Holobiont Nitrogen Control and its Potential for Eutrophication Resistance in an Obligate Photosymbiotic Jellyfish

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Holobiont nitrogen control and its potential for eutrophication resistance in an obligate photosymbiotic jellyfish

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ABSTRACT

Background: Marine holobionts depend on microbial partners for health and nutrient cycling. This is particularly evident amongst cnidarian-Symbiodiniaceae symbioses, where nutrient acquisition is facilitated. However, the symbiosis is sensitive to environmental change - including eutrophication – that cause dysbiosis and host mortality, which contributes to global coral reef decline. Yet, some holobionts exhibit resistance to dysbiosis in eutrophic environments, including the obligate photosymbiotic scyphomedusa *Cassiopea xamachana*.

Methods: Our aim was to assess the mechanisms in *C. xamachana* that stabilize symbiotic relationships. We combined labelled bicarbonate (^{13}C) and nitrate (^{15}N) and metabarcoding approaches to evaluate nutrient cycling and microbial community composition in symbiotic and aposymbiotic medusae.

Results: We found C-cycling within the *C. xamachana* holobiont to be essential as aposymbiotic medusae continuously lost weight even at high heterotrophic feeding rates. Heterotrophically acquired C and N were readily shared among host and algae. This was in sharp contrast to nitrate assimilation, which was strongly restricted from Symbiodiniaceae. Instead, the bacterial microbiome seemed to play a major role in the holobiont's DIN assimilation as uptake rates showed a significant positive relationship with phylogenetic diversity of medusa-associated bacteria. This is corroborated by inferred functional capacity that links the dominant bacterial taxa (~90 %) to nitrogen cycling and particularly denitrification. Observed bacterial community structure differed between apo- and symbiotic *C. xamachana* putatively highlighting enrichment of ammonium oxidizers and denitrifiers and depletion of nitrogen-fixators in symbiotic medusae.

Conclusion: Host, algal symbionts, and bacterial associates contribute to regulated nutrient assimilation and cycling in *C. xamachana*. We found that the bacterial microbiome of symbiotic medusae was

- 46 seemingly structured to increase DIN removal and enforce algal N-limitation - a mechanism that would
- 47 help to stabilize algae-host relationship even under eutrophic conditions.

BACKGROUND

Multicellular life depends on microorganisms for tight symbiosis, for their ability to drive biogeochemical processes thus providing nutrients, or both [1]. Hosts and their associated microbial communities, together referred to as holobionts, have received increasing attention, especially in the context of ongoing global declines in coral reefs [2]. Cnidarian holobionts include bacteria, archaea, fungi, and viruses. Some form additional photosymbioses with endosymbiotic unicellular dinoflagellates from the family *Symbiodiniaceae* [3,4]. The associated microorganisms, in particular bacteria and Symbiodiniaceae, contribute to holobiont nutrient cycling and health [5].

Symbiosis allows the holobiont to directly access autotrophically fixed carbon (C) and thereby thrive in nutrient limited environments where heterotrophic food supply is strongly limited [6].

Photosynthetically fixed C is translocated mostly in the form of glucose from algae to host [7], but also other metabolites are exchanged [8]. In some cnidarian species (e.g. *Cassiopea* spp.), phototrophic C can completely cover and even exceed the host's energetic requirements [9]. In turn, the host provides favorable conditions in respect to light and CO₂ [10]. This symbiosis is the foundation of coral reefs, which provide ecosystem services with an estimated value of US\$375 billion per year [11]. The cnidarian-symbiodinian relationship includes facultative associations, for instance *Exaiptasia* and the stony coral *Astrangia poculata* as well as obligate association in most tropical reef-building corals. The symbiosis is remarkably sensitive to environmental changes and environmental stressors can cause its breakdown resulting into coral bleaching [12]. Such loss of photosynthetic pigments can be accompanied by the dysbiosis of associated microbial communities [2,13]. Obligate hosts may sometimes survive bleaching periods and recover [14], but nonetheless, climate change driven temperature rises and resulting mass bleaching are having critical consequences for coral reefs on a global scale [15]. Consequently, key questions for conservation are what prevents host-algae

71 dissociations and which factors support the reestablishment of disrupted symbiotic relationship once
72 bleaching occurred.

73 Besides temperature, also high nutrient levels can either directly cause bleaching or lead to dysbiosis,
74 disease and host mortality [16]. Naturally, nitrogen (N) is limited in oligotrophic tropical coral reefs. It
75 can be acquired by heterotrophic feeding of the host and passed on to its associates, e.g. as ammonium
76 or amino acids, and further efficiently recycled within the holobiont [17]. N can also be directly obtained
77 from seawater, where it is commonly present at low concentrations in form of dissolved inorganic N
78 (DIN; i.e. nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+)), dissolved organic N, or particulate organic
79 N [8]. Cnidarian hosts are able to incorporate ammonium but often associated microbial communities
80 account for most DIN uptake [17]. Symbiodiniaceae assimilate ammonium and nitrate, many microbial
81 associates are involved in acquiring and efficiently (re)cycling N, and prokaryote diazotrophs can even fix
82 atmospheric nitrogen [17–19]. The host benefits from a limited N availability [8,20] as excess inorganic N
83 can disrupt the host-symbiont partnership by altering the N to phosphate ratio within the holobiont and
84 exacerbate heat-induced bleaching [17,21]. Recently, prokaryote associates have also been linked to
85 contribute to Symbiodiniaceae N limitation in coral holobionts by metabolizing and therefore limiting
86 biologically available N [18,22].

87 Cnidarian-symbiodinian holobionts are diverse and adapted to different trophic environments – not only
88 coral reefs. Consequently, they vary in their heterotrophic feeding capacity and likely in their ability to
89 assimilate and retain N [6,23,24]. A higher heterotrophic feeding capacity seems to correlate with an
90 increased bleaching threshold in coral (i.e. greater bleaching resistance) [23]. The mechanisms
91 regulating nutrient acquisition and N cycling within the holobiont are still poorly resolved, but are key
92 for understanding intricate Cnidaria-Symbiodiniaceae symbiosis [17,22,25].

Of particular interest are organisms adapted to high nutrient environments as they might provide insights on how to effectively mitigate negative consequences of eutrophication. A potential mechanism to thrive under such conditions is the capacity of hosts to restrict N access from their symbionts. Examples of highly adaptive organisms are obligate photosymbiotic jellyfishes of the genus *Cassiopea*, which persist in mangrove environments and seagrass beds with high nutrient loads as well as in more oligotrophic reef flats [9,26,27]. *C. xamachana* has recently gained increasing attention as a suitable organism to study the cnidarian-symbiodinian relationship [28–30]. Differing from most corals, its algal symbiosis can be discontinued for extended periods (>8 weeks), and re-infection with a range of Symbiodiniaceae species is easily facilitated [30,31].

Consequently, *C. xamachana* provides a number of physiological characteristics turning it into a suitable model organisms to study nutrient dynamics in Cnidaria and their resistance to nutrient-facilitated or induced bleaching. However, up to now only comparatively little is known about its energy and nutrient budgets. Similar to other scyphomedusae such as *Linuche unguiculata* [32], *C. xamachana* seems to attain the majority of its C requirements from photosynthesis as bleached or light limited *C. xamachana* fail to maintain their mass even in the presence of high heterotrophic food concentrations [33,34]. Further, adult medusae can utilize different DIN species including ammonium and nitrate [35] that are transferred between host and symbiont [36]. However, quantitative data on C and N acquisition is missing and we do not know in which form and at which rates elements are cycled among members of the holobiont in *C. xamachana*.

In this study our aim was to assess energy and nutrient cycling in *C. xamachana* in order to identify potential mechanisms stabilizing host-Symbiodiniaceae relationships in eutrophic environments. A key objective in this process was the incorporation of the microbiome due to its potential functional importance. Consequently, we combined the application of isotopic labels in pulse-chase experiments with physiological measurements of the host and gene amplicon sequencing of the microbiome. This

allowed us to compare energy cycling in symbiotic and aposymbiotic individuals and relate nutrient uptake and turnover (or assimilation) rates with biodiversity and composition of the microbiome in *C. xamachana*.

MATERIAL AND METHODS

Study organism and preparation of experiments

Cassiopea xamachana specimens employed in this study belong to strain T1A (draft genome available [37]). All individuals were from the same cohort, monoclonal, and propagated asexually as polyps. Associated Symbiodiniaceae were identified as clade A (the representative sequence matched A3 closely) by ITS2 sequencing (tested on 8 representative individuals, primers 'ITS2symbF': 5' TGTGAATTGCAGAACTCCGT 3' and 'ITS2symbR': 5' TTTCCAAAGTCCTTTTCATCTTTC 3' covering the full ITS2 and partial 5.8S and 28S genes, length: ~667 bp), the best hit upon blastn search was considered (query cover: 100 %, Identity: 99 %). Adult medusae were maintained in a 500 L aquarium equipped with filtration system, protein skimmer and reef sand. Two LED lights (Prime 16HD Reef, Aqualllumination) were set to a 12:12 h light:dark cycle at 100-150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The medusae were kept in artificial seawater (ASW) with 35 PSS-78 salinity at 27-29°C temperature and fed *ad libitum* with freshly hatched or frozen *Artemia salina* 3-5 times a week.

For the experimental preparation 35 adult symbiotic medusae (49 ± 7 mm bell diameter) were randomly selected and transferred to a separate tank (~15 L) equipped with filtered ASW (0.22 μm , changed daily) and air stones. Specimens were acclimated for four days. Aposymbiotic medusae were prepared by menthol-bleaching additional 20 individuals employing the modified protocol by Matthews et al. [38]. Briefly, the medusae were incubated in ASW spiked with 0.38 mM menthol (99 %, Sigma-Aldrich; 1.28 M in 95 % ethanol) eight hours per day for four days. Loosely closed incubation chambers were placed in a

plant incubator (MLR-352, Panasonic) at 28°C and ~350 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Medusae were considered fully bleached when no fluorescence was detectable (Imaging-PAM, Waltz, Germany) and the absence of Symbiodiniaceae cells was confirmed under a light microscope (Olympus Optical, mod. CHK at 400 $\times$). All 20 fully bleached medusae were kept in a separate, Symbiodiniaceae-free (0.22 μm ASW) system for 21 days at the same temperature and light regime as in the rearing tank.

Experimental procedure – pulse-chase labelling experiment

In order to assess inorganic N and C assimilation, we performed a pulse-chase isotope labelling experiment under three experimental treatments. Treatments included symbiotic individuals incubated under light conditions (SymL), symbiotic individuals incubated in darkness (SymD), and aposymbiotic individuals incubated in light (ApoL) (Fig.1). Prior to the experiment, five symbiotic and five aposymbiotic specimens were sampled to establish natural stable isotope ratios (T0; Fig.1). The remaining specimens (30 symbiotic and 15 aposymbiotic), were randomly assigned to one of the three treatments. The experiment consisted of an incubation with tracers (pulse), followed by an extended tracer-free incubation (chase). All incubations were performed in glass jars ('incubation chamber'; IKEA Korken, ~1L) in incubators (MLR-352, Panasonic) at 28°C. Light was adjusted to 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

At the start of the experiment, each medusae was transferred to an incubation chamber filled with ^{13}C and ^{15}N enriched seawater (1.18 mM $\text{Na}^{15}\text{NO}_3$; 98 atom % ^{15}N , Sigma-Aldrich and 117 μM $\text{NaH}^{13}\text{CO}_3$; 98 atom % ^{13}C , Sigma-Aldrich). Dissolved oxygen (DO) was measured for each chamber (YSI® ProODO™ optical DO sensor, Yellow Springs, USA), then chambers were closed airtight without any remaining bubbles and randomly arranged in the incubators. After ~5 h, chambers were opened one by one and DO measured (Fig.1). Each medusa was rinsed with filtered ASW and five specimens from each treatment (SymL, SymD, and ApoL; $n = 15$) were sampled (T1). Each sampled individual was measured (bell diameter using a standard caliper), rinsed with abundant MilliQ water, wrapped in sterile aluminum

foil, frozen at -80°C for about 30 min, and stored at -20°C until further processing. Meanwhile, the incubation chambers were carefully cleaned (bleach and MilliQ), and filled with filtered (tracer-free) ASW. The remaining medusae ($n = 30$) were transferred back into the chambers for the chase part of the experiment. Chambers were closed and handled as described above. After ~3 h, five individuals from each treatment ($n = 15$) were sampled (T2, 'chase3h') as described. The remaining individuals ($n = 15$) were sampled after another three hours (T3, 'chase6h').

