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Abstract. Over the past several decades, jellyfish blooms have intensified spatially and temporally, affecting functions and services of ecosystems worldwide. At the demise of a bloom, an enormous amount of jellyfish biomass sinks to the seabed and decomposes. This process entails reciprocal microbial and biogeochemical changes, typically enriching the water column and seabed with large amounts of organic and inorganic nutrients. Jellyfish decomposition was hypothesized to be particularly important in nutrient-impoverished ecosystems, such as the Eastern Mediterranean Sea — one of the most oligotrophic marine regions in the world. Since the 1970s, this region is experiencing the proliferation of a notorious invasive scyphozoan jellyfish, *Rhopilema nomadica*. In this study, we estimated the short-term decomposition effects of *R. nomadica* on nutrient dynamics at the sediment-water interface. Our results show that the degradation of *R. nomadica* has led to increased oxygen demand and acidification of overlying water as well as high rates of dissolved organic nitrogen and phosphate production. These conditions favored heterotrophic microbial activity, bacterial biomass accumulation, and triggered a shift towards heterotrophic bio-degrading bacterial communities, whereas autotrophic pico-phytoplankton abundance was moderately affected or reduced. This shift may further decrease primary production in the water column of the Eastern Mediterranean Sea. Deoxygenation, acidification, nutrient enrichment and microbial community shifts at the sediment-water interface may have a detrimental impact on macrobenthic communities. Based on these findings we suggest that jelly-falls and their decay may facilitate an additional decline in ecosystem functions and services.

1 Introduction

Marine jellyfish often form massive aggregations, known as jellyfish blooms, with profound implications to human health, recreation and tourism, fisheries, aquaculture, and coastal installations (Purcell, 2012; Purcell et al., 2007; Richardson et al., 2009). Over the past three decades, a substantial increase in the frequency and intensity of jellyfish blooms has been documented worldwide (Attrill et al., 2007; Brotz et al., 2012; Licandro et al., 2010; Lynam et al., 2006; Quiñones et al., 2015; Shiganova et al., 2001) and was attributed to the growth in shipping, aquaculture and coastal protection (Duarte et al., 2013) or to natural global oscillations (Condon et al., 2013; Sanz-Martín et al., 2016). These blooms typically occur in 'boom and bust' cycles, where individuals suddenly appear in large numbers and shortly after disappear (Condon et al., 2013; Hamner

and Dawson, 2009; Schnedler-Meyer et al., 2018). This rapid collapse of jellyfish blooms *en masse* and their sinking to the seabed is a process commonly termed as 'jelly-falls' (Lebrato and Jones, 2011; Lebrato et al., 2012; Sweetman and Chapman, 2011).

During the blooms, jellyfish propagate by assimilating organic compounds of their prey, thus acting as a nutrient sink of organic carbon (C), nitrogen (N) and phosphorus (P) (Lebrato and Jones, 2011; Lucas et al., 2011; Pitt et al., 2009). The death and sinking of jellyfish, followed by bacterial decomposition of their carcasses, lead to microbial community shifts (Kramar et al., 2019; Tinta et al., 2012; Titelman et al., 2006), resulting in oxygen depletion and acidification (Qu et al., 2015; Sweetman et al., 2016; West et al., 2008). On the seabed, jellyfish carcasses can be consumed by scavengers, thus acting as a rich carbon source that sustains benthic foodwebs (Hays et al., 2018; Sweetman et al., 2016; Sweetman et al., 2014). Both in the water column and on the sediment, jelly-falls undergo bacterial decomposition, directly affecting ~~and play an important role in~~ 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). 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). The contribution of jellyfish degradation to nutrient cycling was hypothesized to be particularly important in nutrient-depleted, oligotrophic ecosystems (Pitt et al., 2009), such as the ultra-oligotrophic Eastern Mediterranean Sea (EMS), where microbial production is mainly limited by organic carbon (Sisma-Ventura and Rahav, 2019), nitrogen (Rahav et al., 2018b), or co-limited by nitrogen and phosphorus (Kress et al., 2005).

The most prominent jellyfish blooms in the Mediterranean Sea, particularly in its eastern basin, are caused by the scyphozoan *Rhopilema nomadica* (Edelist et al., 2020; Katsanevakis et al., 2014) (Fig. 1). *R. nomadica* was first recorded in Israel in 1977 as a Lessepsian invader, introduced via the Suez Canal (Galil et al., 1990). Since then, it has expanded its distribution westwards with more frequent blooming occurrences (Balistreri et al., 2017; Edelist et al., 2020; Yahia et al., 2013). This species is venomous and its nematocysts contain active toxins, inflicting painful stinging on humans, as well as other adverse health problems, negatively affecting coastal recreation and tourism (Galil, 2018; Ghermandi et al., 2015). During blooms, clogged intake pipes of power and desalination plants were reported in Israel (Angel et al., 2016; Galil, 2012). Reduced fishing harvests were also reported from Israel and Egypt, mostly due to net damage, loss of fishing days, and physical injury to the fishermen (Angel et al., 2016; Madkour et al., 2019; Nakar et al., 2011).

Although labeled as one of the worst invasive species in the Mediterranean Sea (Streftaris and Zenetos, 2006; Zenetos et al., 2010), the post-bloom decomposition dynamics of *R. nomadica* have never been investigated before. Here, we used incubation experiments at the sediment-water interface to estimate the short-term decomposition effects of the invasive jellyfish *R. nomadica*, on (1) organic and inorganic nutrient dynamics and derived benthic fluxes, (2) bacterial abundance and production, and (3) microbial community composition, in the nutrient-impoverished EMS. We hypothesize that decomposed *R. nomadica* will trigger a rapid release of limiting nutrients, leading to enhanced fluxes to the sediment and overlying water, a substantial increase in bacterial abundance and production, and a shift in the microbial community composition and functions.

2 Methods and materials

2.1 Specimen collection and experimental setup

Three individuals of the scyphozoan jellyfish *Rhopilema nomadica* (Galil et al., 1990) of medium size (bell diameter 20-25 cm) were collected on the 29th of July 2019, at Tel-Shikmona, Haifa, near the Israel Oceanographic and Limnological Research Institute, on the shore of the easternmost Mediterranean Sea (Lat. 32°49'32"N, Lon. 34°57'26"E). The specimens were weighed and cut to pieces of 4-5 g to ensure representation of all body parts. Processed 25 g wet weight (ca. 1.25 g dry weight) of *R. nomadica* (including umbrellas, tentacles and oral arms, following Qu et al., 2015) were placed each in three Perspex cylinders (9.45 cm internal diameter; 50 cm length) that were filled up to 10 cm height with coastal sediments (Fig. 2), that were collected one week prior to the experiment, allowing the re-establishment of natural sediment profiles. Three additional cylinders with sediments did not include jellyfish and functioned as controls. The set up was completed by topping off the cylinders with oxygen saturated Mediterranean coastal water (ca. 3.14 L) pumped from 1 m depth and pre-filtered to remove large-size zooplankton (67 µm). The cores were sealed with gas tight sealing caps and placed in a lab with a relatively constant temperature of 27-28 °C, which is similar to the summer mean coastal water temperatures of the easternmost EMS (Raveh et al., 2015). The set up was acclimatized for 24 h to insure similar initial conditions in the chambers before jellyfish addition. 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 ~~is using sealed-core incubations~~ (Glud, 2008; Hammond et al., 2004; Pratihary et al., 2014; Skoog and Arias-Esquivel, 2009). Pre-filtered coastal water was transferred to a reserve tank, and stored under the same conditions as the incubated cores. The incubation cores were connected by tubing to the dedicated reserve tank, which replaced the water in the incubation chambers during each sampling. The cores were incubated under PAR= 100 µmol photons·m-2·s-1 with a photoperiodicity of 14:10 (L:D).

Within each chamber, the overlying water was continuously mixed with a magnetic stirrer fixed 10 cm below the upper cap (75 rpm, Hammond et al., 2004), and were sampled at the following intervals: 0, 5, 10, 18, 26, 34, 44h, with dedicated sampling tubing. The reserve tank was sampled only at three intervals, 0, 20, 44h. At each sampling, 200 ml water samples were transferred to acid-washed transparent Nalgene bottles (250 ml), and sub-sampled by filtering (Minisart® 0.45 µm) for the following chemical analyses: PO_4, NO_2+NO_3 (NO_x), $Si(OH)_4$, NH_4, TDP (DOP), TN (DON). Nutrient samples were immediately frozen after collection for later analysis. Biological measurements were collected using unfiltered water for pico-phytoplankton (*Synechococcus*, *Prochlorococcus*, pico- and nano-eukaryotes), heterotrophic bacterial abundance and bacterial production measurements. Oxygen consumption rates at the sediment-water interface were continuously monitored using oxygen sensor spots (FireSting, PyroScience, Germany) adapted for measuring oxygen in closed containers through a transparent window (plastic or glass). The sensor spots were fixed to the inner side of the window with silicone glue. Four optical fibers continuously measured the oxygen in the three jellyfish chambers and one of the control incubations. The system was calibrated with saturated DIW. pH was measured with a sensor (MultiLine WTW, Germany) calibrated with NBS buffers.

Fluxes (mmol m^{-2} d^{-1}) were determined by regressing the change in overlying water concentration (C) through time multiplied by the chamber height (Volume/Area), following Eq. (1):

$$f = \frac{dC}{dt} \times \frac{V}{A}$$

A correction for water replacement from the reserve tank was not applied, as the consequent error was less than 5%.

2.2 Inorganic and organic nutrients analysis

Nutrient concentrations were determined using a three-channel segmented flow auto-analyzer system (AA-3 Seal Analytical) following Kress et al. (2014). The limit of detection (LOD), measured as three times the standard deviation of 10 measurements of the blank (low nutrient seawater collected from the off-shore EMS), was 8 nM for PO_4, 50 nM for total dissolved phosphorus (TDP) and $Si(OH)_4$, 80 nM for NO_2+NO_3 (NO_x) 90 nM for NH_4, and 0.74 µmol for total dissolved phosphorus (TDN). The accuracy of the analyses was determined using certified reference materials (CRM): MOOS 3 (PO_4, NO_x and $Si(OH)_4$), VKI 4.1 (NO_x) and VKI 4.2 (PO_4 and $Si(OH)_4$). Results were accepted when measured CRMs were within ±5% of the certified values.

TDN and TDP were measured following potassium persulphate digestion and ultraviolet (UV) photo-oxidation, using a digestion block system (Seal Analytical, UK). The reproducibility of the analyses was examined with VKI 4.2 and Deep Sea Reference (DSR) material. One of the TDP samples was lost (t= 44 h). DON concentrations were determined by subtracting NO_x and NH_4 from TDN concentrations and DOP concentrations were determined by subtracting PO_4 from TDP concentrations.

2.3 Pico/nano -phytoplankton and heterotrophic bacterial abundance

Samples (1.8 ml) were fixed with flow-cytometry grade glutaraldehyde (0.02% final concentration, G7651, Sigma-Aldrich, USA), frozen in liquid nitrogen, and stored at -80 °C until analysis within two weeks. *Synechococcus* and *Prochlorococcus* , autotrophic pico/nano-eukaryotes (maximal size ~70 µm), and heterotrophic bacterial abundances were determined using an Attune® Acoustic Focusing Flow Cytometer (Applied Biosystems, USA) as described in Bar-Zeev and Rahav (2015). Samples of *Synechococcus*, *Prochlorococcus* and pico/nano-eukaryotes were run at 100 µL min^{-1}. Their taxonomic discrimination for based on the orange fluorescence of phycoerythrin (585 nm), the red fluorescence of chlorophyll.*a* (630 nm), side-scatter (SSC, a proxy of cell volume), and on forward-scatter (FSC, a proxy of cell size.). Heterotrophic bacterial samples were run at 25 µL min^{-1} using a discrimination threshold of green fluorescence (520 nm) and FSC. Beads (0.93 µm, Polysciences) were run in parallel as a size standard. Blank samples of sterile seawater (0.2 µm) were also run and their reads were removed from the total bacterial counts.

2.4 Bacterial production (BP)

Bacterial production was estimated using the ^{3}H-leucine incorporation method (Perkin Elmer, specific activity 123 Ci mmol^{-1}) followed by micro-centrifugation (Simon, 1990). Samples (1.7 ml) were incubated with 10 nmol leucine L^{-1} for 4-5 h under ambient temperature in the dark. Triplicate additions of trichloroacetic acid (TCA) were performed at each time-point and served as controls. The incubations were terminated with 100 µL of concentrated (100%) TCA. After adding 1 mL of scintillation cocktail (Ultima-Gold, PerkinElmer, USA) to each vial, the samples were counted using a TRI-CARB 2100 TR (Packard Biocience, USA) scintillation counter. A conversion factor of 3 kg C mol^{-1} per every mole leucine incorporated was used, assuming an isotopic dilution of 2.0 (Simon and Azam, 1989).

2.5 DNA extraction and sequencing

Approximately 300 mL of overlying seawater were collected with a sterile syringe and passed through 0.22µm Sterivex filter. The membranes were removed from the cases, cut into pieces under sterile conditions and transferred into the extraction tubes. 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). The V4 region of the 16S rRNA gene was amplified using the modified primer pair 515F-806R (Apprill et al., 2015; Parada et al., 2016) in combination with CS1/CS2 tags (CS1_515Fc 5'-ACACTGACGACATGGTTCTACA GTGYCAGCMGCCGCGGTAA, CS2_806Rc 5'-TACGGTAGCAGAGACTTGGTCT GGACTACNVGGGTWTCTAAT), using the following PCR amplification protocol: initial denaturation at 94 °C for 45 s, 30 cycles of denaturation (94 °C for15 sec), annealing (15 cycles at 50 °C and 15 cycles at 60 °C for 20 sec) and extension (72 °C for 30 s). The 18S rRNA gene sequences were amplified using the 1391f-EukBr primer pair (Amaral-Zettler et al., 2009; Stoeck et al., 2010) in combination with CS1/CS2 tags (1391fc 5'-ACACTGACGACATGGTTCTACA GTACACACCGCCCGTC, EukBr 5'- TACGGTAGCAGAGACTTGGTCT TGATCCTTCTGCAGGTTCACCTAC), using the following PCR amplification protocol: initial denaturation at 94 °C for 45 s, 30 cycles of denaturation (94 °C for15 sec), annealing (60 °C for 20 sec) and extension (72 °C for 30 s). Library preparation from the PCR products and sequencing of 2x250 bp Illumina MiSeq reads was performed at HyLabs (Israel).

