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Evidence of nitrification associated with globally distributed pelagic jellyfish

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Complete List of Authors:	Hubot, Nathan; University of Southampton, Ocean and Earth Science Giering, Sarah L. C.; National Oceanography Centre, Ocean Biogeochemistry and Ecosystems Füssel, Jessika; University of Chicago, Department of Medicine Robidart, Julie; National Oceanography Centre, Ocean Technology and Engineering Birchill , Antony ; University of Plymouth, School of Geography, Earth and Environmental Science Stinchcombe, Mark; National Oceanography Centre, Ocean Biogeochemistry and Ecosystems Dumousseaud, Cynthia; University of Southampton, Ocean and Earth Science Lucas, Cathy; University of Southampton, Ocean and Earth Science
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Abstract:	Bioavailable nitrogen is a scarce resource in most of the surface ocean and often limits primary productivity. Although Pelagic jellyfish excrete substantial amounts of ammonia (the preferred form of nitrogen for most phytoplankton), they are overlooked players in marine nitrogen cycling. Here, we observed high rates of nitrification ($NH4+ \rightarrow NO3-$, 5.7 – 40.8 nM gWW-1 (wet weight) h-1) associated with the scyphomedusae Aurelia aurita, Chrysaora hysoscella and Chrysaora pacifica and low rates of incomplete nitrification ($NH4+ \rightarrow NO2-$, 1-2.7 nM gWW-1 h-1) associated with Chrysaora fulgida, Chrysaora hysoscella and Chrysaora pacifica. These observations indicate that microbes living in association with the jellyfish thrive by oxidizing the readily available ammonia to nitrite and nitrate. The four studied species are abundant over a large geographic distribution and exhibit frequent population outbreaks. We show that, during such outbreaks, jellyfish-associated release of nitrogen can provide more than 100% of the nitrogen required for primary production. These findings reveal a so far overlooked pathway when assessing pelagic nitrification rates that might be of particular relevance in nitrogen depleted surface waters and at high jellyfish population densities.

Scientific Significance Statement Topic

Often considered detrimental to the environment and human activities, jellyfish blooms are increasing in several coastal regions worldwide. Yet, the overall effect of these outbreaks on ecosystem productivity and structure are not fully understood. Here we provide evidence for a so far unanticipated role of jellyfish in marine nitrogen cycling. Our observations suggest a widespread association between jellyfish and nitrifying microorganisms. Via ammonium excretion, jellyfish blooms play a substantial role in cycling nitrogen in the surface ocean, supporting chemolithoautotrophic nitrification (up to 33% of the excreted ammonia is oxidize into nitrite/nitrate) and phototrophic primary production (locally providing up to 463% of the nitrogen required for daily primary production). Our novel observations and allometric equations have implications for both the small- and the large-scale coastal processes and are of relevance for researchers from microbiologists to modellers.

Scientific Significance Statement Outlet

As jellyfish blooms occur in lakes and oceans and are important for understanding both the ecology and biogeochemistry of coastal ecosystems, the results of this study are relevant to the broad community reached by L&O.

Evidence of nitrification associated with globally distributed pelagic jellyfish

Nathan Hubot[1,2*], Sarah L C Giering[1], Jessika Füssel[2,3*], Julie Robidart[1], Antony Birchill[1,4], Mark Stinchcombe[1], Cynthia Dumousseaud[2], Cathy Lucas[2]

Affiliations:

[1] National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK

[2] Ocean and Earth Science, University of Southampton, National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK

[3] Department of Medicine, University of Chicago, Chicago, IL, USA

[4] School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, UK

Emails and ORCID ID numbers:

Nathan Damien Hubot: N.D.Hubot@soton.ac.uk, orcid.org/0000-0001-6917-2255

Sarah Lou Carolin Giering: s.giering@noc.ac.uk, orcid.org/0000-0002-3090-1876

Julie Robidart: j.robidart@noc.ac.uk, orcid.org/0000-0001-9805-3570

Jessika Füssel: ju.fuessel@uchicago.edu, orcid.org/0000-0002-4210-2318

Cathy H. Lucas: cathy.lucas@noc.soton.ac.uk, orcid.org/0000-0002-5929-7481

Antony Birchill: antony.birchill@plymouth.ac.uk, orcid.org/0000-0002-1453-5781

Mark Stinchcombe: mark.stinchcombe@noc.ac.uk

Cynthia Dumousseaud: C.C.Dumousseaud@soton.ac.uk, orcid.org/0000-0001-5995-902X

*Correspondence to:

Nathan Hubot, National Oceanography Centre Southampton (Room: 344/35; phone: Ext. 28724) University of Southampton, Waterfront Campus, European Way, Southampton, SO14 3ZH, United Kingdom. N.D.Hubot@soton.ac.uk

&

Jessika Füssel, Department of Medicine, University of Chicago, Chicago, IL, USA

ju.fuessel@uchicago.edu

Key words: Jellyfish, nitrification, microbiome, nitrifiers, nitrogen cycle

Author Contributions

NDH and SLCG designed the study. NDH carried out the experiments and analysed the samples. SLCG helped with the data analysis and interpretation. JF and CHL contributed to the study design and interpretation of the results. JR contributed to drafting the manuscript. AB helped with the sampling of nutrients and use of the Lab-On-Chip sensor. MS and CD contributed to the data acquisition. NDH wrote the manuscript with support from all authors, which approved the final submitted manuscript.

Abstract

Often considered detrimental to the environment and human activities, jellyfish blooms are increasing in several coastal regions worldwide. Yet, the overall effect of these outbreaks on ecosystem productivity and structure are not fully understood. Here we provide evidence for a so far unanticipated role of jellyfish in marine nitrogen cycling. Pelagic jellyfish release nitrogen as a metabolic waste product in form of ammonium. Yet, we observed high rates of nitrification

($NH_4^+ \rightarrow NO_3^-$, 5.7 – 40.8 nM gWW^{-1} (wet weight) h^{-1}) associated with the scyphomedusae *Aurelia aurita, Chrysaora hysoscella* and *Chrysaora pacifica* and low rates of incomplete nitrification ($NH_4^+ \rightarrow NO_2^-$, 1-2.7 nM gWW^{-1} h^{-1}) associated with *Chrysaora fulgida, Chrysaora hysoscella* and *Chrysaora pacifica.* These observations indicate that microbes living in association with these jellyfish thrive by oxidizing the readily available ammonia to nitrite and nitrate. The four studied species have a large geographic distribution and exhibit frequent population outbreaks. We show that, during such outbreaks, jellyfish-associated release of nitrogen can provide more than 100% of the nitrogen required for primary production. These findings reveal a so far overlooked pathway when assessing pelagic nitrification rates that might be of particular relevance in nitrogen depleted surface waters and at high jellyfish population densities.

Introduction

Jellyfish blooms are increasing in frequency and magnitude in several coastal regions around the world (e.g. Sea of Japan, Black sea, Benguela current, Antarctic; Brotz et al. 2015). The presence of jellyfish blooms in coastal waters can cause severe damage to economic activities such as fisheries (e.g., 2.1 - 25% decrease in annual Korean fishery production every year; Kim et al. 2012), tourism (e.g.: costing the Israeli coastal tourism industry an estimated annual monetary loss of €1.8–6.2 million every year; Ghermandi et al. 2015) and power generation (e.g.: the closure costs of Torness nuclear plant in Scotland due to jellyfish bloom from 28th June to 1st July 2011: approximately £1 million d^{-1}; Kopytko 2015). Simultaneously, jellyfish outbreaks create ecological disturbances by altering the marine food chain structure (Pitt and Purcell 2009). Their voracious predation on zooplankton makes them competitors to planktivorous fish (Condon et al. 2011). The grazing pressure that jellyfish put on zooplankton

grazers releases primary producers from predatory control, causing a trophic cascade that can results in phytoplankton blooms (West et al. 2009; Schnedler-Meyer et al. 2018). By preying on ichthyoplankton (eggs and larvae of fish), jellyfish even exert a top-down control on their competitors and predators (Titelman and Hansson 2006; Gordoa et al. 2013). Overall, the complex interaction of jellyfish with the food web can have large impacts on ecosystem structure, function and resilience (Baum and Worm 2009).

The role of jellyfish as top-down predators has been widely studied (e.g.: Purcell and Decker 2005; Compte et al. 2010; Stone and Steinberg 2018), yet their bottom-up influence as a nutrient source on marine ecosystems is less clear. Ammonium excreted by jellyfish has been estimated to support up to 8 %, 10% and 11% of the phytoplankton nitrogen requirement in the Lake Illawarra (Australia), the Inland Sea of Japan and the Kiel Bight, respectively (Schneider 1989; Pitt et al. 2005; Shimauchi and Uye 2007). Ammonium and phosphate released by jellyfish more than doubled the phytoplankton biomass in a mesocosm experiment conducted in a saline lake (West et al. 2009). In addition, the release of organic matter in the form of mucus provides an extremely labile source of organic carbon for bacterioplankton (Condon et al. 2011). While there is clear evidence that jellyfish can alter both biogeochemical cycles and food web structure, their role in pelagic nitrogen cycling remains understudied.

Ammonia is an intensely contested compound in most of the world's sunlit oceans, where nitrogen availability limits primary productivity (~75% of the surface ocean; Moore et al. 2013; Bristow et al. 2017). Additionally, ammonia provides the substrate for ubiquitous chemolithoautotrophic nitrifying bacteria and archaea that generate energy by the stepwise oxidation of ammonia to nitrite and nitrate. The first step is mediated by ammonia oxidizing bacteria (Kowalchuk and Stephen 2001) and archaea (Könneke et al. 2005; Hallam et al. 2006).

Ammonia oxidizing archaea can reach high abundances especially in the dark ocean (> 30% of the microbial community; Karner et al. 2001) and appear to be the main drivers of marine ammonia oxidation (Francis et al. 2005; Wuchter et al. 2006). As for the second step, all known nitrite oxidizers belong to the bacterial domain (Bock and Wagner 2006; Spieck and Bock 2015) and are characterized by their often remarkable metabolic versatility (Koch et al. 2015; Daims et al. 2016; Füssel et al. 2017). Both ammonia oxidizers and nitrite oxidizers (collectively called nitrifiers) are ubiquitous in pelagic environments, where they contribute substantially to carbon fixation in absence of light (dark carbon fixation), influencing ocean carbon fluxes (Wuchter et al. 2006; Herndl and Reinthaler 2013; Pachiadaki et al. 2017). Nitrifiers have also been shown to live in association with benthic invertebrates such as sponges (Diaz and Ward 1997; Schläppy et al. 2010; Radax et al. 2012; Subina et al. 2018), corals (Beman et al. 2007; Siboni et al. 2008; Hoffmann et al. 2009), zoanthids (Sun et al. 2014), bivalves (Welsh and Castadelli 2004), ascidians (Martínez-García et al. 2008) and insect larvae (Stief et al. 2009). As part of invertebrate microbiomes, nitrifiers can provide a source of nutrition for their host when phagocytosed (Martínez-García et al. 2008), preventing the loss of nitrogen into the environment by recycling the excess of ammonium trapped in the mucus (Siboni et al. 2008; Rädecker et al. 2015). Understanding the role of these associations is important for accurate mapping of marine nitrogen biogeochemistry and may help to improve our ability to predict future change (Pajares and Ramos 2019).

Jellyfish are densely populated with microorganisms (Weiland-Bräuer et al. 2015; Lee et al. 2018; Kramar et al. 2019), which play a beneficial role in the fitness of the host and contribute to the ecological features of the jellyfish (Stabili et al. 2018; Tinta et al. 2019). The epithelial mucus layer of a jellyfish is an attractive niche for microbes, providing them with both

a habitat and a high-quality energy source (Kramar et al. 2019). By attracting profitable bacteria and preventing colonization by potentially harmful microorganisms (via interferences with bacterial quorum sensing), the host maintains a healthy microbiome providing immune system functions (Weiland-Bräuer et al. 2019). In addition, jellyfish microbiomes are production hotspots of chemical compounds (e.g. exopolysaccharides, vitamins, enzymes, toxins, antibiotics; Tinta et al. 2019) and harbour microbes closely related to known drivers of major elemental cycles (e.g. nitrogen cyclers, chemolithoautotrophs, methylotrophs, methane oxidizers, and polycyclic aromatic hydrocarbon degraders; Lee et al. 2018). In terms of the nitrogen cycle, two species of nitrifiers (the ammonium oxidizing bacterium *Nitrosospira multiformis* and the nitrite oxidizing bacterium *Nitrospira moscoviensis*) have been found in association with the jellyfish *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-Bräuer et al. 2015). This discovery leads to the suggestion that these two nitrifiers are ubiquitous members of the microbiome of these two genera (Lee et al. 2018) and indicates that jellyfish could contribute to marine nitrogen cycling beyond the excretion of ammonia via their microbiome.

Cnidarian jellyfish excrete ammonium, a by-product of their metabolism, by diffusion through their body surface (Lőw et al. 2016). Though they are not known to directly produce nitrite or nitrate, low rates of nitrate release have been observed in association with pelagic jellyfish (< 2% of total inorganic nitrogen released; Pitt et al. 2009). For benthic jellyfish that live in symbiosis with zooxanthellae (photosynthetic dinoflagellates), experiments have shown nitrite/nitrate release rates equivalent to 21.5% of the total dissolved inorganic nitrogen release, indicating a substantial colonization by nitrifying microorganisms (Welsh et al. 2009). While the authors suggested the association with nitrifiers to be specific to zooxanthellate jellyfish, we hypothesize that nitrifying microorganisms that benefit from the excreted ammonium are

commonly associated with jellyfish and play a significant role in the nitrogen cycling. To test this hypothesis, we chose four species of non-zooxanthellate scyphozoan jellyfish, *Aurelia aurita, Chrysaora hysoscella, Chrysaora fulgida* and *Chrysaora pacifica* from four contrasting environmental conditions (brackish lake, both North and South Atlantic Ocean coastal waters, and artificial seawater), representing a wide range of environmental conditions (Dawson et al. 2005; Morandini and Marques 2010). All of these species exhibit population outbreaks in coastal areas (Lucas 2001; Lynam et al. 2006; Makabe et al. 2015; Abato 2017) leading to high population biomass ultimately disturbing human activities. We measured the release rates of ammonium, nitrite, nitrate and phosphate in association with all four jellyfish species in order to assess the global prevalence of an association between nitrifiers and jellyfish as well as its potential role in the marine nitrogen cycle.

Materials

Adult medusae of *Aurelia aurita* (n = 5), *Chrysaora hysoscella* (n = 5) and *Chrysaora fulgida* (n = 2) were sampled from Horsea lake (UK), the Rame Peninsula (UK), and Walvis Bay (Namibia), respectively (Table 1). Medusae were collected carefully from near-surface waters using a 10-L bucket and kept in approximately 5 L of ambient water during transportation to the laboratory. The water temperature was kept as close to in-situ conditions as possible (maximum fluctuations: ±2°C from in-situ conditions; Table 1). Maximum transportation time was four hours. All jellyfish survived transportation and were transferred to the lab in good condition, indicated by regular swimming pulse. Once in the lab, jellyfish were transferred to their respective experimental conditions (Table 1). *Chrysaora pacifica* specimen (n = 5) were collected from the London aquarium. The medusae were produced from polyps cultured in

artificial conditions (artificial seawater with continuous UV-treatment and filtering system) and had not been in contact with natural seawater.

Sampling sites and species

Horsea Lake (Portsmouth, United Kingdom; 50° 83’ 68.26” N, 1° 10’ 19.11” W) is an enclosed, shallow (6-7 m), brackish (salinity: 19-23 PSU) lake situated on the south coast of England. The lake is oligotrophic with annual surface temperatures between 5°C and 23°C (Lucas 1996). It lacks a riverine input and is replenished with seawater 2–3 times a year during high water spring tides (Lucas et al. 1997). The moon jellyfish *A. aurita* is found in Atlantic boreal waters and in the Black Sea (Dawson 2003; Figure 1). The species was previously associated with a cosmopolitan distribution and is now known to be formed by many regional “cryptic” species spread globally (Dawson and Jacobs 2001; Scorrano et al. 2016, Figure 1). The medusae of *A. aurita* can reach bell diameters up to 40 cm (Arai 1996) and are often found in high densities in coastal and brackish waters such as estuaries and bays (Lucas 2001). They are present in Horsea Lake throughout the year (Lucas 1996).

The Rame Peninsula (Cornwall, United Kingdom) is located on the south-west coast of England. Medusae of the species *C. hysoscella* were collected in waters characteristic of the English Channel (50° 19’ 54.5’’ N, 4° 11’ 59.2’’ W). The mean monthly surface temperature ranges from 9.2°C to 16.5°C and the mean monthly surface salinity ranges from 35.1 to 35.3 PSU (Smyth et al. 2010). Medusae of *C. hysoscella* are of medium sizes (15–25 cm in bell diameter) and are found in the North Sea, the English Channel and the Mediterranean Sea (Morandini and Marques 2010; Figure 1), where they can form dense populations (Abato 2017). They appear in the English Channel during the summer months (Pikesley et al. 2014).

Walvis Bay is a large bay located on the coast of Namibia (22° 57’ 22’’ S, 14° 30’ 29’’ E). The water conditions of the bay are dictated by the Northern Benguela Upwelling System, which is a highly productive eastern boundary ecosystem. The seawater temperature in Walvis Bay varies between 10°C and 22°C and the salinity mainly ranges between 34.5 and 35.5 PSU (Pryor et al. 2009). *C. fulgida* is an exclusively marine species found along the west coast of Africa (Figure 1) with medusae of medium size (10–20 cm in diameter). This species has previously been identified as *C. hysoscella* due to their morphological similarities (Morandini and Marques 2010). *C. fulgida* medusae are found in Walvis Bay throughout the year and frequently reach significant population densities during the summer months (Skrypzeck 2019).

Medusae of the species *C. pacifica* are slightly smaller (typically 10–15 cm in diameter) than the two studied species of *Chrysaora* described above, and occur in the Northern Pacific Ocean in the vicinity of Japan (Figure 1; Morandini and Marques 2010). Since the beginning of the century, the number of *C. pacifica* medusae in the Inland Sea of Japan has been growing, and the population now has recurring annual blooms (Makabe et al. 2015; Takasu et al. 2019).

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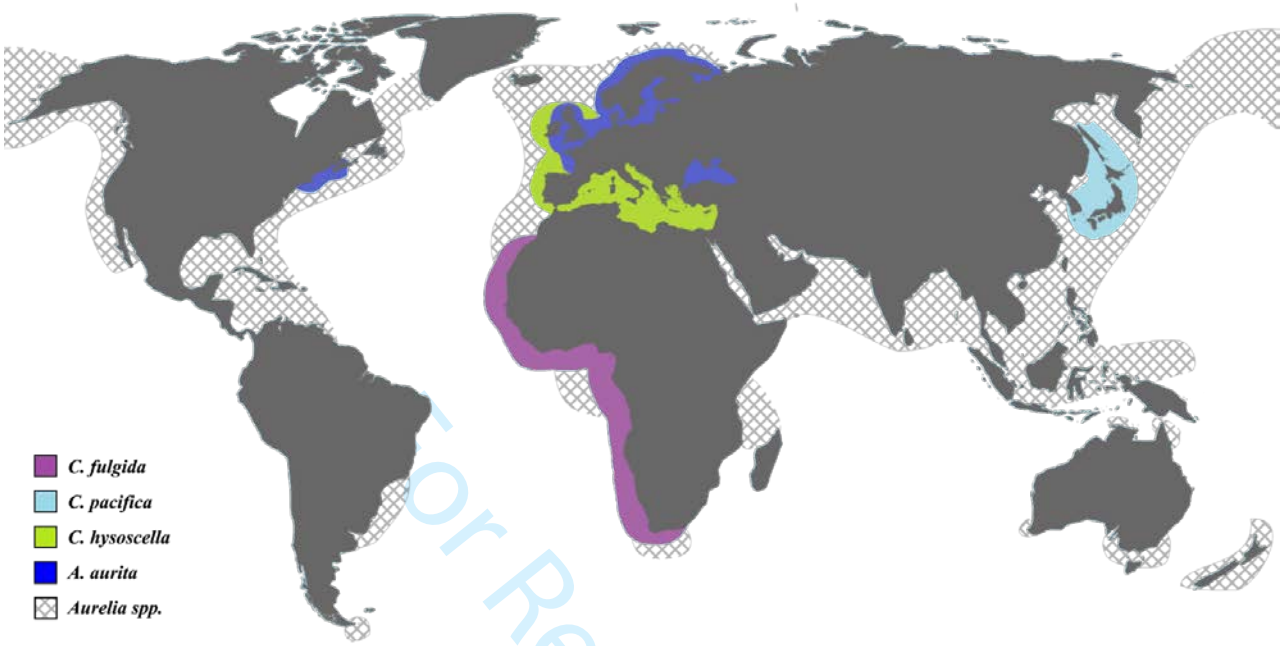


194 **Figure 1.** Geographic distribution of the four jellyfish species investigated in this study (*Aurelia*
195 *aurita, Chrysaora hysoscella, Chrysaora fulgida, Chrysaora pacifica*; based on Dawson et al.
196 2005 and Morandini and Marques 2010) and of the cryptic genus *Aurelia* (based on Dawson and
197 Martin 2001).

198 **Table 1**. Jellyfish collection and incubation details

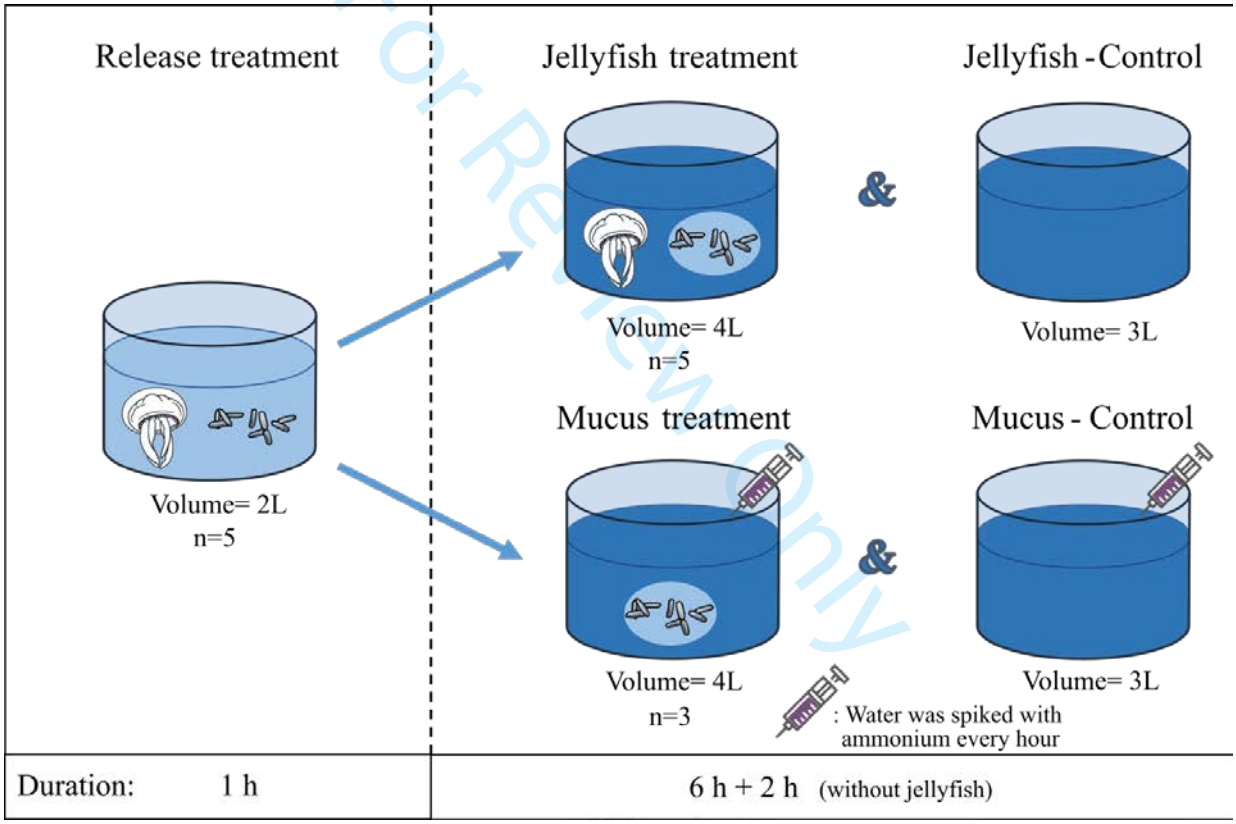
Species	*A. aurita*	*C. hysoscella*	*C. fulgida*	*C. pacifica*
Origin	Horsea Lake (UK)	Rames peninsula (UK)	Walvis Bay (Namibia)	London Aquarium
Date (DD-MM-YY)	23-10-2018	21-08-2018	21-07-2019	27-02-2019
Bell diameter (cm)	12 – 16	9 – 13	12 – 16	6 – 9
Wet weight (g)	73 – 190	100 – 278	180 – 279	35 – 59
Number of specimens	5	5	2	5
In-situ conditions				
Temperature (°C)	14	18	14	16
Salinity	25	35	35	30
Experimental condition				
Temperature (°C)	15	20	14	16
Salinity	25	35	35	30

Experimental structure

Prior to the experiment, all equipment was acid washed in 10% hydrochloric acid and rinsed three times with ultra-high purity water (MilliQ ≥ 18.2 10^6 Ω cm $^{-1}$, Millipore, UK). The incubators consisted of 5-L high-density polyethylene buckets filled with artificial seawater (ASW; ultra-high purity water + Tropic Marin synthetic sea salt; detailed preparation available in Supplemental Information (SI)). A maximum number of five healthy and undamaged adult medusae were selected for each experiment. The health of a jellyfish was evaluated based on the swimming rhythm with active swimming behaviour indicating good health. Two hours before the experiment, selected jellyfish were individually transferred to an incubator filled with 4 L of ASW. The purpose of this first 'acclimation/egestion' phase was to allow the medusae to egest any food they might have held in their gastric pouches. The experiment consisted of an initial Release phase, followed by an incubation phase with four incubation treatments: Jellyfish (ASW + jellyfish), Jellyfish-Control (ASW only), Mucus (ASW + mucus + ammonium), and Mucus-Control (ASW + ammonium; Figure 2). First, the jellyfish were gently transferred by hand to the Release incubators (2 L of ASW) using sterile vinyl gloves, whilst trying to minimise the amount of water transferred with it. The Release phase allowed mucus and its associated microbes to be released into the water. After 1 hour, the jellyfish along with half of the volume of the water in the Release incubator (1 L) were transferred to the Jellyfish incubators (3 L of ASW; final volume = 4 L). The other half of the water was transferred to the Mucus incubators (3 L of ASW; final volume = 4 L). The controls (Jellyfish-Control and Mucus-Control) consisted of incubators containing only ASW (3 L of ASW).

As ammonia is continuously excreted by jellyfish, the nitrification rates associated with jellyfish in ASW (continuously increasing ammonium concentrations) would not be directly

222 comparable to those associated with mucus in ASW (ammonium concentration of <0.1 µM). To
223 allow direct comparison of nitrification rates in the Mucus and Jellyfish treatments, we simulated
224 jellyfish ammonium excretion in both the Mucus and the Mucus-Control treatments by adding
225 ammonium (NH_4Cl, Fisher Scientific, UK) to the incubators after each sample collection. The
226 amount of ammonium added was estimated based on literature (Pitt and Purcell 2009) and
227 previous trial experiments. The expected increase in ammonium concentrations ranged from 0.5
228 to 2.5 µM h^{-1} (SI, Table II) depending on species, size of the jellyfish and temperature.



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230 **Figure 2**: **Experimental setup**. Jellyfish were incubated for one hour in the Release treatment.
231 Subsequently, the jellyfish along with half of the volume from the Release phase were
232 transferred to the Jellyfish treatment; the other half was transferred to the Mucus treatment.
233 Controls for both experiments consisted of incubators containing only ASW. The Mucus and
234 Control-Mucus incubators were spiked with ammonium every hour (SI, Table II).

Rate measurements

Water samples for nutrient analysis were collected every hour. Before collecting each sample, the water was stirred gently to homogenise it. Two sets of 15-mL samples (one for nitrate, nitrite and phosphate, and one for ammonium) were collected using a 20 mL polypropylene syringe. The sample was filtered through a 0.22-µm polyethersulfone sterile syringe filter (33-mm diameter, Millipore, UK) with the first 5 mL discarded to wash the filter. The remaining 10 mL were collected in centrifuge tubes (polypropylene conical centrifuge tubes, 15 mL volume, Fisher Scientific, UK). For each treatment, a dedicated syringe was used to avoid cross-contamination. In between sample collection, the incubators were covered with a lid to avoid contamination. Based on initial experiments and findings of a previous study measuring ammonia release in *C. mosaicus* (Pitt et al. 2005), we decided an incubation volume and duration of 4 L and 6 hours as ideal to measure a significant rate of nutrients release without causing excessive stress to the jellyfish. The jellyfish were then removed from the incubators, and the jellyfish bell diameter and the wet weight (WW) was measured using a ruler and a balance (FireKingdom SF-400, ±1 g). Water sample collection continued for 2 hours after removal of the jellyfish, resulting in a total experiment duration of 8 hours.

Sample analysis

The duplicate sample for ammonium was analysed using the o-phthalaldehyde fluorometric method (Holmes et al. 1999; Taylor et al. 2007). The ammonium measurements were performed the same day using a Turner design Trilogy fluorometer (model 7200, US) with a UV module (7200-047). The duplicate sample for nitrite, nitrate and phosphate was immediately frozen for later analysis. Frozen samples were thawed at room temperature and phosphate, nitrate and nitrite concentrations were measured using standard gas segmented

continuous flow spectrophotometric techniques (QuAAtro, Seal Analytical). The baseline of the auto-analyser was determined using the same ASW as used in the experiment (except for *C. fulgida* samples, for which we used ultra-high purity water as baseline; detailed descriptions of the calibrations and detection limits in SI).

Our hourly sampling regime, which provides a relatively low temporal resolution, was determined by the time it takes to collect the sample and the sample volume removed relative to the incubation volume. To determine the release rates at a higher temporal resolution, for one of the specimen of *A. aurita* incubations, nitrite and nitrate were measured at high-resolution (every 20 minutes) using a microfluidic lab-on-chip analyser (Beaton et al. 2012). This novel application of lab-on-chip microfluidic analysers allowed high-resolution measurements with small sample volumes and avoiding the need for sample storage. The nitrate and nitrite concentrations measured using the "manual" and lab-on-chip method agreed well (SI, Figure V), as shown by a linear regression between the two methods (Auto-Analyzer = 1.04 +/- 0.06 Lab-on-Chip + 0.15 +/- 0.04; $R^2 = 0.98$, $p < 0.001$, $n = 8$; SI, Figure VI). For both techniques, gas segmented continuous flow spectrophotometric and lab-on-chip, the combined (random + systematic) analytical uncertainty associated with nitrate + nitrite and phosphate measurements was <5% (details in SI; Birchill et al. 2019).

Statistical analysis

Contamination, wall effects and production/absorption by microorganisms were accounted for by subtracting the changes in concentrations observed in the ASW controls from the treatments. In order to account for the loss of liquid due to the collection of nutrient samples, the total number of moles of nutrient released at each time point was calculated using the equation:

$$n_{[t]} = n_{[t-1]} + V_{[t-1]} \times (c_{[t]} - c_{[t-1]})$$

where n is the number of moles released at a certain time point (t) since the beginning of the experiment, V is the volume of the incubator, and c the molar concentration of nutrients (Giering et al. 2012).

