

Title: Nitrogen cycling in corals: the key to understanding holobiont functioning?

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1 **Abstract**

2 Corals are animals that form close mutualistic associations with endosymbiotic photosynthetic algae
3 of the genus *Symbiodinium*. Together they provide the three-dimensional calcium carbonate
4 framework of coral reef ecosystems. Only recently, the importance of the microbiome (i.e. bacteria,
5 archaea, fungi, and viruses) to holobiont functioning has been recognized. Given that growth and
6 density of *Symbiodinium* within the coral host is highly dependent on nitrogen availability, nitrogen
7 cycling microbes may be of fundamental importance to the stability of the coral–algae symbiosis and
8 holobiont functioning, in particular under nutrient-enriched and nutrient-depleted scenarios. Here,
9 we summarize what is known about nitrogen cycling in corals and conclude that disturbance of
10 microbial nitrogen cycling may be tightly linked to coral bleaching and disease.

11 **Corals in an oligotrophic environment**

12 Tropical reef-building corals commonly flourish in nutrient-poor environments. The contradiction of
13 high coral productivity on the one hand and limited nutrient availability on the other hand has been
14 coined the 'Darwin Paradox' in reference to its first observer [1,2]. The highly efficient uptake and
15 recycling of nutrients in coral reef organisms can help to explain this paradox [3,4]. Particularly for
16 corals, the close association between the coral animal host and its endosymbiotic dinoflagellate
17 algae of the genus *Symbiodinium* enables an effective use and retention of nutrients and
18 photosynthates, i.e. photosynthetically fixed carbon [5]. Together with their algal symbionts, corals
19 are associated with a variety of other microorganisms, including protozoans, fungi, archaea, and
20 bacteria [6,7], an assemblage termed the coral holobiont (see Glossary). The resulting meta-
21 organism represents a complex interactive system with the potential to extend the physiological
22 capabilities of the coral host [8]. Hence, knowledge of underlying mechanisms and interactions
23 within the holobiont framework is essential to comprehend the response of corals to environmental
24 change, such as ocean acidification, ocean warming, and eutrophication.

25 Distinct microbial assemblages are associated with the surface mucus layer, coral tissue, gastro-
26 vascular cavity, and within the coral skeleton [8]. This variety of habitats results in a highly diverse
27 coral microbiome where associated microbes can potentially perform a multitude of services to the
28 functioning of the coral holobiont including carbon fixation, nitrogen metabolism, sulphur cycling,
29 and anti-microbial defence among many others [6,9-11]. Due to the high importance of different
30 microbial groups for the functioning of the coral holobiont, a variety of mechanisms have evolved to
31 enable an effective intergenerational transfer of specific microbes to ensure offspring fitness [12,13].
32 This vertical transfer presumably facilitated the evolution of host-specific and persistent microbial
33 communities in many corals [14–18]. In this context, Reshef *et al.* [19] proposed the coral probiotic
34 hypothesis, stating that despite the presence of consistent microbial communities, alterations in the
35 microbiome may help corals to rapidly adjust to environmental conditions (Box 1).

36 In particular, nitrogen cycling microbes appear to be ubiquitous and consistent members of the coral
37 microbiome [8,9,16,17,20,21]. Given that symbiont production in corals is highly dependent on
38 nitrogen availability [22,23], nitrogen cycling in the coral holobiont among other factors may be
39 critical for acquisition and retention of nitrogen to sustain primary productivity (i.e. photosynthesis).

40 In this review, we thus summarize the current knowledge of microbial nitrogen cycling within the
41 coral holobiont and its importance for the coral–algae symbiosis. Furthermore, we discuss potential
42 effects of environmental change on these pathways with a focus on their putative role in the
43 occurrence and deleterious effects of coral bleaching and disease.