2.6 Statistical and bioinformatic analyses

Demultiplexed paired-end reads were processed in QIIME2 V2019.7 environment (Bolyen et al., 2018). Reads were truncated based on quality plots, checked for chimeras, merged and grouped into amplicon/environmental sequence variants (A/ESVs) with DADA2 (Callahan et al., 2016), as implemented in QIIME2. After removing the low-quality sequences, a total of 361335 (106169 in 6 and 255166 in 12 seawater and sediment samples, respectively) high-quality 16S rRNA gene amplicon reads with an average length of 260 bp, and a total of 658251 (162313 in 6 and 495938 in 12 seawater and sediment samples, respectively) high-quality 18S rRNA gene amplicon reads with an average length of 207 bp, were generated. The 16S and 18S

amplicons were classified with the Naïve-Bayes classifiers that were trained on the Silva 132 database, clustered at 99% (515F/806R region for the 16S and full-length sequences for the 18S rRNA gene amplicons). Downstream statistical analyses, calculation of alpha diversity indices (Chao1, Shannon and Simpson) and plotting were performed in R (Core Team, 2020), using packages phyloseq (McMurdie and Holmes, 2013), ampvis2 (Andersen et al., 2018) and ggplot2 (Wickham, 2009). Systematic changes across experimental conditions were estimated with DESeq2 (Love et al., 2014). The metabolic functions and pathways of the bacterial communities were predicted using Tax4Fun2 based on the KEGG database (Wemheuer et al., 2018). Pearson correlations and SIMPER analysis (Similarity Percentages, to assess the contribution of KEGG pathways to the dissimilarity between treatments and controls) ~~analyses~~ were performed in R using packages Hmisc (Harrell, 2004) and vegan (Oksanen et al., 2010). Principal component analysis (PCA) of the metabolic functions was performed with PAST V4 (Hammer et al., 2001).

3 Results

3.1 Dissolved oxygen and pH dynamics

Dissolved oxygen (DO) levels in the jellyfish treatments decreased from an initial average concentration of 261.5±4.5 µmol·L^{-1} to null within 40 hours, at an average rate of 5.9±0.1 µmol·L^{-1}·h^{-1}, whereas the DO levels in the control chambers decreased slightly at an average rate of 0.7±0.1 µmol·L^{-1}·h^{-1} (mean ±SD, Fig. 3A). The variability within the treatment replicates and within the controls was small and non-significant (treatment replicates: $F_{(2,18)}$=0.017, p=0.98; controls: $F_{(2,18)}$=0.055, p=0.59). The calculated average DO flux from the water column in the jellyfish treatment was -56.9±1.0 mmol m^{-2} d^{-1} versus -6.7±0.3 mmol m^{-2} d^{-1} in the controls (Table 1). In accordance with the decrease in DO, pH levels in the jellyfish treatments decreased from an initial average level of 8.10±0.02 to 7.88±0.01 and remained relatively stable (8.10-8.15) in the controls (Fig. 3B).

3.2 Nutrient dynamics

Nutrient levels significantly increased in the jellyfish-enriched chambers, whereas in the controls they remained stable and low (Fig. 4). These increases were non-linear and characterized by multiple phases, including in some cases changes of the flux direction. Therefore, nutrient flux rates were calculated in different linear phases over time.

Ammonium was the dominant form of dissolved inorganic nitrogen in the experimental chambers. During the first ~~26~~ 10 hours from the onset of the experiment, NH_4 levels increased at a rate of 0.39~~27~~±0.12 µmol·L^{-1}·h^{-1}, after which (~~26-44~~10-26 h) the rate of NH_4 release slowed to 0.26±0.10 µmol·L^{-1}·h^{-1} and then (26-44 h) sharply increased ~~-~~to 1.33±0.31 µmol·L^{-1}·h^{-1} (Fig. 4A). NO_2 levels steadily increased at a rate of $5.5 \cdot 10^{-3} \pm 2.0 \cdot 10^{-3}$ µmol·L^{-1}·h^{-1}, and decreased to background levels after 34 hours (Fig. 4B). NO_3 levels were generally higher in the jellyfish treatment than in the controls, but did not present any significant trend over time (Appendix A, Fig. A1). 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). Silicic-acid concentrations remained overall stable throughout the experiment, and higher in two of the jellyfish-enriched chambers (Appendix A, Fig. A1).

Within the first 5 hours following the jellyfish enrichment, orthophosphate levels increased by two orders of magnitude from 0.02±0.01 to 1.02±0.13 μmol·L^{-1} (Fig. 4C). Throughout the rest of the experiment, PO_4 was fully consumed and its levels decreased to the background levels within 34 hours (0.04 μmol·L^{-1}), after which an increase was recorded (0.30 μmol·L^{-1}).

The majority of TDN and TDP released from the jellyfish was organic, where 84% of the TDN was DON, (Fig. 4D), and 71% of the TDP was DOP (Fig. 4E). Both organic nutrient levels significantly increased in the jellyfish enriched chambers, whereas their concentrations in the control chambers remained stable and low. During the incubation period, DON concentrations increased 12-fold in the jellyfish treatment compared to the controls (Fig. 4D) and DOP concentrations increased 18-fold (Fig. 4E). The ratio between TDN and TDP (TDN:TDP) decreased from an initial average value of 96±18 :1 to an average value of 23±7 :1 in the jellyfish treatments, whereas in the controls it decreased to 57±3 :1 (Fig. 4F).

The rates of nutrient release (remineralization rates) standardized to jellyfish biomass are detailed in Table 1, and the calculated nutrient fluxes (mmol m^{-2} d^{-1}) in the jellyfish enriched cylinders and in the controls are summarized in Table 2.

3.3 Autotrophic and heterotrophic abundance and bacterial production

Heterotrophic bacterial abundance increased linearly in the jellyfish treatments (R^2=0.98, p<0.01) and reached $1.5 \cdot 10^7 \pm 1.9 \cdot 10^5$ cells·mL^{-1} after 44 hours, whereas the controls remained stable at a concentration of $2.0 \cdot 10^6 \pm 6.7 \cdot 10^4$ (Fig. 5A). *Synechococcus* abundance dropped in both jellyfish-enriched and control cylinders, however, after 44 hours, the number of *Synechococcus* cells in the jellyfish treatment was 5-fold larger compared to the controls (Fig. 5B). *Prochlorococcus* cell numbers increased in both jellyfish-enriched and control cylinders, and after 44 hours was lower in the jellyfish treatment (Fig. 5C). Both cell numbers of pico and nano -eukaryotes dropped throughout the experiment, nonetheless, were higher in the jellyfish treatment than in the controls by 50% (Figs. 5D-E).

Bacterial production remained stable in the jellyfish treatments at a rate of 3.1±0.3 μg C ·L^{-1} ·h^{-1} during the first 26 incubation hours, increased to 4.3±0.1 μg C ·L^{-1} ·h^{-1} and after 34 hours decreased again. Contrary, in the controls the bacterial production decreased ~~immidiately~~immediately from the onset of the experiment, and after 18 hours reached a rate of 0.4±0.2 μg C ·L^{-1} ·h^{-1} that remained stable until the experiment ended (Fig. 5F).

The temporal dynamics of DO and nutrient concentrations strongly correlated with total bacterial abundance, but not with bacterial production (Appendix B, Table B1).

3.4 Microbial diversity

Bacterial alpha diversity (Fig. 6), was significantly lower in the jellyfish-enriched seawater than in the controls (p<0.05), but in the sediment samples there was no significant difference (p>0.05). The vast majority (93-97%) of the 18S sequence variants

in seawater (Appendix C, Fig. C2) belonged to Scyphozoa, hindering alpha diversity evaluation. In the sediment, no significant difference ($p>0.05$) in alpha diversity was observed between treatments (Appendix C, Fig. C1). These findings were confirmed with rarefaction curves (Appendix C, Figs. C3, C4).

The distribution of the 30 most abundant bacterial genera measured in seawater in the jellyfish-enriched and control chambers is presented in a heatmap (as inferred from read abundance estimates, Fig. 7). Lineages for which significant changes in abundance ($p<0.05$) between the treatment and control were detected by DESeq2 (Fig. 7, yellow star symbols: lineages more abundant in the controls, purple star symbols: lineages more abundant in the jellyfish treatment). Nine lineages were significantly more abundant in the jellyfish treatment, whereas 12 lineages were significantly more abundant in the controls. The relative abundance of the common marine bacteria, including the primary producers *Synechoccocus* and (chemo or photo) the heterotrophic bacteria SAR11, HIMB11 and SAR86 (Dupont et al., 2012; Durham et al., 2014; Giovannoni, 2017), have all diminished following jellyfish additions. Mostly opportunistic lineages (*Kordiimonadaceae*, *Pseudoalteromonadaceae*, *Saccharospirillaceae* and *Nitrincolaceae*) that use multiple carbon sources, including xenobiotics, were enriched in jellyfish-amended incubations, and are often associated with oil discharge (Yakimov et al., 2007). *Algicola* (*Pseudoalteromonadaceae*) and *Kordiimonas* (*Kordiimonadaceae*) appear to be the most abundant degraders of the jellyfish biomass based on the marked change observed in the abundance of their relative amplicon sequence variants.

Heatmap showing the distribution of the 30 most abundant genera in the sediment, measured in the 0-1 cm below surface layer and in the 1-2 cm below surface layer (inferred from 16S sequences), in the jellyfish-enriched and control chambers is presented in Fig. C1 (Appendix C). Among the 30 most abundant taxa, only *Fusimonas* and *Algicola* genera were significantly more abundant in the jellyfish treatments in the 0-1 cm layer, however, in the 1-2 cm layer, there was no significant difference between the treatments and controls.

The distribution of the 30 most abundant eukaryotic genera (inferred from the 18S rRNA amplicon read abundance) measured in seawater and sediment in the jellyfish-enriched and control chambers is presented in Fig. C2 (Appendix C). Both sediment layers showed no difference between treatment and controls, whereas in the seawater samples, four lineages of dinoflagellates, *Ciliophora* and Labyrinthulomycetes were more abundant in the jellyfish than the controls.

Predicted functions were classified as KEGG orthologs (KOs) resulting in the identification of 346 KOs across all samples, 160 of which were associated with prokaryotic functions. The principal component analysis (including 324 KOs across all samples, after removal of rare KOs that appear in only one of the replicates to avoid zero-inflated dimensionality) showed that jellyfish-treated and control samples significantly differed based on microbial predicted functions (Fig. 8). Photosynthesis (ko00195) and carbon fixation in photosynthetic organisms (ko00710) were enriched in controls, while catabolic functions, such as fatty acid degradation (ko00071), valine, leucine and isoleucine degradation (ko00362) and xenobiotic degradation pathways, benzoate degradation (ko00650) in particular were enriched in jellyfish additions (Fig. 8). SIMPER analysis (Appendix D, Table D2) showed that the pathways mostly contributing to the difference between the jellyfish treatments and controls were signal transduction 2-component system (ko02020) and ABC transporters (ko02010), contributing to 13% and 10% of the dissimilarity between the groups, respectively.

4 Discussion

4.1 The effects of *R. nomadica* decomposition on oxygen and nutrient fluxes

Jellyfish blooms trigger substantial changes in dissolved oxygen, inorganic carbon and nutrient concentrations in the water column (Condon et al., 2011; Pitt et al., 2009). Post-bloom processes, by comparison, modify the oxygen, carbon and nutrient fluxes in the benthic boundary layer and the sediment-water interface (Chelsky et al., 2015; Lebrato and Jones, 2011; Qu et al., 2015; West et al., 2008). 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. Similarly, increased sediment oxygen demand following jellyfish decomposition was measured by West et al. (2008) in *Catostylus mosaicus* and by Tinta et al. (2016) in the moon jellyfish *Aurelia aurita*. Qu et al. (2015) that studied the effects of *Cyanea nozakii* decomposition in the Yellow Sea using incubations found that oxygen was depleted in both sediment and seawater. They hypothesized that the metabolism and propagation of heterotrophic bacteria led to enhanced oxygen consumption. Indeed, our experimental results support this hypothesis, as bacterial abundance was strongly correlated with oxygen levels, whereas the abundance of autotrophic cyanobacteria decreased as they were likely outcompeted by the heterotrophic bacteria (Sisma-Ventura and Rahav, 2019; Thingstad et al., 2005). Thus, jelly-falls can generate hypoxic areas on the seabed and overlying waters (Pitt et al., 2009), and affect the benthic infauna (Chelsky et al., 2016). Although the Eastern Mediterranean coastal waters are well-oxygenated (Kress et al., 2014), the collapse of massive *R. nomadica* blooms could potentially create local hypoxic or even anoxic hotspots on the seabed, thereby affecting the surrounding biota (Feely et al., 2010).

In addition to deoxygenation, our experiment showed a significant reduction in pH, to levels that are considered detrimental to various organisms, mainly calcifies (Kroeker et al., 2010; Zunino et al., 2017). Acidification as a result of jellyfish decomposition was also observed by Qu et al. (2015) that speculated that the release of amino-acids and fatty-acids from proteins and lipid metabolism of jellyfish tissue is the root cause for the observed decrease in pH. Nonetheless, hypoxia and acidification are biogeochemically coupled via the production of inorganic carbon in the process of respiration (Feely et al., 2010; Gobler and Baumann, 2016). In addition, increase in NH_4, as was measured in our experiment, increases total alkalinity and pH, whereas nitrate and silicate decrease pH, but they were comparably scarce. Based on oxygen to carbon conversion (1:1.3), and alkalinity change due to NH_4 addition, it is estimated that the observed decrease in pH in our experiment can be solely attributed to inorganic carbon and carbonic acid production (due to bacterial respiration) and ammonium release. The combination of hypoxia and acidification may have synergistic additive negative effects on the benthic fauna (Gobler et al., 2014; Melzner et al., 2013). Furthermore, ammonium in high concentrations may have toxic effects on various marine organisms, from bacteria to fish (Brun et al., 2002; Eddy, 2005; Ferretti and Calesso, 2011; Müller et al., 2006).

