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

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Keywords: stable isotope analysis, tracer, bacterial profiling; environmental resilience; 16S rRNA gene
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 holobions, have received increasing attention, especially in the context of ongoing global declines in coral reefs [2]. Cnidarian holobions 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 CO$_2$ [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
dissociations and which factors support the reestablishment of disrupted symbiotic relationship once bleaching occurred.

Besides temperature, also high nutrient levels can either directly cause bleaching or lead to dysbiosis, disease and host mortality [16]. Naturally, nitrogen (N) is limited in oligotrophic tropical coral reefs. It can be acquired by heterotrophic feeding of the host and passed on to its associates, e.g. as ammonium or amino acids, and further efficiently recycled within the holobiont [17]. N can also be directly obtained from seawater, where it is commonly present at low concentrations in form of dissolved inorganic N (DIN; i.e. nitrate (NO$_3^-$), nitrite (NO$_2^-$) and ammonium (NH$_4^+$)), dissolved organic N, or particulate organic N [8]. Cnidarian hosts are able to incorporate ammonium but often associated microbial communities account for most DIN uptake [17]. Symbiodiniaceae assimilate ammonium and nitrate, many microbial associates are involved in acquiring and efficiently (re)cycling N, and prokaryote diazotrophs can even fix atmospheric nitrogen [17–19]. The host benefits from a limited N availability [8,20] as excess inorganic N can disrupt the host-symbiont partnership by altering the N to phosphate ratio within the holobiont and exacerbate heat-induced bleaching [17,21]. Recently, prokaryote associates have also been linked to contribute to Symbiodiniaceae N limitation in coral holobionts by metabolizing and therefore limiting biologically available N [18,22].

Cnidarian-symbodiniarian holobionts are diverse and adapted to different trophic environments – not only coral reefs. Consequently, they vary in their heterotrophic feeding capacity and likely in their ability to assimilate and retain N [6,23,24]. A higher heterotrophic feeding capacity seems to correlate with an increased bleaching threshold in coral (i.e. greater bleaching resistance) [23]. The mechanisms regulating nutrient acquisition and N cycling within the holobiont are still poorly resolved, but are key 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 strive under such conditions is the capacity of hosts to restrict N access from their symbionts. Examples of a 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’ TTTCCAAAGTCCTTTTCATTTTC 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, AquaIllumination) were set to a 12:12 h light:dark cycle at 100-150 µmol photons m⁻² 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 μmol photons m⁻² 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×). 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 μmol photons m⁻² s⁻¹. At the start of the experiment, each medusae was transferred to an incubation chamber filled with ¹³C and ¹⁵N enriched seawater (1.18 mM Na¹⁵NO₃; 98 atom % ¹⁵N, Sigma-Aldrich and 117 μM NaH¹³CO₃; 98 atom % ¹³C, 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

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

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 $^{13}$C and $^{15}$N. Zooplankton was enriched by first culturing Isochrysis galbana (haptophyte alga) for four days in a modified F/2 medium enriched with 750 mg L$^{-1}$ NaH$^{13}$CO$_3$ (98 % heavy isotope) and 75 mg L$^{-1}$ Na$^{15}$NO$_3$ (98 % heavy isotope, accounting for all nitrate present in the medium) to enrichment levels of $\sim$10 AP$^{13}$C and $\sim$15 AP$^{15}$N (atom percent, see below). Isotopically enriched algae were used to feed Artemia salina for 24 - 48 h. Four C. xamachana medusae ($\sim$3 cm BD) were starved for three days under normal light conditions, then fed ad libitum with labelled A. salina and sampled after $\sim$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 $\delta^{15}$N and $\delta^{13}$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 $\delta^{13}$C and 4.1 % for $\delta^{15}$N, while it was 0.2 % for $\delta^{13}$C and 12.8 % for $\delta^{15}$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