The rates of nutrient (ammonium, phosphate, nitrite and nitrate) release per incubator (or per jellyfish for the Jellyfish treatment) were calculated using linear regression for each replicate. The rates were then normalised by the wet weight of the jellyfish and their differences were investigated by an analysis of covariance (ANCOVA; results are presented in SI). The rates of nutrient release per species were calculated by averaging the rates of the replicates for each species. Finally, the differences in weight-specific rates of nutrient release caused by the differences in experimental temperatures were standardized using Q_{10} temperature coefficient factors from the literature. For ammonium and phosphate release, a Q_{10} of 3.1 was used for *A. aurita* (Møller and Riisgård 2007), and the general Q_{10} of 2.66 was used for the other jellyfish species (Ikeda 2014). For nitrite and nitrate release rates, a Q_{10} of 2.2 was used for all species (Zheng et al. 2017), corresponding to the temperature coefficient factor of nitrifying microorganisms. Rates were adjusted to the median temperature of the experimental conditions (16°C) and N:P ratios were calculated as the sum of ammonium, nitrite and nitrite over phosphate. The temperature-corrected nutrient production rates were plotted against the wet weight of the jellyfish, and a linear regression was fitted to investigate the allometric relationships between body weight and nutrient release rates. Finally, estimates of inorganic nitrogen release by jellyfish blooms were calculated using the allometric equations together with jellyfish densities from two case studies. The uncertainty range of these estimates were

303 determined from the error on the allometric exponents and the temperature. All statistical
304 analyses were carried out using R Statistical Software (R Core Team 2019).

305 Results and Discussion

306 Nutrient excretion and nitrification

307 To determine rates of nitrification catalysed by members of the jellyfish microbiome, we
308 performed incubation experiments with four species of non-zooxanthellate scyphozoan jellyfish,
309 *A. aurita*, *C. hysoscella*, *C. fulgida* and *C. pacifica*. We measured rates of ammonium and
310 phosphate excretion along with partial ($NH_4^+ \rightarrow NO_2^-$) and complete ($NH_4^+ \rightarrow NO_3^-$) nitrification
311 associated with these jellyfish species. Ammonium and phosphate concentrations increased
312 continuously in all incubations with jellyfish, whereas nitrite and nitrate concentrations increased
313 only in the presence of three of the four species (see discussion below). For all nutrients,
314 concentrations stabilized or decreased once the jellyfish were removed (Fig. 3, Table 2; see SI
315 for absolute concentrations, Figure I). In the presence of mucus alone, rates of nitrification were
316 negligible for all investigated jellyfish species ($<2.0 \times 10^{-3}$ nmol L^{-1} h^{-1}; SI, Table III), strongly
317 suggesting that the observed rates of nutrient release were directly related to jellyfish metabolism
318 and the associated microbiome. Mass-specific release rates of ammonium ranged from 23 to 86
319 nmol NH_4^+ gWW^{-1} h^{-1} at experimental temperatures (28 - 86 nmol NH_4^+ gWW^{-1} h^{-1} when
320 normalised to 16°C), which falls within the range of previous observations (2 - 111 nmol NH_4^+
321 gWW^{-1} h^{-1}: Pitt et al. 2013). The observed intraspecies variability of ammonium excretion was
322 relatively low, with *C. hysoscella* showing the highest variation (14%) in release rates across
323 specimens. In contrast, excretion rates between different jellyfish species varied widely (up to
324 3.7-fold). Mass-specific release rates of phosphate ranged from 3.2 to 12 nmol PO_4^- gWW^{-1} h^{-1} at

experimental temperatures (3.7 - 12 nmol PO_4^- gWW^{-1} h^{-1} when normalised to 16°C). Excretion rates of phosphate were linearly correlated with ammonium excretion rates (all species included, not taking into account ammonium conversion; $p < 0.001$, $R^2 = 0.60$; $n = 17$; SI, Figure VII). Ammonium:phosphate excretion ratios ranged from 2.7 to 15.2 with an average of 7.4, in accordance with previous reports (8.2 for *A. aurita*, Shimauchi and Uye 2007; 8.7 for *C. mosaicus*, Pitt et al. 2005; 7.5 for *P. noctiluca*, Malej 1991).

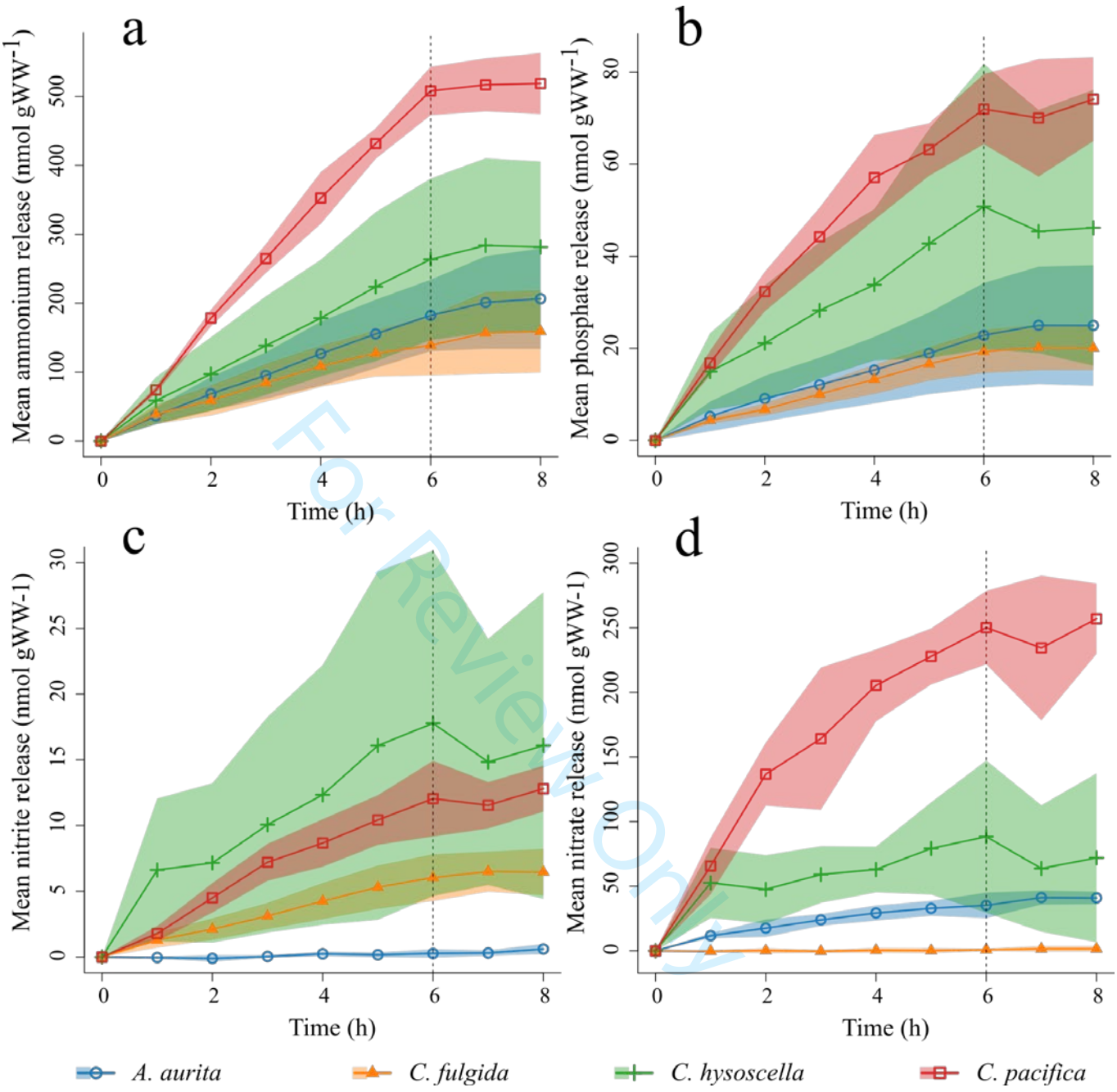


Figure 3. Mean cumulative release of (a) ammonium, (b) phosphate, (c) nitrite and (d) nitrate by *A. aurita* (blue circle), *C. fulgida* (yellow triangle), *C. hysoscella* (green cross) and *C. pacifica* (red square), normalised to the wet weight (WW) of each specimen. Coloured areas indicate uncertainty envelopes (standard deviation) of the mean cumulative release of nutrients. Vertical dotted line corresponds to the time when the jellyfish were removed from the incubators (6 h).

337 **Table 2**. Release rates and regression statistics for the cumulative nutrient release by the four jellyfish species. SD: standard deviation
338 of the slope, *n*: number of observations. Rates at experimental temperatures and adjusted to 16°C are presented, as well as the N:P
339 ratios at 16°C. The rate, SD, $\overline{R^2}$ and $\overline{p}$ are mean values from the replicates individual linear regressions. *, **, and *** indicate
340 levels of statistical significance ($p \leq 0.05$, 0.01, and 0.001, respectively).
341

Species	Nutrient	Rate (nmol gWW^{-1} h^{-1})	SD	*n*	$\overline{R^2}$	$\overline{p}$	*n*	Rate at 16°C (nmol gWW^{-1} h^{-1})	SD	N:P
A. aurita	Ammonium	30	8.1	5	0.99	***	7	34	9.1	10.3
	Phosphate	3.6	1.5	5	0.98	***	7	3.9	1.7	
	Nitrite	0.1	0.0	5	0.31	0.22	7			
	Nitrate	5.7	1.3	5	0.89	**	7	6.2	1.4	
C. fulgida	Ammonium	23	4.5	2	0.97	***	7	28	5.5	7.89
	Phosphate	3.2	0.5	2	0.99	***	7	3.7	0.6	
	Nitrite	1.0	0.2	2	0.99	***	7	1.2	0.2	
	Nitrate	0.1	0.1	2	0.16	0.52	7			
C. hysoscella	Ammonium	43	17	5	0.99	***	7	29	11.5	6.95
	Phosphate	7.9	4.1	5	0.94	***	7	5.7	2.8	
	Nitrite	2.8	1.9	5	0.87	**	7	1.9	1.4	
	Nitrate	12	6.0	5	0.61	*	7	8.7	4.4	
C. pacifica	Ammonium	86	5.0	5	0.99	***	7	86	5.0	10.8
	Phosphate	12	1.2	5	0.96	***	7	12	1.2	
	Nitrite	2.1	0.4	5	0.98	***	7	2.1	0.4	
	Nitrate	41	3.1	5	0.91	***	7	41	3.1	

Ammonia oxidation is usually considered the rate-limiting step in nitrification (Prosser 1990; Heiss and Fulweiler 2016; Zhang et al. 2020): nitrite is immediately oxidized by free-living nitrite-oxidizing bacteria, preventing its accumulation at significant rates. We observed these expected dynamics in the presence of *A. aurita*, when nitrite concentrations did not increase whereas nitrate accumulated (5.7±1.3 nmol NO_3^- gWW^{-1} h^{-1}; Table 2), indicating a tight coupling of both nitrification steps. However, this paradigm did not apply to nitrification in association with the other three jellyfish species that we investigated. In the presence of *C. hysoscella* and *C. pacifica*, accumulation rates were significant for both nitrite (*C. hysoscella*: 2.8±1.9 nmol NO_2^- gWW^{-1} h^{-1}; *C. pacifica* 2.1±0.4 nmol NO_2^- gWW^{-1} h^{-1}) and nitrate (*C. hysoscella*: 12±6.0 NO_3^- nmol gWW^{-1} h^{-1}; *C. pacifica* 41±3.1 nmol NO_3^- gWW^{-1} h^{-1}; Table 2). The decoupling was more pronounced in incubations with *C. hysoscella* (nitrite accumulation rate was 23% of the nitrate accumulation rate), whereas nitrite accumulation in association with *C. pacifica* was lower (5% of nitrate accumulation). During the incubations with C. *fulgida*, ammonia oxidation to nitrite was the only detectable nitrification process (1.0±0.2 nmol NO_2^- gWW^{-1} h^{-1}; Table 2).

To our knowledge, two other studies investigated the nitrite + nitrate (NO_x, no distinction made) release by non-zooxanthellate scyphomedusae: Pitt et al. (2005) found that *C. mosaicus* released <2% of the released nitrogen in form of NO_x, and Shimauchi and Uye (2007) did not observe significant release of NO_x associated with *A. aurita*. The latter study contrasts with our observation that 16% of the released nitrogen by *A. aurita* was in the form of nitrate. This discrepancy indicates a potential effect of past and present environmental conditions on the jellyfish-associated microbial community composition and, subsequently, on the balance of jellyfish-associated nitrification rates. In contrast to the hypothesis that nitrifiers are specific to zooxanthellate jellyfish (Welsh et al. 2009), our results suggest that both, zooxanthellate and non-zooxanthellate, jellyfish are potential hosts for nitrifiers and can thus be a source of nitrite and nitrate to the environment.

Together nitrite and nitrate release rates were 5-50% lower than ammonium excretion rates (Figure 4) and contributed 5-33% of the total inorganic nitrogen release. Under saturating substrate levels (ammonia and nitrite), nitrification reactions follow a zero-order kinetic (Chen et al. 2006), meaning that increases in substrate concentration do not increase the reaction rates. As ammonium excretion exceeded that of nitrite and nitrate substantially, we conclude that nitrification rates were not limited by ammonia availability in any of the experiments. Moreover, since the total ammonia concentrations of the incubators were well below toxicity levels for polyps and ephyrae (2 mg L^{-1}, Jian-Long et al. 2018), we are confident that the observed nitrification rates are reflective of in-situ processes.

Between species, the rates of nitrification varied more than the excretion rates of ammonium and phosphate. For example, we observed a >6-fold difference between the nitrate release rates of *A. aurita* and *C. pacifica* (Table 2, Figure 4). Both the inter- and intraspecies variability observed in ammonia and phosphate excretion as well as nitrification rates can partly be explained by allometric scaling of the mass-specific release rates to the wet weight of each individual (ammonium excretion: 80%, phosphate excretion: 73%, nitrification: 55%, Figure 5; SI, Table VI). The allometric relationships for the ammonium, phosphate and nitrate-specific release (ASR, NSR and PSR, respectively; nmol gWW^{-1} h^{-1}) were:

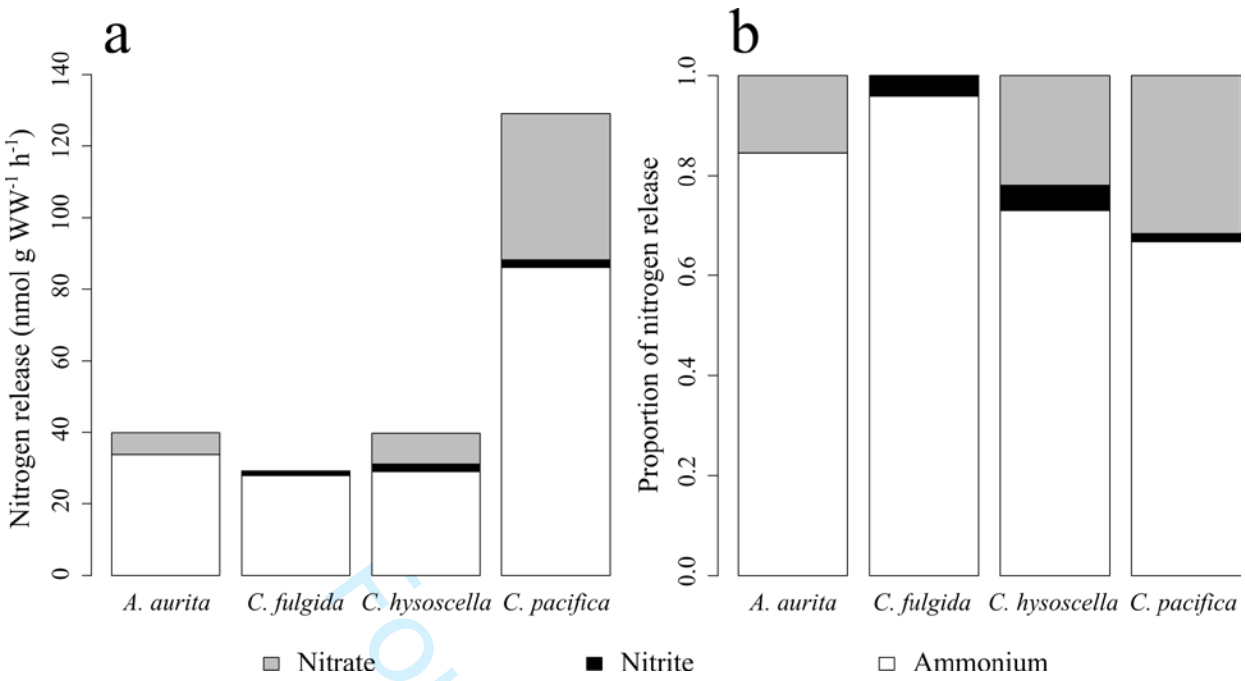
$$\mathbf{ASR = 1.84 \times 10^{3} \pm 1.6\ WW^{-0.82 \pm 0.10}} \quad (p<0.001,\ R^2=0.80,\ n=17) \quad (1)$$

$$\mathbf{PSR = 369 \pm 1.9\ WW^{-0.90 \pm 0.13}} \quad (p<0.001,\ R^2=0.73,\ n=17) \quad (2)$$

$$\mathbf{NSR = 2.84 \times 10^{3} \pm 3.6\ WW^{-1.20 \pm 0.28}} \quad (p<0.001,\ R^2=0.55,\ n=15) \quad (3)$$

The negative scaling exponents indicate that smaller specimens release more nutrients per gram of mass, and hence follow the expected allometric scaling. The high rates of nutrient excretion and nitrification associated with *C. pacifica* can therefore be partly explained by the small size of these specimens (35 – 59 gWW) compared to the other investigated species.

Similarly, the high variability in *C. hysoscella* rates matches the wider range of wet weights per individual (100 – 278 gWW, Table 1). All scaling exponents (Equation 1, 2 and 3; SI: Slope, Table VI) were lower than the -¼ allometric exponent commonly observed for other zooplankton mass-specific physiological processes (Arhonditsis et al. 2019). We suggest that this divergence relates to the jellyfish's high water contents and unique body plans (Pitt et al. 2013). The scaling exponent of the nitrate release allometric equation (-1.20±0.28, Equation 3) being lower than the exponent for the ammonium release (-0.82±0.10, Equation 1) indicates that, when wet weight increases, the nitrate-specific rate decreases faster than the ammonium-specific rate. This difference in scaling exponent is likely to be related to the changes in the jellyfish surface-to-volume ratio: the release of ammonium is likely more depending on the jellyfish's body volume as it is a metabolic waste product, whereas nitrate is likely more dependent on the jellyfish surface owing to the association with the microbiome living on the jellyfish. Our data show that release rates by jellyfish are highly variable between populations, yet, when normalized to wet weight, we observe strong allometric scaling. This observation highlights the potential for these pathways to be incorporated into models.



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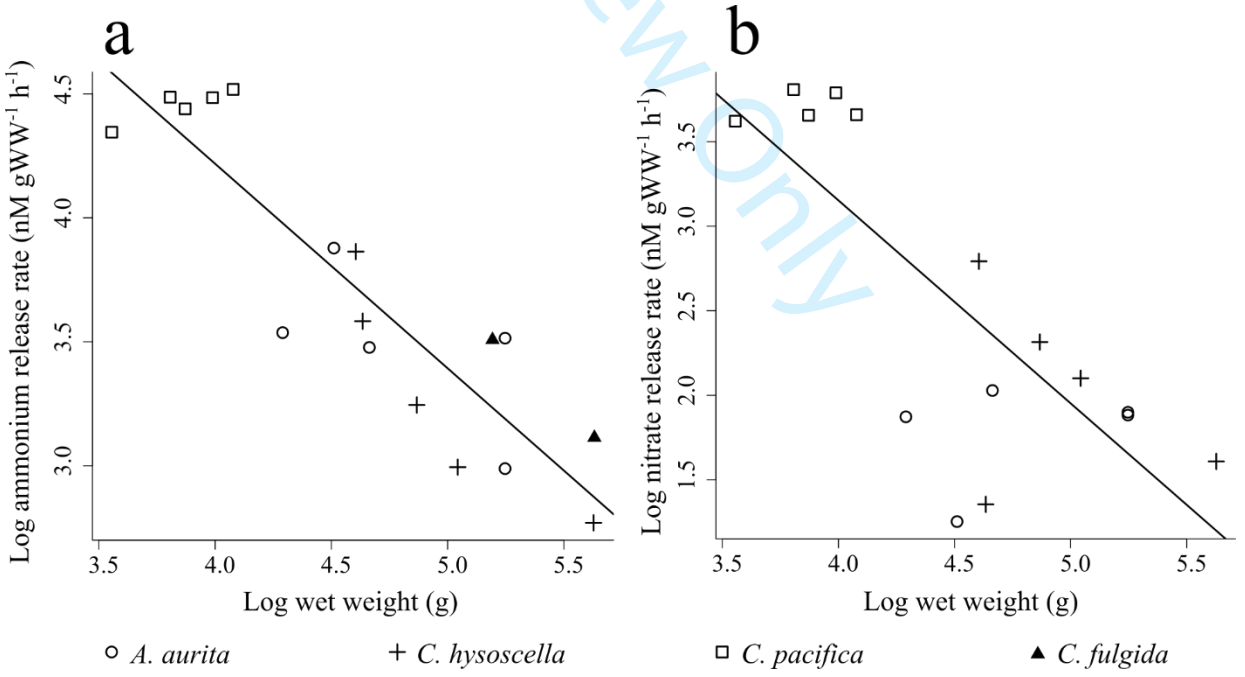
407 **Figure 4**. Inorganic nitrogen release rates of different jellyfish species (a) normalized by the wet

408 weight of the specimens, and (b) as proportion of total inorganic nitrogen release. WW= wet

409 weight.



410

411 **Figure 5**. Effect of wet weight on the mass-specific release rates of ammonium (a; $p<0.001$,

412 $R^2=0.80$, $n=17$) and nitrate (b; $p<0.001$, $R^2=0.55$, $n=15$) for the jellyfish *A. aurita (circle), C.*

413 *hysoscella (cross), C. pacifica (square)* and *C. fulgida* (triangle) at 16°C. The black line is the
414 linear regression. No significant release of nitrate was observed for *C. fulgida*.

415 **Evidence of active nitrifying microorganisms in jellyfish**

416 Jellyfish host diverse microbial communities on their epithelium as their mucus provides
417 an attractive niche for microorganisms (Tinta et al. 2012, 2019; Weiland-Bräuer et al. 2015;
418 Kramar et al. 2019). Two species of nitrifiers, the ammonia-oxidizing bacterium *Nitrosospira*
419 *multiformis* and the nitrite-oxidizing bacterium *Nitrospira moscoviensis*, have been identified as
420 members of the microbiome of jellyfish *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-
421 Bräuer et al. 2015). However, neither of these nitrifiers were highly abundant (<2% of total
422 operational taxonomic unit; Lee et al. 2018). The high nitrification rates we observed strongly
423 supports the presence of either highly active or highly abundant nitrifying microorganisms in the
424 jellyfish microbiome. The low coupling between nitrification rates could be caused by poor
425 diffusional connectivity between nitrifiers (Welsh et al. 2001), i.e., a fraction of the produced
426 nitrite might diffuse directly to the water column rather than to a zone where it can be oxidised to
427 nitrate. The differential production of nitrite and nitrate associated with the four jellyfish
428 populations investigated strongly indicates variable community composition or distribution of the
429 microbiome on the jellyfish depending on jellyfish species or environmental factors. While our
430 findings are representative only of a subset of jellyfish populations, the diverse identity and origin
431 of the investigated specimens strongly supports our hypothesis of a widespread association with
432 nitrifying bacteria and archaea. The detailed nature of this association requires further
433 investigations including molecular approaches to determine the identity and distribution of
434 nitrifiers within the jellyfish microbiome.

435 **Ecological implications**

436 Jellyfish stimulate primary production through the excretion of ammonium and phosphate
437 (Pitt et al. 2005). The average inorganic N:P ratio of the released nutrients (7.3 – 10.9, Table 2;
438 SI, Figure VIII) lies below the Redfield Ratio (N:P = 16; Redfield 1963) and substantially below
439 the N:P ratios of their main diet, zooplankton (N:P > 20; Elser and Hassett 1994). Thus, the
440 gelatinous biomass of these jellyfish appears to retain nitrogen efficiently, which is further
441 supported by their low molar C:N ratio (4.4; Lucas et al. 2011) compare to other marine
442 zooplankton organisms (4.8-6.2 for crustacean zooplankton; Pitt et al. 2013). By storing nitrogen
443 over phosphorus, expanding jellyfish blooms may locally drive the ecosystem toward N-
444 limitation (Sterner 1990). Whereas under starvation, while jellyfish consume up to 85% of their
445 own nitrogen-rich tissues (Pitt et al. 2014; Lilley et al. 2014), the N:P ratio of the excreted
446 nutrients would increase. Starvation, a major cause of jellyfish bloom decline (Pitt et al. 2014),
447 could temporarily drive the ecosystem towards P-limitation. A large jellyfish bloom could thus
448 act as a “nitrogen buffer”, storing nitrogen over phosphorus when food is abundant and releasing
449 nitrogen over phosphorus during its decay.

450 Our findings demonstrate that a substantial fraction of the excreted ammonium is shunted
451 through partial or complete nitrification (ammonium: 80±12%, nitrite: 3±2%, nitrate: 17±13%;
452 Figure 6), thereby fuelling dark carbon fixation in the sunlit surface ocean. An association with
453 jellyfish allows nitrifiers direct access to ammonium in the surface ocean, thereby bypassing
454 competition with phytoplankton for this otherwise scarce resource (Smith et al. 2014; Zakem et
455 al. 2018). During jellyfish blooms, the release of different forms of bioavailable inorganic (nitrite,
456 nitrate and ammonium) has the potential to locally enhance surface primary production and even
457 influence phytoplankton community composition (Figure 6; Shilova et al. 2017). This effect on
458 the community composition, in turn could impact the quantity and quality of organic matter that
459 sinks to depth (Basu and Mackey 2018).

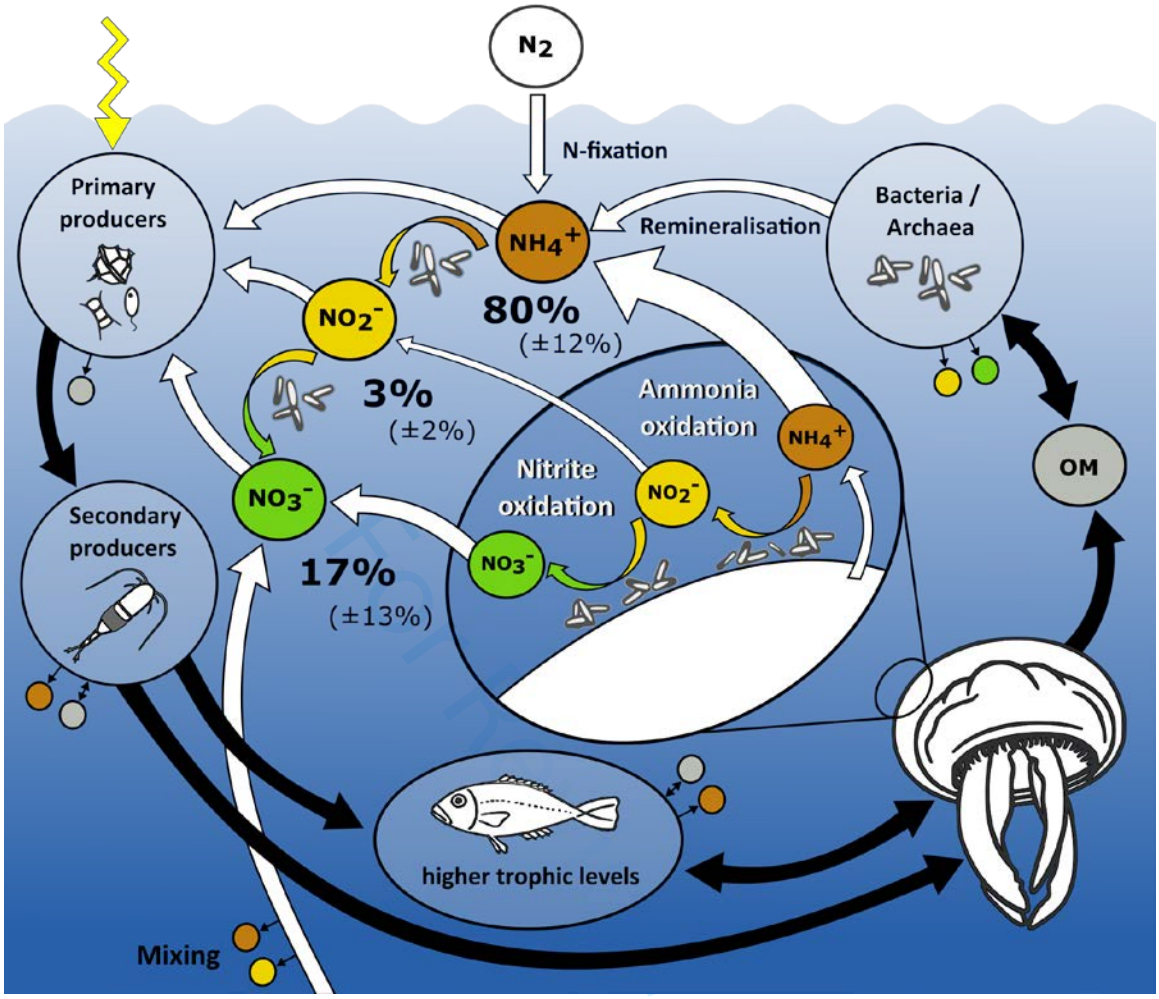


Figure 6. Conceptual diagram of the role and position of jellyfish in the surface marine nitrogen cycle. The flow of organic and inorganic matter is shown by black and white arrows, respectively. Colours indicate ammonium (NH_4^+, orange), nitrite (NO_2^-, yellow), nitrate (NO_3^-, green) and organic matter (OM, grey). Coloured arrows represent ammonium-oxidation (orange-to-yellow) and nitrite-oxidation (yellow-to-green). Components linked to small coloured circles release/assimilate nutrients of the same colour. The average release of nitrogen forms are presented as percentage (± standard deviation) of total dissolved inorganic nitrogen released by jellyfish. The yellow zigzag arrow represent light. The large middle circle zooms in on the jellyfish epithelium.