The decomposition of dead *R. nomadica* tissue generated an immediate rapid release of organic and inorganic phosphate after which the inorganic phosphate (PO_4) was completely consumed, while the efflux of organic and inorganic (mostly ammonium) nitrogenous compounds gradually increased throughout the experiment. Similar dynamics were observed in *C. mosaicus* by

West et al. (2008) and Chelsky et al. (2015), and by Tinta et al. (2010) in *Aurelia solida*, where organic and inorganic phosphate peaked and completely abolished within 24 hours, presumably due to bacterial uptake. The production of NO_x in our experiment was evident only in the jellyfish treatment while oxygen levels were conducive, suggesting that nitrification plays an important role in nutrient dynamics following jellyfish decomposition, as was found in different jellyfish species (Hubot et al., 2020; Welsh et al., 2009). The non-linearity of nutrient fluxes that was evident in our experiment (especially in the multi-rate flux of NH_4 and in the bi-directional flux of NO_2 and PO_4) as well as in other jellyfish degradation studies (Blanchet et al., 2015; Chelsky et al., 2016; Qu et al., 2015; Tinta et al., 2010), indicate a sequential nature of decomposition, likely due to microbial colonization and non-linear growth rates. 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). The stoichiometric relationship between TDN and TDP decreased from 57:1 to 23:1 as a result of *R. nomadica* decomposition, as was also found by West et al. (2009) and Qu et al. (2015). This decrease can be explained by the eElemental 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 1125:1 (Lucas et al., 2011). The higher N:P measured in our experiment indicates a mismatch between the resource (i.e., jellyfish organic matter) and consumers (e.g., bacteria). This elemental imbalance determines ecological interactions and metabolic rates (Sterner and Elser, 2002). Thus, the higher N:P measured here may imply on a preferential bacterial retention of phosphate (West et al., 2008). A recent study from the same area showed that the addition of organic nutrients stimulated heterotrophic microbial biomass and activity (Sisma-Ventura and Rahav, 2019), highlighting the importance of nutrient remineralization in this ecosystem.

The rates of nutrient release from *R. nomadica* decomposition found in this study were comparable to jellyfish decomposition-driven rates found in former studies (e.g., Blanchet et al., 2015; Pitt et al., 2009; Qu et al., 2015; Tinta et al., 2012; Tinta et al., 2016; Titelman et al., 2006; West et al., 2008). Ammonium release rate in *R. nomadica* (1.96 µmol g^{-1} WW d^{-1}) was slightly higher than the rate measured by Tinta et al. (2012) in *Rhizostoma pulmo* (1.6 µmol g^{-1} WW d^{-1}), another common Mediterranean scyphozoan. 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}. We can, therefore, estimate that the collapse of *R. nomadica* bloom potentially releases ammonium and phosphate in concentrations of 2.5 and 0.8 kmol km^{-2}, respectively.

Nutrient remineralization during jelly-fall decomposition, as was found in this study and others, can be inhibitory or toxic to some organisms (e.g., dissolved sulfides and ammonium in Chelsky et al., 2016), but on the other hand, can stimulate primary production and induce algal blooms in the water column and on the sediment. Møller and Riisgård (2007) found that following blooms of *A. aurita*, peak concentrations of chlorophyll-*a* were measured in a heavily eutrophied Danish Fjord. Using mesocosm experiments, West et al. (2009) found that excretion of jellyfish *C. mosaicus* led to a 10-fold increase in diatom

abundance. In the EMS, *R. nomadica* typically peaks in the summer months and collapses at the end of July (Edelist et al., 2020), whereas peak chlorophyll-*a* concentrations in the water column are measured during wintertime (Ignatiades et al., 2009; Rahav et al., 2018a; Raveh et al., 2015). This may result from the competitive exclusion of phytoplankton by heterotrophic bacteria (Sisma-Ventura and Rahav, 2019). Thus, fertilization of the water column due to nutrient release from *R. nomadica* decomposition may fail to trigger an algal bloom in the EMS. In contrast to the water column, maximum chlorophyll concentrations were measured in the sediment of the shallow Israeli coastal shelf during the late spring-summer (Hyams-Kaphzan et al., 2009; Tadir et al., 2017). This discrepancy was explained by the spring bloom of benthic primary producers. However, the results of this study could provide another plausible explanation for the high summer chlorophyll concentrations in the sediment, which may be the post-bloom nutrient boost to the benthic ecosystem.

4.2 Decomposition induced shifts in bacterial community abundance, production, composition and functionality

Heterotrophic bacteria are major consumers of dissolved organic matter (DOM) in marine ecosystems and can, therefore, benefit from jellyfish decomposition. Previous studies have demonstrated a significant increase in bacterial abundance triggered by jellyfish degradation (Blanchet et al., 2015; Condon et al., 2011; Dinasquet et al., 2012; Frost et al., 2012; Kramar et al., 2019; Tinta et al., 2016; Tinta et al., 2010; Titelman et al., 2006; West et al., 2009). Our study found that the decomposition of *R. nomadica* induced an increase in two orders of magnitude in the heterotrophic bacteria abundance. Autotrophic cyanobacteria, on the other hand, decreased (*Synechococcus*~~*Prochlorococcus*~~), or increased to a lower level than the unamended control (~~*Synechococcus*~~*Prochlorococcus*), likely due to deoxygenation (Bagby and Chisholm, 2015) or out-competition by heterotrophic bacteria (Sisma-Ventura and Rahav, 2019; Thingstad et al., 2005).

The fate of jellyfish DOM consumed by bacteria depends on bacterial growth efficiency—the ratio of bacterial production to substrate assimilation (i.e., the sum of bacterial production and respiration) (Condon et al., 2011). While some studies have found that the succession of bacterial production mirrored bacterial abundance and respiration (Blanchet et al., 2015; Titelman et al., 2006), in our study, bacterial production reduced in the controls, whereas under jellyfish enrichment remained at a steady, eightfold higher, level. This decoupling between bacterial abundance and production may indicate a shift in the functional diversity and metabolic demands of the jellyfish-associated bacterial communities ~~along~~ during the experiment. In the shallow coastal waters of the EMS, bacterial production levels peak in winter and ~~in~~ summer (Raveh et al., 2015), coinciding with, and potentially contributed by, the seasonal aggregations of *R. nomadica* (Edelist et al., 2020).

A significant reduction in the microbial α-diversity indices of seawater during jellyfish decomposition was observed in this as well as in former studies (Blanchet et al., 2015; Kramar et al., 2019; Tinta et al., 2012). The decline in diversity can be attributed to the specialization of surface-colonizing bacteria, having the competitive advantage for settling from the surrounding seawater (Kramar et al., 2019), and was thus less evident in the sediment samples. Additionally, changes in bacterial diversity may result from bacterial antagonism, i.e. the production of antagonistic compounds and sensitivity or resilience to them (Titelman et al., 2006). In this study, we found a significant increase in the relative abundance of the Alphaproteobacterium *Kordiimonas* and the Gammaproteobacteria *Algicola* in the seawater enriched with *R. nomadica*. Similarly, the predominance

of Alphaproteobacterium and Gammaproteobacteria stimulated by jellyfish decomposition was found in different studies (Basso et al., 2019; Blanchet et al., 2015; Condon et al., 2011; Dinasquet et al., 2012; Kramar et al., 2019; Tinta et al., 2012; Titelman et al., 2006). Gammaproteobacteria are conspicuous particle colonizers (Bižić-Ionescu et al., 2015; Simon et al., 2002), capable of degrading high molecular weight organic compounds (Cottrell and Kirchman, 2000; Reichenbach, 1992; Woyke et al., 2009), e.g. hydrocarbons (Niepceron et al., 2013). Kramar et al. (2019) found that Alphaproteobacteria and Gammaproteobacteria dominated the body surface of *Aurelia*, especially during the senescent phase. Blanchet et al. (2015) found a succession of bacterial diversity during the degradation of *Aurelia* and concluded that Alphaproteobacteria and Gammaproteobacteria have a major role in the succession of jellyfish DOM degradation. The link between the bacterial diversity of living *R. nomadica* at different life phases and the diversity of bacteria associated with its decomposed DOM is yet to be investigated.

Both genetic and functional diversity analyses of bacterial communities demonstrated a shift under *R. nomadica* degradation. We found that the predicted functions that dominated the decomposed jellyfish communities were signal transduction (2-component system), catabolic functions, such as fatty acid degradation, valine, leucine and isoleucine degradation, xenobiotic degradation pathways, and benzoate degradation. In the control communities, predominating functions were photosynthesis and carbon fixation in photosynthetic organisms. This functional shift can be explained by the fact that autotrophic cyanobacteria may be outcompeted by bio-degrading heterotrophic bacteria. Once the jellyfish bloom decomposes, populations of these intrinsic microbial bio-degraders become dominant and active, exploiting the carbon and nutrients released from the jellyfish. Using 16S rRNA amplicon data for predicting functional profiles is a powerful tool for assessing bacterial functional diversity, nonetheless, its accuracy and resolution are dependent on the representation of sampled organisms in the 16S rRNA and KEGG databases (Sun et al., 2020; Wemheuer et al., 2018). Likely, jellyfish degraders are under-represented in these databases. Further research using omics (e.g., whole-genome sequencing) will elucidate the metabolic potential of microbial degraders of the jellyfish necromass.

Although not to the same extent as bacterial diversity, eukaryotic diversity had too, shifted during the decomposition of *R. nomadica*, to a more flagellate-dominated community. Marine ciliates and parasitic protists (Labyrinthulomycetes) were also more abundant in the jellyfish decomposed community. Flagellate bacterivory represents the primary mechanism for the reintroduction of jellyfish carbon into the planktonic food web (Condon et al., 2011; Gasol and Kirchman, 2018). The increase in ciliates can be attributed to a "bottom-up" effect, where with the increase in flagellates, the abundance of their predators (e.g., ciliates) also increases (Epstein et al., 1992). Since jellyfish consume ciliates (Kamiyama, 2018; Stoecker et al., 1987), the flagellate carbon could be assimilated and recycled by the jellyfish, creating a positive-feedback loop termed as the "jelly-loop" (Condon et al., 2011; Lebrato and Jones, 2011).

5 Conclusions

Our study examined, for the first time, the decomposition effects ~~dynamics~~ of the bloom-forming invasive jellyfish *R. nomadica* on the oxygen and nutrient fluxes and microbial communities at~~in~~ the sediment-water interface~~Mediterranean Sea~~. The geographical distribution of this venomous species is continuously expanding, and its outbreaks are becoming more frequent, large, prolonged, with numerous negative impacts on human health, marine infrastructure, tourism, and fisheries.

We found that ~~the~~ jellyfish degradation had a significant influence on the fluxes of organic and inorganic nutrients at the sediment-water interface, transforming the microbial community composition and functions. The high rates of organic nitrogen and phosphate release favored heterotrophic-dominated metabolism, leading to a shift towards heterotrophic bio-degrading bacterial communities. This shift may further decrease primary production under the ultra-oligotrophic regime of the Eastern Mediterranean Sea. On the seabed, hotspots of deoxygenated, acidified, and nutrient-rich sediment may alter microbial and macrobenthic communities.

Future investigations on the decomposition dynamics of *R. nomadica* should be conducted in larger experimental systems (i.e., mesocosms) or in-situ, under more realistic conditions. The effects of environmental change drivers, such as warming, acidification, or anthropogenic pollution should also be tested. Additionally, the consumption of jelly-falls by scavengers in the Eastern Mediterranean Sea should be explored. This and future studies will shed light on the variable effects of the reoccurring massive blooms on the ecosystem functions and services in this rapidly changing environment.

Data availability

All data were deposited in an Open Access data archiving and publication repository (Pangaea, a member of the ICSU World Data System) and are available at https://doi.pangaea.de/10.1594/PANGAEA.915464. All the 16S and 18S~~A~~ rRNA gene amplicon reads were submitted to NCBI Sequence Read Archive BioProject PRJNA626084.

Author contribution

This work was conceived by all authors. TGH and GSV led the research and performed the experiments, MRB conducted the microbial diversity and bioinformatic analyses, ER and NB analyzed the microbial abundance and production, JS contributed to the study conception. TGH wrote the manuscript with substantial contributions from all co-authors. All authors have read and approved the final submitted manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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References

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., and Huse, S. M.: A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes, PloS one, 4, 2009.

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., and Albertsen, M.: ampvis2: an R package to analyse and visualise 16S rRNA amplicon data, BioRxiv, 2018. 299537, 2018.

Angel, D. L., Edelist, D., and Freeman, S.: Local perspectives on regional challenges: jellyfish proliferation and fish stock management along the Israeli Mediterranean coast, Regional environmental change, 16, 315-323, 2016.

Apprill, A., McNally, S., Parsons, R., and Weber, L.: Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton, Aquatic Microbial Ecology, 75, 129-137, 2015.

Attrill, M. J., Wright, J., and Edwards, M.: Climate-related increases in jellyfish frequency suggest a more gelatinous future for the North Sea, Limnology and Oceanography, 52, 480-485, 2007.

Bagby, S. C. and Chisholm, S. W.: Response of Prochlorococcus to varying CO2: O2 ratios, The ISME journal, 9, 2232-2245, 2015.

Balistreri, P., Spiga, A., Deidun, A., Gueroun, S. K., and Yahia, M. N. D.: Further spread of the venomous jellyfish *Rhopilema nomadica* Galil, Spannier & Ferguson, 1990 (Rhizostomeae, Rhizostomatidae) in the western Mediterranean, BioInvasions Records, 6, 19-24, 2017.

Bar-Zeev, E. and Rahav, E.: Microbial metabolism of transparent exopolymer particles during the summer months along a eutrophic estuary system, Frontiers in microbiology, 6, 403, 2015.

Basso, L., Rizzo, L., Marzano, M., Intranuovo, M., Fosso, B., Pesole, G., Piraino, S., and Stabili, L.: Jellyfish summer outbreaks as bacterial vectors and potential hazards for marine animals and humans health? The case of Rhizostoma pulmo (Scyphozoa, Cnidaria), Science of The Total Environment, 692, 305-318, 2019.