Wet weight and bell diameter

During the experiment, only bell diameter (BD) was measured to minimize handling stress. In order to relate BD to wet weight (WW), we measured both for 35 symbiotic and 12 aposymbiotic individuals that were not employed in the experiments. We then examined changes in mass and size of the 12 aposymbiotic medusae by BD and WW measurements 2, 8, and 25 days after the end of the menthol treatment (Additional File 1: Fig.S1). Measurements after 25 days were selected to calculate the BD to WW relationships for aposymbiotic individuals in the isotopic pulse-chase experiment, which took place 21 days after bleaching the respective medusae.

To measure WW, medusae were individually captured with a fine plastic net, gently shaken to remove excess water and weighted in a cup containing ASW. Immediately after weighing, maximum bell diameter (BD) was measured using a standard caliper. WW-BD relationships were established using linear regressions and the best model was selected based on Akaike information criterion (AIC), resulting in a second-degree polynomial function for the symbiotic and a linear function for the aposymbiotic medusae (Additional File 1: Fig.S2).

Productivity

DO concentrations before and after each incubation were used to calculate respiration (R; for dark incubation and for aposymbiotic medusae) and net primary production (P_n; for light incubation of symbiotic specimens) rates as

$$P_n [mg O_2 g^{-1} h^{-1}] = \frac{\Delta O_2 [mg L^{-1}] * (V_{chamber} - V_{medusa} [L])}{T[h] * WW[g]} \quad (1)$$

where $V_{chamber}$ and V_{medusa} stand for incubation chamber volume and medusa volume, respectively. V_{medusa} was calculated from WW based on the seawater density at 35 PSS-78, 28°C and standard atmospheric pressure. For symbiotic medusae, gross primary production (P_g; P_g = P_n + R) and the P_g:R ratio were calculated for the 5 h light pulse period. We want to note here that these values are approximations as there are systematic differences between light and dark respiration and both can vary over time [39].

Heterotrophic nutrient dynamics

To assess the relative importance of heterotrophic feeding in *C. xamachana*, symbiotic specimens were fed with live zooplankton enriched with ¹³C and ¹⁵N. Zooplankton was enriched by first culturing *Isochrysis galbana* (haptophyte alga) for four days in a modified F/2 medium enriched with 750 mg L⁻¹ NaH¹³CO₃ (98 % heavy isotope) and 75 mg L⁻¹ Na¹⁵NO₃ (98 % heavy isotope, accounting for all nitrate present in the medium) to enrichment levels of ~10 AP¹³C and ~15 AP¹⁵N (atom percent, see below). Isotopically enriched algae were used to feed *Artemia salina* for 24 - 48 h. Four *C. xamachana* medusae (~3 cm BD) were starved for three days under normal light conditions, then fed *ad libitum* with labelled *A. salina* and sampled after ~5 - 6 h to allow for complete digestion (based on empty appearance of the gastric cavity). Each individual was thoroughly rinsed with ASW and MilliQ, and the gastric cavity excised with a clean scalpel to exclude partially undigested food particles. Medusae were then preserved as described above for stable isotope analyses (SIA).

Samples processing

All frozen *C. xamachana* samples were homogenized. Aliquots of the 15 samples after the pulse were taken from all treatments for microbial analyses. The tissue homogenate of each sample was then separated by centrifugation into host and algal symbiont fractions. Algal cell counts were conducted using a hemocytometer under a light microscope. Algal and host fractions were freeze-dried before SIA. For detailed sample processing see Additional File 2: Text S1.

Stable isotope analysis (SIA)

SIA was performed via combustion in a Eurovector EA3028 elemental analyzer coupled to a Nu Instruments Perspective-series stable isotope ratio mass spectrometer in continuous flow mode. SIA results were firstly expressed in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, based on the isotopic composition of atmospheric N_2 and on the Pee Dee Belemnite (PDB) standard, respectively. Acetanilide standards measurements were used to calculate the machine precision as percent relative standard deviation (standard deviation/mean $\times$ 100). Measurement precision in the pulse-chase experiment was 1.4 % for $\delta^{13}\text{C}$ and 4.1 % for $\delta^{15}\text{N}$, while it was 0.2 % for $\delta^{13}\text{C}$ and 12.8 % for $\delta^{15}\text{N}$ for the heterotrophic experiment. For the pulse-chase experiment, each fraction (host and algae) from each medusa was measured in duplicates. The mean of both measurements was used for statistical analysis. Due to the low sample mass for the algal symbiont fraction, only one reading was obtained in three samples (SymD-pulse, SymD-chase6h and SymL-chase6h). Isotope abundances were then converted to atom percent of ^{13}C (AP ^{13}C) and ^{15}N (AP ^{15}N) following Fry [40] after

$$AP^HE = \left[\frac{{}^HE}{{}^LE + {}^HE} \right] \times 100 \quad (2)$$

where H and L refer to the number of light and heavy isotope atoms of the element E (i.e. C or N).

Enrichment of both fractions in the heterotrophic experiment was calculated as atom percent excess

($APE = AP_{\text{sample}} - AP_{\text{controls}}$). Then the enrichment of the symbiont was converted to percentage of enrichment relative to the host's enrichment ($(APE_{\text{Algal}} / APE_{\text{Host}}) \times 100$). When necessary, a certified acetanilide standard was spiked in samples from the heterotrophic experiment to increase sample mass and/or to dilute highly enriched samples. APE values were then derived from mass balance equations.

Data analysis

Data analysis was performed after visually inspecting data for normality and testing for homogeneity using Bartlett tests. Enrichment was tested with pairwise Welch two-sample upper-tailed t-tests with Holm correction for multiple comparisons. Effects of treatment, incubation and fraction were tested using linear mixed effect models or, when failing the assumptions, with Kruskal-Wallis test by rank followed by post-hoc Wilcoxon rank sum exact test with Holm correction. The same non-parametric approach was used to test for difference in productivity (P_n) and respiration (R) among treatments. No test was performed when the significance could already be inferred by a clear lack of overlap between the plotted groups 95 % confidence interval. All test were performed in R version 4.0.2 [40].

Bacterial community analysis

DNA from five medusae per treatment was extracted from homogenized tissue using the modified 2x CTAB chloroform protocol after Coffroth et al. [42]. DNA was quantified on a Multiskan GO (Thermo Fisher Scientific, Waltham, USA). The primers 784F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA-3') and 1061R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCRRCACGAGCTGACGAC-3') [43] with Illumina over-hang adaptor sequences (underlined above) were used to amplify the variable regions 5 and 6 of the 16SrRNA gene. For amplification and sequencing details see Additional File 2: Text S1.

Bacterial community analysis was conducted using Qiime2 [44]. Forward and reverse reads were split according to barcodes and assembled to contigs. Contigs >310 bp and those containing ambiguous bases

250 were discarded. Sequences were quality filtered and categorised in amplicon sequence variants (ASVs)
251 using dada2 [45]. The resulting 303 ASVs were aligned against SILVA [46], release 138.1, using a primer-
252 specific classifier trained in Qiime2. If present, chloroplasts, mitochondria, *archaea*, eukaryotes and at
253 the phylum level unknown sequences were removed. In total, we produced 16 16S rRNA gene libraries
254 containing 221,216 ASVs (252,674 before quality control) from one extraction control, five SymL, five
255 SymD, and five ApoL *C. xamachana* samples. ASVs that occurred more than 10 % in the extraction
256 control compared to the sum of all other samples were removed (i.e., 7 ASVs removed, 6 occurred only
257 in the control; Additional File 3: Table S2) to account for potential contamination. Then stacked column
258 plots representing bacterial community compositions at the family level were constructed, and alpha
259 diversity indices (ASV richness, Simpson and Shannon diversity) were calculated after implementation of
260 a combined rarefaction-extrapolation procedure [46]. Evenness was computed from unrarefied data and
261 phylogenetic diversity was established after Faith [47]. Alpha diversity indices were selected to provide
262 largely independent measures (richness, evenness, and Faith) and comparability with other studies
263 (Shannon and Simpson). Indices were compared among treatments using ANOVA analyses or Kruskal-
264 Wallis tests if assumptions of heteroscedasticity could not be met after eventual data transformations.
265 We used relative ASV densities (i.e. contribution of ASVs to total no. of reads per sample) computed
266 from unrarefied data to establish a Bray-Curtis dissimilarity matrix and visualised sample similarity in a
267 non-metric dimension scaling (NMDS) biplot. Differences between samples and treatments were
268 assessed using PERMANOVAs (Holm correction applied for multiple pairwise comparisons). Further, we
269 assessed differences in within-treatment variability in community similarity, by pooling all pairwise Bray-
270 Curtis similarity scores for within-treatment comparisons of samples and assessing differences among
271 treatments using a Kruskal-Wallis test. All statistical test have been performed in R, the scripts can be
272 found in Additional File 4.

Differences in predicted functional profiles based on phylogenetic inference of the associated bacterial communities were assessed using METAGENassist [49]. We used the cleaned ASV table (Additional File 3: Table S2) to perform ‘taxonomic-to-phenotypic mapping’ in METAGENassist, where all ASVs were taxonomically assigned and mapped, condensed into 184 functional taxa, and filtered based on interquartile range [50]. The remaining 175 functional taxa were normalized across samples by sum and over taxa by autoscaling. We analysed the dataset for ‘metabolism by phenotype’ using the Euclidean distance measure and clustered the 15 most differentially abundant metabolic processes (selected with random forest) using single algorithm.

RESULTS

Bleaching and Productivity

Menthol bleaching successfully produced aposymbiotic *C. xamachana* medusae after ~10 incubation days (Additional File 1: Fig.S1). However, even with regular feeding ($\geq$ thrice a week *ad libitum*) aposymbiotic medusae shrank from 2.54 ± 0.47 to 1.35 ± 0.24 g over 29 days and survived only for little over ten weeks. Symbiotic and aposymbiotic medusae differed in their WW to BD relationship (Additional File 1: Fig.S1, S2). Respiration (R) in aposymbiotic ($49 \pm 8 \mu\text{g O}_2 \text{ g}^{-1} \text{ h}^{-1}$, measured under light conditions) was higher ($p_{\text{Holm}} < 0.001$) than in symbiotic ($28 \pm 14 \mu\text{g O}_2 \text{ g}^{-1} \text{ h}^{-1}$, measured under dark conditions) medusae. Gross primary production (P_g) for symbiotic specimens was $56 \pm 15 \mu\text{g O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and the P_g :R ratio for the 5 h pulse period was 2.0 ± 0.7 .