$$AP^H_E = \left[ \frac{H_E}{L_E + H_E} \right] \times 100$$

(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_{sample} − AP_{controls}). Then the enrichment of the symbiont was converted to percentage of enrichment relative to the host’s enrichment \((\text{APE}_{\text{Algal}} / \text{APE}_{\text{Host}}) \times 100\). 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 preformed 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′-TCGTCGCGCACGTCAGATGTGTATAAAGAGACAG-3′) and 1061R (5′-GTCTCGTGGGCTCGAGATGTGTATAAGAGACAG-3′) [43] with Illumina over-hang adaptor sequences (underlined above) were used to amplify the variable regions 5 and 6 of the 16S rRNA 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.
were discarded. Sequences were quality filtered and categorised in amplicon sequence variants (ASVs) using dada2 [45]. The resulting 303 ASVs were aligned against SILVA [46], release 138.1, using a primer-specific classifier trained in Qiime2. If present, chloroplasts, mitochondria, archaea, eukaryotes and at the phylum level unknown sequences were removed. In total, we produced 16 16S rRNA gene libraries containing 221,216 ASVs (252,674 before quality control) from one extraction control, five SymL, five SymD, and five ApoL C. xamachana samples. ASVs that occurred more than 10% in the extraction control compared to the sum of all other samples were removed (i.e., 7 ASVs removed, 6 occurred only in the control; Additional File 3: Table S2) to account for potential contamination. Then stacked column plots representing bacterial community compositions at the family level were constructed, and alpha diversity indices (ASV richness, Simpson and Shannon diversity) were calculated after implementation of a combined rarefaction-extrapolation procedure [46]. Evenness was computed from unrarefied data and phylogenetic diversity was established after Faith [47]. Alpha diversity indices were selected to provide largely independent measures (richness, evenness, and Faith) and comparability with other studies (Shannon and Simpson). Indices were compared among treatments using ANOVA analyses or Kruskal-Wallis tests if assumptions of heteroscedasticity could not be met after eventual data transformations. We used relative ASV densities (i.e. contribution of ASVs to total no. of reads per sample) computed from unrarefied data to establish a Bray-Curtis dissimilarity matrix and visualised sample similarity in a non-metric dimension scaling (NMDS) biplot. Differences between samples and treatments were assessed using PERMANOVAs (Holm correction applied for multiple pairwise comparisons). Further, we assessed differences in within-treatment variability in community similarity, by pooling all pairwise Bray-Curtis similarity scores for within-treatment comparisons of samples and assessing differences among treatments using a Kruskal-Wallis test. All statistical test have been performed in R, the scripts can be 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 (≥ 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 ± 8 μg O₂ g⁻¹ h⁻¹, measured under light conditions) was higher (p_Holm < 0.001) than in symbiotic (28 ± 14 μg O₂ g⁻¹ h⁻¹, measured under dark conditions) medusae. Gross primary production (P₉) for symbiotic specimens was 56 ± 15 μg O₂ g⁻¹ h⁻¹ and the P₉: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 ¹³C and ¹⁵N after the pulse, chase3h and chase6h (for all p_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 AP¹³C enrichment (2.57 – 3.35),
followed by the respective host fraction (1.48 – 2.23). Owing to the lack of photosynthetic activity, both
SymD and ApoL showed a much smaller enrichment overall (1.09 – 1.13). Enrichment of the SymL host
fraction showed comparatively high across sample variation (Fig.2a) which we found was well explained
by Symbiodiniaceae densities ($r^2_{Adj} = 0.68$; slope = 0.17, $p_{lm} < 0.001$, Fig.2c). Further, we found good
accordance between host AP$^{13}$C and net primary production ($P_n$) computed from O$_2$ measurements ($r^2_{Adj}$
= 0.74, slope = 10.84, $p_{lm} < 0.001$, Additional File 1: Fig.S3). Surprisingly, the highest $^{13}$C enrichment for
both the host and algal fraction of SymL occurred at chase rather than at pulse (AP$^{13}$C$_{Chase3} >$ AP$^{13}$C$_{Pulse}$
and AP$^{13}$C$_{Chase3} >$ AP$^{13}$C$_{Chase6h}$; $p_{LME} < 0.01$). A similar delay in tissue enrichment was also found in the algal
fraction of SymD, with the greatest enrichment at chase6h (AP$^{13}$C$_{Chase6h} >$ AP$^{13}$C$_{Pulse}$, $p_{Wilcox} < 0.05$).
Conversely, SymD host fraction peaked at pulse and showed lowest enrichment levels at chase6h.
Unlike for $^{13}$C, $^{15}$N enrichment was always higher in the host than in the algal fraction. AP$^{15}$N in the algal
fraction of SymL was higher than in SymD indicating a link between nitrate assimilation and
photosynthesis ($p_{LME} < 0.001$). In the host fraction, $^{15}$N enrichment was highest at pulse (0.644 – 0.900)
and dropped below 0.424 at chase for all treatments (Fig.2b). At pulse, there were no significant
differences between treatments ($p_{ANOVA} > 0.05$) (Additional File 3: Table S4), but ApoL samples
experienced a larger drop in AP$^{15}$N (i.e. N turnover rates) during the chase ($p_{LME} < 0.01$; Fig.2b). Notably,
AP$^{15}$N levels significantly correlated with medusa size for both chases when considering the host
fractions from all treatments ($r^2_{Adj} = 0.67$ and 0.86 respectively, $p_{lm} < 0.001$ for both; Fig.2d). However,
the effect of medusa size seemed to be solely related to N turnover rates as there was no relationship
between BD and AP$^{15}$N after the pulse experiment ($r^2_{Adj} = 0.04$, $p_{lm} > 0.05$). AP$^{15}$N values of the algal
fraction in symbiotic individuals from both treatments peaked at the end of the chase (AP$^{15}$N$_{Chase6h} >$
AP$^{15}$N$_{Pulse}$ and AP$^{15}$N$_{Chase6h} >$ AP$^{15}$N$_{Chase3h}$; $p_{LME} < 0.001$), contrasting the pattern observed in the host
fraction.
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 $36.7 \pm 4.6 \%$ of the host $^{13}$C and $70.3 \pm 4.0 \%$ 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 ApoL 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; \( \chi^2=21.463, df = 2, p_{\text{Wilcoxon}} < 0.01 \)). 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 (expect 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 \( \text{O}_2 \) 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<sub>g</sub>: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<sup>13</sup>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 symbodinians maintained high <sup>13</sup>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 <sup>13</sup>CO<sub>2</sub> could diffuse into the host tissue and contribute to a ‘delayed enrichment’ (i.e. highest AP<sup>13</sup>C values after the 3 hour chase) in our inorganic labelling experiment. Such dissolved CO<sub>2</sub> pools utilized for photosynthesis were likely lost during sampling preparation resulting in lower AP<sup>13</sup>C values in pulse samples.