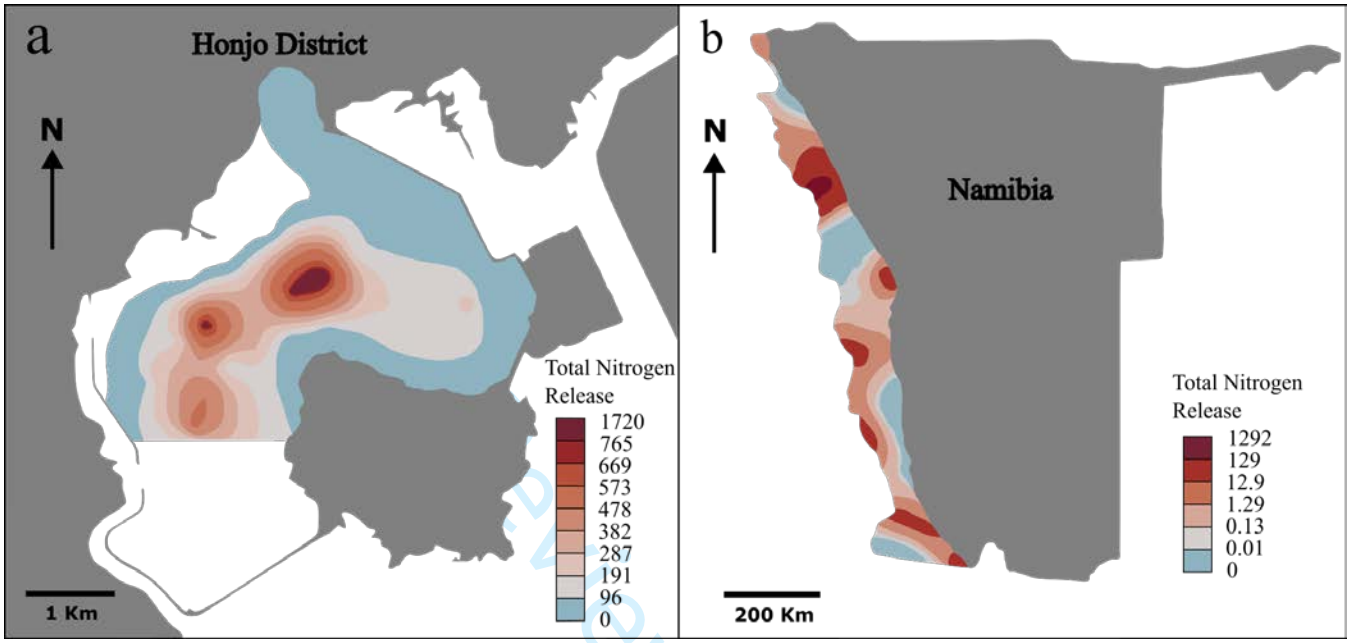
To explore the potential relevance of jellyfish blooms on surface nitrogen cycling, we extrapolated our nitrification rate measurements based on two jellyfish blooms, whose spatial extend was measured in high resolution (Lynam et al. 2006; Han and Uye 2009). The blooms were observed in (1) the shallow eutrophic and brackish Honjo lagoon, northwest of Lake Nakaumi, Japan (Han and Uye 2009) and in (2) the coastal area of Namibia representing the Northern Benguela Upwelling System (Lynam et al. 2006). We applied our allometric equations for ammonium and nitrate release (Equation 1 & 3) to the average body characteristics of the jellyfish (Table 3), corrected for temperature (Table 3 and as described in methods), and multiplied by abundance.

Table 3. Overview of case studies. Surface temperature at sampling time and body characteristics of jellyfish used to estimate inorganic nitrogen release. [a] calculated from Han et al. (2009), [b] mean annual surface temperature in August from Junker et al. (2017), [c] calculated from Houghton et al. (2007).

Location	Species	Surface temperature (C°)	mean WW (g)	mean bell diameter (cm)	References
Honjo District	*A. coerula*	28 - 28.7	92.5[a]	13.1	Han & Uye 2009
Northern Benguela	*C. fulgida*	13[b]	1100[c]	27	Lynam et al. 2006

In the Honjo District Lake, *Aurelia coerula* (a cryptic species to *A. aurita* and until recently named *A. aurita*) is highly abundant (up to 18 medusae m^{-3}) from June to November and are thought to ingest up to 47% of the daily mesozooplankton production (Han et al. 2009; Han and Uye 2009). During these months, average ammonium and nitrate levels are consistently low (≤0.01 mg L^{-1} for both ammonium and nitrate; Chugoku Regional Development Bureau 2018). We estimated that the large aggregation of *A. coerula* could have released up to 1.7 mmoles N m^{-2} h^{-1} (uncertainty: 1.0 - 3.2 mmoles N m^{-2} h^{-1}), of which 85% was in the form of ammonium and 15% in the form of nitrate (Figure 7a). On a daily basis, assuming Redfield ratio (C:N = 106:16;

492 Redfield 1963), this nitrogen release would be able to support a primary production rate of 3.2 g
493 C m^{-2} d^{-1} (uncertainty: 1.9 -6.1 g C m^{-2} d^{-1}), equivalent to 463% (uncertainty: 275 – 884%) of the
494 mean daily primary production of a typical estuarine-coastal ecosystems (global average: 252 g C
495 m^{-2} y^{-1}; Cloern et al. 2014).



496

497 **Figure 7.** Heat map of estimated total inorganic nitrogen release associated with the densities of
498 *A. coerula* in the Honjo District sea lake in Japan (a, linear scale: 0 - 1720 µmoles N m^{-2} h^{-1}; map
499 modified from Han and Uye 2009) and of *C. fulgida* along the coast of Namibia (b, exponential
500 scale: 0 - 1292 µmoles N m^{-2} h^{-1}; map modified from Lynam et al. 2006).

501 The Benguela Upwelling System is one of the four major coastal upwelling regions
502 presenting the highest primary production of the world oceans (Carr 2001). Large jellyfish
503 populations occur sporadically throughout the year with highest abundances observed in June–
504 August (Flynn et al. 2012). The biomass of these blooms can at times exceed the biomass of fish
505 by a factor of three (Lynam et al. 2006). We estimated that the *C. fulgida* blooms in August 2006
506 (Lynam et al. 2006) could have released up to 1.3 mmoles N m^{-2} h^{-1} (uncertainty range: 0.7 - 2.7
507 mmoles N m^{-2} h^{-1}; Figure 7b), of which 95% was in the form of ammonium and 5% in the form

508 of nitrite. Assuming the Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release
509 corresponded to a daily primary production of 2.5 g C m^{-2} d^{-1} (uncertainty: 1.3 – 5.2 g C m^{-2} d^{-1}),
510 which is equivalent to 208% (uncertainty range: 108 – 433) of the average daily primary
511 production of the Northern Benguela ecosystem (1.2 g C m^{-2} d^{-1}; Brown et al. 1991).

512 The densities observed in the Honjo District lake, although high, are not unusual for
513 coastal habitats (e.g.: 36 ± 34 *A. aurita* m^{-3} in Limfjorden; Riisgård et al. 2010). Likewise, the
514 jellyfish densities of the Northern Benguela Upwelling System are to our knowledge the highest
515 currently on record, yet such high densities are predicted to become more common in some
516 coastal areas of our changing ocean (Cheung et al. 2019). For areas experiencing increases in
517 jellyfish blooms, the two case studies provide a guide to understand how jellyfish and their
518 associated microbiomes can impact the nitrogen cycle and supply nutrients for primary
519 production.

520 ## Conclusion

521 Overall, our results suggest a widespread association between jellyfish and nitrifying
522 microorganisms, which can oxidize up to one third of the ammonium excreted by jellyfish. While
523 the identity of the nitrifiers and their distribution on the jellyfish remain unknown, it appears that
524 their activity and abundance are constant in a given jellyfish population but likely vary between
525 different environments. The allometric relationships obtained from our observations allow us to
526 estimate the amount of nutrients released by a jellyfish population via extrapolation of the
527 individual mass-specific release rates based on the abundance and size distribution of a
528 population. This study highlights the importance and complex role of jellyfish blooms in coastal
529 nitrogen cycling, where they can locally support high rates of surface ocean nitrification. Equally,
530 the substantial release of ammonium likely supports phytoplankton growth and may locally
531 impact phytoplankton community composition. Considering the widespread geographic

distribution of bloom forming jellyfish species investigated in this study (Figure 1) and the predicted future increase of jellyfish blooms, our findings point toward an increasing relevance of jellyfish on coastal nitrogen and carbon cycling.

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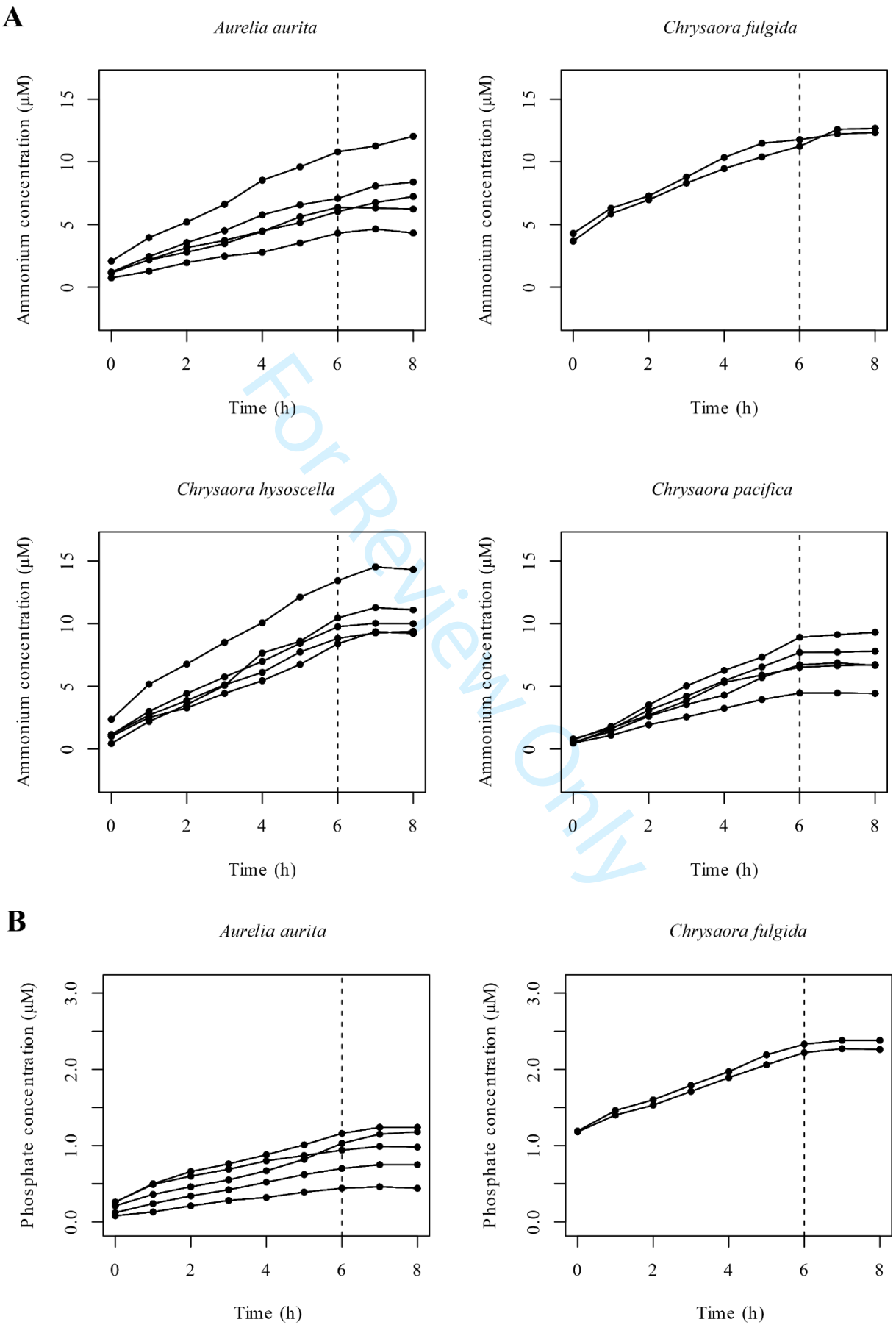
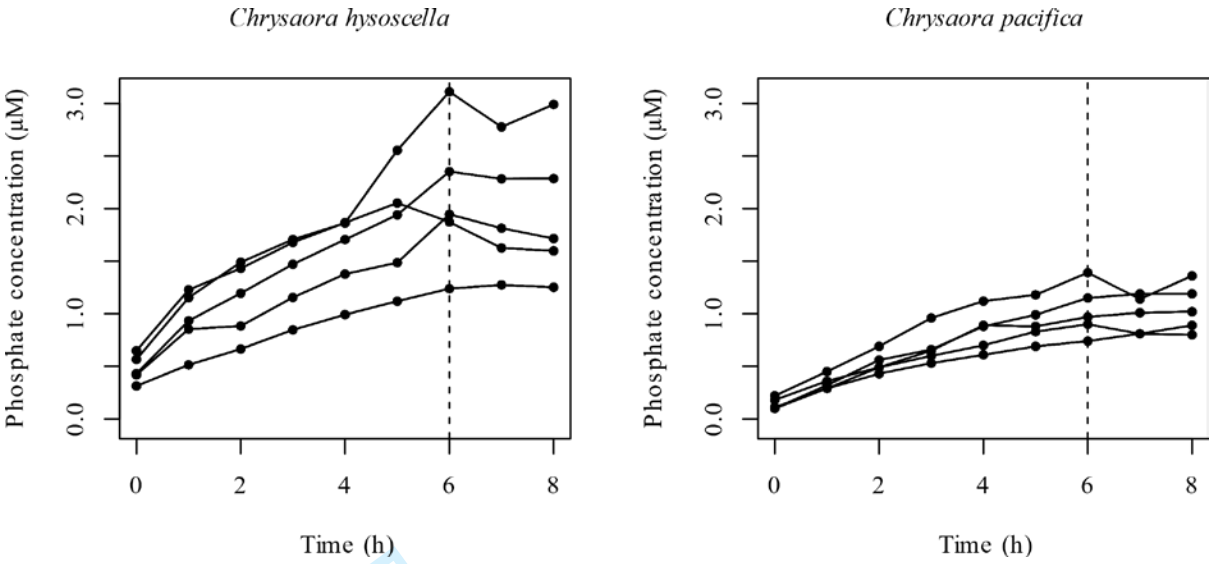
No conflicts of interest

Supplementary Information

Contents

Absolute concentrations of Jellyfish treatment





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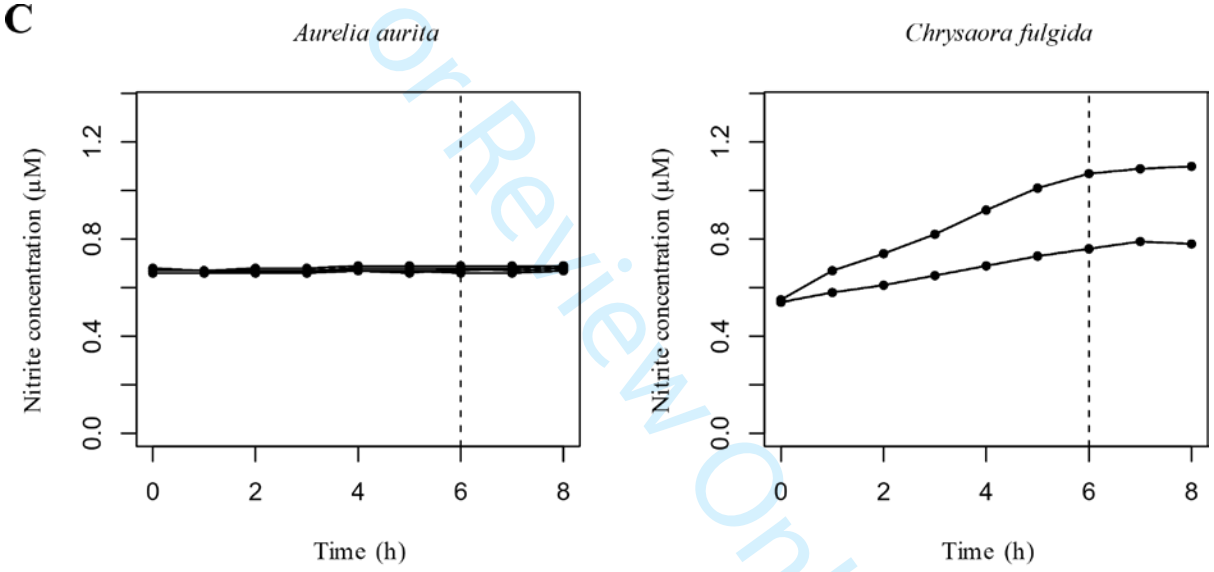

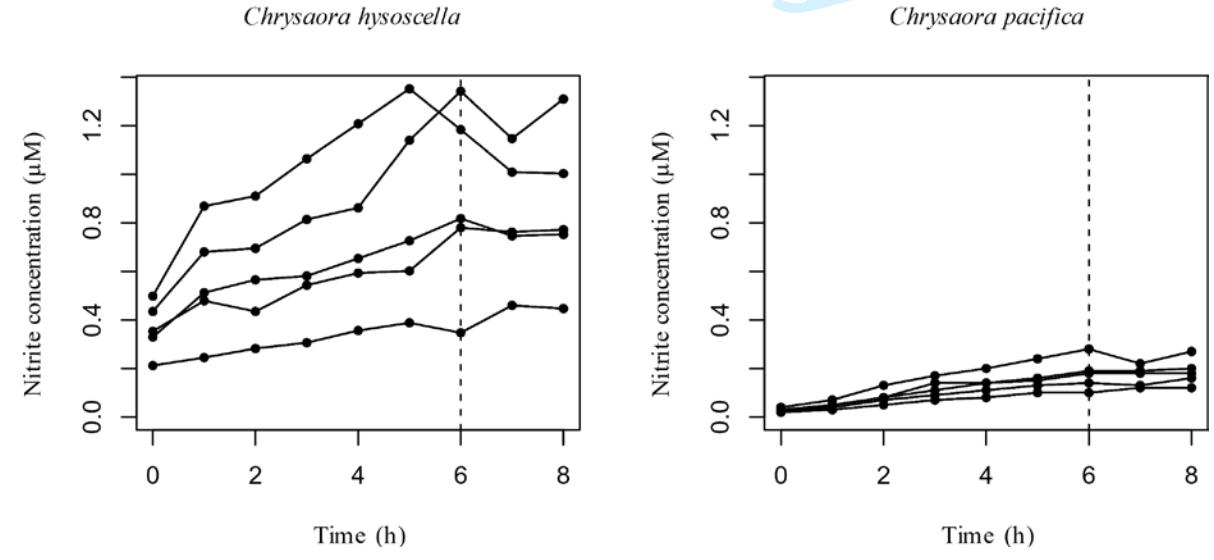

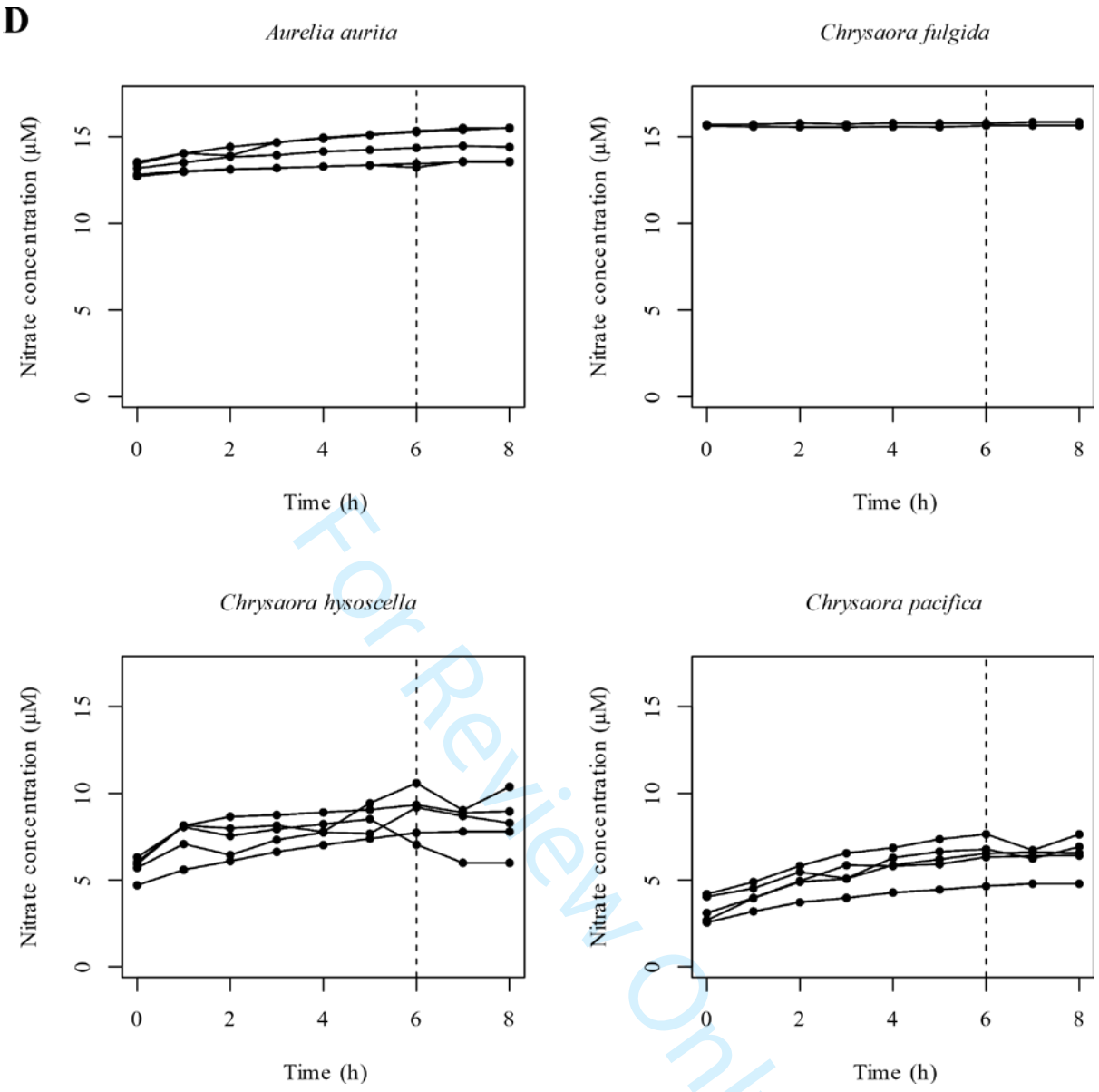

Figure I. Measured concentrations of ammonium (A), phosphate (B), nitrite (C) and nitrate (D) of the Jellyfish treatment incubators for the jellyfish species *A. aurita*, *C. fulgida*, *C. hysoscella* and *C. pacifica*. Vertical dashed line corresponds with time when the jellyfish were removed from the incubators (6 h).

Controls

Jellyfish-Control:

In 8 cases out of 16, the nutrient concentrations of the Jellyfish treatment, which consists of artificial seawater only, showed a small but significant change in concentration with time

(Figure II and table I). An increase in concentration suggests that contamination and/or production by microorganism occurred in the incubators. On the other hand, a decrease in concentration suggests absorption and/or consumption in the incubator. Any processes responsible for the changes in concentration in the Jellyfish-Control treatment were expected to also happen in the Jellyfish treatment incubators. Therefore, the concentrations of the Jellyfish treatment were corrected by subtracting them with the Jellyfish-Control changes in concentration. The corrections did not change the observed results.

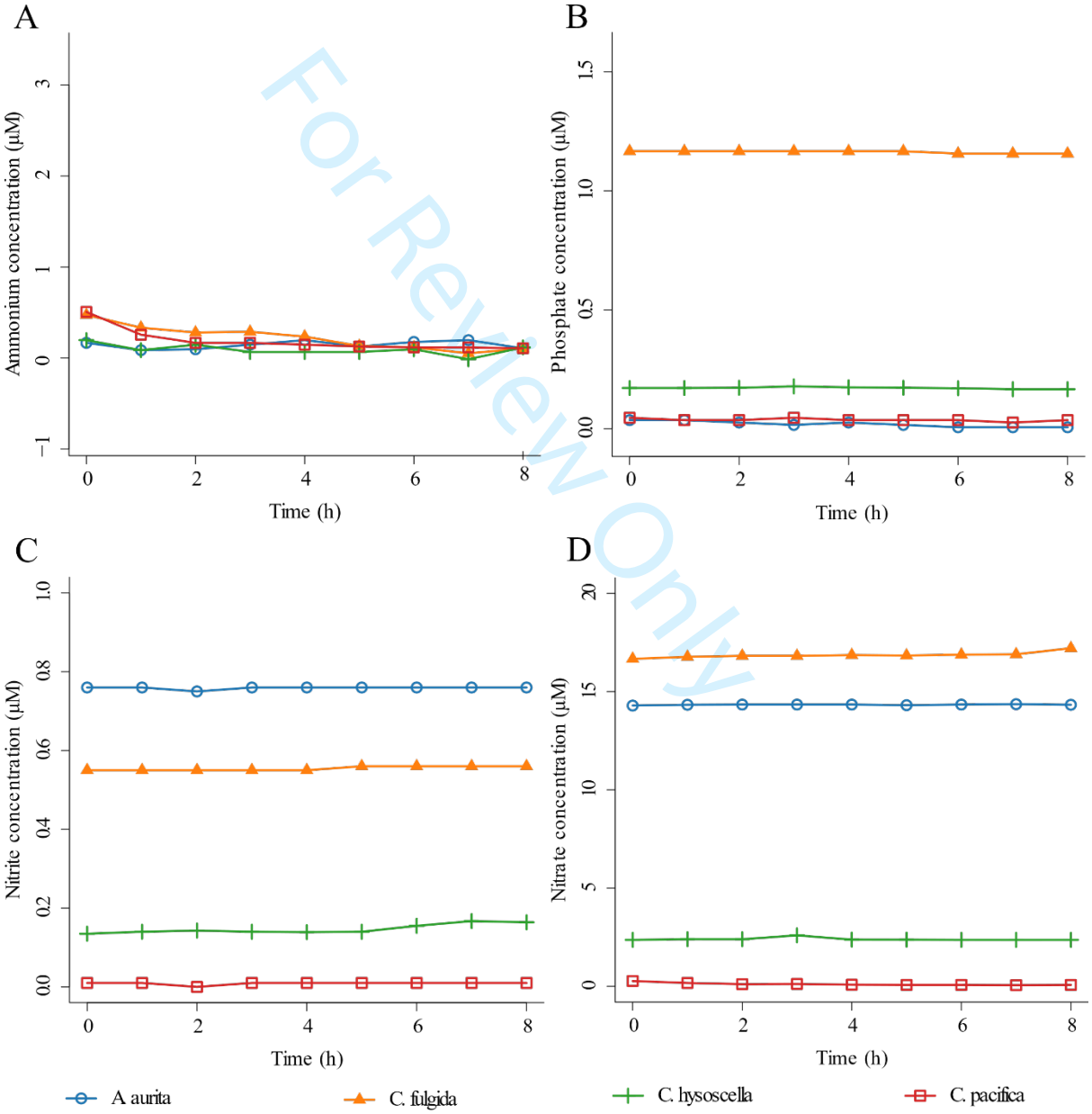

Figure II. Ammonium (A), phosphate (B), nitrite (C) and nitrate (D) concentrations of the Jellyfish-Control treatment of *A. aurita* (blue), *C. hysoscella* (green), *C. pacifica* (red) and *C. fulgida* (orange).

Table I. Summary table of linear regression results from the Jellyfish-Control treatment. The use of *, **, and *** denotes levels of statistical significance (p=0.05, 0.01, and 0.001 respectively).

A. Ammonium:

Species	Intercept	Slope	R^2	P
A. aurita	-0.02	0.00	-0.07	0.52
C. hysoscella	-0.01	-0.01	0.21	0.12
C. pacifica	0.19	-0.04	0.54	*
C. fulgida	-2.31	-0.05	0.89	**

B. Phosphate

Species	Intercept	Slope	R^2	P
A. aurita	-0.0300	-0.0042	0.8492	***
C. fulgida	1.1027	-0.0015	0.6286	*
C. hysoscella	0.1081	-0.0007	0.2050	0.1240
C. pacifica	-0.0236	-0.0013	0.2791	0.0830

C. Nitrite

Species	Intercept	Slope	R^2	P
A. aurita	0.76	0.0003	-0.06	0.48
C. hysoscella	0.13	0.0037	0.68	**
C. pacifica	0.01	0.0003	-0.06	0.48
C. fulgida	0.55	0.0017	0.71	**

D. Nitrate

Species	Intercept	Slope	R^2	P
A. aurita	14.32	0.00	0.14	0.18
C. hysoscella	2.43	-0.01	-0.08	0.54
C. pacifica	0.20	-0.02	0.67	**
C. fulgida	16.68	0.04	0.66	*

Mucus-Control:

The Mucus-Control treatment consisted of artificial seawater spiked every hour during the first 6 hours with different amounts of ammonium (Figure III A; Table II). The phosphate, nitrite and nitrate concentrations did not significantly change with time, except for the phosphate and nitrite of *A. aurita* and the nitrate of *C. hysoscella* (table III B). In these 3 cases, the concentrations of the Mucus treatment were corrected by subtracting them with the

corresponding Mucus-Control changes in concentration. The corrections did not change the observed results.

Table II. Volume of ammonium stock solution (100 mM) and expected increase in concentration of the Mucus and Mucus-Control treatments of *A. aurita*, *C. hysoscella*, *C. pacifica* and *C. fulgida*.

Species	*A. aurita*	*C. hysoscella*	*C. fulgida*	*C. pacifica*
Ammonium spike (µL) in Mucus treatment incubators	20	80	100	40
Ammonium spike (µL) in Mucus-control treatment incubators	15	60	75	30
Expected increase in concentration (µM)	0.5	2	2.5	1

Figure III. Ammonium (A), phosphate (B), nitrite (C) and nitrate (D) concentrations of the Mucus-Control treatment of *A. aurita* (blue), *C. hysoscella* (green), *C. pacifica* (red) and *C. fulgida* (orange).

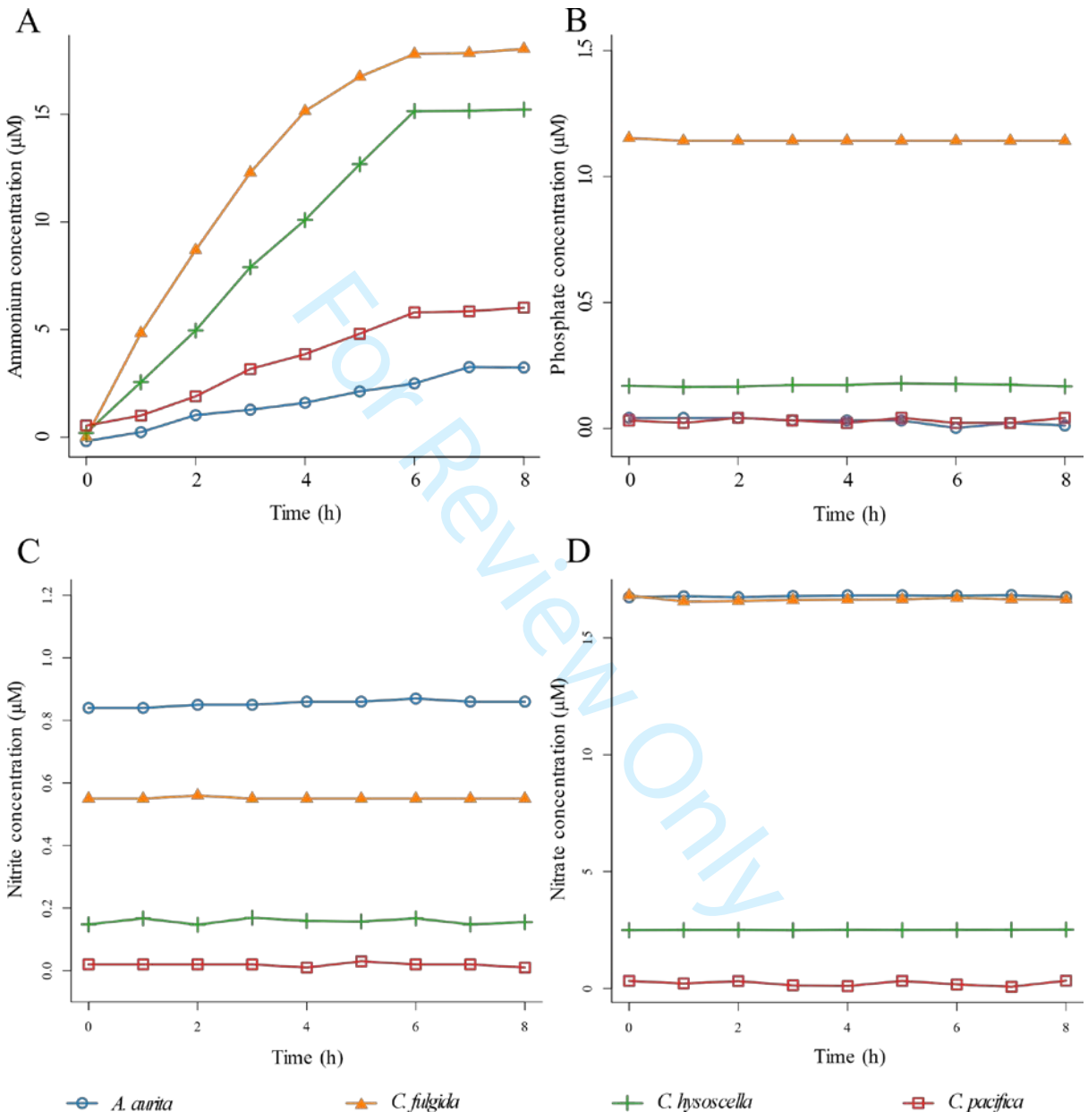

Table III. Summary tables of linear regression results from the Mucus-Control treatment. The use of *, **, and *** denotes levels of statistical significance (p=0.05, 0.01, and 0.001 respectively).