Bižić-Ionescu, M., Zeder, M., Ionescu, D., Orlić, S., Fuchs, B. M., Grossart, H. P., and Amann, R.: Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization, Environmental microbiology, 17, 3500-3514, 2015.

Blanchet, M., Pringault, O., Bouvy, M., Catala, P., Oriol, L., Caparros, J., Ortega-Retuerta, E., Intertaglia, L., West, N., and Agis, M.: Changes in bacterial community metabolism and composition during the degradation of dissolved organic matter from the jellyfish *Aurelia aurita* in a Mediterranean coastal lagoon, Environmental Science and Pollution Research, 22, 13638-13653, 2015.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., and Asnicar, F.: QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science, PeerJ Preprints2167-9843, 2018.

Brotz, L., Cheung, W. W., Kleisner, K., Pakhomov, E., and Pauly, D.: Increasing jellyfish populations: trends in large marine ecosystems. In: Jellyfish Blooms IV, Springer, 2012.

Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G., and Pérez-Lloréns, J. L.: Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*, Marine Ecology Progress Series, 225, 177-187, 2002.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.: DADA2: high-resolution sample inference from Illumina amplicon data, Nature methods, 13, 581, 2016.

Chelsky, A., Pitt, K. A., Ferguson, A. J., Bennett, W. W., Teasdale, P. R., and Welsh, D. T.: Decomposition of jellyfish carrion in situ: Short-term impacts on infauna, benthic nutrient fluxes and sediment redox conditions, Science of The Total Environment, 566, 929-937, 2016.

Chelsky, A., Pitt, K. A., and Welsh, D. T.: Biogeochemical implications of decomposing jellyfish blooms in a changing climate, Estuarine, Coastal and Shelf Science, 154, 77-83, 2015.

Condon, R. H., Duarte, C. M., Pitt, K. A., Robinson, K. L., Lucas, C. H., Sutherland, K. R., Mianzan, H. W., Bogeberg, M., Purcell, J. E., and Decker, M. B.: Recurrent jellyfish blooms are a consequence of global oscillations, Proceedings of the National Academy of Sciences, 110, 1000-1005, 2013.

Condon, R. H., Steinberg, D. K., Del Giorgio, P. A., Bouvier, T. C., Bronk, D. A., Graham, W. M., and Ducklow, H. W.: Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems, Proceedings of the National Academy of Sciences, 108, 10225-10230, 2011.

Core Team, R.: R: A language and environment for statistical computing. . 2020.

Cottrell, M. T. and Kirchman, D. L.: Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low-and high-molecular-weight dissolved organic matter, Appl. Environ. Microbiol., 66, 1692-1697, 2000.

Denis, L., Grenz, C., Alliot, E., and Rodier, M.: Temporal variability in dissolved inorganic nitrogen fluxes at the sediment–water interface and related annual budget on a continental shelf (NW Mediterranean), Oceanologica acta, 24, 85-97, 2001.

Dinasquet, J., Granhag, L. M., and Riemann, L.: Stimulated bacterioplankton growth and selection for certain bacterial taxa in the vicinity of the ctenophore Mnemiopsis leidyi, Frontiers in microbiology, 3, 302, 2012.

Duarte, C. M., Pitt, K. A., Lucas, C. H., Purcell, J. E., Uye, S.-i., Robinson, K., Brotz, L., Decker, M. B., Sutherland, K. R., and Malej, A.: Is global ocean sprawl a cause of jellyfish blooms?, Frontiers in Ecology and the Environment, 11, 91-97, 2013.

Dupont, C. L., Rusch, D. B., Yooseph, S., Lombardo, M.-J., Richter, R. A., Valas, R., Novotny, M., Yee-Greenbaum, J., Selengut, J. D., and Haft, D. H.: Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage, The ISME journal, 6, 1186-1199, 2012.

Durham, B. P., Grote, J., Whittaker, K. A., Bender, S. J., Luo, H., Grim, S. L., Brown, J. M., Casey, J. R., Dron, A., and Florez-Leiva, L.: Draft genome sequence of marine alphaproteobacterial strain HIMB11, the first cultivated representative of a unique lineage within the Roseobacter clade possessing an unusually small genome, Standards in genomic sciences, 9, 632, 2014.

Eddy, F.: Ammonia in estuaries and effects on fish, Journal of Fish Biology, 67, 1495-1513, 2005.

Edelist, D., Guy-Haim, T., Kuplik, Z., Zuckerman, N., Nemoy, P., and Angel, D. L.: Phenological shift in swarming patterns of *Rhopilema nomadica* in the Eastern Mediterranean Sea, Journal of Plankton Research, 2020. 2020.

Epstein, S. S., Burkovsky, I. V., and Shiaris, M. P.: Ciliate grazing on bacteria, flagellates, and microalgae in a temperate zone sandy tidal flat: ingestion rates and food niche partitioning, Journal of experimental marine Biology and Ecology, 165, 103-123, 1992.

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., and Maloy, C.: The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary, Estuarine, Coastal and Shelf Science, 88, 442-449, 2010.

Ferretti, J. A. and Calesso, D. F.: Toxicity of ammonia to surf clam (*Spisula solidissima*) larvae in saltwater and sediment elutriates, Marine environmental research, 71, 189-194, 2011.

Frost, J. R., Jacoby, C. A., Frazer, T. K., and Zimmerman, A. R.: Pulse perturbations from bacterial decomposition of *Chrysaora quinquecirrha* (Scyphozoa: Pelagiidae). In: Jellyfish Blooms IV, Springer, 2012.

Galil, B.: Poisonous and venomous: marine alien species in the Mediterranean Sea and human health, Invasive species and human health, 2018. 1-15, 2018.

Galil, B., Spanier, E., and Ferguson, W.: The Scyphomedusae of the Mediterranean coast of Israel, including two Lessepsian migrants new to the Mediterranean, Zoologische Mededelingen, 64, 95-105, 1990.

Galil, B. S.: Truth and consequences: the bioinvasion of the Mediterranean Sea, Integrative Zoology, 7, 299-311, 2012.

Gasol, J. M. and Kirchman, D. L.: Microbial ecology of the oceans, John Wiley & Sons, 2018.

Ghermandi, A., Galil, B., Gowdy, J., and Nunes, P. A.: Jellyfish outbreak impacts on recreation in the Mediterranean Sea: welfare estimates from a socioeconomic pilot survey in Israel, Ecosystem services, 11, 140-147, 2015.

Giovannoni, S. J.: SAR11 bacteria: the most abundant plankton in the oceans, Annual review of marine science, 9, 231-255, 2017.

Glud, R. N.: Oxygen dynamics of marine sediments, Marine Biology Research, 4, 243-289, 2008.

Gobler, C. J. and Baumann, H.: Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life, Biology letters, 12, 20150976, 2016.

Gobler, C. J., DePasquale, E. L., Griffith, A. W., and Baumann, H.: Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves, PloS one, 9, 2014.

Hammer, Ø., Harper, D. A., and Ryan, P. D.: PAST: Paleontological statistics software package for education and data analysis, Palaeontologia electronica, 4, 9, 2001.

Hammond, D. E., Cummins, K. M., McManus, J., Berelson, W. M., Smith, G., and Spagnoli, F.: Methods for measuring benthic nutrient flux on the California Margin: Comparing shipboard core incubations to in situ lander results, Limnology and Oceanography: Methods, 2, 146-159, 2004.

Hamner, W. M. and Dawson, M. N.: A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages, Hydrobiologia, 616, 161-191, 2009.

Harrell, F.: Hmisc: Harrell Miscellaneous library for R statistical software, R package, 2, 2-3, 2004.

Hays, G. C., Doyle, T. K., and Houghton, J. D.: A paradigm shift in the trophic importance of jellyfish?, Trends in ecology & evolution, 33, 874-884, 2018.

Hubot, N., Giering, S. L. C., Lucas, C., Robidart, J., and Fuessel, J.: EVIDENCE OF NITRIFICATION ASSOCIATED WITH JELLYFISH, 2020.

Hyams-Kaphzan, O., Almogi-Labin, A., Benjamini, C., and Herut, B.: Natural oligotrophy vs. pollution-induced eutrophy on the SE Mediterranean shallow shelf (Israel): Environmental parameters and benthic foraminifera, Marine Pollution Bulletin, 58, 1888-1902, 2009.

Ignatiades, L., Gotsis-Skretas, O., Pagou, K., and Krasakopoulou, E.: Diversification of phytoplankton community structure and related parameters along a large-scale longitudinal east–west transect of the Mediterranean Sea, Journal of Plankton Research, 31, 411-428, 2009.

Kamiyama, T.: Planktonic ciliates as food for the scyphozoan Aurelia coerulea: feeding and growth responses of ephyra and metephyra stages, Journal of Oceanography, 74, 53-63, 2018.

Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppäkoski, E., Çinar, M. E., Oztürk, B., Grabowski, M., Golani, D., and Cardoso, A. C.: Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review, Aquatic Invasions, 9, 391-423, 2014.

Kramar, M. K., Tinta, T., Lučić, D., Malej, A., and Turk, V.: Bacteria associated with moon jellyfish during bloom and post-bloom periods in the Gulf of Trieste (northern Adriatic), PloS one, 14, 2019.

Kress, N., Gertman, I., and Herut, B.: Temporal evolution of physical and chemical characteristics of the water column in the Easternmost Levantine basin (Eastern Mediterranean Sea) from 2002 to 2010, Journal of Marine Systems, 135, 6-13, 2014.

Kress, N., Thingstad, T. F., Pitta, P., Psarra, S., Tanaka, T., Zohary, T., Groom, S., Herut, B., Mantoura, R. F. C., and Polychronaki, T.: Effect of P and N addition to oligotrophic Eastern Mediterranean waters influenced by near-shore waters: a microcosm experiment, Deep Sea Research Part II: Topical Studies in Oceanography, 52, 3054-3073, 2005.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecology letters, 13, 1419-1434, 2010.

Lakkis, S. and Zeidane, R.: Jellyfish swarm along the Lebanese coast (Abstract) Lebanese Association for the Advancement of Science 11th Science Meeting American University of Beirut, 1991. 1991.

Lebrato, M. and Jones, D.: Jellyfish biomass in the biological pump: Expanding the oceanic carbon cycle, The Biochemical Society Journal, 2011, 35-39, 2011.

Lebrato, M., Pitt, K. A., Sweetman, A. K., Jones, D. O., Cartes, J. E., Oschlies, A., Condon, R. H., Molinero, J. C., Adler, L., and Gaillard, C.: Jelly-falls historic and recent observations: a review to drive future research directions, Hydrobiologia, 690, 227-245, 2012.

Licandro, P., Conway, D., Daly Yahia, M., Fernandez de Puelles, M. L., Gasparini, S., Hecq, J.-H., Tranter, P., and Kirby, R.: A blooming jellyfish in the northeast Atlantic and Mediterranean, Biology letters, 6, 688-691, 2010.

Lotan, A., Ben-Hillel, R., and Loya, Y.: Life cycle of *Rhopilema nomadica*: a new immigrant scyphomedusan in the Mediterranean, Marine Biology, 112, 237-242, 1992.

Lotan, A., Fine, M., and Ben-Hillel, R.: Synchronization of the life cycle and dispersal pattern of the tropical invader scyphomedusan *Rhopilema nomadica* is temperature dependent, Marine Ecology-Progress Series, 109, 59-59, 1994.

Love, M. I., Huber, W., and Anders, S.: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome biology, 15, 550, 2014.

Lucas, C. H., Pitt, K. A., Purcell, J. E., Lebrato, M., and Condon, R. H.: What's in a jellyfish? Proximate and elemental composition and biometric relationships for use in biogeochemical studies, Ecology, 92, 1704, 2011.

Lynam, C. P., Gibbons, M. J., Axelsen, B. E., Sparks, C. A., Coetzee, J., Heywood, B. G., and Brierley, A. S.: Jellyfish overtake fish in a heavily fished ecosystem, Current biology, 16, R492-R493, 2006.

MacKenzie, K. M., Trueman, C. N., Lucas, C. H., and Bortoluzzi, J.: The preparation of jellyfish for stable isotope analysis, Marine Biology, 164, 219, 2017.

Madkour, F. F., Safwat, W., and Hanafy, M. H.: Record of Aggregation of Alien Tropical Schyphozoan *Rhopilema nomadica* Galil, 1990 in the Mediterranean Coast of Egypt, International Marine Science Journal, 1, 1, 2019.

McMurdie, P. J. and Holmes, S.: phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data, PloS one, 8, 2013.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A.: Future ocean acidification will be amplified by hypoxia in coastal habitats, Marine Biology, 160, 1875-1888, 2013.

Møller, L. F. and Riisgård, H. U.: Impact of jellyfish and mussels on algal blooms caused by seasonal oxygen depletion and nutrient release from the sediment in a Danish fjord, Journal of Experimental Marine Biology and Ecology, 351, 92-105, 2007.

Müller, T., Walter, B., Wirtz, A., and Burkovski, A.: Ammonium toxicity in bacteria, Current microbiology, 52, 400-406, 2006.

Nakar, N., Disegni, D., and Angel, D.: Economic evaluation of jellyfish effects on the fishery sector—Case study from the eastern Mediterranean, 2011, 11-13.

Niepceron, M., Martin-Laurent, F., Crampon, M., Portet-Koltalo, F., Akpa-Vinceslas, M., Legras, M., Bru, D., Bureau, F., and Bodilis, J.: GammaProteobacteria as a potential bioindicator of a multiple contamination by polycyclic aromatic hydrocarbons (PAHs) in agricultural soils, Environmental pollution, 180, 199-205, 2013.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H.: Vegan: community ecology package. R package version 1.17-4, URL http://CRAN. R-project. org/package= vegan, 2010. 2010.

Parada, A. E., Needham, D. M., and Fuhrman, J. A.: Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples, Environmental microbiology, 18, 1403-1414, 2016.

Pitt, K. A., Welsh, D. T., and Condon, R. H.: Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production, Hydrobiologia, 616, 133-149, 2009.