Inorganic carbon and nitrogen

All fractions in all treatments were significantly enriched in ^{13}C and ^{15}N after the pulse, chase3h and chase6h (for all $p_{\text{Holm}} < 0.01$) compared to the controls (Fig.2a, b; Additional File 3: Table S3, S4). As anticipated, the algal fraction of the SymL treatment yielded the highest AP^{13}C enrichment (2.57 – 3.35),

295 followed by the respective host fraction (1.48 – 2.23). Owing to the lack of photosynthetic activity, both
 296 SymD and ApoL showed a much smaller enrichment overall (1.09 – 1.13). Enrichment of the SymL host
 297 fraction showed comparatively high across sample variation (Fig.2a) which we found was well explained
 298 by Symbiodiniaceae densities ($r^2_{Adj} = 0.68$, slope = 0.17, $p_{lm} < 0.001$, Fig.2c). Further, we found good
 299 accordance between host AP¹³C and net primary production (P_n) computed from O₂ measurements (r^2_{Adj}
 300 = 0.74, slope = 10.84, $p_{lm} < 0.001$, Additional File 1: Fig.S3). Surprisingly, the highest ¹³C enrichment for
 301 both the host and algal fraction of SymL occurred at chase rather than at pulse (AP¹³C_{Chase3} > AP¹³C_{Pulse}
 302 and AP¹³C_{Chase3} > AP¹³C_{Chase6h}, $p_{LME} < 0.01$). A similar delay in tissue enrichment was also found in the algal
 303 fraction of SymD, with the greatest enrichment at chase6h (AP¹³C_{Chase6h} > AP¹³C_{Pulse}, $p_{Wilcox} < 0.05$).
 304 Conversely, SymD host fraction peaked at pulse and showed lowest enrichment levels at chase6h.

 305 Unlike for ¹³C, ¹⁵N enrichment was always higher in the host than in the algal fraction. AP¹⁵N in the algal
 306 fraction of SymL was higher than in SymD indicating a link between nitrate assimilation and
 307 photosynthesis ($p_{LME} < 0.001$). In the host fraction, ¹⁵N enrichment was highest at pulse (0.644 – 0.900)
 308 and dropped below 0.424 at chase for all treatments (Fig.2b). At pulse, there were no significant
 309 differences between treatments ($p_{ANOVA} > 0.05$) (Additional File 3: Table S4), but ApoL samples
 310 experienced a larger drop in AP¹⁵N (i.e. N turnover rates) during the chase ($p_{LME} < 0.01$; Fig.2b). Notably,
 311 AP¹⁵N levels significantly correlated with medusa size for both chases when considering the host
 312 fractions from all treatments ($r^2_{Adj} = 0.67$ and 0.86 respectively, $p_{lm} < 0.001$ for both; Fig.2d). However,
 313 the effect of medusa size seemed to be solely related to N turnover rates as there was no relationship
 314 between BD and AP¹⁵N after the pulse experiment ($r^2_{Adj} = 0.04$, $p_{lm} > 0.05$). AP¹⁵N values of the algal
 315 fraction in symbiotic individuals from both treatments peaked at the end of the chase (AP¹⁵N_{chase6h} >
 316 AP¹⁵N_{pulse} and AP¹⁵N_{chase6h} > AP¹⁵N_{chase3h}; $p_{LME} < 0.001$), contrasting the pattern observed in the host
 317 fraction.

Organic carbon and nitrogen

Medusae were fed ^{13}C and ^{15}N labelled *A. salina* (Additional File 3: Table S5) to assess nutrient dynamics in *C. xamachana* supplemented with organic nutrients. Both the symbiont and the host fraction were enriched (APE host fraction > 1.19 and > 0.46 , APE algal fraction > 1.12 and > 0.43 for ^{13}C and ^{15}N , respectively) and as expected prey enrichment level affected jellyfish enrichment (Additional File 3: Table S3). As direct consumer of the prey, the host showed higher enrichment levels than the algal fraction. Nevertheless, algal enrichment reached 36.7 ± 4.6 % of the host ^{13}C and 70.3 ± 4.0 % of the host ^{15}N enrichment.

Bacterial communities associated with *C. xamachana*

The microbiome of *C. xamachana* was dominated by *Moraxellaceae* (>60 %) and *Pseudomonadaceae* (~ 15 - 25 %) across all samples (Fig.3a). Differences between samples were generally subtle, but ApoL3 contained a higher abundance of ‘others’, i.e. families with a comparably low abundance.

Further, we assessed differences in the bacterial community structure among treatments by computing pairwise sample similarity (Bray-Curtis index) and displaying them in an nMDS biplot (Fig.3b). ApoL samples showed slight differences to non-bleached treatments, which were significant (ApoL and SymL; $p_{\text{PERMANOVA}} = 0.036$) or marginally non-significant (ApoL and SymD; $p_{\text{PERMANOVA}} = 0.068$). A key difference, however, was within treatment heterogeneity in community composition (Fig.3b, c). A comparison of within group community similarity across treatments clearly revealed that ApoL showed a much higher compositional heterogeneity than the two treatments with symbiotic algae (Kruskal-Wallis Test; $\chi^2 = 21.463$, $\text{df} = 2$, $p_{\text{Wilcoxon}} < 0.01$). On average, the number of reads, richness, Shannon and Simpson diversity, phylogenetic diversity (Faith), and evenness were lower in both symbiotic groups than in the aposymbiotic samples, which showed a markedly higher variability (i.e. standard deviation) for all measures (Table 2).

Table 2. Summary statistics detailing bacterial communities associated with *C. xamachana*.

	# of reads	richness	Shannon	Simpson	Faith	evenness
SymLPul1	12437	38	3.76	2.39	0.333	0.364
SymLPul2	13394	39	3.49	2.26	0.367	0.341
SymLPul3	11826	27	3.43	2.26	0.184	0.374
SymLPul4	16425	29	3.27	2.17	0.206	0.352
SymLPul5	13997	39	3.51	2.29	0.255	0.343
Mean	13616	34	3.49	2.27	0.269	0.355
STDV	1780	6	0.18	0.08	0.079	0.014
SymDPul1	13346	37	3.45	2.26	0.255	0.342
SymDPul2	17415	42	3.45	2.24	0.260	0.331
SymDPul3	13342	32	3.41	2.29	0.208	0.353
SymDPul4	14175	37	3.46	2.22	0.224	0.343
SymDPul5	15073	28	3.26	2.22	0.174	0.354
Mean	14670	35	3.40	2.24	0.224	0.345
STDV	1693	5	0.08	0.03	0.036	0.010
ApoLPul1	15829	53	3.95	2.25	0.336	0.345
ApoLPul2	17442	58	4.09	2.26	0.292	0.347
ApoLPul3	14280	140	10.23	3.02	0.618	0.470
ApoLPul4	12120	25	3.12	2.14	0.267	0.353
ApoLPul5	19938	69	4.45	2.26	0.358	0.352
Mean	15922	69	5.17	2.39	0.374	0.373
STDV	2983	43	2.87	0.36	0.141	0.054

Treatments: Apo = aposymbiotic, Sym = symbiotic, L = light, D = dark, Pul = sampled after pulse incubation.

Moreover, we assessed ASVs based on their ubiquity as potential indicator of their functional importance [48]. The core microbiome, i.e. the ASVs present in all 15 medusae, consisted of 10 ASVs, including 9 of the 10 most abundant taxa (Additional File 3: Table S2). The symbiome (ASVs occurring in all SymL and SymD samples) additionally consisted of two ASVs (*Acinetobacter* sp. and *Ruegeria* sp.) of which the former also occurred in four of the five aposymbiotic samples. The apobiome also consisted of 2 ASVs (*Cellvibrio* sp. and *Ralstonia* sp.), both occurring in six and eight symbiotic samples, respectively (Additional File 3: Table S2).

Taxonomy-based functional profiling of bacterial communities in *C. xamachana*

Based on a METAGENassist analysis, we identified the 15 functional categories, which showed the largest differences across all microbiome samples (Fig. 4). Further, we used these categories to cluster all samples, which revealed a clear differentiation between aposymbiotic and symbiotic samples. Only ApoLPul4, the aposymbiotic sample with the lowest alpha diversity indices (except evenness) (Table 2), was clustering more closely with the symbiotic than with the aposymbiotic samples. Functional differences included in particular a number of functions related to N metabolism. Functional traits that would limit inorganic N availability for the symbiotic algae, such as ammonium oxidizers and nitrite reducer were enriched in symbiotic microbiomes. On the other hand, nitrogen fixing, which potentially increases the nitrogen availability for the holobiont was depleted in symbiotic medusae. Further, an enrichment of sulfur oxidizers and a depletion of carbon fixers indicate lower O₂ level for microbiomes of symbiotic medusae (Fig. 4), which is a requirement for effective N removal. Other functions like 'Degrades aromatic hydrocarbon' and 'Xylan degraders' were also more enriched in aposymbiotic sampled compared to 'Atrazine metabolism' and 'Chitin degraders' that were generally more depleted in these samples.

DISCUSSION

In this study, we assessed energy and nutrient dynamics in the *Cassiopea xamachana* holobiont to assess mechanisms that facilitate the maintenance of cnidarian-symbiodinian relationships under high nutrient conditions. The ability of *C. xamachana* to survive prolonged periods in an aposymbiotic state permits to disentangle the role of host and symbionts in nutrient dynamics, turning it into a suitable model organism for such assessments. Our results demonstrate that both autotrophically fixed and heterotrophically acquired C quickly cycled within the holobiont and was incorporated in both host and

Symbiodiniaceae tissue. Nevertheless, heterotrophic feeding alone was not sufficient to cover the host's needs and led to consistent weight loss. N assimilated from zooplankton prey was likewise shared among host and the algae. In contrast, inorganic N present in surrounding water was effectively blocked off from Symbiodiniaceae. An analysis of host associated bacterial communities indicated that bacterial processes involved in N removal through ammonium oxidation and nitrite reductions were presumably enriched in the symbiotic hosts. Restricting the transmission of ambient DIN to Symbiodiniaceae can support stable host-algal relationships in nutrient-rich environments and our data suggests that *Cassiopea xamachana*'s bacterial microbiome might play an important role in this process.

The fate of carbon

The daytime photosynthesis: respiration ratio ($P_g:R = 2$) indicated that C fixation rates are sufficient to cover respiration and corresponds well with previous measurements (2.04) [35]. As anticipated, light conditions aligned with the highest $AP^{13}C$ enrichment in Symbiodiniaceae and the host. Here, a large fraction of C was immediately transferred to the host (during the 5 h pulse), which is coherent with findings from coral holobionts [49]. Both host and symbiodinians maintained high ^{13}C level for at least 6 h, indicating an incorporation of C into cell tissues. In symbiotic holobionts, C transfer to the algae can be realized through respiration or in form of organic C. Dissolved $^{13}CO_2$ could diffuse into the host tissue and contribute to a 'delayed enrichment' (i.e. highest $AP^{13}C$ values after the 3 hour chase) in our inorganic labelling experiment. Such dissolved CO_2 pools utilized for photosynthesis were likely lost during sampling preparation resulting in lower $AP^{13}C$ values in pulse samples.