A. Phosphate

Species	Intercept	Slope	R^2	P
A. aurita	-0.0060	-0.0043	0.6619	**
C. hysoscella	1.0938	-0.0007	0.2000	0.1270
C. pacifica	0.1163	0.0007	0.0645	0.2530
C. fulgida	-0.0218	0.0002	-0.1401	0.9000

B. Nitrite

Species	Intercept	Slope	R^2	P
A. aurita	0.8418	0.0032	0.6934	**
C. hysoscella	0.1575	0.0000	-0.1428	0.9890
C. pacifica	0.0209	-0.0005	-0.0835	0.5550
C. fulgida	0.5524	-0.0003	-0.0571	0.4760

C. Nitrate

Species	Intercept	Slope	R^2	P
A. aurita	16.7642	0.0053	0.0309	0.299
C. hysoscella	2.4931	0.0023	0.5030	*
C. pacifica	0.2491	-0.0078	-0.0949	0.597
C. fulgida	16.6576	-0.0013	-0.1400	0.899

Mucus treatment

The Mucus treatment consisted of artificial seawater that had previously contained jellyfish, this artificial seawater was spiked with ammonium every hour during the first 6 hours. The values of ammonium concentration measured from the Mucus treatment were subtracted with the values obtained in the Mucus-Control treatment (Figure III A) in order to present the differences in between the incubations (figure IV A). The ammonium concentrations of the Mucus treatment - *C. hysoscella*, showed a highly significant decrease with time, while there was a significant increase of ammonium in the Mucus treatment - *C. pacifica* (Table III A). Two species showed a change in nitrite (*A. aurita* & *C. pacifica*; Figure III B, Table III B). One species showed a significant change in nitrate with time (*C. fulgida;* Table 3 C). For nitrate and nitrite, the rates of change in concentrations were extremely low (<0.01 µM h^{-1}; Table III B &

C). The decrease in ammonium concentration in the Mucus treatment – *C. hysoscella*, suggests the presence of microorganisms utilising the ammonium. The absence of nitrite and/or nitrate increase associated with the ammonium decrease supports that the microorganisms are not nitrifiers as they do not release nitrite and nitrate. They seem to be retaining the nitrogen. The changes in nitrite concentrations were close to the detection limit (≤0.02 µM) and could therefore be caused by increasing signal to noise ratios at low concentrations. The low rates of change for nitrite and nitrate suggest that the effect is negligible.

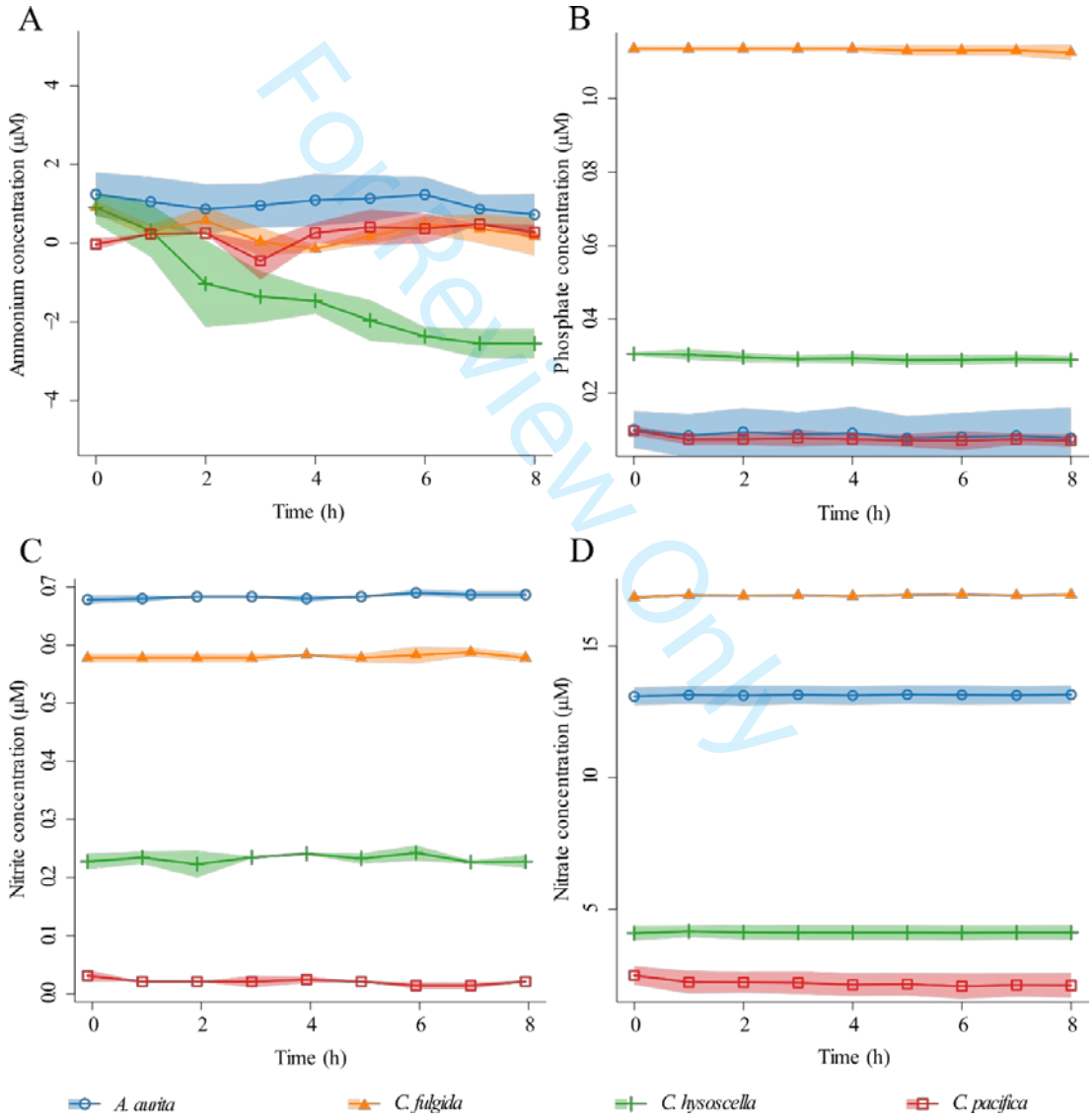

Figure IV. Ammonium (A), phosphate (B), nitrite (C) and nitrate (D) blank corrected concentrations of the Mucus treatment of *A. aurita* (blue), *C. hysoscella* (green), *C. pacifica* (red) and *C. fulgida* (orange). Coloured area = standard deviation of the mean cumulative release of nutrients.

Table IV. Summary tables of linear regression results from the Mucus treatment for ammonium (A), phosphate (B), nitrite (C) and nitrate (D).

A. Ammonium

Species	Intercept	Slope	R^2	p
A. aurita	1.1360	-0.0284	-0.0164	0.4530
C. fulgida	0.5110	-0.0112	-0.0547	0.7350
C. hysoscella	0.3777	-0.4291	0.7644	***
C. pacifica	0.0013	0.0628	0.1659	*

B. Phosphate

Species	Intercept	Slope	R^2	p
A. aurita	0.0942	-0.0022	-0.0289	0.6080
C. fulgida	1.1369	-0.0012	0.0523	0.1830
C. hysoscella	0.3023	-0.0020	0.1813	*
C. pacifica	0.0832	-0.0020	0.0602	0.1150

C. Nitrite

Species	Intercept	Slope	R^2	p
A. aurita	0.6758	0.0011	0.2500	**
C. fulgida	0.5746	0.0007	0.0120	0.2880
C. hysoscella	0.2297	0.0002	-0.0377	0.8150
C. pacifica	0.0249	-0.0012	0.1930	*

D. Nitrate

Species	Intercept	Slope	R^2	p
A. aurita	13.1150	0.0047	-0.0382	0.8380
C. fulgida	16.8923	0.0082	0.2343	*
C. hysoscella	4.1106	-0.0016	-0.0396	0.9230
C. pacifica	2.3264	-0.0365	0.0265	0.2030

ANCOVA

The regression lines were compared between species for each nutrient by analyses of covariance (ANCOVA). The data used for the analyses was lower or equal to time=6, i.e.: when the jellyfish was present in the incubator. The tables below show the p-value of the interaction between time and species. A significative interaction ($p<0.05$) means that there is a high probability that the species influence is real. In order words, the slopes of the regression lines are statistically different.

Table V. Summary tables of ANCOVA analyses in between species of the Jellyfish treatment for ammonium (A), phosphate (B), nitrite (C) and nitrate (D). The use of *, **, and *** denotes levels .05, .01, and .001 of statistical significance, respectively. NS= non-significative.

A. Ammonium

Species	*A. aurita*	*C. fulgida*	*C. hysoscella*	*C. pacifica*
A. aurita	NS	NS	NS	***
C. fulgida	NS	NS	*	***
C. hysoscella	NS	*	NS	***
C. pacifica	***	***	***	NS

B. Phosphate

Species	*A. aurita*	*C. fulgida*	*C. hysoscella*	*C. pacifica*
A. aurita	NS	NS	*	***
C. fulgida	NS	NS	NS	***
C. hysoscella	*	NS	NS	**
C. pacifica	***	***	**	NS

C. Nitrite

Species	*A. aurita*	*C. fulgida*	*C. hysoscella*	*C. pacifica*
A. aurita	NS	***	***	***
C. fulgida	***	NS	NS	***
C. hysoscella	***	NS	NS	NS
C. pacifica	***	***	NS	NS

D. Nitrate

Species	*A. aurita*	*C. fulgida*	*C. hysoscella*	*C. pacifica*
A. aurita	NS	***	***	***
C. fulgida	***	NS	NS	***
C. hysoscella	***	NS	NS	NS
C. pacifica	***	***	NS	NS

Allometric scalling

Both the inter- and intraspecies variability observed in ammonia and phosphate excretion as well as nitrification rates can be partly be explained by allometric scaling of the mass-specific release rates to the wet weight of each individual (ammonium excretion: 80%, phosphate excretion: 73%, nitrification: 55%, Table V; MS, Figure 5)

Table VI. Summary of the linear regressions on the effect of wet-weigh on the mass-specific nutrient releases normalised to 16°C. The use of *, **, and *** denotes levels of statistical significance (p = 0.05, 0.01, and 0.001 respectively).

Nutrient	Intercept	SD	Slope	SD	R^2	n	*p*
Ammonium	7.52	0.48	-0.82	0.10	0.80	17	***
Phosphate	5.91	0.63	-0.90	0.13	0.73	17	***
Nitrite	3.55	3.13	-0.88	0.67	0.05	12	0.20
Nitrate	7.95	1.29	-1.20	0.28	0.55	15	***

Lab-on-Chip analyser

An additional experiment was performed incubating a medusa of the species *A. aurita* in 4L of ASW and measuring nitrate + nitrite and nitrite concentrations using two microfluidic lab-on-chip (LoC) analysers. The LoC analysers used in this study was designed and fabricated at the National Oceanography Centre, Southampton, U.K and described in detail elsewhere (Beaton et al. 2012). Briefly, the LoC analyser is composed of a three layer poly(methyl methacrylate) chip with precision milled microchannels, mixers and optical components consisting of light emitting diodes and photodiodes, electronics, solenoid valves and syringe pumps are mounted on the chip. Reagent preparation details can be found in Birchill et al. (2019), for nitrate + nitrite detection an off-chip cadmium column was used, for nitrite only detection the column was removed. The standards were prepared from the same stock solutions that were used to prepare standards for gas segmented continuous flow (Seal; QuAAtro) analysis, the nitrite LoC analyser was equipped with 1.00 µM NO_2 standard and the NO_3 + NO_2 LoC analyser with a 2 µM NO_3 standard. A 0.45 µm Polyethersulfone Millipore filter was added to the end of the sample inlet tubing to prevent jellyfish mucus clogging the sensor. The incubator was fixed on a stirring table to homogenise the water.

A control and jellyfish incubation experiment were conducted. The spectrophotometric Greiss assay used on the LoC analysers measures NO_2, therefore any NO_3 present in the sample must be reduced prior to colour formation. This was achieved by the use of an off chip cadmium (Cd) column (Beaton et al. 2012). For each experiment, the Cd column reduction efficiency on the NO_3 + NO_2 LoC analyser was determined. The NO_3 reduction efficiency of the Cd column was determined by analysing a 1.00 µM NO_2 sample with the NO_3 + NO_2 LoC analyser that was standardised with a 2.00 µM NO_3 standard (i.e. if the analyser returned a NO_3 + NO_2 > 1.00 µM this would indicate < 100 % reduction efficiency). For the control experiment, the Cd column efficiency was 61-65 % (mean 64 %, n=5). For the jellyfish incubation experiment, the Cd column efficiency was 61-69 % (mean 66 %, n=7). Ideally the NO_3 reduction efficiency would be total (i.e. 100 %), but as the NO_2 concentration was being measured simultaneously, inefficiencies in NO_3 reduction could be accounted for. All reported NO_3 + NO_2 concentrations are therefore corrected for Cd column efficiency.

The control experiment was set up in the same manner as for the jellyfish experiments but without the addition of jellyfish. Firstly, the analysers repeatedly measured the concentration of NO_2 and NO_3 + NO_2 of artificial seawater in the incubation container, which was < 0.025 µM (n= 3) and 0.15 ± 0.02 µM (n= 7) respectively. Following this a 0.70 µM NO_2 spike was added to artificial seawater, therefore for target NO_2 and NO_3 + NO_2 concentration was 0.70 µM and 0.85 µM respectively. The concentration returned by the NO_2 LoC analyser was 0.70 ± 0.01 µM (n=3), whilst the NO_3 + NO_2 LoC analyser returned a lower than expected concentration of 0.72 ± 0.04 µM (n=3). A 0.50 µM NO_3 spike was then added to the same artificial seawater, therefore for target NO_2 concentration remained at 0.70 µM whilst the target NO_3 + NO_2 concentration increased to 1.35 µM. The concentration returned by the NO_2

LoC analyser was 0.70 ± 0.01 µM (n=4) and the NO_3 + NO_2 was 1.33 ± 0.08 µM (n=5) respectively. In summary, the control experiments demonstrated the analytical set up worked well, with the LoC analysers responding as expected to NO_2 and NO_3 additions. The lower than expected concentration returned by the NO_3 + NO_2 LoC analyser after the 0.70 µM NO_2 spike may in part be due to variable Cd-column efficiencies. Future experiments should aim for total NO_3 reduction efficiency.

Prior to the addition of an *A. aurita* specimen, the concentration of NO_2 and NO_3 + NO_2 in the artificial seawater used for the jellyfish incubation experiment was < 0.025 µM (n=6) and 0.06 ± 0.02 µM (n=7) respectively. Following the addition of the *A. aurita* specimen, the nitrate concentration increased linearly with a rate of 0.19 µM h^{-1} (Figure V, left) corresponding to the same average rate observed during the 5 replicate *A. aurita* jellyfish treatments (Figure V, right). The nitrite concentration remained < 0.025 µM (n= 11) throughout the experiment, which is also consistent with the *A. aurita* jellyfish treatments. Eight water samples were taken to compare the value of nitrate concentration measured by the LOC sensor and with concentrations determined by gas segmented continuous flow analysis (QuAAtro). The values are distributed around a linear regression line with a slope of 1.04 and a coefficient of determination of 0.98 (Figure VI).

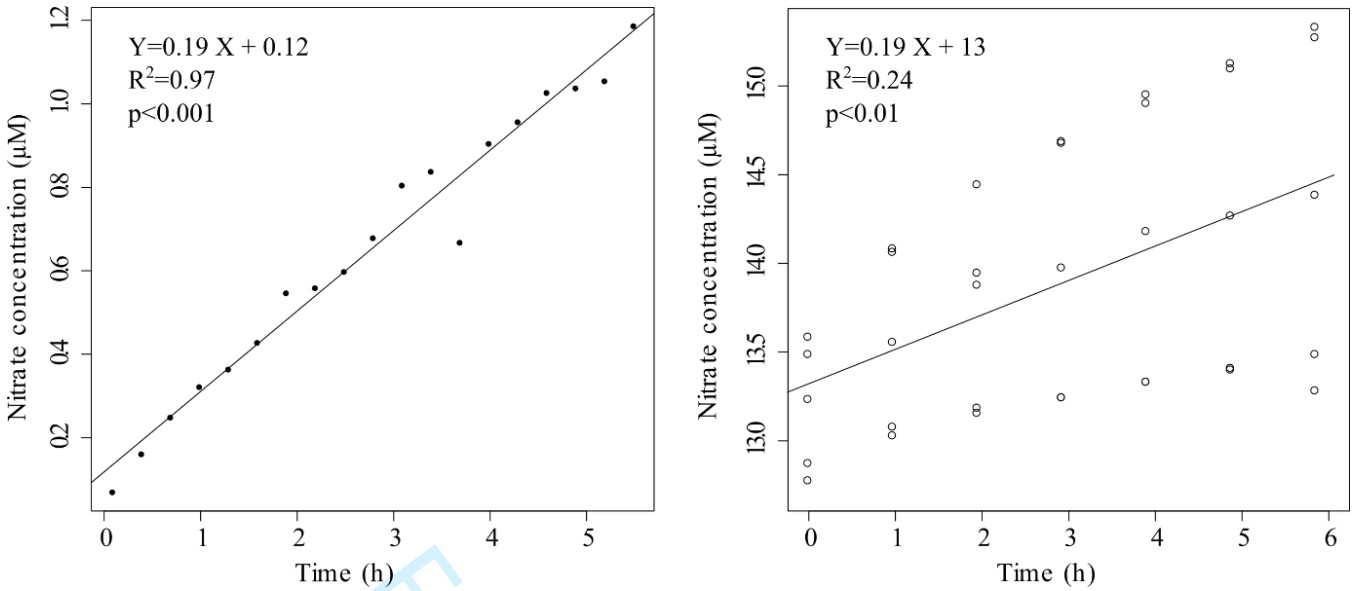

Figure V. Nitrate concentration of an incubator (Volume=4L) with a jellyfish of *A. aurita* measured by a lab-on-chip sensor (left) and nitrate concentrations of the Jellyfish treatment incubator of *A. aurita* measured by gas segmented continuous flow (QuAAtro, right). The ASW for the Jellyfish treatment incubator was made with reverse osmosis water presenting already high concentrations of nitrate (right).

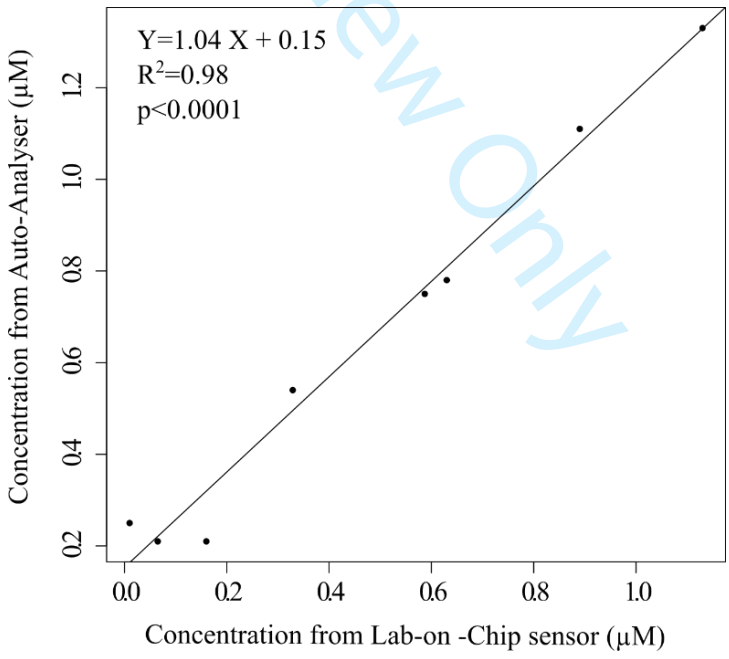

Figure VI. Comparing spectrophotometric methods: Gas segmented continuous flow (QuAAtro) vs Lab-on-Chip sensor. Real time concentrations of nitrate measured by a lab-on-chip sensor and filtered grab samples measured by gas segmented continuous flow.

Phosphate to ammonium

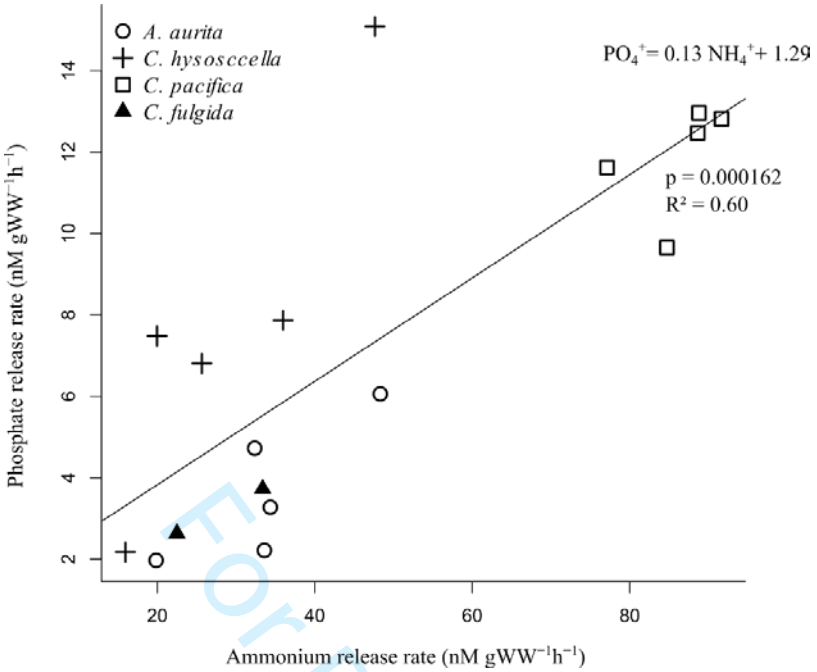

Figure VII. Relationship between the weight-specific phosphate and ammonium release rates for the jellyfish *A. aurita*, *C. hysoscella*, *C. pacifica* and *C. fulgida* at 16°C. The line is the linear regression.

Nitrogen to phosphate

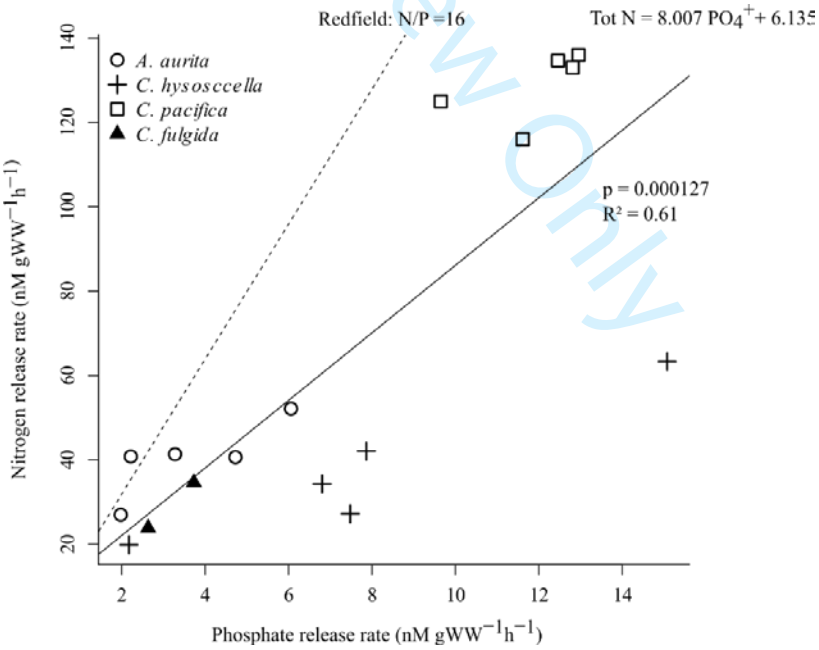

Figure VIII. Relationship between the weight-specific nitrogen and phosphate release rates for the jellyfish *A. aurita*, *C. hysoscella*, *C. pacifica* and *C. fulgida* at 16°C. The continuous line is the linear regression. The dashed line indicates where N:P ratio is 16:1 (Redfield ratio).

Artificial sea water preparation

First, the containers and tools used were cleaned using 10% hydrochloric acid (overnight) and ethanol, and then rinsed 3 times with ultra-high purity (UHP) water (MilliQ ≥ 18.2 MΩ cm $^{-1}$, Millipore). Two 20 L high density polyethylene containers with dispensing tap were filled with 10 L of UHP water. A pre-weighted amount of Tropic Marin PRO-REEF Sea Salt was added and the containers were shaken until dissolution of the salt. Then, the remaining 10L of UHP water was added gradually checking the salinity to reach the experimental salinity (Table 1).

Calibration and limit of detection

In total, three instruments were used to measure nutrients: a Turner design Trilogy fluorometer (model 7200, US) with a UV module (7200-047), an auto-analyser (QuAAtro, Seal Analytics) and a microfluidic lab-on-chip (LoC) analyser (Beaton et al. 2012). Table 6 present details of the calibration and the detection limit of the instrument used.

Table VII. Calibration points and limit of detection (LOD) for the different instrument used for the analysis of samples from the experiment of the jellyfish species *A. aurita*, *C. hysoscella*, *C. fulgida* and *C. pacifica*. C= concentration (µM) and A= absorbance.

Nutrients	Instrument		*A. aurita*	*C. hysoscella*	*C. fulgida*	*C. pacifica*
Ammonium	fluorometer	Calibration points (µM)	0,1,2,3,4	0,2,4,6,8	0,2,4,6,8	0,2,4,6,8
		Coefficient (a; b for C=aA+b)	316319; 549621	307972; 667256	9045; 53464*	391522; 78287
	AA	LOD (µM)	-	0.01	-	-
		Calibration points (µM)	-	2.5, 5, 10, 15, 20	-	-
Phosphate	AA	LOD (µM)	0.01	0.01	0.01	0.01
		Calibration points (µM)	0.5, 1, 1.5, 2, 2.5	0.2, 0.5, 1, 1.5, 2	0.5, 1, 1.5, 2, 2.5	0.5, 1, 1.5, 2, 2.5
Nitrate	AA	LOD (µM)	0.04	0.01	0.04	0.04
		Calibration points (µM)	4.5, 9, 13.5, 18, 22.5	0.5, 1.25, 2.50, 3.7, 5	4.5, 9, 13.5, 18, 22.5	4.5, 9, 13.5, 18, 22.5
	LoC Sensor	LOD (µM)	0.025	-	-	-
		Calibration point (µM)	2	-	-	-
Nitrite	AA	LOD (µM)	0.01	0.02	0.01	0.01
		Calibration points (µM)	0.5, 1, 1.5, 2, 2.5	0.2, 0.5, 1, 1.5, 2	0.5, 1, 1.5, 2, 2.5	0.5, 1, 1.5, 2, 2.5

*Different Turner Trilogy fluorometer used

References

Beaton, A. D., C. L. Cardwell, R. S. Thomas, V. J. Sieben, F.-E. Legiret, E. M. Waugh, P. J. Statham, M. C. Mowlem, and H. Morgan 2012. Lab-on-Chip Measurement of Nitrate and Nitrite for In Situ Analysis of Natural Waters. Environ. Sci. Technol. **46**: 9548–9556. doi:10.1021/es300419u

Birchill, A. J., G. Clinton-Bailey, R. Hanz, and others. 2019. Realistic measurement uncertainties for marine macronutrient measurements conducted using gas segmented flow and Lab-on-Chip techniques. Talanta **200**: 228–235. doi:10.1016/j.talanta.2019.03.032

Response to reviewers' comments

Manuscript ID: LO-20-0383

Title: Evidence of nitrification associated with globally distributed pelagic jellyfish

Authors: Nathan Hubot, Sari Giering, Jessika Füssel, Julie Robidart, Antony Birchill, Mark Stinchcombe, Cynthia Dumousseaud, Cathy Lucas

REVISION SUMMARY: We thank the reviewers for their constructive criticism and their thorough review of our manuscript. Please find below our point-by-point response to all comments. We have welcomed all comments and modified the manuscript in accordance with the suggestions. The changes include a new figure (Figure 6) to improve the understanding of the implications of our results for the nitrogen cycle. We also condensed the text and the data tables to improve the readability and clarity of our manuscript. We hope that we could address all concerns and comments to your satisfaction.

Reviewer #1:

This manuscript shows nice data and deals with the interesting topic of exploring if nitrifying microorganisms associated with jellyfish play a significant role in marine nitrogen cycling. To test this, they measured rates of ammonium and phosphate excretion as well as partial and complete nitrification in four species of non-zooxanthellate scyphozoan jellyfish from contrasting environmental conditions. The authors found that nitrifying microorganisms can oxidize up to a third of the ammonium excreted by the jellyfish, suggesting that jellyfish blooms may play an important role in surface ocean nitrogen cycling, supporting nitrification and phototrophic primary production via ammonia excretion.

The manuscript is very well written and organized, and I found the study to be of great potential interest because it provides new important information for "a so-far unanticipated role of jellyfish in marine nitrogen cycling". In addition, the experimental design is well established (with enough replicates per treatment) and I have really liked the introduction and discussion sections, which contains valuable information. From my point of view, there are only minor observations that the authors should address to the manuscript (please, see below). The only issue that I see in this article to make it a more complete study is that the abundance of the genes involved in the two nitrification processes (amoA and nxrAB) was not analyzed. It would have been very easy to filter the water from the incubators at the end of the experiment, extract the DNA, and quantify the genes by qPCR (is there still a possibility of doing it?). At least it would be interesting to know if it would be possible to repeat this type of experiment incorporating molecular biology techniques in the future.

Thank you for your positive feedback. We totally agree that quantifying the amoA and nxrAB genes present in the jellyfish microbiome is the next step forward and are planning to address this in the future. We have been collecting some samples for molecular analyses but unfortunately, these were not collected consistently for all the incubation experiments performed in this study. In the future, our samples will be merged with other sample to form

a larger collection. We will than perform 16S rRNA amplicon sequencing, qPCR and Card-FISH on this collection of sample. The results of these analyses will be presented in a separated publication.

To acknowledge this point on the manuscript, we added the following sentence:

L432: “The detailed nature of this association requires further investigations including molecular approaches to determine the identity and distribution of nitrifiers within the jellyfish microbiome.”

Minor observations:

- The title could be more specific, such as: “Evidence of nitrification associated with globally distributed pelagic jellyfish”.