Pratihary, A., Naqvi, S., Narvenkar, G., Kurian, S., Naik, H., Naik, R., and Manjunatha, B.: Benthic mineralization and nutrient exchange over the inner continental shelf of western India, 2014. 2014.

Purcell, J. E.: Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations, Annual Review of Marine Science, 4, 209-235, 2012.

Purcell, J. E., Uye, S.-i., and Lo, W.-T.: Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review, Marine Ecology Progress Series, 350, 153-174, 2007.

Qu, C.-F., Song, J.-M., Li, N., Li, X.-G., Yuan, H.-M., Duan, L.-Q., and Ma, Q.-X.: Jellyfish (*Cyanea nozakii*) decomposition and its potential influence on marine environments studied via simulation experiments, Marine pollution bulletin, 97, 199-208, 2015.

Quiñones, J., Mianzan, H., Purca, S., Robinson, K. L., Adams, G. D., and Acha, E. M.: Climate-driven population size fluctuations of jellyfish (*Chrysaora plocamia*) off Peru, Marine biology, 162, 2339-2350, 2015.

Rahav, E., Belkin, N., Paytan, A., and Herut, B.: Phytoplankton and Bacterial Response to Desert Dust Deposition in the Coastal Waters of the Southeastern Mediterranean Sea: A Four-Year In Situ Survey, Atmosphere, 9, 305, 2018a.

Rahav, E., Raveh, O., Hazan, O., Gordon, N., Kress, N., Silverman, J., and Herut, B.: Impact of nutrient enrichment on productivity of coastal water along the SE Mediterranean shore of Israel-A bioassay approach, Marine pollution bulletin, 127, 559-567, 2018b.

Raveh, O., David, N., Rilov, G., and Rahav, E.: The temporal dynamics of coastal phytoplankton and bacterioplankton in the Eastern Mediterranean Sea, PLoS One, 10, 2015.

Reichenbach, H.: The order cytophagales. In: The prokaryotes, Springer, 1992.

Richardson, A. J., Bakun, A., Hays, G. C., and Gibbons, M. J.: The jellyfish joyride: causes, consequences and management responses to a more gelatinous future, Trends in ecology & evolution, 24, 312-322, 2009.

Sanz-Martín, M., Pitt, K. A., Condon, R. H., Lucas, C. H., Novaes de Santana, C., and Duarte, C. M.: Flawed citation practices facilitate the unsubstantiated perception of a global trend toward increased jellyfish blooms, Global Ecology and Biogeography, 25, 1039-1049, 2016.

Schnedler-Meyer, N. A., Kiørboe, T., and Mariani, P.: Boom and Bust: Life History, Environmental Noise, and the (un) Predictability of Jellyfish Blooms, Frontiers in Marine Science, 5, 257, 2018.

Shiganova, T., Mirzoyan, Z., Studenikina, E., Volovik, S., Siokou-Frangou, I., Zervoudaki, S., Christou, E., Skirta, A., and Dumont, H.: Population development of the invader ctenophore Mnemiopsis leidyi, in the Black Sea and in other seas of the Mediterranean basin, Marine biology, 139, 431-445, 2001.

Simon, M.: Improved assessment of bacterial production: combined measurements of protein synthesis via leucine and cell multiplication via thymidine incorporation, Ergebnisse der Limnologie ERLIA 6, 34, 1990.

Simon, M. and Azam, F.: Protein content and protein synthesis rates of planktonic marine bacteria, Marine ecology progress series. Oldendorf, 51, 201-213, 1989.

Simon, M., Grossart, H.-P., Schweitzer, B., and Ploug, H.: Microbial ecology of organic aggregates in aquatic ecosystems, Aquatic microbial ecology, 28, 175-211, 2002.

Sisma-Ventura, G. and Rahav, E.: DOP stimulates heterotrophic bacterial production in the oligotrophic southeastern Mediterranean coastal waters, Frontiers in microbiology, 10, 1913, 2019.

Skoog, A. C. and Arias-Esquivel, V. A.: The effect of induced anoxia and reoxygenation on benthic fluxes of organic carbon, phosphate, iron, and manganese, Science of the total environment, 407, 6085-6092, 2009.

Sterner, R. W. and Elser, J. J.: Ecological stoichiometry: the biology of elements from molecules to the biosphere, Princeton university press, 2002.

Stief, P.: Stimulation of microbial nitrogen cycling in aquatic ecosystems by benthic macrofauna: mechanisms and environmental implications, Biogeosciences, 10, 2013.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D., BREINER, H. W., and Richards, T. A.: Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water, Molecular ecology, 19, 21-31, 2010.

Stoecker, D. K., Michaels, A. E., and Davis, L. H.: Grazing by the jellyfish, *Aurelia aurita*, on microzooplankton, Journal of Plankton Research, 9, 901-915, 1987.

Streftaris, N. and Zenetos, A.: Alien marine species in the Mediterranean-the 100 'Worst Invasives' and their impact, Mediterranean Marine Science, 7, 87-118, 2006.

Sun, S., Jones, R. B., and Fodor, A. A.: Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories, Microbiome, 8, 1-9, 2020.

Sweetman, A. K. and Chapman, A.: First observations of jelly-falls at the seafloor in a deep-sea fjord, Deep Sea Research Part I: Oceanographic Research Papers, 58, 1206-1211, 2011.

Sweetman, A. K., Chelsky, A., Pitt, K. A., Andrade, H., van Oevelen, D., and Renaud, P. E.: Jellyfish decomposition at the seafloor rapidly alters biogeochemical cycling and carbon flow through benthic food-webs, Limnology and Oceanography, 61, 1449-1461, 2016.

Sweetman, A. K., Smith, C. R., Dale, T., and Jones, D. O.: Rapid scavenging of jellyfish carcasses reveals the importance of gelatinous material to deep-sea food webs, Proceedings of the Royal Society B: Biological Sciences, 281, 20142210, 2014.

Tadir, R., Benjamini, C., Almogi-Labin, A., and Hyams-Kaphzan, O.: Temporal trends in live foraminiferal assemblages near a pollution outfall on the Levant shelf, Marine pollution bulletin, 117, 50-60, 2017.

Thingstad, T. F., Krom, M., Mantoura, R., Flaten, G. F., Groom, S., Herut, B., Kress, N., Law, C., Pasternak, A., and Pitta, P.: Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean, Science, 309, 1068-1071, 2005.

Tinta, T., Kogovšek, T., Malej, A., and Turk, V.: Jellyfish modulate bacterial dynamic and community structure, PloS one, 7, 2012.

Tinta, T., Kogovšek, T., Turk, V., Shiganova, T. A., Mikaelyan, A. S., and Malej, A.: Microbial transformation of jellyfish organic matter affects the nitrogen cycle in the marine water column—A Black Sea case study, Journal of experimental marine biology and ecology, 475, 19-30, 2016.

Tinta, T., Malej, A., Kos, M., and Turk, V.: Degradation of the Adriatic medusa *Aurelia* sp. by ambient bacteria. In: Jellyfish Blooms: New Problems and Solutions, Springer, 2010.

Titelman, J., Riemann, L., Sørnes, T. A., Nilsen, T., Griekspoor, P., and Båmstedt, U.: Turnover of dead jellyfish: stimulation and retardation of microbial activity, Marine Ecology Progress Series, 325, 43-58, 2006.

Welsh, D. T.: It's a dirty job but someone has to do it: the role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column, Chemistry and Ecology, 19, 321-342, 2003.

Welsh, D. T., Dunn, R. J., and Meziane, T.: Oxygen and nutrient dynamics of the upside down jellyfish (*Cassiopea* sp.) and its influence on benthic nutrient exchanges and primary production, Hydrobiologia, 635, 351-362, 2009.

Wemheuer, F., Taylor, J. A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T., and Wemheuer, B.: Tax4Fun2: a R-based tool for the rapid prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene marker gene sequences, BioRxiv, 2018. 490037, 2018.

West, E. J., Pitt, K. A., Welsh, D. T., Koop, K., and Rissik, D.: Top-down and bottom-up influences of jellyfish on primary productivity and planktonic assemblages, Limnology and oceanography, 54, 2058-2071, 2009.

West, E. J., Welsh, D. T., and Pitt, K. A.: Influence of decomposing jellyfish on the sediment oxygen demand and nutrient dynamics. In: Jellyfish Blooms: Causes, Consequences, and Recent Advances, Springer, 2008.

Wickham, H.: ggplot2: elegant graphics for data analysis, Springer, New York, 2009.

Woyke, T., Xie, G., Copeland, A., Gonzalez, J. M., Han, C., Kiss, H., Saw, J. H., Senin, P., Yang, C., and Chatterji, S.: Assembling the marine metagenome, one cell at a time, PloS one, 4, 2009.

Xiao, W., Zeng, Y., Liu, X., Huang, X., Chiang, K.-P., Mi, T., Zhang, F., Li, C., Wei, H., and Yao, Q.: The impact of giant jellyfish Nemopilema nomurai blooms on plankton communities in a temperate marginal sea, Marine pollution bulletin, 149, 110507, 2019.

Yahia, M. N. D., Yahia, O. K.-D., Gueroun, S. K. M., Aissi, M., Deidun, A., Fuentes, V., and Piraino, S.: The invasive tropical scyphozoan *Rhopilema nomadica* Galil, 1990 reaches the Tunisian coast of the Mediterranean Sea, BioInvasions Records, 2, 319-323, 2013.

Yakimov, M. M., Timmis, K. N., and Golyshin, P. N.: Obligate oil-degrading marine bacteria, Current opinion in biotechnology, 18, 257-266, 2007.

Zenetos, A., Gofas, S., Verlaque, M., Çinar, M. E., Raso, J. G., Bianchi, C., Morri, C., Azzurro, E., Bilecenoglu, M., and Froglia, C.: Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution, Mediterranean marine science, 11, 381, 2010.

Zunino, S., Canu, D. M., Bandelj, V., and Solidoro, C.: Effects of ocean acidification on benthic organisms in the Mediterranean Sea under realistic climatic scenarios: a meta-analysis, Regional Studies in Marine Science, 10, 86-96, 2017.

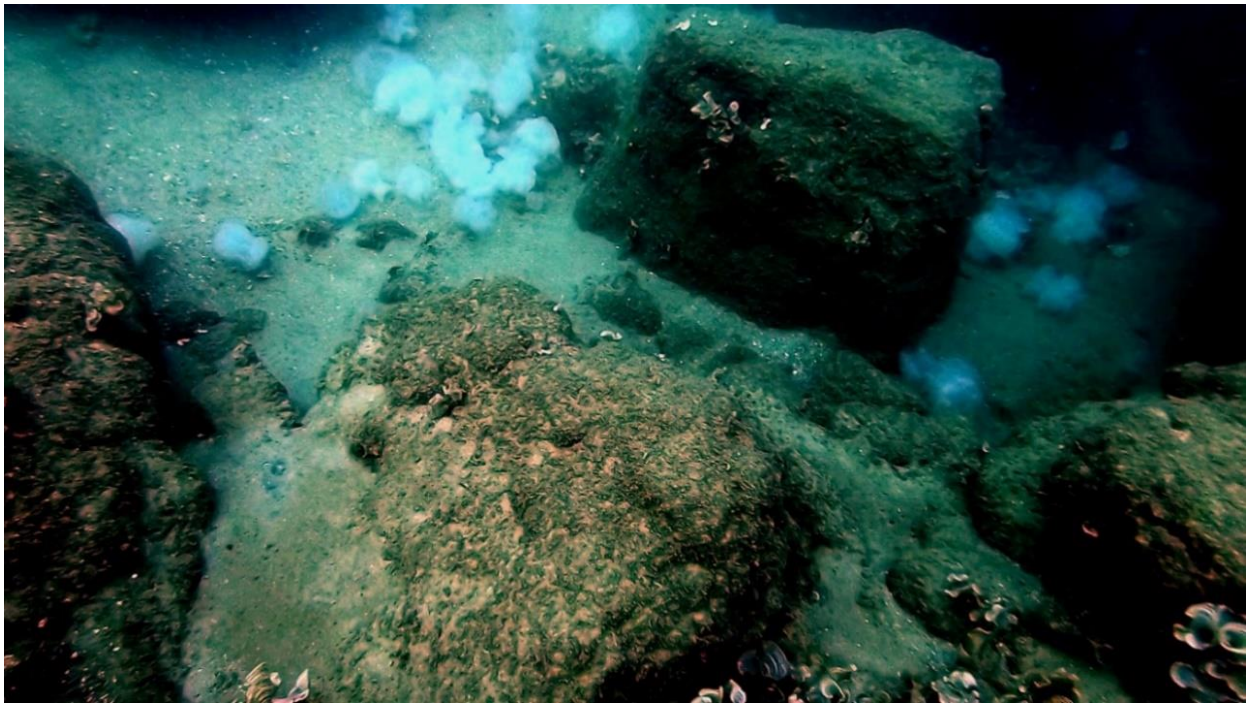

Figure 1. Jelly-falls (carcasses) of ca. 30 *Rhopilema nomadica* in the Mediterranean coast of Caesarea, Israel. 8-9 m depth, photographed on 27 July 2019 after the typical peak summer bloom (Photo: Zvika Fayer).

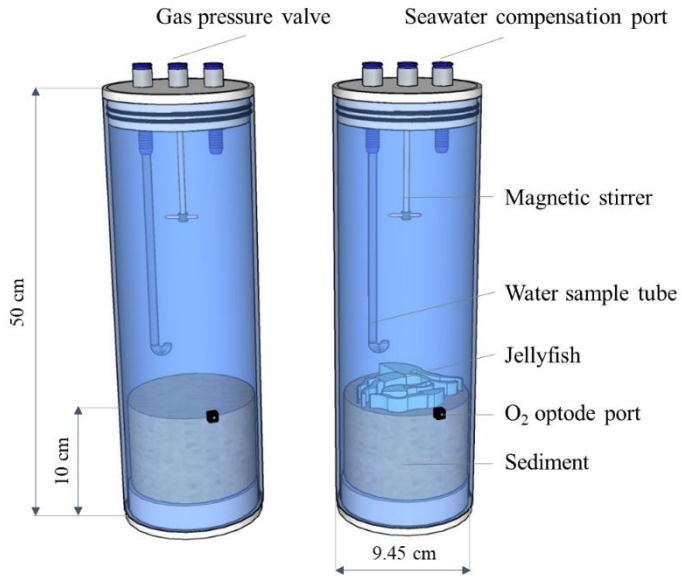

Figure 2. Experimental set-up. Incubation cylinders including jellyfish treatment (right, N=3) and controls (left, N=3).