In treatments lacking photosynthesis (SymD and ApoL treatments), <sup>13</sup>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 ($^{15}$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.
Importantly, the algal symbionts were unable to capitalize on available DIN indicating an effective control mechanism. N restriction as a mechanism of symbiont control has been suggested in coral and Exaiptasia; it is thought to maintain algal cell densities and ensure high rates of photosynthate translocation [20]. In corals, Tanaka et al. [49] found AP$^{15}$N in the associated algae to be 4.7 times higher than in the host which corroborates that only the algae are able to assimilate nitrate. In C. xamachana, however, the algal partners enrich slowly throughout the experiment, largely independent of the hosts $^{15}$N level. Overall low nitrate assimilation is in line with other ‘nutrient resistant’ cnidarian-symbiodiniian associations. L. unguiculata [53] and Exaiptasia pulchella [54] removed none or only minimal nitrate from their surrounding water and $\delta^{15}$N enrichment from nitrate was not detectable in Exaiptasia [55] (notably, all of these are non-calcifying hosts and Exaiptasia spp. are not obligate photosymbiotic). In other photosymbioses adapted to high nutrient concentrations, for instance Hydra viridissima [56] or Paramecium bursaria [57] some Chlorella endosymbionts have lost their assimilatory nitrate reduction pathway as an adaptation to symbiotic conditions. Such inability for nitrate uptake remains to be tested for C. xamachana’s algal partners. However, the host’s capacity to associate with several Symbiodiniaceae strains originating from ‘nutrient sensitive’ corals [30] argues for alternative mechanisms. Studies on such calcifying corals demonstrate that the photosymbionts were able to effectively assimilate nitrate and translocate it to their hosts in Acropora pulchra [58], A. tenuis [59], Orbicella faveolata [60], Porites cylindrica [49], and Stylophora pistillata [61]. Compared to C. xamachana, in a similar experimental setup (at 26°C with similar C assimilation rates) the algal fraction of O. faveolata showed $^{15}$N enrichment two orders of magnitude greater (0.784 APE$^{15}$N compared to 0.002 APE$^{15}$N) and was remarkably higher than the host fraction enrichment [61]. In C. xamachana, Symbiodiniaceae are located in a symbiosome, a membrane complex wrapping the algal cells, which are situated in amoebocytes. While little is known about the function of (likely mobile) amoebocytes [31], 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 $^{15}$N 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
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 $^{15}$N that was assimilated by their associated prokaryotic community. However, based on the quick release of $^{15}$N 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 $^{15}$N 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 NaHCOO expressed as AP/C and (b) N enrichment from NaNOO expressed as AP/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 AP/C and 0.368 AP/N). Further shown are (c) the regression between host C enrichment and symbiont density expressed as AP/C for SymL (*n* = 15; *y* = 1.24 + 0.17*x*; *r*²Adj = 0.68, *p*<0.001) and (d) the regressions between host N enrichment and medusae size expressed as AP/N from all treatments for chase3h (*y* = 0.3381 + 0.0014*x*; *r*²Adj = 0.67, *p*<0.001) and chase6h (*y* = 0.3583 + 0.0004*x*; *r*²Adj = 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 (chi² = 21.5, df = 2, *p*<0.001). Letters indicate significant differences of pairwise comparisons (*p*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*²Adj = 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*ₚ: dark-adapted minimal fluorescence yields; *F*ₘ: dark-adapted maximal fluorescence yield; *F*ₚ/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*ₚ, *F*ₘ, and *F*ₚ/ₘ).
Figure S2. Relationship between wet weight (WW) and bell diameter (BD) in (a) symbiotic (n = 35) [WW\textsubscript{symbiotic} = 8.540 - 0.494*BD + 0.009*BD\textsuperscript{2}] and (b) aposymbiotic C. xamachana (n = 12) [WW\textsubscript{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\textsubscript{n}) and host carbon enrichment. Host fraction enrichment expressed as AP\textsuperscript{13}C 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. \textsuperscript{13}C and \textsuperscript{15}N 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.

Funding
Funding was provided by the University of Hong Kong, Faculty of Science, Division of Ecology and Biodiversity.

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.

Acknowledgements
We thank Acacia Tsz So Tang, Jordan Pierce and Jonathan Cybulski for valuable support during the experiments and James Hagan for advice on the statistical analysis. The ITS2 primer were designed by Jean-François Flot and Monica Medina supplied the C. xamachana strain (polyps). We acknowledge Aki Ohdera and Victoria Sharp’s support in establishing DNA extraction protocols. We thank two anonymous reviewer for their helpful comments on the manuscript.

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