44 **Nitrogen uptake and symbiont control in the coral holobiont**

45 The coral holobiont is highly efficient in the assimilation of nitrogen. Heterotrophic feeding by the
46 coral can meet a large part of its nitrogen requirements if sufficient food is available [24]. In
47 addition, corals acquire nutrients from their symbiotic algae from the genus *Symbiodinium*. This
48 symbiotic relationship forms the foundation of coral holobionts and shallow-water coral reefs. In this
49 association, the phototrophic dinoflagellates provide photosynthates to the coral host. However, the
50 translocated photosynthates have been referred to as “junk food” as they show a high C:N ratio and
51 therefore require additional nitrogen supplementation to sustain coral growth [25]. The symbiotic
52 algae benefit from inorganic nutrients which are released as metabolic waste products by the host
53 [5,26] Their capacity for efficient uptake and utilization of dissolved inorganic nitrogen (DIN) helps
54 also the acquisition of nitrogen from the surrounding seawater. Although both the coral host and
55 associated *Symbiodinium* have the enzymatic machinery to incorporate ammonium, the algae
56 account for most of the uptake of dissolved inorganic nitrogen (DIN) from the environment, mainly
57 in the form of ammonium (NH_4^+) and nitrate (NO_3^-) [27]. This nitrogen, together with host-derived
58 nitrogen compounds, is either stored by the algae or used in their metabolism and may be partially
59 translocated to the coral host in form of organic nitrogen compounds, e.g. amino acids [28-30].

60 Together with an efficient recycling of coral metabolic waste products within the holobiont, this
61 symbiosis thus enables efficient utilization of nitrogen compounds from surrounding seawater.

62 The availability of nitrogen sources in coral reefs however underlies strong seasonal and diel
63 variations and can be impacted by anthropogenic activities [31]. Consequently, internal regulation
64 mechanisms need to be in place in order to control for these fluctuations.

65 At least three mechanisms have been identified by which corals exert control over their algal
66 symbionts: (i) compounds of the coral host tissue, so-called host release factors (HRFs), trigger the
67 release of photosynthetically fixed carbon in freshly isolated *Symbiodinium* [32–34]. These HRFs are
68 likely specific amino acids and crucial to ensure the nutrition of the coral host and to increase
69 photosynthetic rates (via unknown mechanisms). (ii) Control of algal numbers by
70 degradation/digestion [35]. Via this process the host may benefit from the nutrients contained in the
71 symbionts in organic forms. (iii) Control of *Symbiodinium* density by limiting nutrient availability [22].
72 Nitrogen limitation in particular may be essential to regulate cell division rates of the faster
73 proliferating *Symbiodinium* to match those of the host [36,37]. Furthermore, the translocation of
74 photosynthates to the host can help the symbionts to maintain a favorable carbon to nitrogen ratio,
75 since carbon fixation by the algae proceeds in particular in high light environments despite nutrient
76 limitation [38]. Wiedenmann *et al.* [39] showed that a shift away from nitrogen limitation by excess
77 nitrogen provision can ultimately results in phosphate starvation, which can increase the
78 susceptibility of the corals to a heat and light stress-mediated loss of the algal symbionts (coral
79 bleaching). Hence, low internal nutrient availability, specifically of nitrogen, seems crucial to
80 maintain high primary production, while simultaneously controlling algae growth.

81 In this context, adjustment of internal nitrogen availability may help to control algal growth. The
82 ability of the coral host to assimilate ammonium has been suggested to allow for a more efficient
83 uptake of ammonium from seawater by maintaining the diffusion gradient into the tissue, whilst
84 enabling an internal limitation of nitrogen availability to *Symbiodinium* at the same time [27,36,40].

85 At the same time, regulation of symbiosome *pH* surrounding *Symbiodinium* may help to control the
86 diffusion of ammonium ions into the symbiosome [41]. Furthermore, nitrogen cycling by coral-
87 associated microbes may be an additional mechanism contributing to the stabilization, or conversely
88 destabilization, of the coral–algae symbiosis.

89 **Nitrogen fixation**

90 Coral reefs are net sources of fixed nitrogen [42]. Nitrogen fixation, i.e. the conversion of elemental
91 dinitrogen (N₂) into ammonium, is associated with many substrates (e.g. sand, coral rock, and
92 rubble) and benthic organisms (e.g. corals, macroalgae, and sponges) [21, 43]. This input of new
93 fixed nitrogen into the reef ecosystem helps to sustain net productivity under oligotrophic
94 conditions and to compensate for net nitrogen export from the system, for instance, by currents.
95 [44]. Nitrogen fixation in hermatypic corals has been reported for several different species [45–48],
96 suggesting a high relevance of this process for the coral holobiont. However, reported nitrogen
97 fixation rates in corals are about a magnitude lower than those found in reef sediments and bare
98 rock [43]. Hence, corals appear to be minor contributors to the overall nitrogen budget of coral
99 reefs.