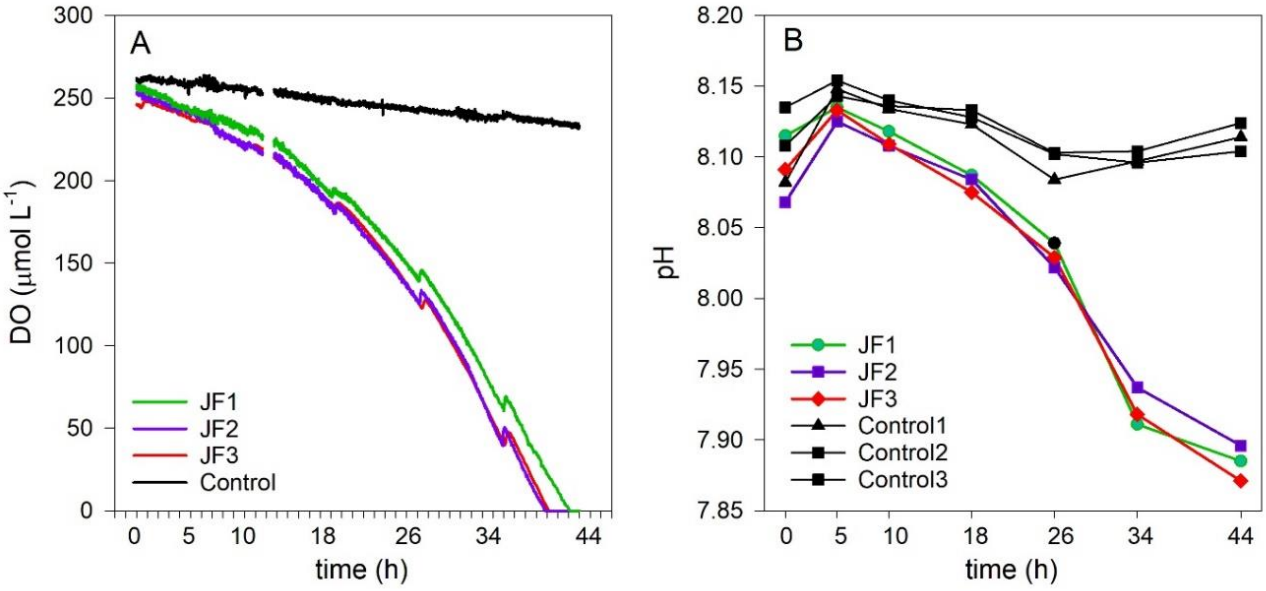

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Figure 3. The decomposition of the jellyfish *R. nomadica* leads to oxygen depletion and acidification in the seawater overlying the sediment. A. Continuous dissolved oxygen (DO) record in the experimental cylinders enriched with carcasses of the jellyfish *R. nomadica* (JF1-JF3) and in the controls. B. pH dynamics in the experimental cylinders, including jellyfish and in the controls. N=3. The temperature was kept relatively constant at 27-28°C. The slight 690 **increases in DO concentrations throughout the incubation period indicate water compensation during discrete sampling events.**

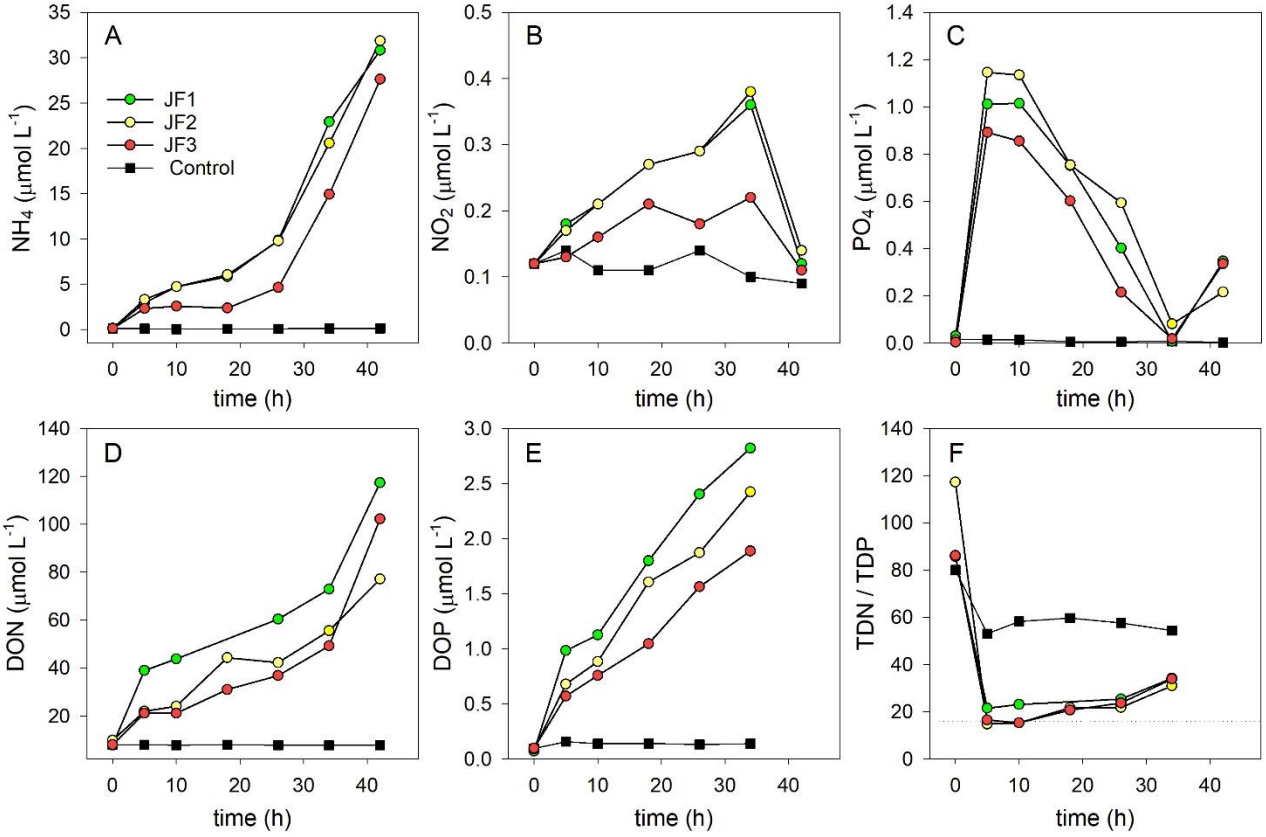

Figure 4. Changes in the concentrations (µmol L^{-1}) of organic and inorganic nutrients in the experimental cylinders enriched with carcasses of the jellyfish *R. nomadica* and in the controls. A. ammonium. B. nitrite. C. orthophosphate. D. DON. E. DOP. F. TDN/TDP ratio. (N=3).

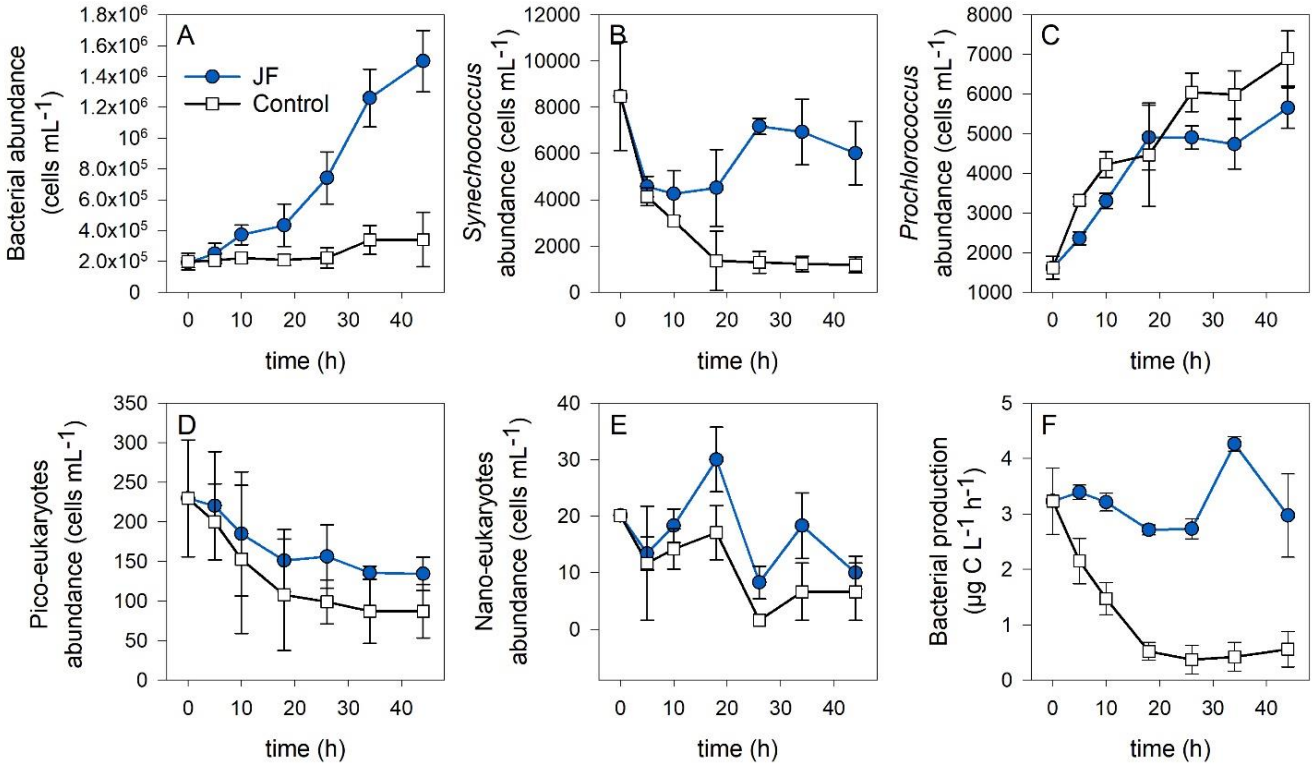

Figure 5. Microbial abundance (cell·mL^{-1}) and production (µg C·L^{-1}·h^{-1}) in the jellyfish *R. nomadica* -enriched (blue) and control (black) experimental cylinders over the experimental period. A. total bacterial abundance. B. *Synechococcus*. C. *Prochlorococcus*. D. Pico-eukaryotes. E. Nano-eukaryotes. F. bacterial production. N=3, the error bars denote standard deviation.

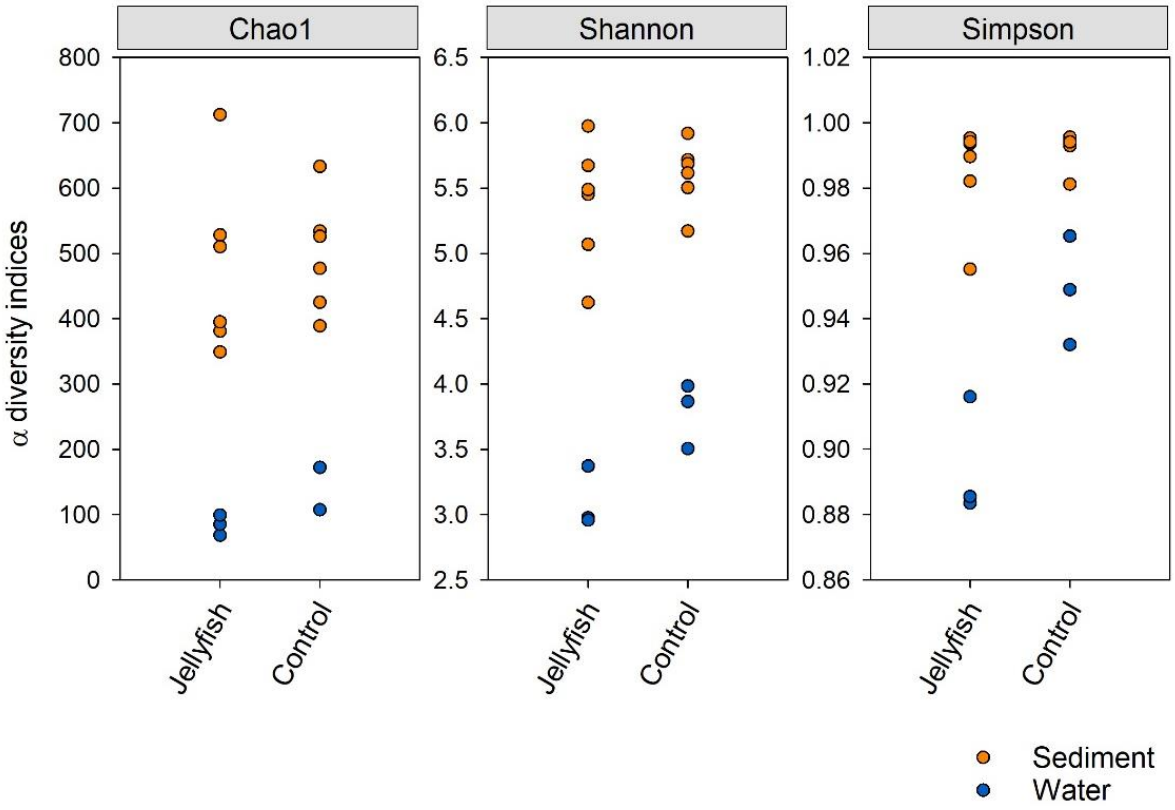

Figure 6. Bacterial alpha diversity indices (Chao, Shannon, Simpson) in water and sediment samples from experimental cylinders enriched with carcasses of the jellyfish and in the controls (n=3).