In treatments lacking photosynthesis (SymD and ApoL treatments), ^{13}C labelling also resulted in C assimilation, albeit at much lower rates (Fig.2a). Baker et al. [24] explained host C enrichment in the dark by anaplerotic reactions that form intermediates of metabolic pathways including lipid synthesis and oxidation which could be performed by host and/or microbiome [50]. In the octocoral species

assessed by Baker et al., however, the algal symbionts were not enriched after dark incubation. In *C. xamachana*, Symbiodiniaceae continued to accumulate C in the dark even after the pulse suggesting it originated from other holobiont members, in particular the host. Such C transfer from host to algae was also apparent from labelled *Artemia* and has been shown in coral from heterotrophic food sources [51,52]. When bleached, the aposymbiotic holobiont enters a starvation mode that is characterized by a collapse in DIC uptake and cycling. Overall, C assimilation and (re)cycling was only effective in symbiotic *C. xamachana* holobionts under light conditions.

The fate of nitrogen

The uptake of inorganic N ($^{15}\text{NO}_3^-$) showed distinctly different patterns from C assimilation, including much lower DIN uptake rates compared to DIC and a continuous AP^{15}N decline in the host after the pulse for all treatments. All host samples (independent of state and treatment) showed a similarly strong initial enrichment after the pulse. Interestingly, this enrichment was followed by a pronounced drop in AP^{15}N in all treatments, which correlated with medusa size (i.e. larger medusae maintained higher AP^{15}N level during both chases; Fig.2d) and hence surface-to-volume ratio. A similarly quick increase and depletion in ^{15}N in *C. xamachana* was found by Freeman et al. [36] who speculated that N-fixing microbial communities could drive such pronounced nitrate uptake. The here characterized bacterial communities do not seem to include particularly abundant N-fixers. Our data, however, indicate abundant denitrifying bacterial communities that could contribute to the observed AP^{15}N pattern, specifically to the AP^{15}N drop (see below). This pattern is in stark contrast to coral host tissue, where ^{15}N did not decrease over 14 days in a similar pulse-chase experiment [49] which indicates coral to be highly effective in N retention [17]. We argue that initial enrichment and subsequent size-correlated depletion in AP^{15}N indicates a passive, concentration gradient driven movement of nitrate in and out of the host tissue that might work in concert with the associated prokaryotic community.

421 Importantly, the algal symbionts were unable to capitalize on available DIN indicating an effective
422 control mechanism. N restriction as a mechanism of symbiont control has been suggested in coral and
423 *Exaiptasia*; it is thought to maintain algal cell densities and ensure high rates of photosynthate
424 translocation [20]. In corals, Tanaka et al. [49] found AP¹⁵N in the associated algae to be 4.7 times higher
425 than in the host which corroborates that only the algae are able to assimilate nitrate. In *C. xamachana*,
426 however, the algal partners enrich slowly throughout the experiment, largely independent of the hosts
427 ¹⁵N level. Overall low nitrate assimilation is in line with other 'nutrient resistant' cnidarian-symbiodinian
428 associations. *L. unguiculata* [53] and *Exaiptasia pulchella* [54] removed none or only minimal nitrate
429 from their surrounding water and $\delta^{15}\text{N}$ enrichment from nitrate was not detectable in *Exaiptasia* [55]
430 (notably, all of these are non-calcifying hosts and *Exaiptasia* spp. are not obligate photosymbiotic). In
431 other photosymbioses adapted to high nutrient concentrations, for instance *Hydra viridissima* [56] or
432 *Paramecium bursaria* [57] some *Chlorella* endosymbionts have lost their assimilatory nitrate reduction
433 pathway as an adaptation to symbiotic conditions. Such inability for nitrate uptake remains to be tested
434 for *C. xamachana*'s algal partners. However, the host's capacity to associate with several
435 Symbiodiniaceae strains originating from 'nutrient sensitive' corals [30] argues for alternative
436 mechanisms. Studies on such calcifying corals demonstrate that the photosymbionts were able to
437 effectively assimilate nitrate and translocate it to their hosts in *Acropora pulchra* [58], *A. tenuis* [59],
438 *Orbicella faveolata* [60], *Porites cylindrica* [49], and *Stylophora pistillata* [61]. Compared to *C.*
439 *xamachana*, in a similar experimental setup (at 26°C with similar C assimilation rates) the algal fraction
440 of *O. faveolata* showed ¹⁵N enrichment two orders of magnitude greater (0.784 APE¹⁵N compared to
441 0.002 APE¹⁵N) and was remarkably higher than the host fraction enrichment [61]. In *C. xamachana*,
442 Symbiodiniaceae are located in a symbiosome, a membrane complex wrapping the algal cells, which are
443 situated in amoebocytes. While little is known about the function of (likely mobile) amoebocytes [31],
444 the symbiosome is actively involved in nutrient transfer in *Exaiptasia pallida* and *Acropora digitifera* [62]

and could be involved in restricting N access of the photosymbionts. Unlike nitrate, organic ¹⁵N from labelled *Artemia* appeared freely transferable within *C. xamachana* indicating that the N restriction might be specific for certain chemical forms. Heterotrophic or organic N, in contrast to nitrate, tends to improve health and metabolism in coral and may not harm the cnidarian-symbiodinian relationship [63,64]. Ammonium, a metabolic waste product of the host, can also stimulate photosynthesis and carbon translocation to the host even under environmental stress and, importantly, as the preferred N source inhibit nitrate uptake in Symbiodiniaceae [61,65]. By restricting access of external DIN for the algal symbionts and exerting control on the transfer of organic N and ammonium the host presumably improves the holobionts (nutrient) resilience, which might be supported by the prokaryotic community (see below). Taken together, N cycling within the *C. xamachana* holobiont suggests an effective internal nitrate restriction of its algal associates, which might contribute to the high nutrient tolerance in *C. xamachana*.

The associated bacterial microbiome shares characteristics with other cnidarians

Prokaryotic associates contribute to nutrient cycling and interact directly with host and Symbiodiniaceae. Dysbiosis, an imbalance of the associated microbial communities caused for instance by poor water quality and high nutrient level, can lead to coral disease, bleaching, and mortality [2]. Data on the associated bacterial communities (and disease) in *C. xamachana* are lacking. Other, aposymbiotic scyphozoans host largely species-specific bacterial communities that differ across body parts and life stages [66,67]. The bacterial associates are distinct from the environment, potentially selected via antimicrobial peptides, and involved in asexual reproduction, health, and fitness of the host [68,69].

The bacterial communities associated with *C. xamachana* are dominated by two ASVs (*Acinetobacter* sp. and *Pseudomonas* sp.) which make up 85 ± 2 % of all sequences in all samples but ApoLPul3 (66 %). The

most abundant taxa have been found to be associated with cnidarians before (e.g. *Acinetobacter* [70], *Pseudomonas* [71–73], *Massilia* [74], *Sphingobium* [75]). However, employing a blastn search based on representative sequences on NCBI including the eleven most abundant ASVs (each >500 sequence counts across all samples) did not yield associations with marine hosts. We next performed an OTU (operational taxonomic units, 97 % cut-off) based analysis (Additional File 2: Text S2) for comparison with other cnidarian microbiomes as to date most published data are based on this approach. Briefly, each *C. xamachana* sample hosts between 51 and 300 distinct OTUs which is in the same range (~100) as the cnidarian anemones *E. pallida* [73] and *Hydra vulgaris* [76] (Additional File 3: Table S6). In coral, the numbers are more variable depending on species and environmental conditions, but are generally at the order of tens to hundreds of OTUs [71,77,78]. Considering the best blastn hits for each OTU, we found that 9 of the 15 core microbiome member OTUs have previously been identified in corals and five in *E. pallida* (Additional File 3: Table S7). This highlights similarities in cnidarian holobiont and points towards bacterial taxa that are potentially conserved within the phylum.

Aposymbiotic samples seemed to host more variable bacterial communities illustrated by larger variations in the alpha diversity indices and a significantly larger within-treatment similarity value compared to both symbiotic groups (Table 2, Fig.3c). Stressed coral tend to display higher community dissimilarity [79], though that is not always the case [80]. Stress may leave them more vulnerable to invasion and thus be associated with otherwise untypical residents which may increase alpha diversity [81]. Here, a lack of nutrients in the aposymbiotic medusae could have similar results. In particular a lack of the obligate photosymbionts and the main energy source might cause an unbalance of the associated microorganisms. Members of the symbiome may also be linked to the cnidarian-symbiodinian association as suggested for *E. pallida* [73]. Interestingly, in the symbiome we identified only two members (*Acinetobacter* sp. and *Ruegeria* sp.), one of which was only absent in a single aposymbiotic sample. This is surprising as Symbiodiniaceae are thought to maintain core microbiomes of their own

[82], even *in hospite* [18,83]. In this context, *C. xamachana* provides a system to readily test Symbiodiniaceae-*Bacteria* associations in an obligate symbiosis as the host can be infected with different algal species [30].

The role of the bacterial microbiome in holobiont nutrient cycling

The importance of bacteria to multicellular organism's function has led to a surge of marine microbiome research [84]. Yet, the complexity of marine holobionts still hampers our understanding of functional interactions in these inter-kingdom relationships. Model organisms for host-microbe interactions are employed to overcome these hurdles, for instance the freshwater cnidarian *Hydra vulgaris* [85]. *C. xamachana* is a model for the marine cnidarian-symbiodinian association, but currently lacking data on its prokaryotic associates and their function [28]. This is an essential shortcoming as bacteria also directly interact with Symbiodiniaceae [82,86]. Further corroborating a functional importance of these associations, coral holobionts host conserved algal-bacterial partnerships [83] and bacteria derived carotenoids have been shown to protect the algal endosymbionts (and thereby the holobiont) from environmental stress [87].

The *Bacteria*-Symbiodiniaceae interactions has been hypothesized to be a hidden key for coral reef resilience [18]. Our data suggests that the majority of *C. xamachana* associated *Bacteria* are directly involved in nitrogen cycling and in particular denitrification. Based on a literature search the 20 most abundant ASVs (Additional File 3, Table S2) belong to genera that can be linked to denitrification processes including *Acinetobacter* [88], *Pseudomonas* [89], *Massilia* [90], *Sphingobium* [91], *Stenotrophomonas* [92], *Cellvibrio* [93], *Brevundimonas* [94], *Sphingomonas* [95], *Ralstonia* [96], *Cutibacterium* (KEGG pathway *Cutibacterium acnes* KPA171202 [97]), *Flavobacterium* [98], and *Ruegeria* [99]. Further, *Acinetobacter* [88], *Pseudomonas* [89], *Massilia* [100], *Sphingomonas* [101] and *Flavobacterium* [98] have also been associated to nitrification processes and nitrifying bacteria have

previously been linked to *Cassiopea* sp. [35]. These findings were substantiated by a sequence based NCBI blastn search. For the 20 most abundant ASVs we identified 12 complete genomes at 100 % similarity, 6 at >99 %, and 2 at >97 %, which all included gene homologs presumably involved in N-cycling (Additional File 3: Table S8). Considering only genomes that match 100 %, 10 taxa contained at least one gene homolog linked to denitrification making up >88 % of all ASVs for all samples but ApoL3 (>73 %). We further identified several gene homologs for nitrogen transport, ammonification, nitrification, and nitrogen fixation (Additional File 3: Table S8). Amplicon sequencing data are not quantitative and - like inferred functionality - should be interpreted with caution, however, in comparison to coral [72,102] the dominance of taxa presumably involved in nitrogen cycling and particularly denitrification is striking. Both processes could be further supported by archaeal communities that were not assessed in this study [103]. While the pathways involved remain to be elucidated (particularly considering oxygen sensitivity of nitrogenase), a combination of denitrifying, nitrifying, and ammonifying communities could effectively remove bioavailable N from the holobiont and support algal N limitation [22,103,104]. Future studies should target specifically functional genes to quantify N cycling processes (e.g. nitrite reductase nir, nitric oxide reductase nor, ammonia monooxygenase amo, and dinitrogenase reductase nif).