Thank you for this suggestion. We agree and have changed the title accordingly. It is now: “Evidence of nitrification associated with globally distributed pelagic jellyfish”

- As the words nitrogen and phosphorus are used extensively in the document, I suggest using their abbreviations.

Thank you for the comment. We agree that the abbreviation could be used. However, while we use the words ‘nitrogen’ and ‘phosphate’ 39 and 20 times, respectively (‘phosphorus’ was only used 3 times), we feel that using abbreviations would affect the readability and would prefer to spell these words out. We leave this, however, to the discretion of the editor.

- L59: please, change “Pelagic” for “pelagic”.

Thank you for spotting this mistake. The change has been made.

- L243: Why was it decided to do an 8-hour experiment and not longer? Please justify.

This is a very good point. From repeated trial experiments, we learnt that the release of ammonium is linear over several hours even after starving the jellyfish for up to a day. These observations are consistent with Pitt et al. (2005), showing constant rates of ammonium excretion for *C. mosaicus* over 7 hours. Based on our trial experiments, we aimed for an incubation period that balances both incubation time and volume of incubator with achieving a significant, linear and reliable signal. Thus, we decided to collect seven samples over a period of six hours from 4-L incubators. That duration of incubation minimize the stress caused by starvation. A volume of 4 L allows the detection of small releases of nutrient (for example nitrite) without causing too much stress to the jellyfish. The level of stress caused to the jellyfish was estimated based on the swimming pulse and post-incubation survival. No jellyfish died following our trial experiments, which reassured us that the incubation conditions were not causing excessive stress to the jellyfish. After removing the jellyfish, we collected 2 more samples over 2 h to confirm that nutrients would stop accumulating upon the removal of the jellyfish from the incubators.

To reflect this in the manuscript, we added the following sentences:

L244 “. Based on initial experiments and findings of a previous study measuring ammonia release in *C. mosaicus* (Pitt et al. 2005), we decided an incubation volume and duration of 4 L and 6 hours as ideal to measure a significant rate of nutrients release without causing excessive stress to the jellyfish. The jellyfish were then removed from the incubators, and the jellyfish bell diameter and the wet weight (WW) was measured using a ruler and a balance

(FireKingdom SF-400, ±1 g). Water sample collection continued for 2 hours after removal of the jellyfish, resulting in a total experiment duration of 8 hours."

- L432: Change this subtitle for a more appropriate one, since this article does not study the microbiome of jellyfish, but nitrification. It could go something like this: "Evidence of active nitrifying microorganisms in jellyfish".

Thank you for suggesting a more appropriate subtitle. The subtitle is now: "Evidence of active nitrifying microorganisms in jellyfish".

We again thank the reviewer for his/her valuable time and constructive suggestions.

Reviewer #2:

The manuscript "Evidence of nitrification associated with jellyfish" by Hubot et al presents a study that investigates ammonium, nitrite and nitrate production by four jellyfish species and their respective microbiomes. In general, the manuscript is very well written and describes carefully designed experiments that support the conclusion that all four jellyfish species release ammonium and, dependent on the species, some of this ammonium is oxidized to either nitrite or ammonium. The authors then discuss the ecological implications of their study, using two case studies as examples. This part of the manuscript raised some questions for me concerning how jellyfish fit into the bigger picture of nitrogen cycling in the environment, which should be addressed.

We thank the reviewer for his/her valuable time and the thoughtful comments. We welcomed all the raised questions and addressed them below. We produced a new figure (figure 6) to better describe how jellyfish fit into the marine nitrogen cycle.

1) I was very surprised that the authors considered that the estimated nitrogen release by jellyfish in the Benguela upwelling system was realistic considering that it could support primary production rates three times higher than those in the respective area (L553). This should be addressed in the text.

Your point is a very valid one, and it highlights that we were not clear enough in the text.

The Benguela Upwelling System case study relies on jellyfish density estimates calculated by Lynam et al. (2006) based on data collected during a bloom period between August 20th and 31st in 2003 over the Namibian shelf. These densities represent a snapshot of a jellyfish bloom at that time. Based on these maximal densities, we estimated the potential release of nitrogen by the jellyfish. The resulting values (presented on figure 7b) are hence 'snapshot' rates for that particular bloom. We compared these to the mean daily primary production of the area (Brown et al. 1991). You are correct that this situation does not represent a steady state system: jellyfish blooms appear sporadically and primary production varies over time. Based on our calculation, we observed that – during a jellyfish bloom – the "mismatch" between the primary production and nutrient release by jellyfish can be substantial. In other words, the comparison between our estimates of nitrogen release during jellyfish blooms and the mean daily primary production of the ecosystem aims to show the maximum effect that a jellyfish bloom could have in this region over a limited period of time.

To estimate the overall impact on the ecosystem productivity throughout a year, high-resolution time series of jellyfish distribution and densities would be needed. We hope that our study will encourage future research to provide such data.

We changed the text to provide clarifications on this point and hope that it is much clearer now:

L501: “The Benguela Upwelling System is one of the four major coastal upwelling regions presenting the highest primary production of the world oceans (Carr 2001). Large jellyfish populations occur sporadically throughout the year with highest abundances observed in June–August (Flynn et al. 2012). The biomass of these blooms can at times exceed the biomass of fish by a factor of three (Lynam et al. 2006). We estimated that the *C. fulgida* blooms in August 2006 (Lynam et al. 2006) could have released up to 1.3 mmoles N m^{-2} h^{-1} (uncertainty range: 0.7 - 2.7 mmoles N m^{-2} h^{-1}; Figure 7b), of which 95% was in the form of ammonium and 5% in the form of nitrite. Assuming the Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release corresponded to a daily primary production of 2.5 g C m-2 d-1 (uncertainty: 1.3 – 5.2 g C m^{-2} d^{-1}), which is equivalent to 208% (uncertainty range: 108 – 433) of the average daily primary production of the Northern Benguela ecosystem (1.2 g C m^{-2} d^{-1}; Brown et al. 1991).

Considering that in the previous section, it is stated that ammonium release might be stimulated by starvation conditions (and I would therefore assume also under stress), can the authors really be confident enough in the N-release estimates from jellyfish to make these extrapolations? Please note that if the ammonium release is overestimated, I do not believe this would affect the nitrification rates that the authors measured as these were lower than the ammonium release rates. In fact, it would mean that in situ, they are of more relative importance to N-transformation.

Thank you for pointing this out, we were not sufficiently clear in our discussion on this point.

Based on our measurements of the release rates of nitrogen compounds and phosphate, we calculated the N:P ratio of the nutrients released by jellyfish (i.e. 7.3 – 10.9). We then compared that ratio with the ratio of marine zooplankton biomass (N:P > 20; Elser and Hassett 1994) which constitute the major diet of the jellyfish. Based on that comparison and the low molar C:N ratio (4.5 ± 1.1; Pitt et al. 2009) of jellyfish body, we concluded that jellyfish retain nitrogen in their tissue as already postulate by Pitt et al. (2009). Taking this though a bit further, we speculated that starving jellyfish, as they start consuming their body mass, would induce an increase in the N:P ratio of excreted nutrients, reflecting the high nitrogen content of the tissue consumed. Whether this change induces an increase of ammonium excretion rates is not known and still needs to be investigated.

To reflect this point, we changed the text and hope it is now clearer:

L437: “The average inorganic N:P ratio of the released nutrients (7.3 – 10.9, Table 2; SI, Figure VIII) lies below the Redfield Ratio (N:P = 16; Redfield 1963) and substantially below the N:P ratios of their main diet, zooplankton (N:P > 20; Elser and Hassett 1994). Thus, the gelatinous biomass of these jellyfish appears to retain nitrogen efficiently, which is further supported by their low molar C:N ratio (4.4; Lucas et al. 2011) compare to other marine zooplankton organisms (4.8-6.2 for crustacean zooplankton; Pitt et al. 2013). By storing nitrogen over phosphorus, expanding jellyfish blooms may locally drive the ecosystem

toward N-limitation (Sterner, 1990). Whereas under starvation, while jellyfish consume up to 85% of their own nitrogen-rich tissues (Pitt et al. 2014, Lilley et al. 2014), the N:P ratio of the excreted nutrients would increase. Starvation, a major cause of jellyfish bloom decline (Pitt et al. 2014), could temporarily drive the ecosystem towards P-limitation. A large jellyfish bloom could thus act as a "nitrogen buffer", storing nitrogen over phosphorus when food is abundant and releasing nitrogen over phosphorus during its decay."

Further, the collapse of a jellyfish bloom is a slow process as jellyfish can be starved for weeks (Hamner and Jenssen 1974; Lilley et al. 2014). The jellyfish used in our experiments were last feeding the day prior the experiment at maximum. Thus, although they could have been starving, we do not expect that the N:P ratio of excreted nutrients would be greatly affected at such an early stage of starvation. The low N:P ratio (7.3 – 10.9) of released nutrients, consistent with previous experiment (6.9 – 8.7; Pitt et al. 2009) of nutrients released by the incubated jellyfish supports that hypothesis. In addition, our excretion rates of ammonium (23 to 86 nmol NH_4^+ gWW^{-1}) are in line with previous observations (2.4 - 111.1 nmol NH_4^+ gWW^{-1} h^{-1}; reviewed by Pitt et al. 2013). As you pointed out we cannot rule out that the incubation conditions might induce stress and could have affected the ammonia release. Nevertheless, we are confident that our ammonium excretion rates can be used to estimate the release of ammonium by jellyfish blooms but agree that these extrapolations should be taken with caution.

We modified the method description to reflect that care was taken to prevent excessive stress to the jellyfish.

L244: "Based on initial experiments and findings of a previous study measuring ammonia release in *C. mosaicus* (Pitt et al. 2005), we decided an incubation volume and duration of 4 L and 6 hours as ideal to measure a significant rate of nutrients release without causing excessive stress to the jellyfish."

2) The results in the manuscript do indicate a role for jellyfish in marine nitrogen cycling, but more emphasis could be placed on the cycling aspect of this. A figure and some text describing the position of the jellyfish in the food web in terms of nitrogen would be useful – particularly on where the jellyfish are getting their nitrogen from (I assume feeding on zooplankton), where that nitrogen is coming from (primary production) and then completing the cycle of what percentage of that nitrogen in either the form of ammonium or nitrate is cycled back to support regenerated PP. I think that this is an important aspect of the authors results which is currently underrepresented in the manuscript.

Thank you for this very constructive comment. Following your suggestion, we have included a new figure (Figure 6) representing the role of jellyfish in the surface ocean nitrogen cycle. See below:

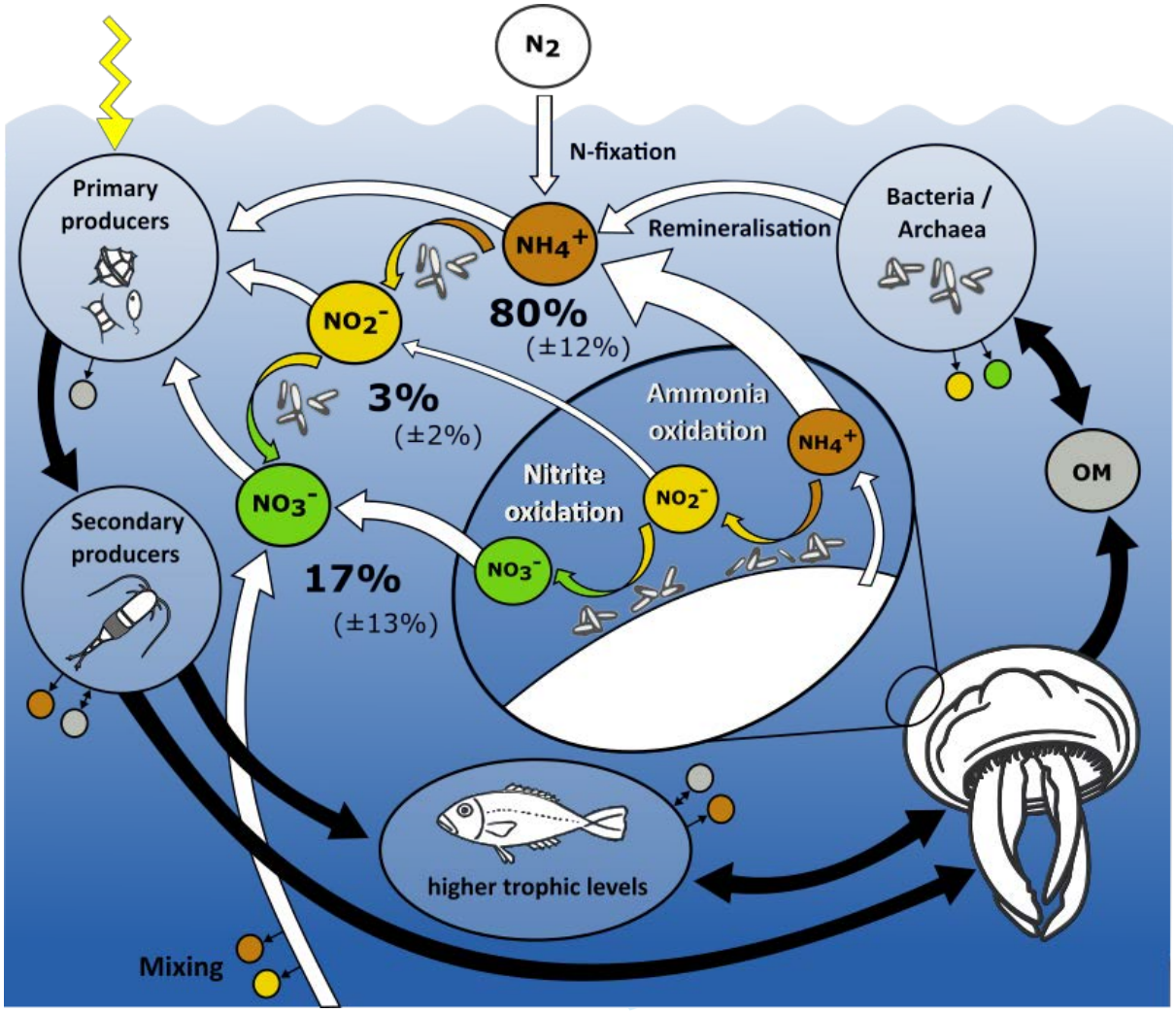

Figure 6. Conceptual diagram of the role and position of jellyfish in the surface marine nitrogen cycle. The flow of organic and inorganic matter is shown by black and white arrows, respectively. Colours indicate ammonium (NH_4^+, orange), nitrite (NO_2^-, yellow), nitrate (NO_3^-, green) and organic matter (OM, grey). Coloured arrows represent ammonium-oxidation (orange-to-yellow) and nitrite-oxidation (yellow-to-green). Components linked to small coloured circles release/assimilate nutrients of the same colour. The average release of nitrogen forms are presented as percentage (± standard deviation) of total dissolved inorganic nitrogen released by jellyfish. The yellow zigzag arrow represent light. The large middle circle zooms in on the jellyfish epithelium.

In support of the figure, we have added the following paragraph:

L450: “Our findings demonstrate that a substantial fraction of the excreted ammonium is shunted through partial or complete nitrification (ammonium: 80±12%, nitrite: 3±2%, nitrate: 17±13%; Figure 6), thereby fuelling dark carbon fixation in the sunlit surface ocean. An association with jellyfish allows nitrifiers direct access to ammonium in the surface ocean, thereby bypassing competition with phytoplankton for this otherwise scarce resource (Smith et al. 2014; Zakem et al. 2018). During jellyfish blooms, the release of different forms of bioavailable inorganic (nitrite, nitrate and ammonium) has the potential to locally enhance surface primary production and even influence phytoplankton community composition (Figure 6; Shilova et al. 2017). This effect on the community composition, in turn could

impact the quantity and quality of organic matter that sinks to depth (Basu and Mackey 2018)."

3) The section on the nitrifying microbiome could be considerable shortened or even removed as the authors add no additional data in this section. Figure 6 could then be replaced of expanded to show the source of the nitrogen to the jellyfish and the implications for nitrogen release from them on primary production.

Thank you for the comment, as we do not present any molecular data we agree that this section should be shortened, however, we consider the discussion on potentially responsible microorganisms as relevant, especially since previous studies investigating the taxonomic composition of the jellyfish mucus microbiome have not found nitrifiers to be highly abundant. We aim to investigate this conundrum in the near future. Therefore, we condensed this section (by 55%) and expanded figure 6 as suggested.

4) It is unclear which experiments were spiked with ammonium from Table 1 and the text, please clarify.

Thank you for pointing that out. To improve clarity regarding the ammonium spikes, we have removed the information on the ammonium spikes from table 1, added a new table in the SI document (Table II), moved the paragraph describing the ammonium spikes to L228 and complemented the text. The changes are presented below:

- L209: "The experiment consisted of an initial Release phase, followed by an incubation phase with four incubation treatments: Jellyfish (ASW + jellyfish), Jellyfish-Control (ASW only), Mucus (ASW + mucus + ammonium), and Mucus-Control (ASW + ammonium; Figure 2)".
- L220: "As ammonia is continuously excreted by jellyfish, the nitrification rates associated with jellyfish in ASW (continuously increasing ammonium concentrations) would not be directly comparable to those associated with mucus in ASW (ammonium concentration of <0.1 µM). To allow direct comparison of nitrification rates in the Mucus and Jellyfish treatments, we simulated jellyfish ammonium excretion in both the Mucus and the Mucus-Control treatments by adding ammonium (NH_4Cl, Fisher Scientific, UK) to the incubators after each sample collection. The amount of ammonium added was estimated based on literature (Pitt and Purcell 2009) and previous trial experiments. The expected increase in ammonium concentrations ranged from 0.5 to 2.5 µM h^{-1} (SI, Table II) depending on species, size of the jellyfish and temperature".

In addition to the text and table, Figure 2 allows the identification of the treatments including ammonium spikes by the use of a syringe symbol. We are now confident that the manuscript is providing enough information to prevent confusion regarding the treatments spikes with ammonium and thank the reviewer for asking clarifications.

We again thank the reviewer for his/her valuable time and constructive suggestions.

Reviewer #3:

The manuscript describes a study of rates of ammonium excretion and bacterial nitrification associated with various jellyfish species. The collected data are then used to estimate the potential contribution but theseof jellyfish N-excretion to primary production in two case studies. This is a novel study and the first to specifically focus on the association between jellyfish and nitrifying bacteria and would be of interest to readers of L&O.

The manuscript is generally well written, but the authors could be more succinct in parts and the overall length could easily be reduced by at least 10%.

We very much appreciate the positive feedback and the constructive criticism. While adding clarification, we condensed the overall manuscript from 5946 to 5747 words (-4%) mostly focusing on the sections:

- Evidence of active nitrifying microorganisms in jellyfish: - 55%
- Ecological implications & Case study: -32%

Similarly, there is quite a bit of overlap in the data presented in the figures and tables. For example, Fig 3 shows the accumulation of nutrients per biomass over time, but afterwards the rates of nutrient production per biomass are shown in both tables 2 and 3, and Fig 4. Thus again a significant amount of space could be saved by combining figs and tables, and/or moving some of them to the SI.

Thank you for pointing that out. Table 3 has been removed from the manuscript as the data presented was already available in the supplementary information documents (Table II). We moved table 4 to the SI document (Table V).

Specific comments

114-118. I believe the first studies to demonstrate an association between nitrifiers and various taxonomic ranges of benthic fauna were Welsh & Castadelli, 2004 and Stief et al. 2009.

Thank you for pointing us to these studies. We included both studies as references in the relevant context:

L97: “Nitrifiers have also been shown to live in association with benthic invertebrates such as sponges (Diaz and Ward 1997; Schläppy et al. 2010; Radax et al. 2012; Subina et al. 2018), corals (Beman et al. 2007; Siboni et al. 2008, Hoffman et al. 2009), zoanthids (Sun et al. 2014), bivalves (Welsh & Castadelli 2004), ascidians (Martínez-García et al. 2008) and insect larvae (Stief et al. 2009).”

Table 1. Do not need to include the volume of stock solution added in this table.

Thank you for that comment. We agree and realized that the values describing the ammonium additions are not needed in that table. We decided to move both the “ammonium spike” and the “expected increase in concentration" data to a new table now presented in the SI document (Table II).

226-228. Fourth treatment, ASW alone has been omitted.

Thank you for your comment. The Jellyfish-Control treatment consist of ASW only. To make this clear we have changed the sentence as follow:

L209: “The experiment consisted of an initial Release phase, followed by an incubation phase with four incubation treatments: Jellyfish (ASW + jellyfish), Jellyfish-Control (ASW only), Mucus (ASW + mucus + ammonium), and Mucus-Control (ASW + ammonium; Figure 2).”

317. g WW of jellyfish or mucus? There is no description of any method to quantify mucus in the methods.

Thank you for pointing that out. There was a mistake in the unit. The amount of mucus in the incubator was indeed not quantified. The changes in concentration of the water containing jellyfish mucus (from the Mucus-treatment) are now expressed in nmol L^{-1} h^{-1} as follow:

L315: “In the presence of mucus alone, rates of nitrification were negligible for all investigated jellyfish species (<2.0 x 10^{-3} nmol L^{-1} h^{-1}; SI, Table III),…”

441-444. Low coupling between processes really indicates poor diffusional connectivity, which can be due to a number of reasons. Basically means that is easier for nitrite to diffuse to the water column than to a zone where it can be oxidised to nitrate. This can also at least partially explain differences in % ammonium that is oxidised to nitrate.

Thank you. This is a really interesting point which had not been considered. We added a sentence:

L424: “The low coupling between nitrification rates could be caused by poor diffusional connectivity between nitrifiers (Welsh et al. 2001), i.e., a fraction of the produced nitrite might diffuse directly to the water column rather than to a zone where it can be oxidised to nitrate.”

Case studies. This is a useful section to show the potential contribution of N-excretion on primary production. But it could be improved by including the errors in your measurements and the other data sets used and propagating these in the model so that the contribution of N excretion could be expressed as a % range of primary production.

First, we are glad to hear that you find the case studies useful. An error propagation is indeed helpful to put the data into a global context. We now calculate uncertainty ranges and changed the manuscript accordingly.

In Methods:

L300: “Finally, estimates of inorganic nitrogen release by jellyfish blooms were calculated using the allometric equations together with jellyfish densities from two case studies. The uncertainty range of these estimates were determined from the error on the allometric exponents and the temperature.”

In Discussion:

L475: “We applied our allometric equations for ammonium and nitrate release (Equation 1 & 3) to the average body characteristics of the jellyfish (Table 3), corrected for temperature (Table 3 and as described in methods), and multiplied by abundance.

L489: “We estimated that the large aggregation of *A. coerula* could have released up to 1.7 mmoles N m^{-2} h^{-1} (uncertainty: 1.0 - 3.2 mmoles N m^{-2} h^{-1}), of which 85% was in the form of ammonium and 15% in the form of nitrate (Figure 7a). On a daily basis, assuming Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release would be able to support a primary production rate of 3.2 g C m^{-2} d^{-1} (uncertainty: 1.9 -6.1 g C m^{-2} d^{-1}), equivalent to 463% (uncertainty: 275 – 884%) of the mean daily primary production of a typical estuarine-coastal ecosystems (global average: 252 g C m^{-2} y^{-1}; Cloern et al. 2014).”

L505: “We estimated that the *C. fulgida* blooms in August 2006 (Lynam et al. 2006) could have released up to 1.3 mmoles N m^{-2} h^{-1} (uncertainty range: 0.7 - 2.7 mmoles N m^{-2} h^{-1}; Figure 7b), of which 95% was in the form of ammonium and 5% in the form of nitrite. Assuming the Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release corresponded to a daily primary production of 2.5 g C m^{-2} d^{-1} (uncertainty: 1.3 – 5.2 g C m^{-2} d^{-1}), which is equivalent to 208% (uncertainty range: 108 – 433) of the average daily primary production of the Northern Benguela ecosystem (1.2 g C m^{-2} d^{-1}; Brown et al. 1991).”

We again thank the reviewer for his/her valuable time and constructive suggestions.

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Evidence of nitrification associated with globally distributed pelagic jellyfish

Nathan Hubot[1,2*], Sarah L C Giering[1], Jessika Füssel[2,3*], Julie Robidart[1], Antony Birchill[1,4], Mark Stinchcombe[1], Cynthia Dumousseaud[2], Cathy Lucas[2]

Affiliations:

[1] National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK

[2] Ocean and Earth Science, University of Southampton, National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK

[3] Department of Medicine, University of Chicago, Chicago, IL, USA

[4] School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, UK

Emails and ORCID ID numbers:

Nathan Damien Hubot: N.D.Hubot@soton.ac.uk, orcid.org/0000-0001-6917-2255

Sarah Lou Carolin Giering: s.giering@noc.ac.uk, orcid.org/0000-0002-3090-1876

Julie Robidart: j.robidart@noc.ac.uk, orcid.org/0000-0001-9805-3570

Jessika Füssel: ju.fuessel@uchicago.edu, orcid.org/0000-0002-4210-2318

Cathy H. Lucas: cathy.lucas@noc.soton.ac.uk, orcid.org/0000-0002-5929-7481

Antony Birchill: antony.birchill@plymouth.ac.uk, orcid.org/0000-0002-1453-5781

Mark Stinchcombe: mark.stinchcombe@noc.ac.uk

Cynthia Dumousseaud: C.C.Dumousseaud@soton.ac.uk, orcid.org/0000-0001-5995-902X

*Correspondence to:

Nathan Hubot, National Oceanography Centre Southampton (Room: 344/35; phone: Ext. 28724)

University of Southampton, Waterfront Campus, European Way, Southampton, SO14 3ZH,

United Kingdom. N.D.Hubot@soton.ac.uk

&

Jessika Füssel, Department of Medicine, University of Chicago, Chicago, IL, USA

ju.fuessel@uchicago.edu

Key words: Jellyfish, nitrification, microbiome, nitrifiers, nitrogen cycle

Statement of significance

Often considered detrimental to the environment and human activities, jellyfish blooms are increasing in several coastal regions worldwide. Yet, the overall effect of these outbreaks on ecosystem productivity and structure are not ~~yet~~ fully understood. Here we provide evidence for a so far unanticipated role of jellyfish in marine nitrogen cycling. Our observations suggest a widespread association between jellyfish and nitrifying microorganisms. Via ammonium excretion, ~~J~~jellyfish blooms ~~may p~~play a substantial role in ~~surface ocean~~cycling nitrogen ~~cycling~~in the surface ocean, supporting chemolithoautotrophic nitrification (up to 33% of the excreted ammonia is oxidize into nitrite/nitrate) and phototrophic primary production (locally providing up to 463% of the nitrogen required for daily primary production)~~via ammonia excretion~~. ~~These~~ Our novel observations and allometric equations have implications for both the small- and the large-scale coastal processes and are of relevance for researchers from microbiologists to modellers. As jellyfish blooms occur in ~~both~~ lakes and oceans and are

important for understanding both ecology and biogeochemistry, the results of this study are relevant to the broad community reached by L&O.

Author Contributions

~~Nathan Damien Hubot~~NDH~~B~~ and SLCG designed the study. NDH~~B~~ carried out the experiments and ~~analyzed~~analysed the samples. SLCG helped with the data analysis and interpretation. JF and CHL contributed to the study design and interpretation of the results. ~~contributed substantially to the study's conception, drafting the manuscript, the data acquisition and analysis, and approved the final submitted manuscript.~~

JR contributed to drafting the manuscript~~??~~. AB helped with the sampling of nutrients and use of the Lab-On-Chip sensor.~~,~~ MS and CD ~~Sarah Lou Giering contributed substantially to the study's conception, drafting the manuscript, data analysis, and approved the final submitted manuscript.~~

~~Jessika Füssel: contributed substantially to drafting the manuscript, to the study's conception and approved the final submitted manuscript.~~

~~Cathy H. Lucas: contributed substantially to drafting the manuscript, to the study's conception and approved the final submitted manuscript.~~

~~Julie Robidart: contributed to drafting the manuscript and approved the final submitted manuscript.~~

~~Antony Birchill: contributed~~contributed to the data acquisition. NDH~~B~~ wrote the manuscript with support from all authors, which approved the final submitted manuscript.~~, drafting the manuscript and approved the final submitted manuscript.~~

~~Mark Stinchcombe: contributed to data acquisition and approved the final submitted manuscript.~~

~~Cynthia Dumousseaud: contributed to data acquisition and approved the final submitted manuscript.~~

Abstract

Often considered detrimental to the environment and human activities, jellyfish blooms are increasing in several coastal regions worldwide. Yet, the overall effect of these outbreaks on ecosystem productivity and structure are not fully understood. Here we provide evidence for a so far unanticipated role of jellyfish in marine nitrogen cycling~~Bioavailable nitrogen, particularly ammonia, is a scarce resource in most of the surface ocean and often limits primary productivity. Although Pelagic jellyfish excrete substantial amounts of ammonia (the preferred form of nitrogen for most phytoplankton), they are overlooked players in marine nitrogen cycling~~. Pelagic jellyfish release nitrogen as a metabolic waste product in form of ammonium. ~~However~~Yet, ~~Here,~~ we observed high rates of nitrification ($NH_4^+ \rightarrow NO_3^-$, 5.7 – 40.8 nM gWW^{-1} (wet weight) h^{-1}) associated with the scyphomedusae *Aurelia aurita, Chrysaora hysoscella* and *Chrysaora pacifica* and low rates of incomplete nitrification ($NH_4^+ \rightarrow NO_2^-$, 1-2.7 nM gWW^{-1} h^{-1}) associated with *Chrysaora fulgida, Chrysaora hysoscella* and *Chrysaora pacifica.* These observations indicate that microbes living in association with these jellyfish thrive by oxidizing the readily available ammonia to nitrite and nitrate. The four studied species ~~are abundant over~~have a large geographic distribution and exhibit frequent population outbreaks. We show that, during such outbreaks, jellyfish-associated release of nitrogen can provide more than 100% of the nitrogen required for primary production. These findings reveal a so far overlooked

pathway when assessing pelagic nitrification rates that might be of particular relevance in nitrogen depleted surface waters and at high jellyfish population densities.

Introduction

Jellyfish blooms are increasing in frequency and magnitude in several coastal regions around the world (e.g. Sea of Japan, Black sea, Benguela current, Antarctic; Brotz et al. 2015). The presence of jellyfish blooms in coastal waters can cause severe damage to economic activities such as fisheries (e.g., 2.1 - 25% decrease in annual Korean fishery production every year; Kim et al. 2012), tourism (e.g.: costing the Israeli coastal tourism industry an estimated annual monetary loss of €1.8–6.2 million every year; Ghermandi et al., 2015) and power generation (e.g.: the closure costs of Torness nuclear plant in Scotland due to jellyfish bloom from 28th June to 1st July 2011: approximately £1 million d^{-1}; Kopytko 2015). Simultaneously, jellyfish outbreaks create ecological disturbances by altering the marine food chain structure (Pitt et al. 2009). Their voracious predation on zooplankton makes them competitors toof planktivorous fish (Condon et al. 2011). In turn,The grazing pressure that jellyfish control put on zooplankton grazers populations and thereby releases primary producers from predatory control, causing a trophic cascade that often can resulting results in phytoplankton blooms (West et al. 2009; Schnedler-Meyer et al. 2018). By preying on ichthyoplankton (eggs and larvae of fish), jellyfish even exert a top-down control on both their competitors and predators (Titelman and Hansson 2006; Gordoa et al. 2013).; Overall, the complex interaction of jellyfish with the food web which can have large impacts on ecosystem structure, function and resilience (Baum and Worm 2009).