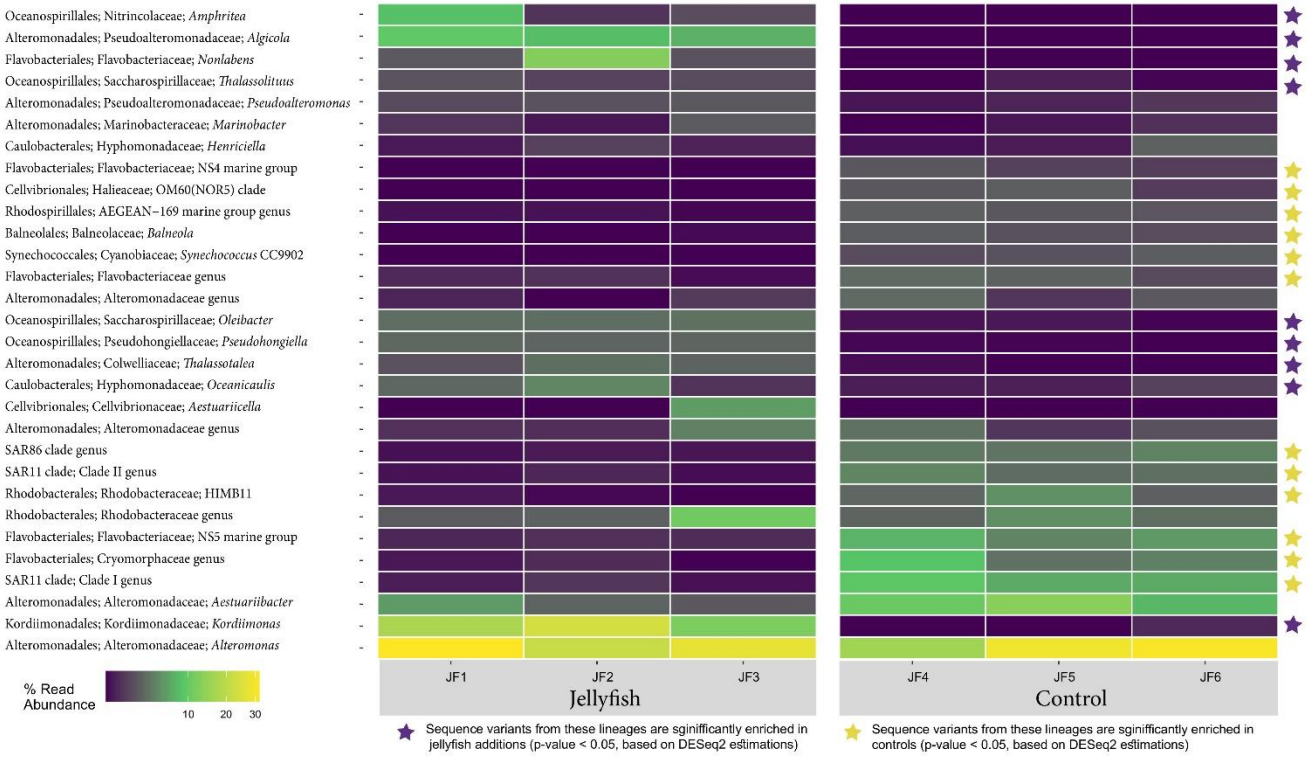

Figure 7. Diversity of bacteria in the jellyfish-enriched and control experimental cylinder seawater. The 30 most abundant lineages are presented and organized by hierarchical clustering. Color scale denotes the relative abundance of reads (%). The star symbols on the right-side panel indicate lineages significantly more abundant in the jellyfish treatment (in purple) or the controls (in yellow) based on DESeq2 estimations.

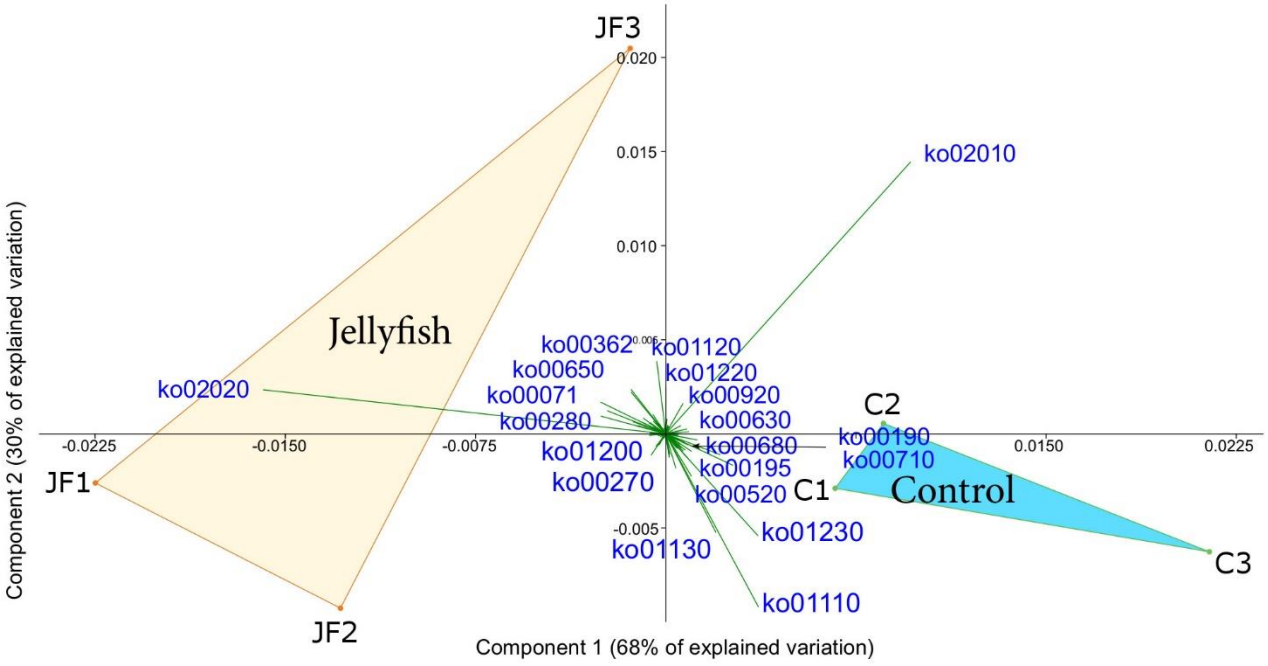

Figure 8. Principle Component Analysis biplot of functional diversity based on taxonomy-based functional predictions using Tax4Fun2. JF = jellyfish samples; C = control samples. The vectors present KEGG pathways. The within-group similarity is 95% between the jellyfish treatments (red) and 97% between the controls (light blue). The following KEGG pathways are shown: ko00071 Fatty acid degradation, ko00190 Oxidative phosphorylation, ko00195 Photosynthesis, ko00270 Cysteine and methionine metabolism, ko00280 Valine, leucine and isoleucine degradation, ko00362 Benzoate degradation, ko00520 Amino sugar and nucleotide sugar metabolism, ko00630 Glyoxylate and dicarboxylate metabolism, ko00650 Butanoate metabolism, ko00680 Methane metabolism, ko00710 Carbon fixation in photosynthetic organisms, ko00920 Sulfur metabolism, ko01110 Biosynthesis of secondary metabolites, ko01120 Microbial metabolism in diverse environments, ko01130 Biosynthesis of antibiotics, ko01200 Carbon metabolism, ko01220 Degradation of aromatic compounds, ko01230 Biosynthesis of amino acids, ko02010 ABC transporters, ko02020 Two-component system.

Table 1: Daily oxygen consumption and nutrient release rates standardized to jellyfish (*R. nomadica*) biomass (µmol·g $WW^{-1}·d^{-1}$). The average wet weight of the whole jellyfish was 1.5±0.4 kg. N=3.

	rate (µmol·g $WW^{-1}·d^{-1}$)	SD
DO	-17.9	0.3
NH_4	2.0	0.2
PO_4	0.6	0.1
DON	4.0	0.7
DOP	0.2	0.04

730 **Table 2: Calculated oxygen and nutrient fluxes in the seawater of jellyfish (*R. nomadica*) -enriched and control experimental cylinders. Positive flux represents water column enrichment (source), negative flux represent removal from the water column (sink). N=3. SD denotes standard deviation. N.A – not available.**

	Jellyfish (mmol m^{-2} d^{-1})		Control (mmol m^{-2} d^{-1})	
	Mean	SD	Mean	SD
DO	-56.9	1.0	-6.7	0.3
NH_4 (0-36 h)	6.9	0.4	$1·10^{-2}$	$8·10^{-3}$
PO_4 (0-5 h)	1.9	0.2	$-5·10^{-3}$	$1·10^{-2}$
DON	12.7	2.4	$-4·10^{-2}$	N.A.
DOP	0.6	0.1	$5·10^{-3}$	N.A.

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Appendix A: Additional nutrient data

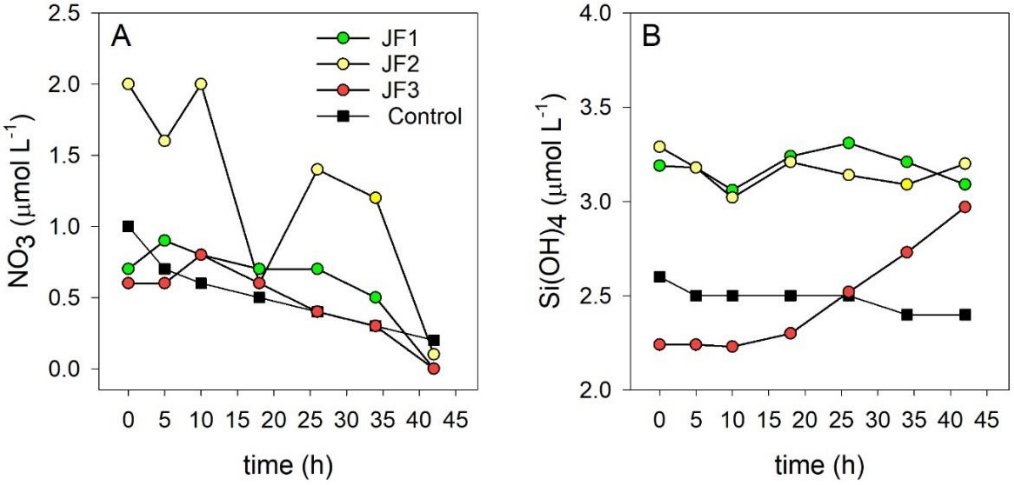

Figure A1. Changes in the concentrations (µmol L^{-1}) of A. NO_3 and, B. $Si(OH)_4$ in the experimental cylinders enriched with carcasses of the jellyfish *R. nomadica* (JF1-JF3) and in the controls (N=3).

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Appendix B: Nutrient--bacteria correlations

Table B1: Pearson correlation coefficients (*r*) between nutrient concentrations, bacterial abundance and production rates. Averages of three replicates per time step were used (N=7). Significant correlations are marked in bold (p<0.05).

	Bacterial abundance	Bacterial production
DO	**-0.995**	-0.211
NH_4	**0.979**	0.236
NO_x	**-0.765**	0.213
PO_4	-0.485	-0.323
$Si(OH)_4$	**0.841**	0.055
DON	**0.944**	0.038
DOP	**0.912**	0.164
TDN	**0.954**	0.355
TDP	0.632	-0.027

Appendix C: Bacterial and eukaryotic diversity in water and sediment samples

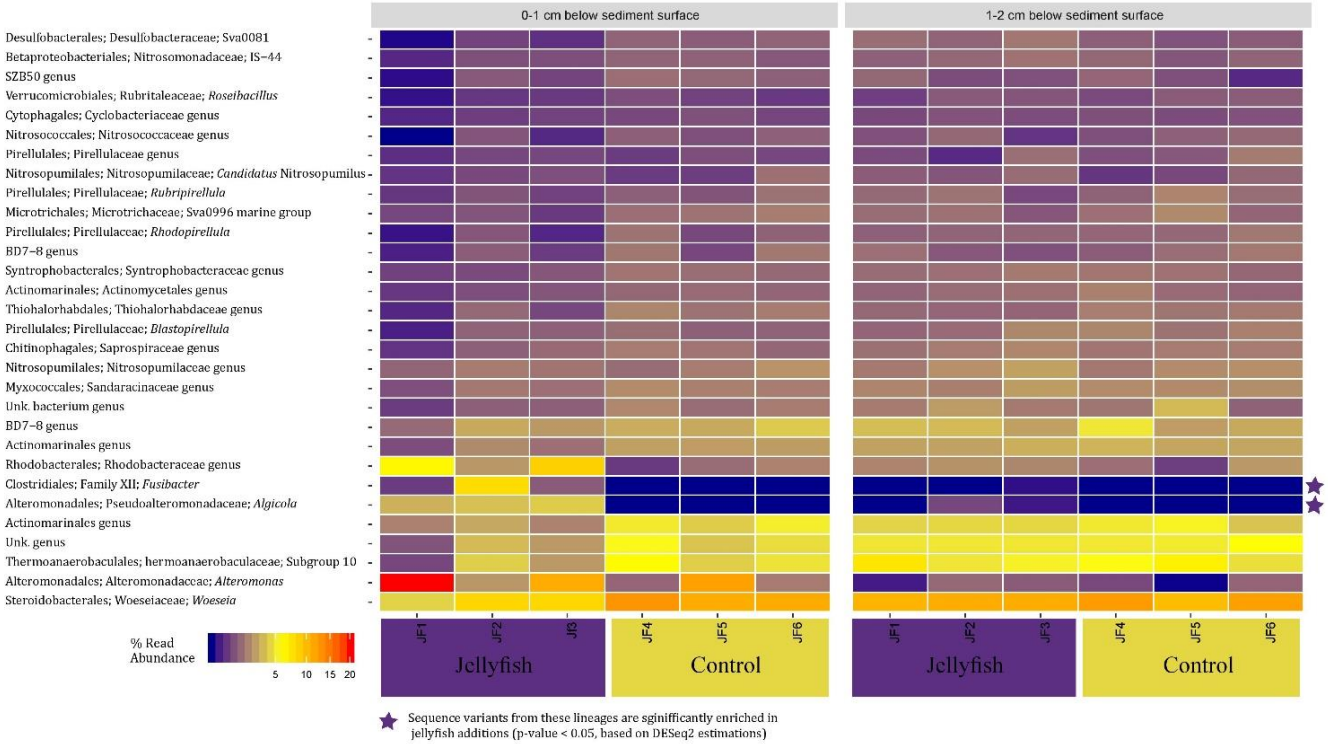


Figure C1. Microbial diversity in sediment samples from the jellyfish-enriched and control experimental cylinders, from 0-1 cm (left) and 1-2 cm (right) depth layers. The 30 most abundant lineages are presented and organized by hierarchical clustering. Color scale denotes the relative abundance of reads (%). The star symbols on the right-side panel indicate lineages significantly more abundant in the jellyfish treatment based on DESeq2 estimations.