The associated bacterial communities further seem to contribute to the holobionts N assimilation. N enrichment in the host tissue correlated positively with phylogenetic diversity of the associated bacterial community (Fig.5). Of note, host (and algal) fraction measurements may include ¹⁵N that was assimilated by their associated prokaryotic community. However, based on the quick release of ¹⁵N after the chase (Fig.2b) the holobionts overall assimilation of DIN is low and points towards a contribution of the microbial community. Interestingly, the positive relationship between bacterial phylogenetic diversity and ¹⁵N uptake rates fits with the biodiversity - ecosystem functioning theory [105]. It has been shown that higher species numbers and phylogenetic diversity can enhance nutrient uptake and storage on an

ecosystem level [106]. The here presented data hint that such a relationship might also exist within the holobiont/metaorganism concept.

Holobiont nutrient cycling changed fundamentally upon the cnidarian-symbiodinian dissociation. Without Symbiodiniaceae, the supply of autotrophic C collapsed, the holobionts nutrient cycling and internal composition changed towards a starvation mode. These physiological changes were also reflected by the associated *Bacteria*. Differences in the predicted functional profiles indicated that the bacterial communities changed from an N ('Ammonia oxidizer', 'Nitrite' reducer', 'Sulfate reducer') towards a sulfur ('Sulfur oxidizer', 'Sulfide oxidizer', 'Sulfur metabolism') based metabolism. This trend contrasts findings on *Exaiptasia* where symbiotic polyps indicated higher sulfur metabolic activities, which was suggested to originate from Symbiodiniaceae derived dimethylsulfoniopropionate (DMSP) [73]. DMSP, however, is also present in (symbiotic) *C. andromeda* [107]. The enrichment of 'Ammonia oxidizer' and 'Nitrite reducer' in symbiotic *C. xamachana* indicates biologically available N, which could be removed by the respective bacterial taxa supporting the algae's nutrient limitation. This is also in contrast to findings in *Exaiptasia* ('Nitrite reducer' enriched in aposymbiotic samples). While the predicted functional profiles corroborate nutrient deprivation in the medusae, the results should be interpreted with caution, as bacterial taxa putatively involved in N cycling were also dominating in aposymbiotic individuals (see above) and functional predictions remain hypothetical at large. Future work employing metagenomics and metatranscriptomics may provide more direct assessment of this relationship and the role of each holobiont member.

CONCLUSIONS

Our data indicate that all holobiont members are contributing to nutrient cycling in *C. xamachana*. The host assimilates organic C and N and transfers both to its algal associates which are restricted in DIN access. The obligate symbiosis with Symbiodiniaceae provides access to autotrophically fixed C that may

contribute to energetically costly DIN assimilation. The cnidarian-symbiodinian association further facilitates effective C and N cycling. The associated bacterial communities contribute to DIN uptake (correlated to phylogenetic diversity) and the host to its turnover (correlated to size). Nitrate levels in the host and algal partner indicate an internal strategy to limit algal N access that differs from other cnidarians (especially coral) and might help to explain the high nutrient tolerance peculiar to *Cassiopea* spp. While N stemming from DIN can quickly enrich and deplete in the host tissue, the photosymbionts' access is limited. Nitrate restriction could be realized at the symbiosome which should be addressed in future research. The holobiont hosts a striking abundance of putatively denitrifying (and N-cycling related) bacteria that presumably remove N from the host tissue. The combination of host DIN control and microbial DIN removal might enable the jellyfish to thrive in nutrient rich waters in contrast to coral. Recent 'coral probiotics' approaches could test whether these bacteria are suitable to increase coral resilience in eutrophied habitats [65].

Future research may further focus on several aspects in regard to internal DIN control including (a) the assimilation, recycling, and internal control of other DIN species (e.g. ammonium); the role of the associated microbial communities by (b) identifying the spatial niche that they occupy; (c) quantifying the interplay of N cycling capacities (e.g. denitrification, N₂ fixation, ANAMMOX) by targeting marker genes via qPCR or transcriptomics; and (d) characterizing N-cycling processes in *C. xamachana* affected by environmental change, potentially including manipulation of symbiodinian and/or prokaryotic communities.

Figure details

Figure 1. Experimental set-up. Boxes depict treatments (color) and number of specimens (n). Treatments include symbiotic-light (SymL, orange), symbiotic-dark (SymD, grey), and aposymbiotic-light (ApoL, pink). Each vertical line represents a sampling event (T0, T1, T2, and T3) for 5 specimens from each treatment.

Figure 2. Nutrient dynamics in *C. xamachana*. Displayed are mean values ($n = 5$) per group for **(a)** C enrichment from $\text{NaH}^{13}\text{CO}_3$ expressed as AP^{13}C and **(b)** N enrichment from $\text{Na}^{15}\text{NO}_3$ expressed as AP^{15}N after the pulse (5 h), chase3h (3 h after pulse) and chase6h (6 h after pulse). Both panels are depicted with broken y-axes to facilitate comparisons between less enriched samples. Treatments included light-incubated symbiotic medusae (SymL), dark-incubated symbiotic medusae (SymD) and light-incubated aposymbiotic medusae (ApoL). Error bars represent 95 % confidence intervals and dashed line natural isotope ratios ($n = 5$; $1.081 \text{ AP}^{13}\text{C}$ and $0.368 \text{ AP}^{15}\text{N}$). Further shown are **(c)** the regression between host C enrichment and symbiont density expressed as AP^{13}C for SymL ($n = 15$; $y = 1.24 + 0.17x$; $r^2_{\text{Adj}} = 0.68$, $p < 0.001$) and **(d)** the regressions between host ^{15}N enrichment and medusae size expressed as AP^{15}N from all treatments for chase3h ($y = 0.3381 + 0.0014x$; $r^2_{\text{Adj}} = 0.67$, $p < 0.001$) and chase6h ($y = 0.3583 + 0.0004x$; $r^2_{\text{Adj}} = 0.86$, $p < 0.001$; each $n = 15$).

Figure 3. *C. xamachana* associated bacterial communities. **(a)** Stacked column plot representing the bacterial community composition associated with *C. xamachana* on the family level (SILVA database). Each color represents one of the eight most abundant families, all 85 other families are grouped as 'others'. **(b)** Clustering of *C. xamachana* samples based on Bray-Curtis dissimilarity of microbial community abundances in a non-metric multi-dimensional scaling plot (NMDS; stress value < 0.078). Circles indicate 95% confidence ranges. **(c)** Boxplots of pairwise Bray-Curtis dissimilarities of samples within treatments. Statistical comparisons are based on Kruskal-Wallis rank sum test ($\chi^2 = 21.5$, $\text{df} = 2$, $p < 0.001$). Letters indicate significant differences of pairwise comparisons ($p_{\text{Holm}} < 0.01$). Treatment abbreviations are same as in Fig.2.

Figure 4. Predicted taxonomy-based functional differences of bacterial communities in *C. xamachana*. Enrichment is depicted in red and depletion in blue on a relative scale. Treatment abbreviations are same as in Fig.2.

Figure 5. Bacterial phylogenetic diversity affects host N assimilation. Regression: $y = 0.667 + 0.368 x$, $r^2_{\text{Adj}} = 0.39$, $p < 0.01$. $n = 15$, all sampled after pulse incubation. Treatment abbreviations are the same than in Fig.2.

Additional files

Additional File 1:

Figure S1. Effects of menthol bleaching on *C. xamachana*. **(a)** Photosynthetic efficiency throughout menthol exposure (day 1-4) and bleaching process. $n = 12$; Imaging-PAM (Walz, Germany); F_o : dark-adapted minimal fluorescence yields; F_m : dark-adapted maximal fluorescence yield; F_v/F_m : maximum quantum yield. **(b)** Response of bell diameter (BD) and wet weight (WW) to bleaching (mean, $n = 12$). **(c)** Visualization of photosynthetic efficiency (i.e. F_o , F_m , and F_v/F_m).

Figure S2. Relationship between wet weight (WW) and bell diameter (BD) in (a) symbiotic ($n = 35$) [WW_{symbiotic} = 8.540 - 0.494*BD + 0.009*BD²] and (b) aposymbiotic *C. xamachana* ($n = 12$) [WW_{aposymbiotic} = -1.440 + 0.100*BD]. Different colors indicate the number of days after start of the four day menthol bleaching. Dashed vertical lines indicate minimum and maximum BD of medusae employed in the pulse-chase experiment.

Figure S3. Net primary production (P_n) and host carbon enrichment. Host fraction enrichment expressed as AP¹³C light-incubated medusae (SymL) from all incubations (pulse, chase 3h and chase 6h) ($n = 15$).

Additional File 2:

Text S1. Extended Material and Methods

Text S2. OTU based bacterial analysis.

Additional File 3:

Table S2. ASV abundance table detailing ASV numbers over samples, including taxonomy and representative sequences. Number of ASVs 303, of which 7 are contaminants (marked with *), core microbiome members in bold, 'symbiome' members underlined for symbiotic samples and 'apobiome' members underlined for aposymbiotic samples.

Table S3. Detailed stable isotope measurements of *Cassiopea xamachana* and its associated Symbiodiniaceae in symbiotic-light (SymL), symbiotic-dark (SymD), aposymbiotic-light (ApOL) treatments and upon heterotrophic feeding.

Table S4. Stable isotope analysis enrichment statistics.

Table S5. Stable isotope labelling of *Isochrysis galbana* and *Artemia salina*. ¹³C and ¹⁵N enriched *Isochrysis galbana* algae were employed to label *Artemia salina* as a heterotrophic food source for *C. xamachana*.

Table S6. OTU abundance table detailing OTU numbers over samples, including taxonomy and representative sequences. Number of OTUs 697, of which 9 are contaminants (marked with *), core microbiome members in bold, 'symbiome' members underlined for symbiotic samples and 'apobiome' members underlined for aposymbiotic samples

Table S7. Closest blastn hits considered for presumably important OTUs (including core microbiome, symbiome, and apobiome; assessed June 2020).

Table S8. Closest complete genomes of the 20 most abundant ASVs based on an NCBI blastn search (assessed November 2020). Considered gene homologs are related to nitrogen cycling and transport.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Sequences determined in this study have been deposited in the NCBI Sequence Read Archive under accession no. PRJNA627421 (<https://www.ncbi.nlm.nih.gov/bioproject/627421>).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GP, JCYW, TR, and WM designed and conceived the experiments. GP, TR, AB, JCYW, and WM generated, analyzed and interpreted the data. DMB contributed reagents/materials/analysis tools. TR, GP, and JCYW wrote the first draft, all authors contributed to the final manuscript.