The role of jellyfish as top-down predators has been widely studied (e.g.: Purcell and Decker 2005; Compte et al. 2010; Stone and Steinberg 2018), yet their bottom-up influence as a nutrient source on marine ecosystems is less clear. Ammonium excreted by jellyfish ~~was~~has been estimated to support up to 8 %, 10% and 11% of the phytoplankton nitrogen requirement in the Lake Illawarra (Australia), the Inland Sea of Japan and the Kiel Bight, respectively (Schneider 1989; Pitt et al. 2005; Shimauchi and Uye 2007). Ammonium and ~~/~~phosphate released by jellyfish more than doubled the phytoplankton biomass in a mesocosm experiment conducted in a saline lake (West et al. 2009). In addition, the release of organic matter in the form of mucus provides an extremely labile source of organic carbon for bacterioplankton~~, which quickly metabolize it~~ (Condon et al. 2011). While there is clear evidence that jellyfish can alter both biogeochemical cycles and food web structure, ~~few studies have explored~~ their role in pelagic nitrogen cycling ~~is largely unknown~~remains understudied.

Ammonia is an intensely contested compound in most of the world's sunlit oceans, where nitrogen availability limits primary productivity (~75% of the surface ocean; Moore et al. 2013; Bristow et al. 2017). Additionally, ammonia provides the substrate for ubiquitous chemolithoautotrophic nitrifying bacteria and archaea that generate energy by the stepwise oxidation of ammonia to nitrite and nitrate. The first step is mediated by ammonia oxidizing bacteria (Kowalchuk and Stephen 2001) and archaea (Könneke et al. 2005; Hallam et al. 2006). Ammonia oxidizing archaea can reach high abundances especially in the dark ocean (> 30% of the microbial community; Karner, DeLong, and Karl 2001) and appear to be the main drivers of marine ammonia oxidation (Francis et al. 2005; Wuchter et al. 2006). As for the second step, all known nitrite oxidizers belong to the bacterial domain (Spieck and Bock 2015; Bock and Wagner 2006) and are characterized by their often remarkable metabolic versatility (Koch et al.

2015; Daims, Lücker, and Wagner 2016; Füssel et al. 2017). Both ammonia oxidizers and nitrite oxidizers (collectively called nitrifiers) are ubiquitous in p ~~most p~~elagic environments, where they contribute substantially to carbon fixation in absence of light (dark carbon fixation)~~dark carbon fixation and sequestration~~, influencing ocean carbon fluxes (Herndl and Reinthaler 2013; Pachiadaki et al. 2017; Wuchter et al. 2006). ~~They~~Nitrifiers have ~~also~~also been shown to live in association with ~~several~~ benthic invertebrates such as sponges (Diaz and Ward 1997; Schläppy et al. 2010; Radax et al. 2012; Subina et al. 2018), corals (Beman et al. 2007; Siboni et al. 2008, Hoffman et al. 2009), ~~-~~zoanthids (Sun et al. 2014), bivalves (Welsh & Castadelli, 2004), ~~and~~ ascidians (Martínez-García et al. 2008) and insect larvae (Stief et al. 2009). As ~~members~~ part of invertebrate microbiomes, nitrifiers ~~detoxify ammonia~~ ~~by XXXX~~can provide a source of nutrition for their host when phagocytosed (Martínez-García et al. 2008), preventing the loss of nitrogen into the environment by recycling the excess of ammonium trapped in the mucus (Siboni et al. 2008; ~~), provide a source of nutrition for the host through XXX (Martínez-García et al. 2008), and prevent the loss of ammonium~~ ~~when XXXX~~ ~~from corals (~~Rädecker et al. 2015). Understanding the role of these associations is important~~essential~~ for accurate mapping of marine nitrogen biogeochemistry and may help to ~~, which is required to successfully~~improve our ability to predict future change (Pajares and Ramos 2019).

Jellyfish are densely populated with microorganisms (Weiland-Bräuer et al. 2015; Lee et al. 2018; Kramar et al. 2019), which play a beneficial role in the fitness of the host and contribute to the ecological features of the jellyfish (Stabili et al. 2018; Tinta et al. 2019). The epithelial mucus layer of a jellyfish is an attractive niche for microbes, providing them with both a habitat and a high-quality energy source (Kramar et al., 2019). By attracting profitable bacteria and preventing colonization by potentially harmful microorganisms (via interferences with

bacterial quorum sensing), the host maintains a healthy microbiome providing immune system functions (Weiland-Bräuer et al. 2019). In addition, jellyfish microbiomes are production hotspots of chemical compounds (e.g. exopolysaccharides, vitamins, enzymes, toxins, antibiotics; Tinta et al. 2019) and harbour microbes closely related to known drivers of major elemental cycles (e.g. nitrogen cyclers, chemolithoautotrophs, methylotrophs, methane oxidizers, and polycyclic aromatic hydrocarbon degraders; Lee et al. 2018). In terms of the nitrogen cycle, two species of nitrifiers (the ammonium oxidizing bacterium *Nitrosospira multiformis* and the nitrite oxidizing bacteriuma *Nitrospira moscoviensis*) have been found in association with the jellyfish species *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-Bräuer et al. 2015). This discovery, leadsing to the suggestion that these two nitrifiersthey are ubiquitous members of the microbiome of these two genera (Lee et al. 2018), and indicates that . Hence, jellyfish likely could contribute to marine nitrogen cycling beyond the excretion of ammonia via nitrification processes catalyzedcatalysed by members withof in their microbiome.

Cnidarian jellyfish excrete dissolved inorganic nitrogenammonium, a by-product of their metabolism, by diffusing ammonium usion through their body surface as a by-product of metabolism (Lőw et al. 2016). Though They they are not known to directly produce nitrite or nitrate. Yet, low rates of small nitrate releases associated with jellyfish have been observed in association with pelagic jellyfish (< 2% of total inorganic nitrogen released; Pitt et al. 2009). In experiments withFor benthic zooxanthellate jellyfish, that live in symbiosis with zooxanthellae (photosynthetic dinoflagellates), experiments have shown nitrite/nitrate release rates equivalent in contrast corresponded to 21.5% of the total dissolved inorganic nitrogen effluxrelease, indicating a substantial colonization by nitrifying microorganisms (Welsh et al. 2009). While the authors suggested the association with nitrifiers to be specific to zooxanthellate jellyfish, we

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hypothesize that nitrifying microorganisms that benefit from the excreted ammonium are commonly associated with jellyfish and play a significant role in the~~ir~~ nitrogen cycling. To test this hypothesis, we chose four species of non-zooxanthellate scyphozoan jellyfish, *Aurelia aurita, Chrysaora hysoscella, Chrysaora fulgida* and *Chrysaora pacifica* from four contrasting environmental conditions (brackish lake, both North and South Atlantic Ocean coastal waters, and artificial seawater), representing a ~~large~~ wide range of ~~geographic distribution~~environmental conditions (Dawson et al. 2005, Morandini and Marques 2010). ~~Moreover, all~~All of these species ~~have been shown to~~ exhibit population outbreaks in coastal areas (Lucas 2001; Lynam et al. 2006; Makabe et al. 2015; Abato 2017) leading to high population biomass ultimately disturbing human activities. We measured the release rates of ammonium, nitrite, nitrate and phosphate ~~excretion as well as partial and complete nitrification~~ in association with all four jellyfish species in order to assess the global prevalence of ~~this~~ an association between nitrifiers and jellyfish as well as ~~association and~~ its potential role in the marine nitrogen ~~cycling~~cycle.

Materials

Adult medusae of *Aurelia aurita* ($n = 5$), *Chrysaora hysoscella* ($n = 5$) and *Chrysaora fulgida* ($n = 2$) were sampled from Horsea lake (UK), the Rame Peninsula (UK), and Walvis Bay (Namibia), respectively (Table 1). Medusae were collected carefully from near-surface waters using a 10-L bucket and kept in approximately 5 L of ambient water during transportation to the laboratory. The water temperature was kept as close to in-situ conditions as possible (maximum fluctuations: ±2°C from in-situ conditions; Table 1). Maximum transportation time was four hours. All jellyfish survived transportation and were transferred to the lab in good condition, indicated by regular swimming pulse. Once in the lab, jellyfish were transferred to their respective experimental conditions (Table 1). *Chrysaora pacifica* specimen ($n = 5$) were

collected from the London aquarium. The medusae were produced from polyps cultured in artificial conditions (artificial seawater with continuous UV-treatment and filtering system) and had not been in contact with natural seawater.

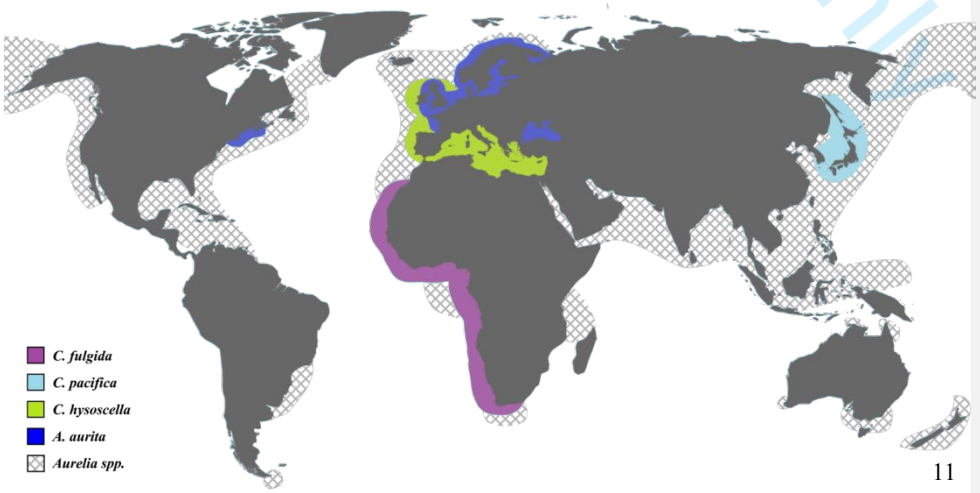
Sampling sites and species

Horsea Lake (Portsmouth, United Kingdom; 50° 83' 68.26" N, 1° 10' 19.11" W) is an enclosed, shallow (6-7 m), brackish (salinity: 19-23 PSU) lake situated on the south coast of England. The lake is oligotrophic with annual surface temperatures between 5°C ~~to~~ and 23°C (Lucas 1996). It lacks a riverine input and is replenished with seawater 2–3 times a year during high water spring tides (Lucas et al. 1997). The moon jellyfish *A. aurita* is found in Atlantic boreal waters and in the Black Sea (Dawson 2003; Figure 1). The species was previously associated with a cosmopolitan distribution and is now known to be formed by many regional "cryptic" species spread globally (Dawson and Jacobs 2001, Scorrano et al. 2016~~;~~ Figure 1). The medusae of *A. aurita* can reach bell diameters up to 40 cm (Arai 1996) and are often found in high densities in coastal and~~or~~ brackish waters such as estuaries and bays (Lucas 2001). They are present in Horsea ~~l~~Lake throughout the year (Lucas 1996).

The Rame Peninsula (Cornwall, United Kingdom) is located on the south-west coast of England. Medusae of the species *C. hysoscella* were collected in waters characteristic of the English Channel (50° 19' 54.5'' N, 4° 11' 59.2'' W). The mean monthly surface temperature ranges from 9.2°C to 16.5°C and the mean monthly surface salinity ranges from 35.1 to 35.3 PSU (Smyth et al. 2010). Medusae of *C. hysoscella* are of medium sizes (15–25 cm in bell diameter) and are found in the North Sea, the English Channel and the Mediterranean Sea (Morandini and Marques 2010; Figure 1), where they can form dense populations (Abato 2017). They appear in the English Channel during the summer months (Pikesley et al. 2014).

Walvis Bay is a large bay located on the coast of Namibia (22° 57’ 22’’ S, 14° 30’ 29’’ E). The water conditions of the bay are dictated by the Nnorthern Benguela Uupwelling Ssystem, which is a highly productive eastern boundary ecosystem. The seawater temperature in Walvis Bay varies between 10°C and 22°C and the salinity mainly ranges between 34.5 and 35.5 PSU (Pryor et al. 2009). *C. fulgida* is an exclusively marine species found along the ~~West~~ west coast of Africa (Figure 1) ~~and presenting~~with medusae of medium size~~s~~ (10–20 cm in diameter). This species has, previously been identified as *C. hysoscella* due to their morphological similarities (Morandini and Marques 2010). *C. fulgida* medusae are found in Walvis Bay throughout the year and frequently reach significant population densities during the summer months (Skrypzeck 2019).

Medusae of the species *C. pacifica* are slightly smaller (typically 10–15 cm in diameter) than the two~~other~~ studied species of *Chrysaora* described above, ~~(typically 10–15 cm in diameter)~~ and occur in the Nnorthern Pacific Ocean, in the vicinity of Japan (Figure 1; Morandini and Marques 2010). Since the beginning of the century, the number of *C. pacifica*



235 medusae in the Inland Sea of Japan has been growing, and the population has now has recurring
236 annual blooms (Makabe et al. 2015; Takasu et al. 2019).

237 **Figure 1.** Geographic distribution of the four studied jellyfish species investigated in this study
238 (*Aurelia aurita, Chrysaora hysoscella, Chrysaora fulgida, Chrysaora pacifica*; based on
239 Dawson et al. 2005 and Morandini and Marques 2010) and species of the cryptic genus *Aurelia*
240 (based on Dawson and Martin 2001).

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246 **Table 1**. Jellyfish collection and incubation details

Species	*A. aurita*	*C. hysoscella*	*C. fulgida*	*C. pacifica*
Origin	Horsea ~~l~~Lake (UK)	Rames peninsula (UK)	Walvis Bab~~a~~ay (Namibia)	London Aquarium
Date (DD-MM-YY)	23-10-2018	21-08-2018	21-07-2019	27-02-2019
Bell diameter (cm)	1~~1.5~~2 – 16	9 – 13	12 – 16	6 – 9
Wet weight (g)	73 – 190	100 – 278	180 – 279	35 – 59
Number of specimens	5	5	2	5
In-situ conditions				
Temperature (°C)	14	18	14	16
Salinity	25	35	35	30
Experimental condition				
Temperature (°C)	15	20	14	16
Salinity	25	35	35	30
~~Ammonium spike (µL)~~	~~20~~	~~80~~	~~100~~	~~40~~
~~Expected increase in concentration (µM)~~	~~0.5~~	~~2~~	~~2.5~~	~~1~~

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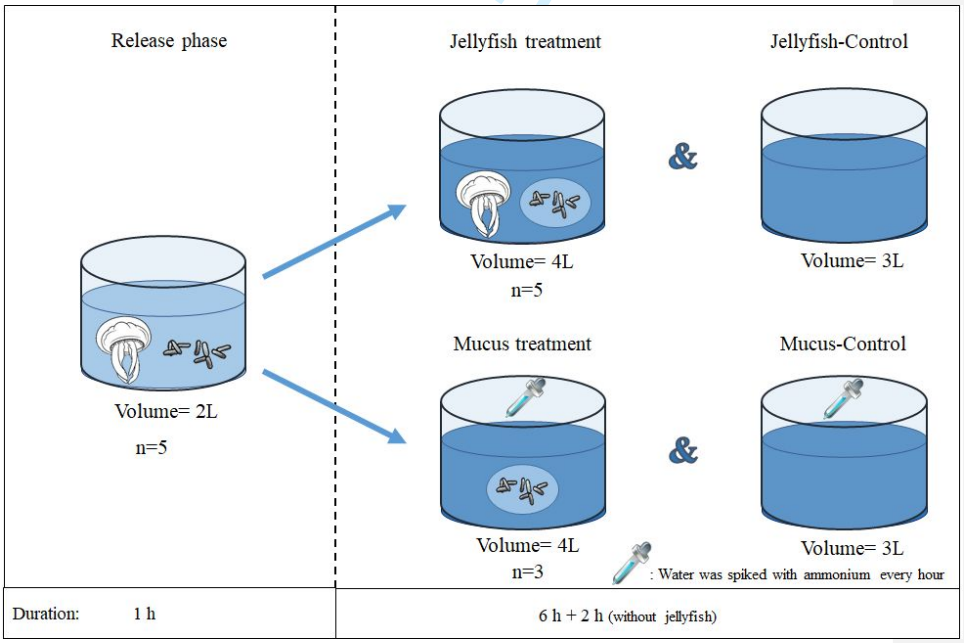
Experimental structure

Prior to the experiment, all equipment was acid washed in 10% hydrochloric acid and rinsed three times with ultra-high purity water (MilliQ ≥ 18.2 10^6 Ω cm $^{-1}$, Millipore, UK). The incubators consisted of 5-L ~~high density~~high-density polyethylene buckets filled with artificial seawater (ASW; ultra-high purity water + Tropic Marin synthetic sea salt; detailed preparation available in Supplemental Information (SI)). A maximum number of five healthy and undamaged adult medusae were selected for each experiment. The health of a jellyfish was evaluated based on the swimming rhythm with active swimming behaviour indicating good health. Two hours before the experiment, selected jellyfish were individually transferred to an incubator filled with 4 L of ASW. The purpose of this first 'acclimation/egestion' phase was to allow the medusae to egest any food they might have held in their gastric pouches. The experiment consisted of an initial Release phase, followed by an incubation phase with four incubation treatments: Jellyfish (ASW + jellyfish), Jellyfish-Control (ASW only), Mucus (ASW + mucus + ammonium), ~~Jellyfish-Control (ASW only)~~ and Mucus-Control (ASW + ammonium; Figure 2)~~and Mucus-Control (Figure 2)~~. First, the jellyfish were gently transferred by hand to the Release incubators (2 L of ASW) using sterile vinyl gloves, whilst trying to minimise the amount of water transferred with it. The Release phase allowed mucus and its associated microbes to be released into the water. After 1 hour, the jellyfish along with half of the volume of the water in the Release incubator (1 L) were transferred to the Jellyfish incubators (3 L of ASW; final volume = 4 L). The other half of the water was transferred to the Mucus incubators (3 L of ASW; final volume = 4 L). The controls (Jellyfish-Control and Mucus-Control) consisted of incubators containing only ASW (3 L of ASW).

As~~While~~ ammonia is continuously excreted by jellyfish, the nitrification rates associated with jellyfish in ASW (continuously increasing ammonium concentrations) would not be directly comparable to those associated with mucus in ASW (ammonium concentration of <0.1 µM). ~~the ammonia concentrations in the ASW were undetectable (<0.1 µM).~~ To allow direct comparison of nitrification rates in~~between~~ the Mucus and Jellyfish treatments, we simulated jellyfish ammonium excretion in both the Mucus and the Mucus-Control treatments by adding ammonium (NH_4Cl, Fisher Scientific, UK) to the incubators after each sample collection. The amount of ammonium added was estimated based on literature (Pitt and Purcell 2009) and previous trial experiments. The expected increase in ammonium concentrations ranged from 0.5 to 2.5 µM h^{-1} (SI, Table II) depending on species, size of the jellyfish and temperature.



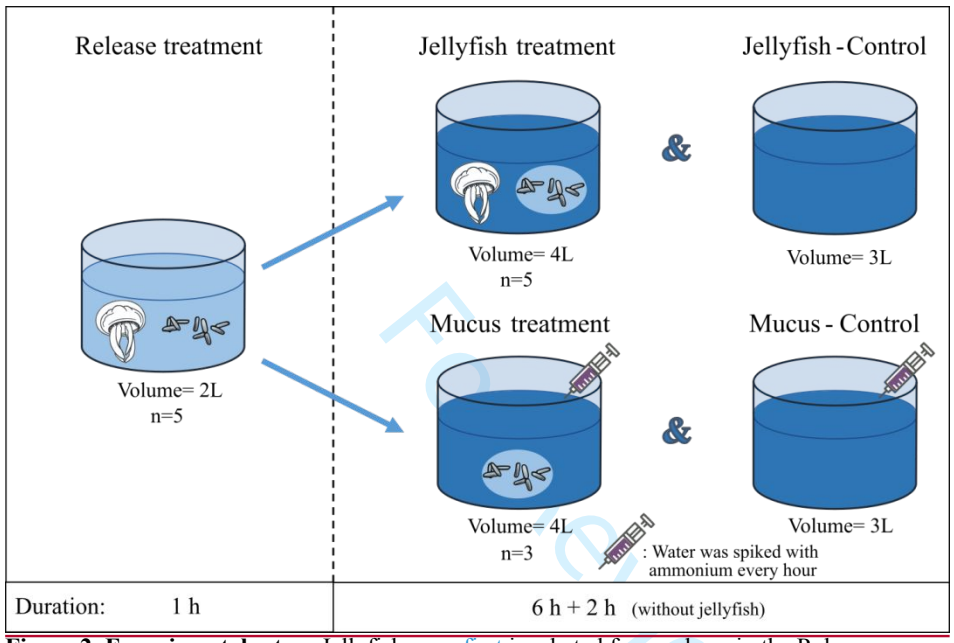


Figure 2: **Experimental setup**. Jellyfish were ~~first~~ incubated for one hour in the Release treatment. Subsequently, the jellyfish along with half of the volume from the Release phase were transferred to the Jellyfish treatment; the other half was transferred to the Mucus treatment. Controls for both experiments consisted of incubators containing only ASW. The Mucus and Control-Mucus incubators were spiked with ammonium every hour (SI, Table II~~I~~).

Rate measurements

~~The total incubation time of the Jellyfish, Mucus and Control treatments was 8 hours.~~

Water samples for nutrient analysis were collected every hour. Before collecting each sample, the water was stirred gently to homogenise it. Two sets of 15-mL samples (one for nitrate, nitrite and phosphate, and one for ammonium) were collected using a 20 mL polypropylene syringe. The sample was filtered through a 0.22-µm polyethersulfone sterile syringe filter (33-mm diameter, Millipore, UK) with the first 5 mL discarded to wash the filter. The remaining 10 mL

were collected in centrifuge tubes (polypropylene conical centrifuge tubes, 15 mL volume, Fisher Scientific, UK). For each treatment, a dedicated syringe was used to avoid cross-contamination. In between sample collection, the incubators were covered with a lid to avoid contamination.

~~While ammonia is continuously excreted by jellyfish, the ammonia concentrations in the ASW were undetectable (<0.1 µM). To allow direct comparison of nitrification rates between the Mucus and Jellyfish treatments, we simulated jellyfish ammonium excretion in the Mucus treatment by adding ammonium (NH_4Cl, Fisher Scientific, UK) to the incubators after each sample collection. The amount of ammonium added was estimated based on literature (Pitt and Purcell 2009) and previous trial experiments. The expected increase in ammonium concentrations ranged from 0.5 to 2.5 µM h^{-1} depending on species, size of the jellyfish and temperature (Table 1).~~ Based on ~~trial~~initial experiments and ~~previous~~findings of a previous study measuring ammonia release in *C. mosaicus* ~~literature~~ (Pitt et al. 2005), we ~~set up and~~decided ~~on~~ an incubation volume and duration of 4 L~~itres~~ and 6 hours~~, respectively,~~ as ~~sufficient~~ideal to measure a ~~constant~~linear and significant rate ~~release~~ of nutrients release without causing excessive stress to the jellyfish. ~~After 6 h of incubation,~~ T~~t~~he jellyfish were then removed from the incubators, and the jellyfish bell diameter and the wet weight (WW) was measured using a ruler and a ~~scale~~ balance (FireKingdom SF-400, DETAIL? ±1 g). ~~The,~~ W~~w~~ater sample collection continued for ~~another two~~ 2 hours after ~~the~~ removal of the jellyfish ~~in order to confirm the halt of nutrient release, bringing~~resulting ~~the~~in a total experiment duration ~~of the experiment to a total of~~~~to~~of 8 hours.~~.~~

Sample analysis

The duplicate sample for ammonium~~One of the duplicate water samples was immediately frozen. The other~~ was ~~used to measure ammonium~~~~analyzed~~analysed using the o-phthalaldehyde fluorometric method (Taylor et al. 2007; Holmes et al. 1999). The ammonium measurements were performed the same day using a Turner design Trilogy fluorometer (model 7200, US) with a UV module (7200-047). The duplicate sample for nitrite, nitrate and phosphate was immediately frozen for later analysis. Frozen samples were thawed at room temperature and phosphate, nitrate and nitrite concentrations were measured using standard gas segmented continuous flow spectrophotometric techniques (QuAAtro, Seal Analytical). The ~~ASW used to define the~~ baseline of the auto-analyser was determined using the same ASW ~~the same~~ as used in the ~~incubation~~ experiment (except for *C. fulgida* samples, for which we used ultra-high purity water ~~WHAT?~~ as baseline; detailed descriptions of the calibrations and detection limits in ~~the~~ SI).

Our hourly sampling regime, which provides a relatively low temporal resolution, was determined by the time it takes to collect the sample and the sample volume removed relative to the incubation volume. To determine the release rates at a higher temporal resolution, for one of the ~~Additionally, one~~ specimen of *A. aurita* incubations, ~~was incubated in 4 L of ASW and~~ nitrite and ~~/~~nitrate were measured ~~directly~~ at high-resolution (every 20 minutes) using a microfluidic lab-on-chip analyser (Beaton et al. 2012). ~~The combined (random + systematic) analytical uncertainty associated with nitrate + nitrite and phosphate measurements made using gas segmented continuous flow spectrophotometric techniques and lab-on-chip techniques is ≤ 5% (Birchill et al. 2019; details in SI).~~ This novel application of lab-on-chip microfluidic analysers allowed high-resolution measurements with small sample volumes and avoiding the

need for sample storage. The nitrate and nitrite concentrations measured using the "manual" and lab-on-chip method~~The combined (random + systematic) analytical uncertainty associated with nitrate + nitrite and phosphate measurements made using gas segmented continuous flow spectrophotometric techniques and lab-on-chip techniques is < 5% (Birchill et al. 2019; details in SI).~~ ~~The results~~ agreed ~~with the time-series samples measurements performed with the auto-analyser~~well (SI, Figure ~~SI-~~V) ~~(SI, Figure V, as shown by a linear), resulting in a~~ regression between the two methods (Auto-Analyzer = ~~line with a slope of~~ 1.04 +/- ~~SD~~0.06 Lab-on-Chip + 0.15 +/- ~~SD~~0.04; ~~*and a coefficient of determination (R^2) of*~~ = 0.98, $p < 0.001$, $n = 8$; SI, ~~(SI,~~ Figure ~~SI-~~VI). For both techniques, gas segmented continuous flow spectrophotometric and lab-on-chip, ~~T~~the combined (random + systematic) analytical uncertainty associated with nitrate + nitrite and phosphate measurements ~~made using gas segmented continuous flow spectrophotometric techniques and lab-on-chip techniques~~ was <5% (details in SI; Birchill et al. 2019).

Statistical analysis

Contamination, wall effects and production/absorption by microorganisms were accounted for by subtracting the changes in concentrations observed in the ASW controls from the treatments. In order to account for the loss of liquid due to the collection of nutrient samples, the total number of moles of nutrient released at each time point was calculated using the equation:

$$~~N~~n_{[t]} = ~~N~~n_{[t-1]} + V_{[t-1]} \times ~~*~~ (~~C~~c_{[t]} - ~~C~~c_{[t-1]})$$

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where ~~N~~ n is the number of moles released at a certain time point (t) since the beginning of the experiment, V is the volume of the incubator, and ~~C~~ c the molar concentration of nutrients (Giering et al. 2012).

The rates of nutrient (ammonium, phosphate, nitrite and nitrate) release per incubator (or per jellyfish for the Jellyfish treatment) were calculated using~~by~~ linear regression for each replicate. The rates were then normalised by the wet weight of the jellyfish and their differences were investigated by an analysis of covariance (ANCOVA; results are presented in SI)~~.~~. The rates of nutrient release per species were calculated by averaging the rates of the replicates for each species. Finally, the differences in weight-specific rates of nutrient release caused by the differences in experimental ~~temperatures,~~temperatures were standardized using Q_{10} temperature coefficient factors from the literature.~~:~~ F~~f~~or ammonium and phosphate release, a Q_{10} of 3.1 was used for *A. aurita* (Møller and Riisgård 2007), and the general Q_{10} of 2.66 was used for the other jellyfish species (Ikeda 2014). For nitrite and nitrate release rates, a Q_{10} of 2.2 was used for all species (Zheng et al. 2017), corresponding to the temperature coefficient factor of nitrifying microorganisms. Rates were adjusted to the median temperature of the experimental conditions (16°C) and N:P ratios were calculated as the sum of ammonium, nitrite and nitrite over phosphate. The temperature-corrected nutrient production rates were ~~then~~ plotted against the wet weight of the jellyfish, and a linear regression was fitted to investigate the allometric relationships between body weight and nutrient release rates~~excretion~~. ~~Lastly~~Finally, estimates of inorganic nitrogen release by jellyfish blooms were calculated using the allometric equations together with jellyfish densities from two case studies. The uncertainty range of these estimates were determined from the error on the allometric exponents and the temperature. All statistical analyses were carried out using R Statistical Software (R Core Team 2019).

Results and Discussion

Nutrient excretion and nitrification

To determine~~measure~~ rates of nitrification ~~catalyzed~~catalysed by members of the jellyfish microbiome, we performed incubation experiments with four species of non-zooxanthellate scyphozoan jellyfish, *A.~~urelia~~ aurita, ~~Chrysaora~~ C. hysoscella, ~~Chrysaora~~ C. fulgida* and *~~Chrysaora~~ C. pacifica.* We measured rates of ammonium and /phosphate excretion along with partial ($NH_4^+ \rightarrow NO_2^-$) and complete ($NH_4^+ \rightarrow NO_3^-$) nitrification associated with these jellyfish species. ~~-~~

~~**Nutrient excretion and nitrification**~~

Ammonium and ~~and~~ phosphate~~, nitrite and/or nitrate~~ concentrations ~~(see discussion below)~~ increased continuously in all incubations with jellyfish, whereas nitrite and nitrate concentrations increased only in the presence of three of the four species (see discussion below). For all nutrients, concentrations ~~and s~~stabilized or decreased once the jellyfish were removed (Fig. 3, Table 2; see SI for absolute concentrations, Figure I). In the presence of mucus alone, rates of nitrification were negligible for all investigated jellyfish species (<2.0 x 10^{-3} nmol L~~gWW~~$^{-1}$ h^{-1}; SI, Table III~~3~~), strongly suggesting that the observed rates of nutrient release were directly related to jellyfish metabolism and the associated microbiome. Mass-specific release rates of ammonium ranged from 23 to 86 nmol NH_4^+ gWW^{-1} h^{-1} at experimental temperatures ~~and from~~ (28 ~~to~~ - 86 nmol NH_4^+ gWW^{-1} h^{-1} when normalised to 16°C), which falls within the range of previous observations (2 - 111 nmol NH_4^+ gWW^{-1} h^{-1}: Pitt et al. 2013)~~, all species considered~~. The observed intraspecies variability of ammonium excretion was relatively low,

with *C. hysoscella* showing the highest variation (14%) in release rates across specimens. In contrast, ~~E~~excretion rates between different jellyfish species ~~in contrast~~ varied widely (up to 3.7-fold) ~~in accordance with previous observations (2.4 - 111.1 nmol NH_4^+ gWW^{-1} h^{-1}: Pitt et al. 2013)~~. Mass-specific release rates of phosphate ranged from 3.2 to 12~~1.9~~ nmol PO_4^- gWW^{-1} h^{-1} at experimental temperatures ~~(and from~~ 3.7 ~~to -~~ 12~~1.9~~ nmol PO_4^- gWW^{-1} h^{-1} when normalised to 16°C~~)~~ ~~across species~~. Excretion rates of phosphate were linearly correlated with ammonium excretion rates (all species included, not taking into account ammonium conversion; $p < 0.001$, $R^2 = 0.60$; $n = 17$~~???~~; SI, ~~SI,~~ Figure ~~SI~~ VII)~~; and were consistently lower than the ammonium release rates~~. ~~Net~~ A~~a~~mmonium: phosphate excretion ratios ranged from 2.7 to 15.2 with an average of 7.4, ~~comparable~~ in accordance with previous reports (~~e.g.~~ 8.2 for *A. aurita*, Shimauchi and Uye 2007; 8.7 for *C. mosaicus*, Pitt et al., 2005; 7.5 for *P. noctiluca,* Malej, 1991).