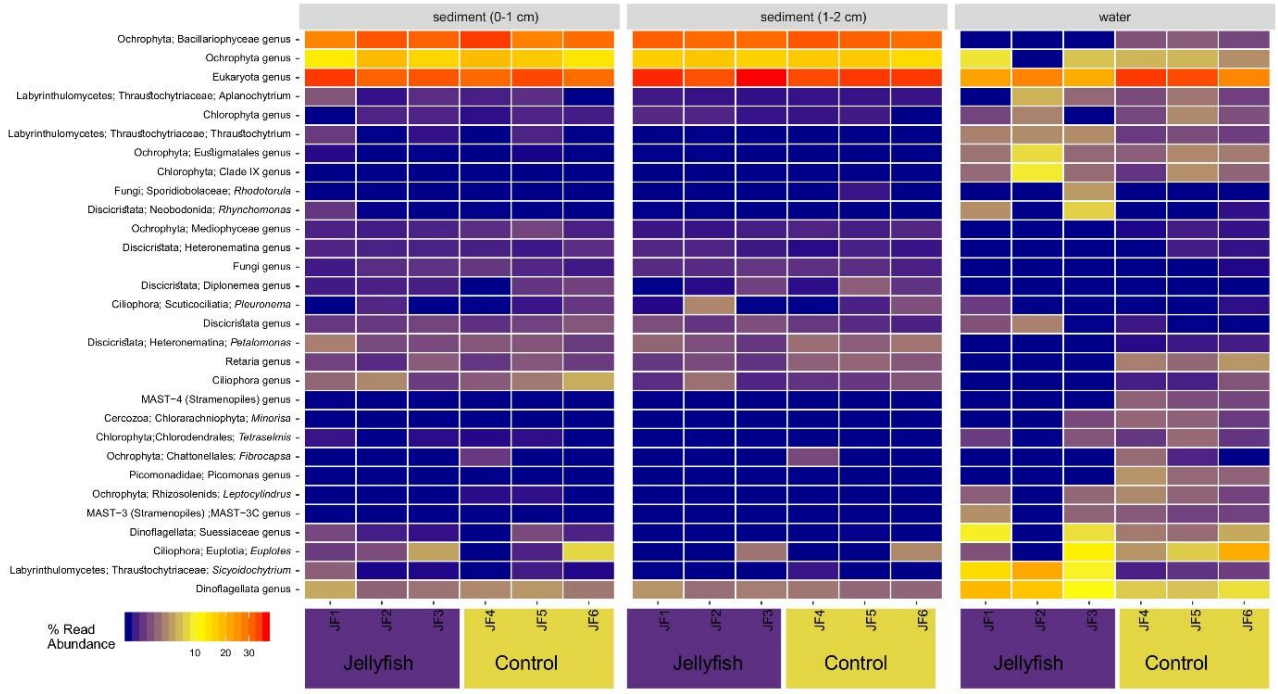


Figure C2. Eukaryote diversity of seawater and sediment samples collected from jellyfish-enriched and control experimental cylinders. The 30 most abundant lineages are presented and organized by hierarchical clustering.. Color scale denotes the relative abundance of reads (%).

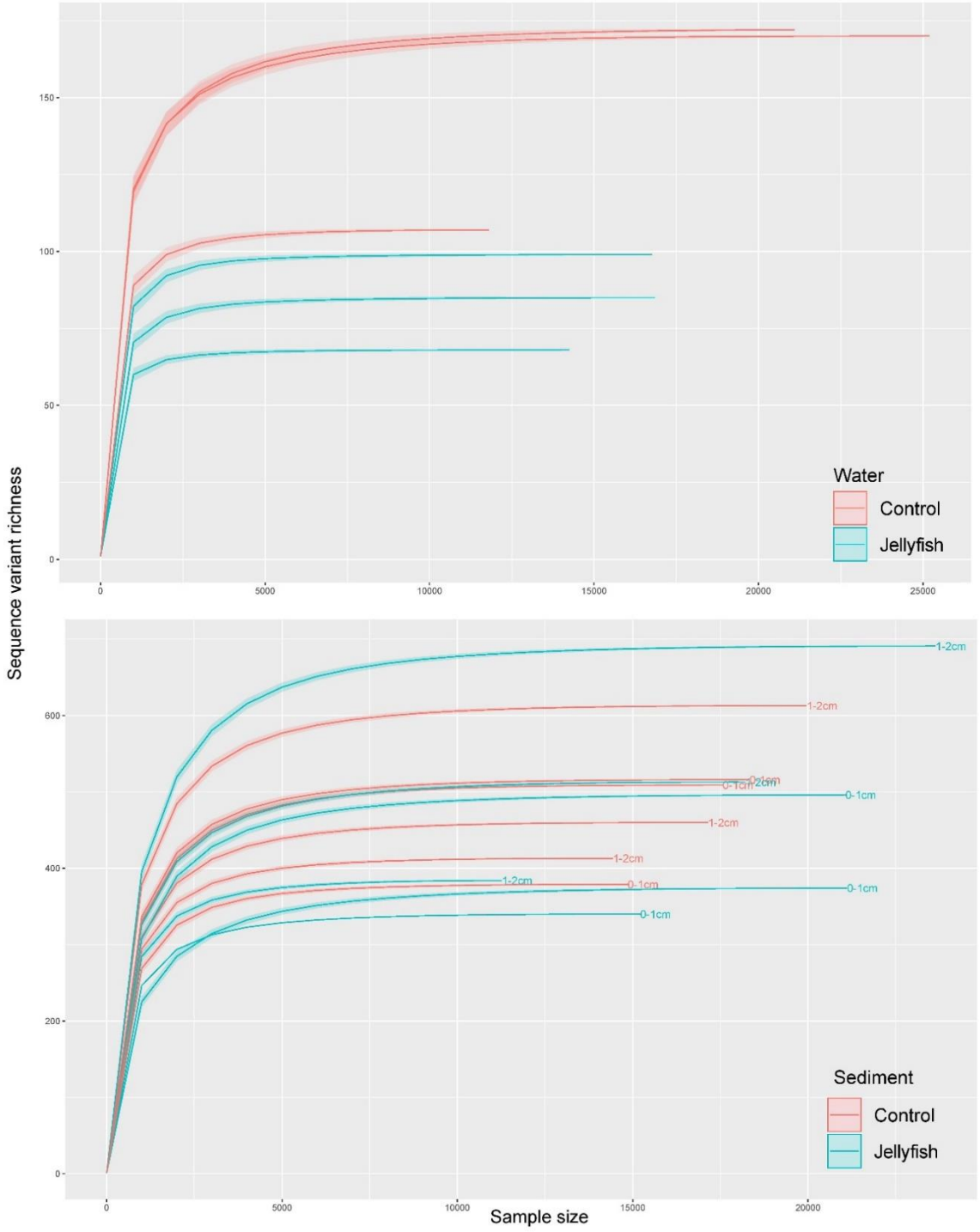
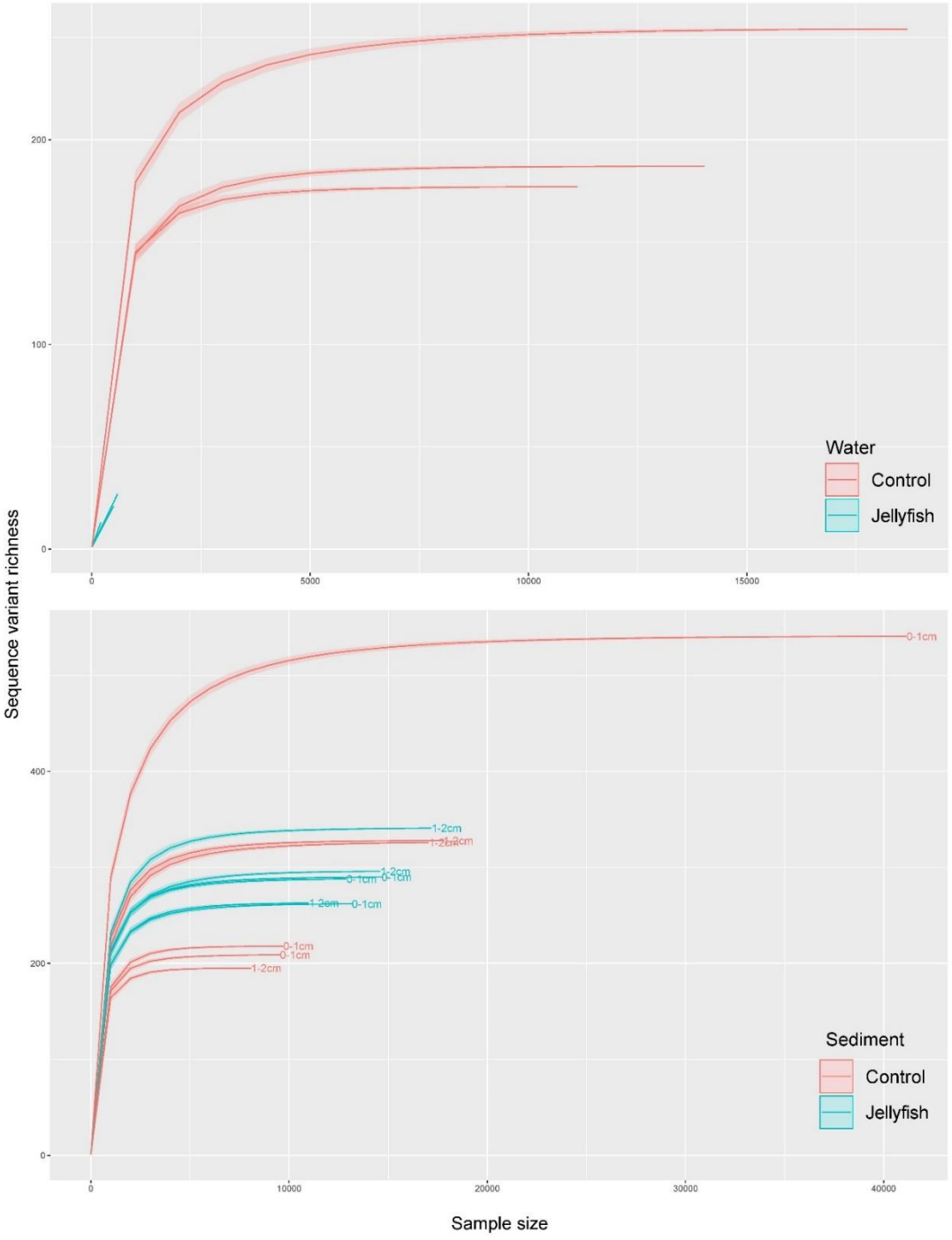


Figure C3. Rarefaction curves of observed 16S rRNA sequence variants retrieved from the seawater (upper graph) and sediment (lower graph) samples.



765 **Figure C4. Rarefaction curves of observed 18S rRNA sequence variants retrieved from the seawater (upper graph) and sediment (lower graph) samples.**

Appendix D: SIMPER analysis of main predicted functions based on KEGG orthologs

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Table D1: Similarity Percentage (SIMPER) analysis indicating the main predicted functions characterizing the jellyfish and control communities (N=3). Av. Abund = Average abundance, Av. Sim = Average similarity, Sim/SD = similarity standard deviation, Contrib% = percent contribution, Cum.% = Cumulative contribution.

Jellyfish treatments

Average similarity: 95.03

KEGG ortholog	Predicted function	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
ko01110	Biosynthesis of secondary metabolites	0.09	8.19	20.04	8.62	8.62
ko01120	Microbial metabolism in diverse environments	0.08	7.42	458.33	7.81	16.43
ko01130	Biosynthesis of antibiotics	0.07	6.87	33.95	7.23	23.66
ko02020	Two-component system	0.07	6.41	25.99	6.74	30.4
ko02010	ABC transporters	0.05	3.92	40.15	4.13	34.53
ko01200	Carbon metabolism	0.04	3.87	44.28	4.08	38.61
ko01230	Biosynthesis of amino acids	0.03	3.27	18.35	3.44	42.05

Controls

Average similarity: 97.47

KEGG ortholog	Predicted function	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
ko01110	Biosynthesis of secondary metabolites	0.09	9.08	91.13	9.32	9.32
ko01120	Microbial metabolism in diverse environments	0.07	7.37	959.26	7.57	16.88
ko01130	Biosynthesis of antibiotics	0.07	7.37	242.75	7.56	24.45

ko02010	ABC transporters	0.06	5.48	31.5	5.62	30.07
ko02020	Two-component system	0.05	4.63	6.87	4.75	34.81
ko01230	Biosynthesis of amino acids	0.04	3.94	129	4.04	38.85
ko01200	Carbon metabolism	0.04	3.88	1602.51	3.98	42.83

Jellyfish treatments & controls

Average dissimilarity: 7.01

KEGG ortholog	Predicted function	Jellyfish Av.Abund	Control Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
ko02020	Two-component system	0.07	0.05	0.93	2	13.19	13.19
ko02010	ABC transporters	0.05	0.06	0.7	3.51	10.02	23.21
ko01110	Biosynthesis of secondary metabolites	0.09	0.09	0.35	1.35	5.05	28.27
ko01230	Biosynthesis of amino acids	0.03	0.04	0.3	2.11	4.34	32.6
ko01130	Biosynthesis of antibiotics	0.07	0.07	0.19	1.34	2.77	35.37
ko00260	Glycine, serine and threonine metabolism	0.01	0.02	0.19	3.46	2.68	38.05
ko00071	Fatty acid degradation	0.01	0.01	0.19	3.94	2.66	40.71

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