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REFERENCES

1. McFall-Ngai M, Hadfield MG, Bosch TCG, Carey H V, Domazet-Lošo T, Douglas AE, et al. Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci*. 2013;110:3229–36. <https://doi.org/10.1073/pnas.1218525110> National Academy of Sciences.
2. Sweet MJ, Bulling MT. On the Importance of the Microbiome and Pathobiome in Coral Health and Disease. *Front Mar Sci*. 2017;4. <https://doi.org/10.3389/fmars.2017.00009> Frontiers Media SA.
3. Rohwer F, Seguritan V, Azam F, Knowlton N. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser*. 2002;243:1–10. <https://doi.org/10.3354/meps243001> Inter Research.
4. LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, et al. Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Curr Biol*. Cell Press; 2018;28:2570–2580.e6. <https://doi.org/10.1016/j.cub.2018.07.008> Cell Press.
5. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* . 2007;5:355–62. <http://dx.doi.org/10.1038/nrmicro1635> Nature Publishing Group.
6. Muscatine L, Porter JW. Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *Bioscience* . 1977;27:454–60. <https://doi.org/10.2307/1297526> Oxford Academic.
7. Burriesci MS, Raab TK, Pringle JR. Evidence that glucose is the major transferred metabolite in dinoflagellate – cnidarian symbiosis symbiosis. *J Exp Biol*. 2012;215:3467–77. <https://doi.org/10.1242/jeb.070946> Company of Biologists Ltd.
8. Davy SK, Allemand D, Weis VM. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol Mol Biol Rev* . School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand. 2012;76:229–61. <https://doi.org/10.1128/membr.05014-11> American Society for Microbiology.
9. Verde E, McCloskey L. Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and season. *Mar Ecol Prog Ser* . Inter-Research. 1998;168:147–62. <https://doi.org/10.3354/meps168147> Inter-Research.
10. Muscatine L, Falkowski PG, Porter JW, Dubinsky Z. Fate of photosynthetic fixed carbon in light-adapted and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proc R Soc Ser B-Biological Sci*. 1984.222:181–202. <https://doi.org/10.1098/rspb.1984.0058> The Royal Society London.
11. Costanza R, D’Arge R, De Groot R, Farber S, Grasso M, Hannon B, et al. The value of the world’s ecosystem services and natural capital. *Nature*. 1997 387:253–60. <https://doi.org/10.1038/387253a0> Nature Publishing Group.
12. Weis VM. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol*. 2008;211:3059–66. <https://doi.org/10.1242/jeb.009597> Company of Biologists Ltd.
13. Brown BE. Coral bleaching: causes and consequences. *Coral Reefs*. 1997;16:S129–38. <http://dx.doi.org/10.1007/s003380050249> Springer Berlin Heidelberg.
14. Buddemeier RW, Fautin DG. Coral bleaching as an adaptive mechanism - a testable hypothesis. *Bioscience*. 1993;43:320–6. <https://doi.org/10.2307/1312064> Oxford Academic.
15. Hughes TP, Kerry JT, Baird AH, Connolly SR, Dietzel A, Eakin CM, et al. Global warming transforms

731 coral reef assemblages. *Nature*. 2018;556:492–6. <https://doi.org/10.1038/s41586-018-0041-2> Nature
732 Publishing Group.

733 16. Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR. Chronic nutrient
734 enrichment increases prevalence and severity of coral disease and bleaching. *Glob Chang Biol* .
735 2014;20:544–54. Available from: <http://dx.doi.org/10.1111/gcb.12450> John Wiley & Sons, Ltd.

736 17. Rådecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C. Nitrogen cycling in corals: The key to
737 understanding holobiont functioning? *Trends Microbiol*. 2015. p. 490–7.
738 <https://doi.org/10.1016/j.tim.2015.03.008> Elsevier.

739 18. Matthews JL, Raina J, Kahlke T, Seymour JR, Oppen MJH, Suggett DJ. Symbiodiniaceae-bacteria
740 interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic
741 networks. *Environ Microbiol*. 2020;22:1675–87. <https://doi.org/10.1111/1462-2920.14918> Blackwell
742 Publishing Ltd.

743 19. Benavides M, Bednarz VN, Ferrier-Pagès C. Diazotrophs: Overlooked key players within the coral
744 symbiosis and tropical reef ecosystems? *Front Mar Sci*. 2017;4:10.
745 <https://doi.org/10.3389/fmars.2017.00010> Frontiers Media SA.
746
747

748 20. Cui G, Liew YJ, Li Y, Kharbatia N, Zahran NI, Emwas A-H, et al. Host-dependent nitrogen recycling as a
749 mechanism of symbiont control in *Aiptasia*. *PLOS Genet*. 2019;15:e1008189.
750 <https://doi.org/10.1371/journal.pgen.1008189> Krediet CJ, editor. Public Library of Science.

751 21. Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, et al. Nutrient enrichment can
752 increase the susceptibility of reef corals to bleaching. *Nat Clim Chang*. 2013;3:160–4.
753 <https://doi.org/10.1038/nclimate1661> Nature Publishing Group.

754 22. Tilstra A, El-Khaled YC, Roth F, Rådecker N, Pogoreutz C, Voolstra CR, et al. Denitrification Aligns with
755 N₂ Fixation in Red Sea Corals. *Sci Rep*. 2019;9:1–9. <https://doi.org/10.1038/s41598-019-55408-z> Nature
756 Publishing Group.
757

758 23. Conti-Jerpe IE, Thompson PD, Wong CWM, Oliveira NL, Duprey NN, Moynihan MA, et al. Trophic
759 strategy and bleaching resistance in reef-building corals. *Sci Adv*. 2020;6:eaaz5443.
760 <https://doi.org/10.1126/sciadv.aaz5443> American Association for the Advancement of Science.

761 24. Baker DM, Freeman CJ, Knowlton N, Thacker RW, Kim K, Fogel ML. Productivity links morphology,
762 symbiont specificity and bleaching in the evolution of Caribbean octocoral symbioses. *ISME J*.
763 2015;9:2620–2629 <http://dx.doi.org/10.1038/ismej.2015.71> Nature Publishing Group.

764 25. Wooldridge SA. Breakdown of the coral-algae symbiosis: towards formalising a linkage between
765 warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae.
766 *Biogeosciences*. 2013;10:1647–58. <https://dx.doi.org/10.5194/bg-10-1647-2013> Copernicus
767 Publications.

768 26. Stoner EW, Yeager LA, Sweatman JL, Sebilian SS, Layman CA. Modification of a seagrass community
769 by benthic jellyfish blooms and nutrient enrichment. *J Exp Mar Bio Ecol*. 2014;461:185–92.
770 <https://doi.org/10.1016/j.jembe.2014.08.005> Elsevier.

771 27. Niggel W, Wild C. Spatial distribution of the upside-down jellyfish *Cassiopea* sp. within fringing coral
772 reef environments of the Northern Red Sea: implications for its life cycle. *Helgol Mar Res*. 2010;64:281–

7. <https://dx.doi.org/10.1007/s10152-009-0181-8> Springer Berlin Heidelberg.
28. Ohdera AH, Abrams MJ, Ames CL, Baker DM, Suescún-Bolívar LP, Collins AG, et al. Upside-Down but Headed in the Right Direction: Review of the Highly Versatile *Cassiopea xamachana* System. *Front Ecol Evol.* 2018;6:35. <https://doi.org/10.3389/fevo.2018.00035> Frontiers Media SA.
29. Rädercker N, Pogoreutz C, Wild C, Voolstra CR. Stimulated Respiration and Net Photosynthesis in *Cassiopeia* sp. during Glucose Enrichment Suggests *in hospite* CO₂ Limitation of Algal Endosymbionts. *Front Mar Sci.* 2017;4:267. <https://doi.org/10.3389/fmars.2017.00267> Frontiers Media SA.
30. Newkirk CR, Frazer TK, Martindale MQ, Schnitzler CE. Adaptation to Bleaching: Are Thermotolerant Symbiodiniaceae Strains More Successful Than Other Strains Under Elevated Temperatures in a Model Symbiotic Cnidarian? *Front Microbiol.* 2020;11:822. <https://doi.org/10.3389/fmicb.2020.00822> Frontiers Media SA.
31. Estes AM, Kempf SC, Henry RP. Localization and quantification of carbonic anhydrase activity in the symbiotic scyphozoan *Cassiopea xamachana*. *Biol Bull.* 2003;204:278–89. <https://doi.org/10.2307/1543599> University of Chicago Press.
32. Kremer P. Ingestion and elemental budgets for *Linuche unguiculata*, a scyphomedusa with zooxanthellae. *J Mar Biol Assoc.* 2005;85:613–25. <https://doi.org/10.1017/S0025315405011549> Cambridge University Press.
33. McGill CJ, Pomory CM. Effects of bleaching and nutrient supplementation on wet weight in the jellyfish *Cassiopea xamachana* (Bigelow) (Cnidaria: Scyphozoa). *Mar Freshw Behav Physiol.* 2008;41:179–89. <https://doi.org/10.1080/10236240802369899> Taylor & Francis Group.
34. Mortillaro JM, Pitt KA, Lee SY, Meziane T. Light intensity influences the production and translocation of fatty acids by zooxanthellae in the jellyfish *Cassiopea* sp. *J Exp Mar Bio Ecol.* 2009;378:22–30. <https://doi.org/10.1016/j.jembe.2009.07.003> Elsevier.
35. Welsh DT, Dunn RJK, Meziane T. Oxygen and nutrient dynamics of the upside-down jellyfish (*Cassiopea* sp.) and its influence on benthic nutrient exchanges and primary production. *Hydrobiologia.* 2009;635:351–62. <https://doi.org/10.1007/s10750-009-9928-0> Springer Berlin Heidelberg.
36. Freeman C, Stoner E, Easson C, Matterson K, Baker D. Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggests a coupling of host and symbiont metabolism in the Symbiodinium-Cassiopea mutualism. *Mar Ecol Prog Ser.* 2017;571:245–51. <https://doi.org/10.3354/meps12138> Inter-Research.
37. Ohdera A, Ames CL, Dikow RB, Kayal E, Chiodin M, Busby B, et al. Box, stalked, and upside-down? Draft genomes from diverse jellyfish (Cnidaria, Acraspeda) lineages: *Alatina alata* (Cubozoa), *Calvadosia cruxmelitensis* (Staurozoa), and *Cassiopea xamachana* (Scyphozoa). *GigaScience.* 2019;8:giz069. <https://doi.org/10.1093/gigascience/giz069> Oxford Academic.
38. Matthews JL, Sproles AE, Oakley CA, Grossman AR, Weis VM, Davy SK. Menthol-induced bleaching rapidly and effectively provides experimental aposymbiotic sea anemones (*Aiptasia* sp.) for symbiosis investigations. *J Exp Biol.* 2016;219:306–10. <https://dx.doi.org/10.1242/jeb.128934> Company of Biologists Ltd.
39. Al-Horani FA, Al-Moghrabi SM, Beer D. The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. *Mar Biol.* 2003;142:419–426. <https://doi.org/10.1007/s00227-002-0981-8> Springer Berlin Heidelberg.