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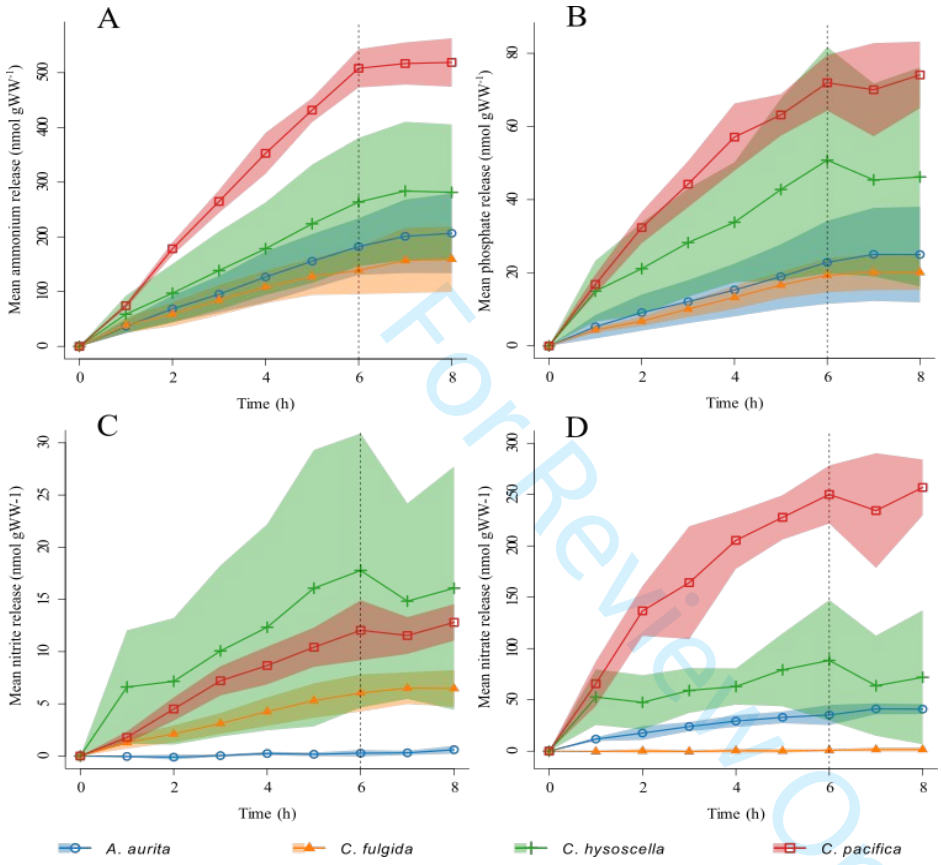

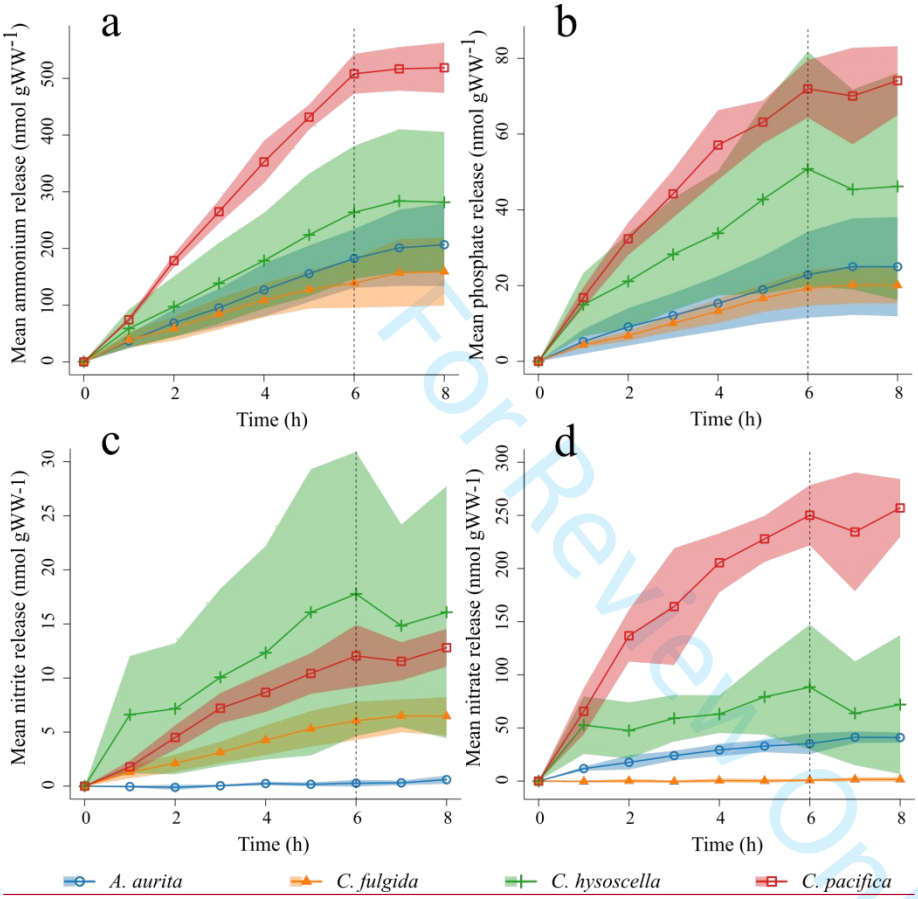

Figure 3. Mean cumulative release of (a~~A~~) ammonium ~~(A)~~, (b~~B~~) phosphate ~~(B)~~, (c~~C~~) nitrite ~~(C)~~ and (d~~D~~) nitrate ~~(D)~~ by ~~of the~~ ~~*Jellyfish*~~ *A. aurita* (blue circle), *C. fulgida* (yellow triangle), *C. hysoscella* (green cross) and *C. pacifica* (red square), ~~treatment~~ normalised ~~by~~to the wet weight (WW) of ~~the~~ each specimen~~s~~. ~~WW = wet weight.~~ Coloured area~~s~~ =indicate uncertainty envelopes (standard deviation) of the mean cumulative release of nutrients. Vertical dotted line corresponds to the~~with~~ time when the jellyfish were removed from the incubators (6 h).

Table 2. Release rates and regression statistics for the cumulative nutrient release by ~~Summary of results from the~~ the four jellyfish ~~treatments~~species. ~~Table presenting the results of l~~SD: ~~inear regressions from cumulative nutrient release from the different jellyfish species incubations, both the rates and~~ standard deviation ~~(SD)~~of the slope, *n*: number of observations. Rates at ~~both~~ experimental temperatures and adjusted to 16°C are presented, as well as ~~using Q_{10} law and~~ the N:P ratios ~~of the total release of nutrient~~ at 16°C. The rate, SD, ~~The values of R^2~~ and ~~*p* values~~ are ~~the~~ mean values from ~~values from~~ the replicates individual linear regressions ~~of the replicates. The values of rate, SD and number of observations (*n*) are from linear regressions on all replicates~~. ~~The use of~~ *, **, and *** denotes indicate levels of statistical significance (p <= 0.05, 0.01, and 0.001, respectively).

~~Species~~	~~Nutrient~~	~~Rate (nmol gWW^{-1} h^{-1})~~	~~SD~~	~~R^2~~	~~*p*~~	~~*n*~~	~~Rate (nmol gWW^{-1} h^{-1})~~	~~SD~~	~~N:P~~
~~*A. aurita*~~	~~Ammonium~~	~~30~~	~~2~~	~~0.99~~	~~***~~	~~35~~	~~34~~	~~3~~	~~10.1~~
	~~Phosphate~~	~~3.6~~	~~0.5~~	~~0.98~~	~~***~~	~~35~~	~~3.9~~	~~0.6~~	
	~~Nitrite~~	~~0.1~~	~~0.1~~	~~0.31~~	~~0.22~~	~~35~~			
	~~Nitrate~~	~~5.7~~	~~0.5~~	~~0.89~~	~~**~~	~~35~~	~~6.2~~	~~0.5~~	
~~*C. fulgida*~~	~~Ammonium~~	~~23~~	~~3~~	~~0.97~~	~~***~~	~~14~~	~~28~~	~~4~~	~~7.84~~
	~~Phosphate~~	~~3.2~~	~~0.3~~	~~0.99~~	~~***~~	~~14~~	~~3.7~~	~~0.3~~	
	~~Nitrite~~	~~1.0~~	~~0.1~~	~~0.99~~	~~***~~	~~14~~	~~1.2~~	~~0.1~~	
	~~Nitrate~~	~~0.1~~	~~0.1~~	~~0.15~~	~~0.26~~	~~14~~			
~~*C. hysoscella*~~	~~Ammonium~~	~~43~~	~~6~~	~~0.99~~	~~***~~	~~35~~	~~29~~	~~4~~	~~6.89~~
	~~Phosphate~~	~~7.9~~	~~1.4~~	~~0.94~~	~~***~~	~~35~~	~~5.7~~	~~1.0~~	
	~~Nitrite~~	~~2.8~~	~~0.7~~	~~0.87~~	~~**~~	~~35~~	~~1.9~~	~~0.5~~	
	~~Nitrate~~	~~11.9~~	~~2.6~~	~~0.61~~	~~**~~	~~35~~	~~8.7~~	~~1.9~~	
~~*C. pacifica*~~	~~Ammonium~~	~~86~~	~~2~~	~~0.99~~	~~***~~	~~35~~	~~86~~	~~2~~	~~10.85~~
	~~Phosphate~~	~~11.9~~	~~0.6~~	~~0.96~~	~~***~~	~~35~~	~~11.9~~	~~0.6~~	
	~~Nitrite~~	~~2.1~~	~~0.1~~	~~0.97~~	~~***~~	~~35~~	~~2.1~~	~~0.1~~	
	~~Nitrate~~	~~40.8~~	~~2.8~~	~~0.91~~	~~***~~	~~35~~	~~40.8~~	~~2.8~~	

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Species	Nutrient	Rate (nmol gWW^{-1} h^{-1})	SD	*n*	R^2	*p*	*n*	Rate at 16°C (nmol gWW^{-1} h^{-1})	SD	N:P
A. aurita	Ammonium	3030	8.12	355	0.99	***	7	34	9.13	10.31
	Phosphate	3.63.6	1.50.5	35	0.98	***	7	3.9	1.70.6	
	Nitrite	0.10.1	0.00.1	35	0.31	0.22	7			
	Nitrate	5.75.7	1.30.5	35	0.89	**	7	6.2	1.40.5	
C. fulgida	Ammonium	2323	4.53	142	0.97	***	7	28	5.54	7.894
	Phosphate	3.23.2	0.50.3	214	0.99	***	7	3.7	0.60.3	
	Nitrite	1.01.0	0.20.1	142	0.99	***	7	1.2	0.20.1	
	Nitrate	0.10.1	0.10.1	142	0.165	0.5226	7			
C. hysoscella	Ammonium	4343	176	355	0.99	***	7	29	11.54	6.9589
	Phosphate	7.97.9	4.11.4	355	0.94	***	7	5.7	2.81.0	
	Nitrite	2.82.8	1.90.7	355	0.87	**	7	1.9	1.40.5	
	Nitrate	1211.9	6.02.6	355	0.61	**	7	8.7	4.41.9	
C. pacifica	Ammonium	8686	5.02	355	0.99	***	7	86	5.02	10.85
	Phosphate	1211.9	1.20.6	355	0.96	***	7	11.912	1.20.6	
	Nitrite	2.12.1	0.40.1	355	0.987	***	7	2.1	0.40.1	

Nitrate	4140.8	3.12.8	355	0.91	***	7	410.8	3.12.8

Table 3. Summary of the linear regressions on nitrite and nitrate release from the Mucus treatment. Table shows significant results only ($p<0.05$). The use of *, **, and *** denotes levels of statistical significance ($p = 0.05$, 0.01, and 0.001 respectively).

Species	Nutrient	Rate (nmol gWW^{-1} h^{-1})	R^2	p
A. aurita	Nitrite	0.001	0.25	**
C. hysoscella	Nitrate	-0.002	0.18	*
C. pacifica	Nitrite	-0.001	0.19	*

Ammonia oxidation is usually considered the rate-limiting step in nitrification (Prosser 1990; Heiss and Fulweiler 2016; Zhang et al. 2020): nitrite is immediately oxidized by free-living nitrite-oxidizing bacteria, preventing its accumulation at significant rates. We observed these expected dynamics in the presence of *A. aurita*, when nitrite concentrations did not increase whereas nitrate accumulated (5.7±10.35 nmol NO_3^- gWW^{-1} h^{-1};; Table 2), indicating a tight coupling of both nitrification steps. However, this paradigm did not apply to nitrification in association with the other three jellyfish species that we investigated. In the presence of *C. hysoscella* and *C. pacifica,* accumulation rates were significant for both nitrite (*C. hysoscella*: 2.87±10.97 nmol NO_2^- gWW^{-1} h^{-1}; *C. pacifica* 2.1±0.41 nmol NO_2^- gWW^{-1} h^{-1}) and nitrate (*C. hysoscella*: 121.9±62.06 NO_3^- nmol gWW^{-1} h^{-1}; *C. pacifica* 410.8±32.18 nmol NO_3^- gWW^{-1} h^{-1};) (Table 2). The decoupling was more pronounced in incubations with *C. hysoscella* (nitrite accumulation rate was ~230% of the nitrate accumulation rate), whereas nitrite accumulation in association with *C. pacifica* was lower (5< 3% of nitrate accumulation). During the incubations with C. *fulgida*, ammonia oxidation to nitrite was the only detectable nitrification process (1.0±0.21 nmol NO_2^- gWW^{-1} h^{-1};; Table 2).

In the presence of *C. hysoscella* and *C. pacifica* both nitrite (*C. hysoscella*: 2.7±0.7 nmol NO_2^- gWW^{-1} h^{-1}; *C. pacifica* 2.1±0.1 nmol NO_2^- gWW^{-1} h^{-1}) and nitrate (*C. hysoscella*:11.9±2.6 NO_3^- nmol gWW^{-1} h^{-1}; *C. pacifica* 40.8±2.8 nmol NO_3^- gWW^{-1}h^{-1}) accumulated at significant rates (Table 2). The accumulation of nitrite suggests a decoupling of the two steps of nitrification as nitrite is produced faster than consumed. The decoupling was more pronounced in incubations with *C. hysoscella*, in which the amount of nitrite accumulated to > 20% of the amount of nitrate, while nitrite accumulation in association with *C. pacifica* was much lower (< 3% of nitrate production). In contrast, during the incubations with C. *fulgida*, ammonia oxidation to nitrite was the only detectable nitrification process and occurred at comparably low rates (1.0±0.1 nmol NO_2^- gWW^{-1} h^{-1}, Table 2). In the presence of *A. aurita*, only nitrate accumulated (5.7±0.5 nmol NO_3^- gWW^{-1} h^{-1}, Table 2), indicating a tight coupling of both nitrification steps. Ammonia oxidation is usually considered as the rate- limiting step in nitrification (Prosser 1990; Heiss and Fulweiler 2016; Zhang et al. 2020). Thus, under environmental conditions, the nitrite would likely be immediately oxidized by free-living nitrite-oxidizing bacteria, preventing its accumulation at significant rates. However, this paradigm does not appear fully transferable to nitrification in association with most of the jellyfish species that we investigated here. To our knowledge, two other studies investigated the nitrite + /nitrate (NO_x, no distinction made) release by non-zooxanthellate scyphomedusae: Pitt et al. (2005) found that *C. mosaicus* released <2% of the released ~~N~~ nitrogen in form of NO_x ~~nitrite/nitrate~~, and Shimauchi & Uye (2007) did not observe significant release of NO_x ~~nitrite/nitrate~~ associated with *A. aurita*. The latter study contrasts with our observation that 16% of the released ~~the~~ nitrogen ~~released~~ by *A. aurita* was in the form of nitrate. ~~We suggest that this~~This discrepancy indicates a potential effect of ~~life history or~~past and present ~~that the environment~~environmental ~~al effects~~conditions on the jellyfish-associated microbial community composition ~~in which a jellyfish lives influences the community composition of the jellyfish's microbiome~~ and, ~~hence~~ subsequently, on the balance of jellyfish-

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associated nitrification rates. In ~~addition~~ contrast to the hypothesis that nitrifiers are specific to zooxanthellate jellyfish (Welsh et al. 2009), our results ~~further~~ instead suggest that both, zooxanthellate and non-zooxanthellate, jellyfish are potential hosts for nitrifier ~~hosts~~ and can thus, ~~providing~~ ~~representing~~ abe a source of nitrite and /nitrate to the environment.

Together ~~N~~nitrite and nitrate release rates were 5-~~ to~~ 50% ~~both~~ lower than ammonium excretion rates ~~by 5 to 50% each~~ (Figure 4) and ~~together~~ contributed~~. Together, nitrite and nitrate made up~~ 5-~~ to~~ 33% of the total inorganic nitrogen release~~d~~. Under ~~sufficient~~ saturating substrate ~~concentration~~ levels (ammonia and nitrite), ~~the~~ nitrification reactions follow a zero-order kinetic (Chen et al. 2006), meaning that increases in substrate concentration do not increase the reaction rates. As ~~there was much more~~ ammonium excretion exceeded that of~~released than~~ nitrite and ~~or~~ nitrate substantially, we conclude that nitrification rates were not limited by ammonia availability ~~was not limiting the nitrification rates~~ in any of the experiments. Moreover, since the total ammonia concentrations of the incubators were well ~~much lower than the safe concentrations~~below toxicity levels ~~measured~~ for polyps and ephyrae (2 mg L^{-1}, Jianlong et al. 2018), we are confident that the observed nitrification rates ~~observed here are true reflections~~are reflective of in-situ processes.

Between species, the rates of nitrification varied more than the excretion rates of ammonium and phosphate. For example, we observed a >-6-fold difference between the nitrate release rates of *A. aurita* and *C. pacifica* (Table 2, Figure 4). Both the inter- and intraspecies variability observed in ammonia and phosphate excretion as well as nitrification rates can ~~be~~ partly be explained by allometric scaling of the mass-specific release rates to the wet weight of each individual (ammonium excretion: 80%, phosphate excretion: 73%, nitrification: 55%, ~~Table 4;~~ Figure 5; SI, Table VI). The allometric relationships for the ammonium, phosphate and ~~specific release and the~~ nitrate-specific release (ASR, ~~and~~ NSR and PSR, respectively; nmol gWW^{-1} h^{-1}) were:

513 **ASR=1.84 ×~~x~~ 10^3 ±1.6 $WW^{-0.82\pm0.10}$** ——— ($p<0.001$, $R^2=0.80$, $n=17$) ———(1)

514 **PSR=369 ±1.9 $WW^{-0.90\pm0.13}$** ($p<0.001$, $R^2=0.73$, $n=17$) (2)

515

516 **NSR=2.84 ×~~x~~ 10^3 ± 3.6 $WW^{-1.20\pm0.28}$** ($p<0.001$, $R^2=0.55$, $n=15$)————

517 (3~~2~~)

518 The negative scaling exponents indicate that smaller specimens release more nutrients
519 ~~(ammonium and nitrate)~~ per gram of mass, and hence follow the expected allometric scaling. The
520 high rates of nutrient excretion and nitrification associated with *C. pacifica* can therefore be
521 partly explained by the small size of these specimens (~~i.e.:~~ 35 – 59 gWW) compared to the other
522 investigated species. Similarly, the high variability in *C. hysoscella* rates ~~is associated~~
523 ~~with~~matches the wider range of ~~specimen~~ wet weights per individual (~~i.e.:~~ 100 – 278 gWW,
524 Table 1). All scaling exponents (Equation 1, 2 and 3; SI: Slope, Table VI4) were lower than the -
525 ¼ allometric exponent commonly observed for other zooplankton mass-specific physiological
526 processes (Arhonditsis et al. 2019). We suggest that this divergence relates to the jellyfish's high
527 water contents and unique body plans (Pitt et al. 2013). The scaling exponent of the nitrate
528 release allometric equation (-1.20±0.28, ~~equation~~ Equation 3~~2~~) being lower than the exponent for
529 the ammonium release (-0.82±0.10, E~~e~~quation 1) indicates that, ~~while~~ when wet weight~~WW~~
530 increases, the nitrate-specific rate decreases faster than the ammonium-specific rate. This
531 difference in scaling exponent is likely to be related to the changes in the jellyfish surface-to-
532 volume ratio~~:~~, ~~with~~ the release of ammonium is likely more depending on the jellyfish's body
533 volume as it is a metabolic waste product, whereas nitrate is likely more dependent on the
534 jellyfish surface owing to the association with the microbiome living on the jellyfish ~~and the~~
535 ~~release of ammonium being more dependent on the jellyfish surface and body volume,~~
536 ~~respectively~~. Our data show that release rates by jellyfish are highly variable between

populations,. yYet, when normalized to wet weight, we observe a strong allometric scaling. This observation is promising as it indicates that thesehighlights the potential for thesely important pathways mayto be reasonably easy to (see next sections) can be easily representedincorporated into models.

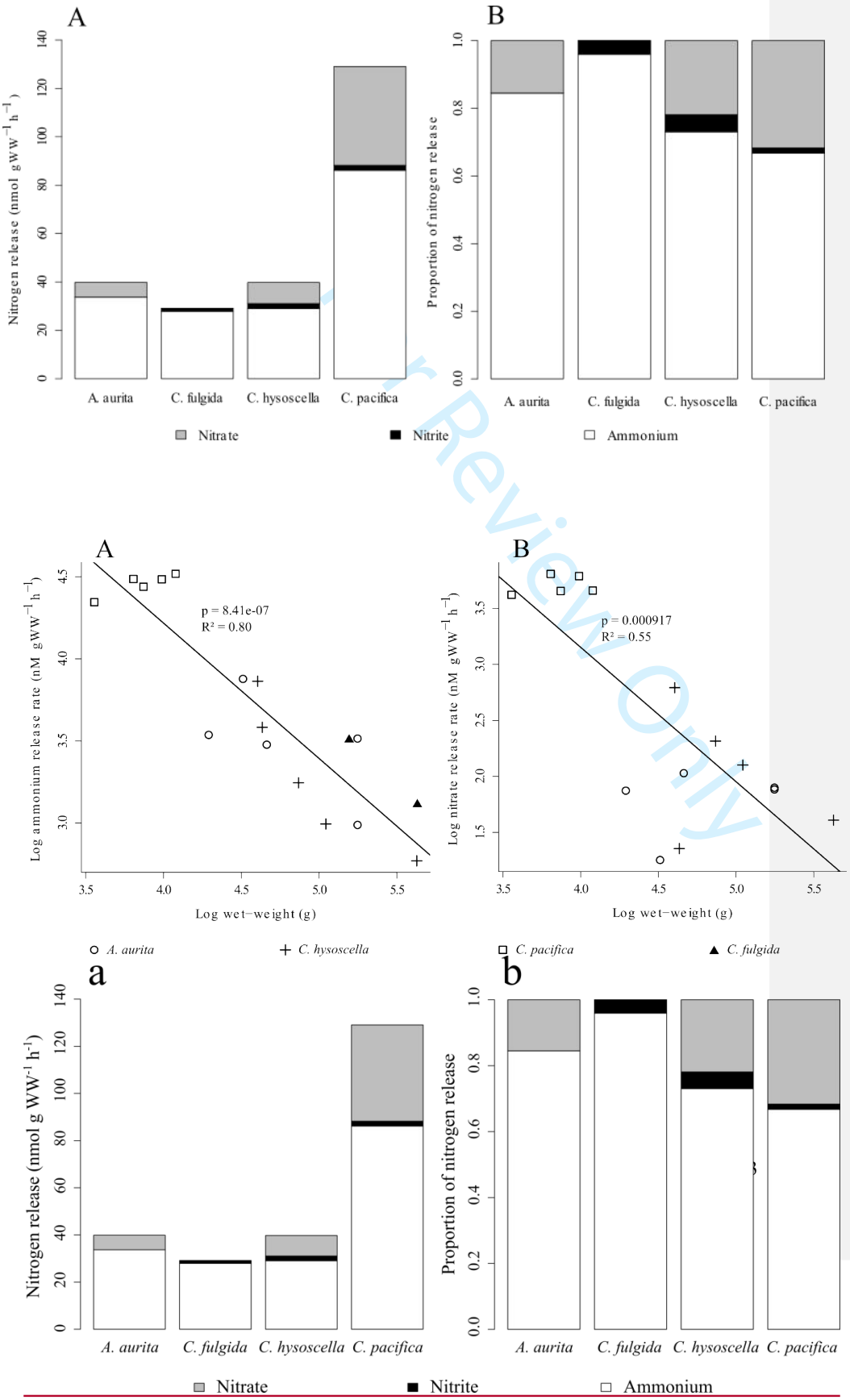

Figure 4. ~~A: Inorganic nNitrogennn compounds~~ release rates of different jellyfish species (a~~A~~) normalized by the wet weight of the specimens, and (~~b~~B) as proportion of total ~~:~~ ~~Relative~~inorganic nitrogen ~~compounds~~ release. WW= wet weight.

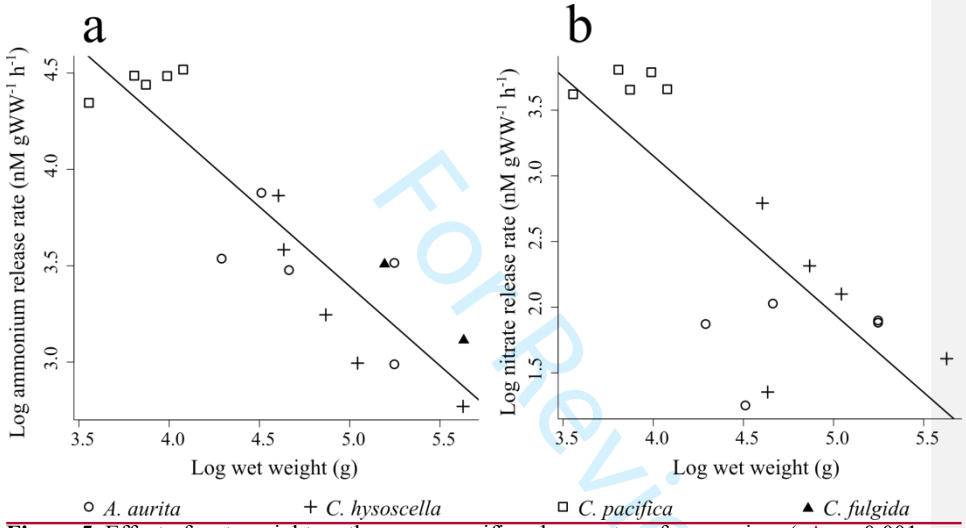

Figure 5. Effect of wet ~~-~~weight on the mass-specific release rates of ammonium (a~~A~~; $p<0.001$, $R^2=0.80$, $n=17$) and nitrate (b~~B~~; $p<0.001$, $R^2=0.55$, $n=15$) for the jellyfish *A. ~~urelia~~ aurita* (circle), *C. hysoscella* (cross), *C. pacifica* (square) and *C. fulgida* (triangle) at 16°C. The black line is the linear regression. No significant release of nitrate was observed for *C. fulgida*.

~~**Table 4.** Summary of the linear regressions on the effect of wet-weigh on the mass-specific nutrient releases normalised to 16°C. The use of *, **, and *** denotes levels of statistical significance (p = 0.05, 0.01, and 0.001 respectively).~~

~~Nutrient~~	~~Intercept~~	~~Slope~~	~~SD~~	~~R^2~~	~~p~~
~~Ammonium~~	~~7.52~~	~~-0.82~~	~~0.10~~	~~0.80~~	~~***~~
~~Phosphate~~	~~5.91~~	~~-0.90~~	~~0.13~~	~~0.73~~	~~***~~
~~Nitrite~~	~~3.55~~	~~-0.88~~	~~0.67~~	~~0.05~~	~~0.20~~

~~Nitrate 7.95 -1.20 0.28 0.55 ***~~

Evidence of active nitrifying microorganisms in jellyfish~~Nitrifying microbiome~~

~~Recent studies have shown that~~ jJellyfish host diverse microbial communities on their epithelium as their mucus provides an attractive niche for ~~bacteria~~ microorganisms (Tinta et al. 2012~~,~~ 2019; Weiland-Bräuer et al. 2015; Kramar et al. 2019). Two species of nitrifiers, ~~T~~the ammonia-oxidizing bacterium *Nitrosospira multiformis* and the nitrite-oxidizing bacterium *Nitrospira moscoviensis*, ~~These communities are different from the surrounding seawater microbial communities and can vary between species and organs (Kramar et al. 2019). The presence of nitrifying microorganisms on zooxanthellate jellyfish species has been suggested previously based on nitrate release (Welsh et al. 2009) were previously~~have been identified as members of the microbiome of jellyfish *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-Bräuer et al. 2015). However, neither of these nitrifiers were highly abundant (<2% of total operational taxonomic unit; Lee et al. 2018). The high nitrification rates we observed ~~, but no supporting molecular data on the jellyfish microbiome were available~~Our observations strongly ~~indicate~~ supports the presence of either highly active ~~and/~~or highly abundant nitrifying microorganisms in the jellyfish microbiome. The low coupling between nitrification rates could be caused by poor diffusional connectivity between nitrifiers (Welsh et al. 2001), i.e., a fraction of the produced nitrite might diffuse directly to the water column rather than to a zone where it can be oxidised to nitrate. ~~The~~ The differential production of nitrite and~~/or~~ nitrate associated with ~~the~~ the four~~studied~~ jellyfish populations ~~may that we~~ investigated strongly indicates variable community composition or distribution of the microbiome on the jellyfish depending on jellyfish species or ~~as a result because of~~ environmental factors. ~~Equally, the low coupling between ammonia and nitrite oxidation could indicate poor diffusional connectivity between zones of~~

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ammonia and nitrite oxidation within the mucus layer (Welsh et al. 2001)suggests that the composition and activity of the nitrifying community is variable across these populations. *C. fulgida* induced the production of nitrite only, indicating the absence or inactivity of nitrite-oxidizing bacteria. Nitrate produced in incubations with *A. aurita* in contrast suggests the presence of ammonia and nitrite oxidizers, and the partial uncoupling of nitrification observed in incubations with *C. hysoscella* and *C. pacifica* indicate low abundance or activity of nitrite-oxidizing bacteria. In Figure 6, we present our interpretation of the pathways of nitrogen cycling associated with the jellyfish and its microbiome: the ammonia excreted by the jellyfish is partially utilized by ammonia-oxidizers located on the jellyfish to produce nitrite, which can then be partially or totally oxidized by nitrite-oxidizers on the jellyfish. Depending on the environment the jellyfish lives in, the balance between ammonia oxidizers and nitrite oxidizers may vary.

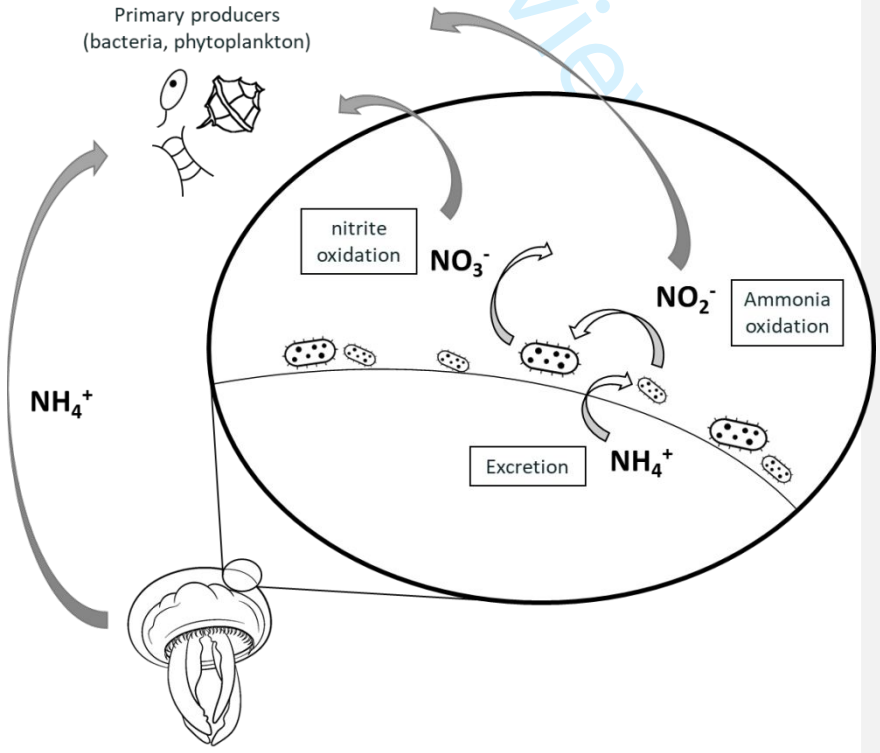

Figure 6. Schematic on the role of jellyfish in the surface marine nitrogen cycle and the hypothesized nitrification pathways associated with its microbiome. White arrows represent processes that are associated with the jellyfish; grey arrows represent processes happening in the water column.

To date, only two species of potential nitrifiers have been observed in association with both the jellyfish *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-Bräuer et al. 2015), the ammonia-oxidizing bacteria *Nitrosospira multiformis* and the nitrite-oxidizing bacteria *Nitrospira moscoviensis*. However, neither of these nitrifiers were highly abundant on the jellyfish (<2% of total operational taxonomic unit; Lee et al. 2018). In addition, several studies failed to identify any ammonia or nitrite oxidizers in jellyfish microbiomes, including Thaumarchaeota (Tinta 2016, Daley 2016, Kramer 2019) which are often associated with sponges and corals (Beman et al. 2007; Radax et al. 2012; Feng et al. 2016). The consistency of the nitrite and nitrate release rates across biological replicates indicate that jellyfish specimens from the same population share similar nitrifying communities. In addition, the absence of nitrate releases associated with *A. aurita* from the Inland Sea of Japan (Shimauchi and Uye, 2007) suggests that the nitrifying community harboured by a jellyfish is in parts determined by environmental factors. The role of the host and the environmental and biotic factors in determining the association with nitrifiers remains to be investigated. The presence of nitrifying microorganisms on zooxanthellate jellyfish species has been suggested previously based on nitrate release (Welsh et al. 2009), but no supporting molecular data on the jellyfish microbiome were available. Our observations suggest that nitrifying activity is widespread amongst the microbiome of a variety of jellyfish populations. While our findings are representative only of a subset of jellyfish populations, the diverse identity and origin of the investigated specimens strongly supports our hypothesis of a widespread association with nitrifying bacteria and archaea. However, the intriguing contradiction between the apparent absence or low abundance of

nitrifiers reported in previous molecular studies and the high rates of nitrification observed in our study suggests that we currently cannot identify the responsible nitrifiers. The detailed nature of this association requires further investigations Future research should use molecular tools andincluding molecular omic technologies approaches to investigate determine the identity and distribution of nitrifiers on within the jellyfish microbiome.

Evidence of active nitrifying microorganisms in jellyfish

Our observations strongly indicate the presence of highly active nitrifying microorganisms in the jellyfish microbiome and suggest that this association is widespread amongst jellyfish populations. The nitrate produced in incubations with *A. aurita* indicates the presence of both ammonia and nitrite oxidizers. The partial uncoupling of nitrification observed in incubations with *C. hysoscella* and *C. pacifica* suggests low abundance or activity of nitrite-oxidizing bacteria while the production of nitrite only, induced by *C. fulgida* indicates the absence or inactivity of nitrite-oxidizing bacteria. Low coupling between nitrification rates could also be caused by poor diffusional connectivity between nitrifiers (Welsh et al. 2001). For example, a fraction of the nitrite produced might be diffused directly to the water column rather than to a zone where it can be oxidised to nitrate. Nevertheless, the consistency of the nitrite and nitrate release rates across biological replicates show that jellyfish specimen from the same population share similar nitrifying communities. In addition, the absence of nitrate releases associated with *A. aurita* from the Inland Sea of Japan (Shimauchi and Uye, 2007) suggests that the nitrifying community harboured by a jellyfish is in parts determined by environmental factors. While our findings are representative only of a subset of jellyfish populations, the diverse identity and origin of the investigated specimens strongly supports our hypothesis of a widespread association. The intriguing contradiction between the apparent absence or low abundance of nitrifiers reported in previous molecular studies (<2% of total operational taxonomic unit; Lee et al. 2018) and the high rates of nitrification observed in our study suggests

that we currently cannot identify the responsible nitrifiers. Future research should use molecular tools and omic technologies to investigate the identity and distribution of nitrifiers on jellyfish.

Ecological implications

During jellyfish blooms, the release of different forms of bioavailable inorganic nitrogen (nitrite, nitrate and ammonium) could be substantial (see "Case studies" section below) and would influence primary producer community composition in the surface ocean (Figure 6; Shilova et al. 2017). Organic nitrogen fixed by primary producers is partly incorporated into gelatinous biomass through the food chain (black arrows, figure 6). Organic and inorganic nitrogen is then excreted by jellyfish in the form of mucus and ammonium, respectively. The ammonium excreted diffuses through the mucus layer where it is party oxidized into nitrite and nitrate by ammonia-oxidizers and nitrite-oxidizers. Ultimately, the different forms of nitrogen released by the jellyfish (ammonium: 80±12%, nitrite: 3±2%, nitrate: 17±13%) are used as a source of both nutrient and energy by primary producers, which supports regeneration of organic matter. While nitrification in the photic zone has been widely demonstrated (e.g. Yool et al. 2007, Smith et al. 2014, Beman et al. 2012; Dehairs et al. 2015), nitrification rates are usually relatively low near the surface and increase toward the deep chlorophyll maximum (Smith 2014). These low rates occur because ammonia is scarce and primarily taken up by phytoplankton, which are more efficient in ammonia uptake than prokaryotes. An association with jellyfish would allow prokaryotic nitrifiers direct access to ammonium, thereby bypassing competition for this otherwise scarce resource. The effect of jellyfish-mediated nitrogen release on the community composition of primary producers, in addition to the partial or complete nitrification fuelling dark carbon fixation in the sunlit surface ocean, indirectly influences the quantity and quality of organic matter that sinks to depth (Basu and Mackey, 2018).

The is shown by black and white arrowsColours indicate ammonium (NH_4^+, orange), nitrite (NO_2^-, yellow), nitrate (NO_3^-, green) and organic matter (OM). Cammonium (orange-to-yellow)

and nitrite-oxidation (yellow-to-green) to. Fluxes to the nitrogen pool from organisms other than jellyfish are represented by small coloured circles.What are the numbers? Can you please change the circles to have little arrows? What is the yellow zig-zag arrow? What is the blow-up bubble in the middle?

Ecological implications

Jellyfish have been recognized to stimulate primary production through the excretion of ammonium and phosphate (Pitt et al. 2005). The average inorganic N:P ratio of the released nutrients (7.3 – 10.9, Table 2; SI, Figure VIII) lies below the Redfield Ratio (N:P = 16; Redfield 1963) and substantially below the N:P ratios of their main diet, zooplankton (N:P > 20; Elser and Hassett 1994). Thus, the gelatinous biomass of these jellyfish appears to retain nitrogen efficiently, which is further supported by their -low molar C:N ratio (4.4; (Lucas Cathy H. et al. 2011) 5 ± 1.1; Pitt et al. 2009)compare to other marine zooplankton organisms (4.8-6.2 for crustacean zooplankton; (Pitt et al. 2013). -By storing nitrogen over phosphorus, COMPARED TO WHAT, also why would they want to retain N? What in their body composition would explain that?. An expanding jellyfish blooms may locally drive the ecosystem toward N-limitation (Sterner, 1990). On the other handWhereas, under starvation, while jellyfish consume up to 85% of their own nitrogen-rich tissues under starvation (Pitt et al. 2014, Lilley et al. 20146), the which is potentially reflected in an increased N:P ratio of the excreted nutrients would increaseammonia release. Starvation, a major cause of jellyfish bloom decline (Pitt et al. 2014), could temporarily drive the ecosystem towards P-limitation. A large jellyfish bloom could thus act as a "nitrogen buffer", storing nitrogen over phosphorus when food is abundant and releasing nitrogen over phosphorus during its decay. However, the relevance of this hypothetical mechanism remains to be investigated.

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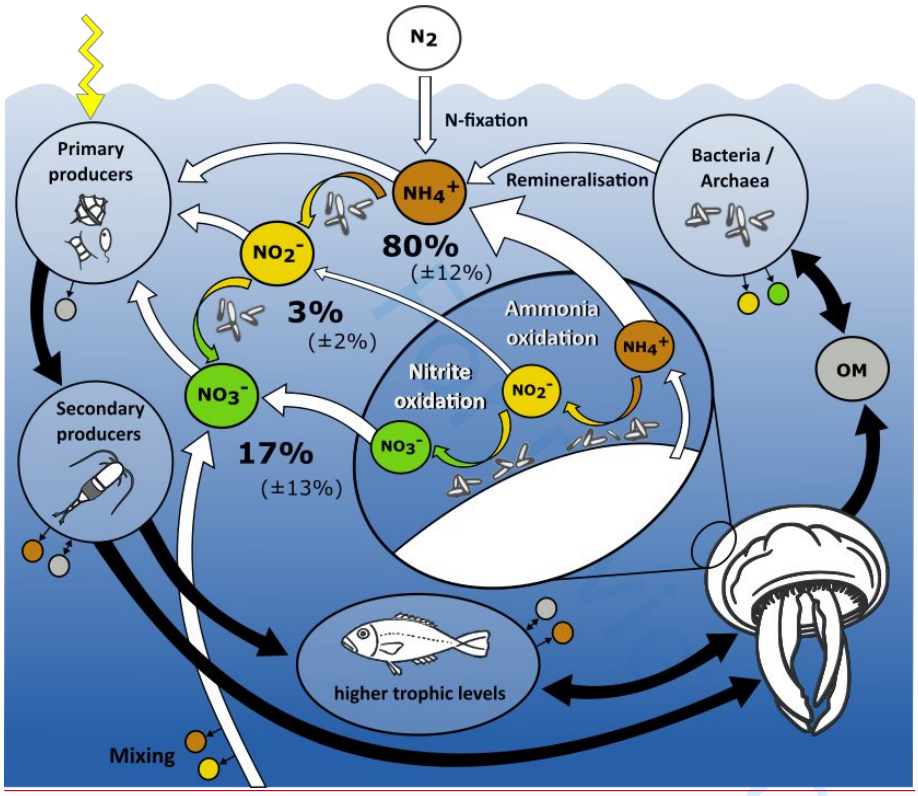
Our findings demonstrate that a significant substantial fraction (ammonium: 80±12%, nitrite: 3±2%, nitrate: 17±13%) of the released excreted ammonium is shunted through partial or

688 complete nitrification (ammonium: 80±12%, nitrite: 3±2%, nitrate: 17±13%; Figure 6), thereby
689 fuelling dark carbon fixation in the sunlit surface ocean ~~while producing the more stable, but~~
690 ~~assimilatory more costly nitrate~~. ~~While nitrification in the photic zone has been widely~~
691 ~~demonstrated (e.g. Yool et al. 2007, Smith et al. 2014, Beman et al. 2012; Dehairs et al. 2015),~~
692 ~~nitrification rates are usually relatively low near the surface and increase toward the deep~~
693 ~~chlorophyll maximum (Smith 2014). These low rates occur because ammonia is scarce and~~
694 ~~primarily taken up by phytoplankton, which are more efficient in ammonia uptake than~~
695 ~~prokaryotes.~~ An association with jellyfish ~~would~~ allows ~~prokaryotic~~ nitrifiers direct access to
696 ammonium in the surface ocean, thereby bypassing competition with phytoplankton for this
697 otherwise scarce resource (Smith et al. 2014; Zakem et al. 2018). D ~~D~~uring jellyfish blooms~~,~~
698 ~~which occuring frequently in some coastal areas (e.g.: Sea of Japan, Black sea, Benguela current,~~
699 ~~Antarctic; Brotz et al. 2015)~~, the release of different forms of bioavailable inorganic ~~nitrogen~~
700 (nitrite, nitrate and ammonium) ~~or organic nitrogen (mucus) nitrogen~~ has the potential to locally
701 enhance surface primary production and even influence phytoplankton community composition
702 (Figure 6; Shilova et al. 2017). ~~could be substantial (see "Case studies" section below) and~~
703 ~~would influence phytoplankton community composition in the surface ocean (Figure 6; Shilova~~
704 ~~et al. 2017).~~ This effect on the community composition, ~~which~~ in turn~~,~~ could ~~determines~~ impact
705 the quantity and quality of organic matter that sinks to depth (Basu and Mackey~~,~~ 2018).
706
707
708 ~~**Case studies**~~

Commented [SLCG9]: Is this something you added in response to a reviewer comment? You have not talked much about organic nitrogen, so I am not sure it is needed here. Reading this, I wondered: if nitrogen in mucous is important, why did you not measure it?

709



710

711 **Figure 6**. Conceptual diagram of the role and position of jellyfish in the surface marine nitrogen
712 cycle. The flow of organic and inorganic matter is shown by black and white arrows,
713 respectively. Colours indicate ammonium (NH_4^+, orange), nitrite (NO_2^-, yellow), nitrate (NO_3^-,
714 green) and organic matter (OM, grey). Coloured arrows represent ammonium-oxidation (orange-
715 to-yellow) and nitrite-oxidation (yellow-to-green). Components linked to small coloured circles
716 release/assimilate nutrients of the same colour. The average release of nitrogen forms are
717 presented as percentage (± standard deviation) of total dissolved inorganic nitrogen released by
718 jellyfish. The yellow zigzag arrow represent light. The large middle circle zooms in on the
719 jellyfish epithelium.

~~Jellyfish are known to frequently form blooms in estuaries and coastal habitats, especially in those impacted by human activities (Purcell 2012).~~ To explore the potential relevance of ~~such~~ jellyfish blooms ~~on~~ surface nitrogen cycling, we extrapolated our nitrification rate measurements based on two jellyfish blooms, whose spatial extend was measured in high -resolution ~~measurementdatasets on the scale and densities of jellyfish blooms~~ (Lynam et al. 2006, Han & Uye, 2009) ~~(Lynam et al. 2006, Han & Uye, 2009), used two of the very few available datasets presenting high-resolution measurement on the scale and densities of jellyfish blooms (Lynam et al. 2006, Han & Uye, 2009). Both studies used acoustic measurements, providing estimates of jellyfish abundance in the shallow eutrophic and brackish Honjo lagoon northwest of Lake Nakaumi, Japan (Han & Uye, 2009) and the coastal area of Namibia representing the Northern Benguela Upwelling System (Lynam et al. 2006).~~. The blooms were observed in (1)

~~In the Honjo District Lake,~~ the shallow~~a~~ eutrophic and brackish Honjo lagoon, northwest of Lake Nakaumi, Japan (Han & Uye, 2009), and in (2) the coastal area of Namibia representing the Northern Benguela Upwelling System (Lynam et al. 2006). We applied our allometric equations for ammonium and nitrate release (Equation 1 & 3) to the average body characteristics of the jellyfish (Table 3), corrected for temperature (Table 3 and as described in methods), and multiplied by abundance.

~~water lake connected with the Sea of Japan, *Aurelia coerula* (a cryptic species to *A. aurita* and until recently named as *A. aurita*) is highly abundant (up to 18 medusae m^{-3}) from June to November (Han et al., 2009; Han & Uye, 2009). During these months, average ammonium and nitrate levels are consistently low (0.01 mg L^{-1} for both ammonium and nitrate; Chugoku Regional Development Bureau 2018) and jellyfish are thought to ingest up to 29% of the mesozooplankton biomass per day, corresponding to 47% of the daily mesozooplankton production rate (Han et al. 2009). The Benguela Upwelling System, one of the most productive coastal upwelling systems, (Carr 2001) harbours large *C. fulgida* populations (previously~~

identified as *C. hysoscella)* in its northern region throughout the year, peaking in June–August (Flynn et al. 2012). density and distribution data of jellyfish are available for both ecosystems (Honjo District Lake, August 2007, Han & Uye, 2009; Northern Benguela upwelling, August 2003, Lynam et al. 2006). Based on these data, average bell diameter and wet weight of *A. aurita* and *C. fulgida* and the mean *in-situ* temperature for each region (tTable….), we estimated the total nitrogen release associated with these populations using our allometric relationship for ammonium-specific release and nitrate-specific release (equations 1 and 2) and by applying a temperature correction (as described in the methods).**Table 3**. Overview of case studies. Surface temperature at sampling time and body characteristics of jellyfish used to estimate inorganic nitrogen release. [a] calculated from Han et al. (2009), [b] mean annual surface temperature in August from Junker et al. (2017), [c] calculated from Houghton et al. (2007).

Location	Species	Surface temperature (C°)	mean WW (g)	mean bell diameter (cm)	References
Honjo District	*A. coerula*	28 - 28.7	92.5[a]	13.1	Han & Uye. 2009
Northern Benguela	*C. fulgida*	13[b]	1100[c]	27	Lynam et al.. 2006

In the Honjo District Lake, *Aurelia coerula* (a cryptic species to *A. aurita* and until recently named *A. aurita*) is highly abundant (up to 18 medusae m^{-3}) from June to November and are thought to ingest up to 47% of the daily mesozooplankton production (Han et al. 2009; Han & Uye 2009). During these months, average ammonium and nitrate levels are consistently low (≤0.01 mg L^{-1} for both ammonium and nitrate; Chugoku Regional Development Bureau 2018). We estimated based on the details in Table 3 that the large aggregation of We estimated the rates of ammonium release and nitrification associated with *A. coerula* in this lake based on population densities obtained in August 2007 (Han & Uye, 2009), the mean *in-situ* temperature of 28.3°C (see Materials, Han & Uye, 2009), and our allometric relationship for ammonium-specific release

and nitrate-specific release (equations 1 and 2), assuming a bell diameter of 13.1 cm and a corresponding wet weight of 92.5 g (Han and Uye 2009). The We found that the large aggregation of *A. coerula* in the Honjo District Lake could potentially have released up to 1.7 mmoles N m^{-2} h^{-1} (uncertainty range: 1.0 - 3.2 mmoles N m^{-2} h^{-1}), of which (85% was in the form of ammonium and 15% in the form of nitrate (; 477 µmoles m^{-2} h^{-1}, (of which 103 µmoles m^{-2} h^{-1} (22%), xxx ammonium) were in the form of nitrate (Figure 7aA). On a daily basis, assuming Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release would be able to support a primary production rate of 3.2 g C m^{-2} d^{-1} (uncertainty range: 1.9 -6.1 g C m^{-2} d^{-1}~ 910 mg C m^{-2} d^{-1}),; amountingequivalent to. 463% (uncertainty range: 275 – 884%) Considering of the mean average annualdaily primary production of a typical estuarine-coastal ecosystems (global average:of 252 g C m^{-2} y^{-1}; (Cloern et al. 2014) (i.e. 690 mg C m^{-2} d^{-1}) this jellyfish aggregation could locally support up to 463% (uncertainty range: 275 – 884)of the primary production. , which suggests that, it is apparent that nitrogen release by jellyfish blooms could play a key role in recycling nutrients in on-shore coastal ecosystems.

The Benguela Upwelling System is one of the four major coastal upwelling regions presenting the highest primary production of the world oceans (Carr 2001). Large jellyfish populations occur sporadically throughout the year with highest abundances observed in June–August (Flynn et al. 2012). The biomass of these blooms can at times exceed the biomass of fish by a factor for three (Lynam et al. 2006). We estimated that In the Benguela upwelling region, high-density *C. fulgida* blooms in August 2006 (Lynam et al. 2006) could have can locally released up to 1.3 mmoles N m^{-2} h^{-1} (uncertainty range: 0.7 - 2.7 mmoles N m^{-2} h^{-1}; Figure 7b), of which 95% was in the form of ammonium and 5% in the form of nitrite. –Assuming the Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release supportscorresponded to a daily primary production of 2.5 3.4 g C m^{-2} d^{-1} (uncertainty: 1.3 – 5.2 g C m^{-2} d^{-1}), whichand is

equivalent ~~corresponds~~ to 208% (uncertainty range: 108 – 433) of the average daily primary production of for the N~~n~~orthern Benguela ecosystem (1.2 g C m^{-2} d^{-1};~~,~~ Brown et al. 1991).

~~Both case studies indicate that jellyfish blooms could locally play an important role in coastal carbon and nitrogen cycling. Yet, jellyfish blooms appear sporadically and are temporarily and spatially limited. Implications at the ecosystem level would require H~~high-resolution time ~~-series of jellyfish distribution, which, to our knowledge, are not currently available. N~~evertheless, these case studies illustrate the potential role of jellyfish and their associated microbiomes as a source of nutrients for primary production in coastal and estuarine ecosystems. The densities observed in the Honjo District lake, although high, are not unusual for coastal habitats (e.g.: 36 ± 34 *A. aurita* m^{-3} in Limfjorden;~~,~~ Riisgård et al. 2010). Likewise, ~~T~~the jellyfish densities of the N~~n~~orthern Benguela U~~u~~pwelling S~~s~~ystem ~~in contrast~~ are to our knowledge the highest currently on record, ~~however,~~yet such high densities are predicted to become ~~increasinglymore~~ common in some coastal areas of our changing ocean (Cheung et al. 2019). For areas experiencing increases in jellyfish blooms~~Given these future scenarios~~, the two~~se~~ case studies provide a guide to understand how jellyfish and their associated microbiomes can~~first insights into the potentially increasing~~ impact ~~of jellyfish blooms on~~the nitrogen cycle~~ing~~ and ~~surface ocean~~supply nutrients for primary production~~vity~~.

Commented [SLCG10]: Why did you add these caveats? Is that in response to one of the reviewers? To me, these sentences just told me that these case studies are not particularly useful.

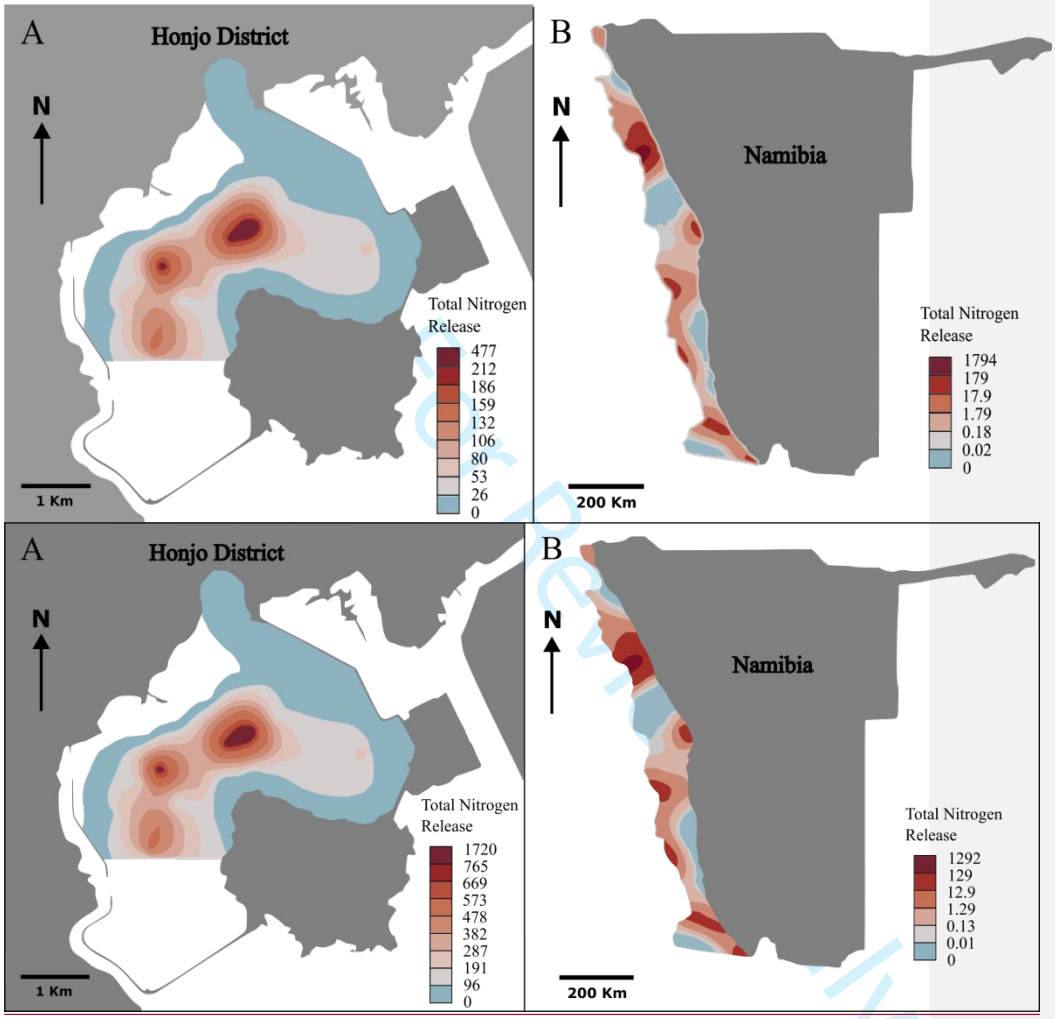


Figure 7. Heat map of estimated total inorganic nitrogen releases associated with the densities of *A.Aurelia coerula* in the Honjo District sea lake in Japan (aA, linear scale: 0 - 1720 0 - 477 µmoles N m^{-2} h^{-1}; map modified from Han and Uye 2009) and of *Chrysaora C. fulgida* along the coast of Namibia (bB, exponential scale: 0 - 1292 0 - 1,794 µmoles N m^{-2} h^{-1}; map modified from Lynam et al. 2006).

The Benguela Upwelling System is one of the four major coastal upwelling regions that present the highest primary production of the world oceans (Carr 2001). Simultaneously, the northern Benguela ecosystem has large jellyfish populations occurring in patchy masses throughout the year and peaking in June–August (Flynn et al. 2012). The distribution and biomass of these blooms was estimated in August 2003 to 12.2 million tonnes for the Namibian coast, corresponding to more than three times the biomass of the dominant fish in the area (3.6 million tonnes; Lynam et al. 2006). Based on biomass estimates of the jellyfish *C. fulgida* (previously identified as *C. hysoscella*), an average bell diameter of 27.0 cm (Lynam et al. 2006) corresponding to 1100 g of WW (Houghton et al. 2007), the ammonium allometric equation (equation 1) and assuming ammonium makes up 96% of total released inorganic N, we estimated the total nitrogen release associated with the population. We found that high-density *C. fulgida* blooms can locally release nitrogen up to 1.79 mmol N m^{-2} h^{-1} (Figure 7B), with 76 µmol N m^{-2} h^{-1} in the form of nitrite. Assuming Redfield ratio (C:N = 106:16; Redfield 1963), this nitrogen release corresponds to a daily primary production of 3.4 g C m^{-2} d^{-1}. Considering an average primary production of 1.2 g C m^{-2} d^{-1} for the northern Benguela ecosystem (Brown et al. 1991), our calculations indicate that high-density bloom of the population of *C. fulgida* can have a substantial but local impact on the nitrogen cycling of the Namibian shelf. Yet, jellyfish blooms appear sporadically and primary production varies over time. Implications at the ecosystem level would require High-resolution time series of jellyfish distribution. Nevertheless, Further, this case study demonstrates the potential impact of jellyfish blooms on offshore ecosystems.

These case studies illustrate the potential impact of jellyfish and their associated microbiomes in the nitrogen cycle and as a source of nutrients for primary production in coastal ecosystems. The densities observed in the Honjo District lake, although high, are not unusual for coastal habitats (e.g.: 36 ± 34 *A. aurita* m^{-3} in Limfjorden, Riisgård et al. 2010). Although the jellyfish densities of the northern Benguela upwelling system are to our knowledge the highest currently on record,

~~such high densities are predicted to become more common in some coastal areas of our changing ocean (Cheung et al. 2019). For areas experiencing increases in jellyfish blooms, these cases provide a guide to understand how jellyfish might impact the nitrogen cycle from small onshore habitats to large offshore ecosystems.~~

Conclusion

Overall, our results suggest a widespread association between jellyfish and nitrifying microorganisms, which can oxidize ~~up to almost~~up to one~~a~~ third of the ammonium excreted by ~~the~~ jellyfish. While the identity of the nitrifiers and their distribution on the jellyfish ~~are still~~remain unknown, it appears that their activity and abundance ~~remain~~ are constant ~~within~~ in~~a~~ a given jellyfish population but ~~is~~ likely ~~to~~ vary ~~with the~~between different environments. The allometric relationships obtained from our observations ~~now~~ allow us to estimate the ~~release of nutrients~~amount of nutrients released by a jellyfish population ~~by~~ via ~~scaling up~~extrapolation of the individual ~~nutrient~~ mass-specific release rates based on the abundance and size distribution of a population. This study highlights the importance and complex role of jellyfish blooms in ~~the nitrogen cycle~~coastal nitrogen cycling, ~~where they can locally support high rates of surface ocean nitrification~~~~supporting pelagic nitrification~~. Equally, the substantial release of ammonium likely supports phytoplankton growth and may locally impact phytoplankton community composition. Considering t~~T~~he widespread geographic distribution of ~~the~~ bloom forming jellyfish species investigated in this study (Figure 1) ~~together with~~and the predicted future increase of jellyfish ~~densities~~blooms, ~~their~~ our findings point toward ~~tendency to form blooms suggest~~ an increasing ~~global~~ relevance of jellyfish-~~associated nitrification~~ on coastal nitrogen and carbon cycling.

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~~Suggested Associate Editors~~

- ~~Robinson (Wally) Fulweiler - Boston University, USA~~

Considering her interest in coastal ecology and biogeochemical cycling of nitrogen, Dr. Fulweiler has the scientific expertise to judge the quality and appropriateness of the study.

- Bo Thamdrup - University of Southern Denmark, Denmark

Our study meets Professor Bo Thamdrup area of expertise in microbial ecology and aquatic biogeochemistry, making him a good candidate to judge the quality of our work.

Suggested Reviewers:

- Kylie Pitt - Griffith University, Australia - k.pitt@griffith.edu.au

Kylie Pitt is one of the world experts on the ecology of jellyfish and has published papers on jellyfish excretion and their role in biogeochemical cycle and primary production. We cited her work many times in this manuscript on thus believe she is the most appropriated researcher to review this study.

- David T. Welsh - Griffith University, Australia - d.welsh@griffith.edu.au

David T. Welsh has expertise in jellyfish ecology and microbiology, among other fields. He has studied the role of jellyfish in biogeochemical cycles and has specifically studied nitrification associated with benthic organisms. He is undoubtedly qualified to assess the quality of our work and review it.

- Silvia Pajares Moreno - National Autonomous University, Mexico - spajares@cmarl.unam.mx

The work and interest of microbiologist Silvia Pajares Moreno in microbiomes, nitrogen cycle and ecosystem functioning make her particularly adapted to judge our work.