- 814 40. Fry B. Stable isotope ecology. Springer; 2006.
- 815 41. Team RDC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation
816 for Statistical Computing; 2020. Available from: <http://www.r-project.org>
- 817 42. Coffroth MA, Lasker HR, Diamond ME, Bruenn JA, Bermingham E. DNA fingerprints of a gorgonian
818 coral: a method for detecting clonal structure in a vegetative species. *Mar Biol.* 1992;114:317–25.
819 <https://doi.org/10.1007/BF00349534> Springer Berlin Heidelberg.
- 820 43. Andersson AF, Lindberg M, Jakobsson H, Backhed F, Nyren P, Engstrand L. Comparative analysis of
821 human gut microbiota by barcoded pyrosequencing. *PLoS One.* 2008;3:e2836.
822 <https://doi.org/10.1371/journal.pone.0002836> Public Library of Science.
- 823 44. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible,
824 interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.*
825 2019;37:852–7. <https://doi.org/10.1038/s41587-019-0209-9> Nature Publishing Group.
- 826 45. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution
827 sample inference from Illumina amplicon data. *Nat Methods.* 2016;13:581–3. Available from:
828 <https://doi.org/10.1038/nmeth.3869> Nature Publishing Group.
- 829 46. Hsieh TC, Ma KH, Chao A. iNEXT: an R package for rarefaction and extrapolation of species diversity
830 (Hill numbers). *Methods Ecol Evol.* 2016;7:1451–6. <https://doi.org/10.1111/2041-210X.12613> British
831 Ecological Society.
- 832 47. Faith DP. Conservation evaluation and phylogenetic diversity. *Biol Conserv.* Elsevier; 1992;61:1–10.
833 [https://doi.org/10.1016/0006-3207\(92\)91201-3](https://doi.org/10.1016/0006-3207(92)91201-3). Elsevier.
- 834 48. Ainsworth T, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, et al. The coral core microbiome
835 identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J.* 2015;9:2261–74.
836 <https://doi.org/10.1038/ismej.2015.39> Nature Publishing Group.
- 837 49. Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H. Translocation and conservation of organic
838 nitrogen within the coral-zooxanthella symbiotic system of *Acropora pulchra*, as demonstrated by dual
839 isotope-labeling techniques *J Exp Mar Bio Ecol.* 2006;336:110–9.
840 <https://doi.org/10.1016/j.jembe.2006.04.011> Elsevier.
- 841 50. Alonso-Sáez L, Galand PE, Casamayor EO, Pedrós-Alió C, Bertilsson S. High bicarbonate assimilation
842 in the dark by Arctic bacteria. *ISME J.* 2010;4:1581–90. <https://doi.org/10.1038/ismej.2010.69> Nature
843 Publishing Group.
- 844 51. Tremblay P, Maguer JF, Grover R, Ferrier-Pages C. Trophic dynamics of scleractinian corals: Stable
845 isotope evidence. *J Exp Biol.* 2015;218:1223–34. <https://doi.org/10.1038/ismej.2010.69> Company of
846 Biologists Ltd.
- 847 52. Hughes A, Grottoli A, Pease T, Matsui Y. Acquisition and assimilation of carbon in non-bleached and
848 bleached corals. *Mar Ecol Prog Ser.* 2010;420:91–101. <https://doi.org/10.3354/meps08866> Inter
849 Research.
- 850 53. Wilkerson FP, Kremer P. DIN, DON and PO₄ flux by a medusa with algal symbionts. *Mar Ecol Ser.*
851 1993;90:237-50. <https://doi.org/10.3354/meps090237> Inter Research.

54. Wilkerson FP, Muscatine L. Uptake and assimilation of dissolved inorganic nitrogen by a symbiotic sea anemone. *Proc R Soc London Ser B Biol Sci.* 1984;221:71–86.
<https://doi.org/10.1098/rspb.1984.0023> The Royal Society London.

55. Sproles AE. Nutritional Interactions in the Cnidarian-Dinoflagellate Symbiosis and their Role in Symbiosis Establishment. 2017. *PhD Thesis.* Victoria University of Wellington.
<http://hdl.handle.net/10063/6646>

56. Hamada M, Schröder K, Bathia J, Kürn U, Fraune S, Khalturina M, et al. Metabolic co-dependence drives the evolutionarily ancient Hydra–Chlorella symbiosis. *Elife.*; 2018;7.
<https://doi.org/10.7554/eLife.35122.001> eLife Sciences Publications Ltd.

57. Kamako SI, Hoshina R, Ueno S, Imamura N. Establishment of axenic endosymbiotic strains of Japanese *Paramecium bursaria* and the utilization of carbohydrate and nitrogen compounds by the isolated algae. *Eur J Protistol.* 2005;41:193–202. <https://doi.org/10.1016/j.ejop.2005.04.001> Elsevier.

58. Tanaka Y, Suzuki A, Sakai K. The stoichiometry of coral-dinoflagellate symbiosis: Carbon and nitrogen cycles are balanced in the recycling and double translocation system. *ISME J.* 2018;12:860–8.
<https://doi.org/10.1038/s41396-017-0019-3> Nature Publishing Group.

59. Baker DM, Andras JP, Jordán-Garza AG, Fogel ML. Nitrate competition in a coral symbiosis varies with temperature among Symbiodinium clades. *ISME J.* 2013;7:1248–51.
<https://doi.org/10.1038/ismej.2013.12> Nature Publishing Group.

60. Baker DM, Freeman CJ, Wong JCY, Fogel ML, Knowlton N. Climate change promotes parasitism in a coral symbiosis. *ISME J.* 2018;12:921–30. <https://doi.org/10.1038/s41396-018-0046-8> Nature Publishing Group.

61. Grover R, Maguer J-F, Allemand D, Ferrier-Pagès C. Nitrate uptake in the scleractinian coral *Stylophora pistillata*. *Limnol Oceanogr.* 2003;48:2266–74. <https://doi.org/10.4319/lo.2003.48.6.2266> American Society of Limnology and Oceanography Inc.

62. Hambleton EA, Jones VAS, Maegele I, Kvaskoff D, Sachsenheimer T, Guse A. Sterol transfer by atypical cholesterol-binding NPC2 proteins in coral-algal symbiosis. *eLife.* 2019;8:e43923.
<https://doi.org/10.7554/eLife.43923> eLife Sciences Publications Ltd.

63. Grottoli AG, Rodrigues LJ, Palardy JE. Heterotrophic plasticity and resilience in bleached corals. *Nature.* 2006;440:1186–9. <https://doi.org/10.1038/nature04565> Nature Publishing Group.

64. Ferrier-Pagès C, Rottier C, Beraud E, Levy O. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. *Exp Mar Bio Ecol.* 2010;390:118–24. <https://doi.org/10.1016/j.jembe.2010.05.007> Elsevier.

65. Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK. Nutrient Availability and Metabolism Affect the Stability of Coral–Symbiodiniaceae Symbioses. *Trends Microbiol.* 2019;27:678–689.
<https://doi.org/10.1016/j.tim.2019.03.004> Elsevier.

66. Lee MD, Kling JD, Araya R, Ceh J. Jellyfish Life Stages Shape Associated Microbial Communities, While a Core Microbiome Is Maintained Across All. *Front Microbiol.* 2018;9:1534.
<https://doi.org/10.3389/fmicb.2018.01534> Frontiers Media SA.

67. Tinta T, Kogovšek T, Klun K, Malej A, Herndl GJ, Turk V. Jellyfish-Associated Microbiome in the Marine Environment: Exploring Its Biotechnological Potential. *Mar Drugs.* 2019;17:94.

892 <https://doi.org/10.3390/md17020094> MDPI AG.

893 68. Ovchinnikova T V., Balandin S V., Aleshina GM, Tagaev AA, Leonova YF, Krasnodembsky ED, et al.
 894 Aurelin, a novel antimicrobial peptide from jellyfish *Aurelia aurita* with structural features of defensins
 895 and channel-blocking toxins. *Biochem Biophys Res Commun.* 2006;348:514–23.
 896 <https://doi.org/10.1016/j.bbrc.2006.07.078> Academic Press.

897 69. Weiland-Bräuer N, Pinnow N, Langfeldt D, Roik A, Güllert S, Chibani CM, et al. The Native
 898 Microbiome is Crucial for Offspring Generation and Fitness of *Aurelia aurita*. *MBio.* 2020;11:e02336-20.
 899 <https://doi.org/10.1128/mBio.02336-20> American Society for Microbiology.

900 70. Koren O, Rosenberg E. Bacteria associated with the bleached and cave coral *Oculina patagonica*.
 901 *Microb Ecol.*2008;55,523–529. <https://doi.org/10.1007/s00248-007-9297-z> Springer Berlin Heidelberg.

902 71. Röthig T, Yum LK, Kremb SG, Roik A, Voolstra CR. Microbial community composition of deep-sea
 903 corals from the Red Sea provides insight into functional adaption to a unique environment. *Sci*
 904 *Rep.*2017;7:44714. <https://doi.org/10.1038/srep44714> Nature Publishing Group.

905 72. Röthig T, Ochsenkühn MA, Roik A, van der Merwe R, Voolstra CR. Long-term salinity tolerance is
 906 accompanied by major restructuring of the coral bacterial microbiome. *Mol Ecol.* 2016;25:1308–23.
 907 <https://doi.org/10.1111/mec.13567> John Wiley & Sons, Ltd.

908 73. Röthig T, Costa RM, Simona F, Baumgarten S, Torres AF, Radhakrishnan A, et al. Distinct Bacterial
 909 Communities Associated with the Coral Model *Aiptasia* in Aposymbiotic and Symbiotic States with
 910 *Symbiodinium*. *Front Mar Sci.* 2016;3:23. <https://doi.org/10.3389/fmars.2016.00234> Frontiers Media SA.

911 74. Yang S-H, Tseng C-H, Huang C-R, Chen C-P, Tandon K, Lee STM, et al. Long-Term Survey Is Necessary
 912 to Reveal Various Shifts of Microbial Composition in Corals. *Front Microbiol.* *Front. Microbiol.*
 913 2017;8:1094. <https://doi.org/10.3389/fmicb.2017.01094> Frontiers Media S.A.

914 75. Hadaidi G, Röthig T, Yum LK, Ziegler M, Arif C, Roder C, et al. Stable mucus-associated bacterial
 915 communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep.*
 916 2017;7:45362. <http://dx.doi.org/10.1038/srep45362> Nature Publishing Group.

917 76. Franzenburg S, Fraune S, Altrock PM, Künzel S, Baines JF, Traulsen A, et al. Bacterial colonization of
 918 *Hydra* hatchlings follows a robust temporal pattern. *ISME J.* 2013;7:781–90.
 919 <https://doi.org/10.1038/ismej.2012.156> Nature Publishing Group.

920 77. Hernandez-Agreda A, Leggat W, Bongaerts P, Herrera C, Ainsworth TD. Rethinking the Coral
 921 Microbiome: Simplicity Exists within a Diverse Microbial Biosphere. *MBio.* 2018;9:e00812-18.
 922 <https://doi.org/10.1128/mBio.00812-18> American Society for Microbiology.

923 78. Röthig T, Bravo H, Corley A, Prigge T-L, Chung A, Yu V, et al. Environmental flexibility in *Oulastrea*
 924 *crispata* in a highly urbanised environment: a microbial perspective. *Coral Reefs.* 2020; 9:649–662.
 925 <https://doi.org/10.1007/s00338-020-01938-2> Springer Berlin Heidelberg.

926 79. Zaneveld JR, McMinds R, Vega Thurber R. Stress and stability: applying the Anna Karenina principle
 927 to animal microbiomes. 2017;2:17121. *Nat. Microb.* <http://dx.doi.org/10.1038/nmicrobiol.2017.121>
 928 Nature Publishing Group.

929 80. Sweet M, Burian A, Fifer J, Bulling M, Elliott D, Raymundo L. Compositional homogeneity in the
 930 pathobiome of a new, slow-spreading coral disease. *Microbiome* 2019;7:139.
 931 <https://doi.org/10.1186/s40168-019-0759-6> Springer Berlin Heidelberg.

81. McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL. Responses of Coral-Associated Bacterial Communities to Local and Global Stressors. *Front Mar Sci*. 2017;4:262. <https://doi.org/10.3389/fmars.2017.00262> Frontiers Media SA.

82. Lawson CA, Raina J-B, Kahlke T, Seymour JR, Suggett DJ. Defining the core microbiome of the symbiotic dinoflagellate, *Symbiodinium*. *Environ Microbiol Rep*. 2018;10:7–11. <https://doi.org/10.1111/1758-2229.12599> John Wiley & Sons, Ltd.

83. Bernasconi R, Stat M, Koenders A, Huggett MJ. Global Networks of *Symbiodinium*-Bacteria Within the Coral Holobiont. *Microb Ecol*. 2018;1–14. <https://doi.org/10.1007/s00248-018-1255-4> Springer Berlin Heidelberg.

84. Moran MA. The global ocean microbiome. *Science* (80-). American Association for the Advancement of Science; 350:aac8455–aac8455. <https://doi.org/10.1126/science.aac8455> American Association for the Advancement of Science.

85. Bosch TCG, Guillemin K, McFall-Ngai M. Evolutionary “Experiments” in Symbiosis: The Study of Model Animals Provides Insights into the Mechanisms Underlying the Diversity of Host–Microbe Interactions. *BioEssays*. 2019;1800256. <https://doi.org/abs/10.1002/bies.201800256> John Wiley & Sons, Ltd.

86. Lawson CA, Seymour JR, Possell M, Suggett DJ, Raina J-B. The Volatilomes of *Symbiodiniaceae*-Associated Bacteria Are Influenced by Chemicals Derived From Their Algal Partner. *Front Mar Sci*. 2020;7:106. <https://doi.org/10.3389/fmars.2020.00106> Frontiers Media SA.

87. Motone K, Takagi T, Aburaya S, Miura N, Aoki W, Ueda M. A zeaxanthin-producing bacterium isolated from the algal phycosphere protects coral endosymbionts from environmental stress. *MBio*. 2020; 11: :e01019-19. <https://doi.org/10.1128/MBIO.01019-19> American Society for Microbiology.

88. Yao S, Ni J, Ma T, Li C. Heterotrophic nitrification and aerobic denitrification at low temperature by a newly isolated bacterium, *Acinetobacter* sp. HA2. *Bioresour Technol*. 2013;139:80–6. <https://doi.org/10.1016/j.biortech.2013.03.189> Elsevier.

89. Zhang J, Wu P, Hao B, Yu Z. Heterotrophic nitrification and aerobic denitrification by the bacterium *Pseudomonas stutzeri* YZN-001. *Bioresour Technol*. 2011;102:9866–9. <https://doi.org/10.1016/j.biortech.2011.07.118> Elsevier.

90. Zhao X, Li X, Qi N, Gan M, Pan Y, Han T, et al. *Massilia neuiana* sp. nov., isolated from wet soil. *Int J Syst Evol Microbiol*. 2017;67:4943–7. <https://doi.org/10.1099/ijsem.0.002333> Microbiology Society.

91. Fang W, Wang X, Huang B, Zhang D, Liu J, Zhu J, et al. Comparative analysis of the effects of five soil fumigants on the abundance of denitrifying microbes and changes in bacterial community composition. *Ecotoxicol Environ Saf*. 2020;187:109850. <https://doi.org/10.1016/j.ecoenv.2019.109850> Elsevier.

92. Yu L, Liu Y, Wang G. Identification of novel denitrifying bacteria *Stenotrophomonas* sp. ZZ15 and *Oceanimonas* sp. YC13 and application for removal of nitrate from industrial wastewater. *Biodegradation*. 2009;20:391–400. <https://doi.org/10.1007/s10532-008-9230-2> Springer Berlin Heidelberg.

93. Li J, Wang JT, Hu HW, Cai ZJ, Lei YR, Li W, et al. Changes of the denitrifying communities in a multi-stage free water surface constructed wetland. *Sci Total Environ*. 2019;650:1419–25. <https://doi.org/10.1016/j.scitotenv.2018.09.123> Elsevier.

974 94. Tsubouchi T, Koyama S, Mori K, Shimane Y, Usui K, Tokuda M, et al. *Brevundimonas denitrificans* sp.
975 nov., a denitrifying bacterium isolated from deep seafloor sediment. *Int J Syst Evol Microbiol*.
976 2014;64:3709–16. <https://doi.org/10.1099/ij.s.0.067199-0> Microbiology Society.

977 95. Cua LS, Stein LY. Characterization of denitrifying activity by the alphaproteobacterium,
978 *Sphingomonas wittichii* RW1. *Front Microbiol*. 2014;5:404. <https://doi.org/10.3389/fmicb.2014.00404>
979 Frontiers Media SA.

980 96. Zumft WG. Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev*. 1997;61:533–
981 616. PMID: PMC232623 American Society for Microbiology.

982 97. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes . *Nucleic Acids Res*. Oxford
983 University Press; 2000 [cited 2020 Nov 18]. p. 27–30. Available from: <http://www.genome.ad.jp/kegg/>

984 98. Castignetti D, Hollocher TC. Heterotrophic nitrification among denitrifiers. *Appl Environ Microbiol*.
985 1984;47:620–3. <https://doi.org/10.1128/aem.47.4.620-623.1984> American Society for Microbiology.

986 99. Arahal DR, Lucena T, Rodrigo-Torres L, Pujalte MJ. *Ruegeria denitrificans* sp. Nov., a marine
987 bacterium in the family rhodobacteraceae with the potential ability for cyanophycin synthesis. *Int J Syst*
988 *Evol Microbiol*. 2018;68:2515–22. <https://doi.org/10.1099/ijsem.0.002867> Microbiology Society.

989 100. Qiao Z, Sun R, Wu Y, Hu S, Liu X, Chan J. Microbial Heterotrophic Nitrification-Aerobic
990 Denitrification Dominates Simultaneous Removal of Aniline and Ammonium in Aquatic Ecosystems.
991 *Water Air Soil Pollut*. 2020;231:1–14. <https://doi.org/10.1007/s11270-020-04476-3> Springer Berlin
992 Heidelberg.

993 101. Xie CH, Yokota A. *Sphingomonas azotifigens* sp. nov., a nitrogen-fixing bacterium isolated from the
994 roots of *Oryza sativa*. *Int J Syst Evol Microbiol*. 2006;56:889–93. <https://doi.org/10.1099/ij.s.0.64056-0>
995 Microbiology Society.

996 102. Röthig T, Roik A, Yum LK, Voolstra CR. Distinct Bacterial Microbiomes Associate with the Deep-Sea
997 Coral *Eguchipsammia fistula* from the Red Sea and from Aquaria Settings. *Front Mar Sci*. 2017;4:259.
998 <http://doi.org/10.3389/fmars.2017.00259> Frontiers Media SA.

999 103. Siboni N, Ben-Dov E, Sivan A, Kushmaro A. Global distribution and diversity of coral-associated
1000 *Archaea* and their possible role in the coral holobiont nitrogen cycle. *Environ Microbiol*. 2008;10:2979–
1001 90. <http://doi.wiley.com/10.1111/j.1462-2920.2008.01718.x> John Wiley & Sons, Ltd.

1002 104. Yoon S, Cruz-García C, Sanford R, Ritalahti KM, Löffler FE. Denitrification versus respiratory
1003 ammonification: Environmental controls of two competing dissimilatory NO₃⁻/NO₂⁻ reduction
1004 pathways in *Shewanella loihica* strain PV-4. *ISME J*. 2015;9:1093–104.
1005 <https://doi.org/10.1038/ismej.2014.201> Nature Publishing Group.

1006 105. Tilman D, Wedin D, Knops J. Productivity and sustainability influenced by biodiversity in grassland
1007 ecosystems. *Nature*. 1996;379:718–20. <https://doi.org/10.1038/379718a0> Nature Publishing Group.

1008 106. Cardinale BJ. Biodiversity improves water quality through niche partitioning. *Nature*. 2011;472:86–
1009 91. <https://doi.org/10.1038/nature09904> Nature Publishing Group.

1010 107. Yancey PH, Heppenstall M, Ly S, Andrell RM, Gates RD, Carter VL, et al. Betaines and
1011 dimethylsulfoniopropionate as major osmolytes in cnidaria with endosymbiotic dinoflagellates. *Physiol*
1012 *Biochem Zool*. 2010;83:167–73. <https://doi.org/10.1086/644625> University of Chicago Press.

Figures

Figure 1

Experimental set-up. Boxes depict treatments (color) and number of specimens (n). Treatments include symbiotic-light (SymL, orange), symbiotic-dark (SymD, grey), and aposymbiotic-light (ApoL, pink). Each vertical line represents a sampling event (T₀, T₁, T₂, and T₃) for 5 specimens from each treatment.

Figure 2

Nutrient dynamics in *C. xamachana*. Displayed are mean values ($n = 5$) per group for (a) C enrichment from $\text{NaH}^{13}\text{CO}_3$ expressed as AP¹³C and (b) N enrichment from $\text{Na}^{15}\text{NO}_3$ expressed as AP¹⁵N after the pulse (5 h), chase3h (3 h after pulse) and chase6h (6 h after pulse). Both panels are depicted with broken y-axes to facilitate comparisons between less enriched samples. Treatments included light-incubated symbiotic medusae (SymL), dark-incubated symbiotic medusae (SymD) and light-incubated aposymbiotic medusae (ApoL). Error bars represent 95 % confidence intervals and dashed line natural isotope ratios ($n = 5$; 1.081 AP¹³C and 0.368 AP¹⁵N). Further shown are (c) the regression between host C enrichment and symbiont density expressed as AP¹³C for SymL ($n = 15$; $y = 1.24 + 0.17x$; $r^2_{Adj} = 0.68$, $p < 0.001$) and (d) the regressions between host ¹⁵N enrichment and medusae size expressed as AP¹⁵N from all treatments for chase3h ($y = 0.3381 + 0.0014x$; $r^2_{Adj} = 0.67$, $p < 0.001$) and chase6h ($y = 0.3583 + 0.0004x$; $r^2_{Adj} = 0.86$, $p < 0.001$; each $n = 15$).

Figure 3

C. xamachana associated bacterial communities. (a) Stacked column plot representing the bacterial community composition associated with *C. xamachana* on the family level (SILVA database). Each color represents one of the eight most abundant families, all 85 other families are grouped as 'others'. (b) Clustering of *C. xamachana* samples based on Bray-Curtis dissimilarity of microbial community abundances in a non-metric multi-dimensional scaling plot (NMDS; stress value < 0.078). Circles indicate

95% confidence ranges. (c) Boxplots of pairwise Bray-Curtis dissimilarities of samples within treatments. Statistical comparisons are based on Kruskal-Wallis rank sum test ($\chi^2 = 21.5$, $df = 2$, $p < 0.001$). Letters indicate significant differences of pairwise comparisons ($p_{\text{Holm}} < 0.01$). Treatment abbreviations are same as in Fig.2.

Figure 4

Predicted taxonomy-based functional differences of bacterial communities in *C. xamachana*. Enrichment is depicted in red and depletion in blue on a relative scale. Treatment abbreviations are same as in Fig.2.

Figure 5

Bacterial phylogenetic diversity affects host N assimilation. Regression: $y = 0.667 + 0.368 x$, $r^2_{Adj} = 0.39$, $p < 0.01$. $n = 15$, all sampled after pulse incubation. Treatment abbreviations are the same than in Fig.2.

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