100 For a long time, cyanobacteria were believed to be the main drivers of nitrogen fixation in corals [49-
101 51], but recent studies revealed that corals harbor ubiquitous diverse communities of diazotrophs,
102 i.e. nitrogen-fixing bacteria and archaea, consisting of mostly heterotrophic bacteria [16,17,20,52].
103 These communities are coral species-specific and highly persistent over space and time [16,17].
104 Moreover, Lema *et al.* [18] found a vertical transfer of diazotrophs from parental colonies of the
105 coral *Acropora millepora* to their larvae, mostly Alphaproteobacteria of the group Rhizobiales. This
106 vertical transfer of diazotrophs further suggests a beneficial role of this group for holobiont
107 functioning. Although the magnitude of transfer of fixed nitrogen from diazotrophs into other
108 compartments of the coral holobiont (e.g. *Symbiodinium*) has not been quantified yet, recent studies
109 show that bacterial symbionts contribute to the nitrogen supply of the holobiont [48,53]. However, a

110 recent stable isotope ($^{15}\text{N}_2$ gas) tracing application in Red Sea corals revealed no direct incorporation
111 of fixed nitrogen in the coral tissue within the first 24 hours of measurement, suggesting coral–
112 diazotroph interactions may be more complex than previously thought [47].

113 Nitrogen fixation is a highly energy-consuming process, which requires 16 mol of ATP for the
114 reduction of 1 mol of dinitrogen [54]. Therefore, nitrogen fixation is energetically more costly than
115 other mechanisms of ammonium assimilation. Hence, other sources of fixed nitrogen may be
116 favored over nitrogen fixation, if available. The depressing effect of combined nitrogen availability
117 on nitrogen fixation by terrestrial, planktonic, and benthic diazotrophs has been reported [55]. It is
118 thus likely that similar regulatory mechanisms to reduce nitrogen fixation rates at times of excess
119 nitrogen availability exist in coral-associated diazotrophs (Figure 1). Nitrogen fixation activity in
120 corals is highly dynamic and can be rapidly affected by changes in environmental conditions [46,48].
121 Consequently, nitrogen fixation may serve as a mechanism to counteract shortages of
122 environmental nitrogen availability, and maintain a constant nitrogen supply for symbiont-based
123 primary production in corals. This view is further supported by the findings of Olson *et al.* [52] and
124 Lesser *et al.* [48], who reported a positive correlation of diazotroph abundance with density and DNA
125 content of *Symbiodinium* cells.

126 Despite the overall comparatively small contribution to the nitrogen budget of the coral holobiont,
127 nitrogen fixation may be still essential to the stability of the coral–algae symbiosis. Consequently, it
128 may constitute an important functional link between carbon and nitrogen fixation within the
129 holobiont and thus contribute to the success of corals in highly oligotrophic reef environments.

130 **Nitrification**

131 In contrast to nitrogen fixation, other pathways of the nitrogen cycle in corals have received little
132 attention to date. Nonetheless, particularly nitrification and denitrification may potentially be of
133 importance for holobiont functioning. High rates of nitrification, i.e. the oxidation of ammonium into
134 nitrite (NO_2^-) and nitrate (NO_3^-), have been measured in coral reef environments [42,56]. Nitrification

135 associated with corals occurs in their skeletons [57], living tissues [58], mucus [59], and the
136 interstitial waters of coral branches [60], likely rendering nitrification a ubiquitous coral-associated
137 process. Consequently, recent studies revealed that nitrifying bacteria and archaea are highly
138 abundant in the microbiomes of many coral species [10,59,61,62]. Among these at least mucus-
139 associated ammonia-oxidizing archaeal communities appear to depend rather on location and
140 environmental conditions than the coral host [59,63]. The metabolic activity of these communities
141 however, remains yet to be determined, and the role of nitrification in the holobiont is largely
142 unknown. *Symbiodinium* prefer uptake of ammonium over other forms of DIN [64,65], and
143 ammonium may inhibit the uptake of nitrate [66]. Nitrification in corals may thus ultimately reduce
144 the amount of nitrogen available for *Symbiodinium* growth. Wafar *et al.* [58] reported that bacterial
145 nitrification almost exclusively utilized ammonium derived from coral metabolism and that this
146 process occurred at rates effectively competing with the autotrophic uptake of ammonium.
147 Consequently, nitrogen cycling may serve as a way to retain nitrogen within the coral holobiont, with
148 nitrification preventing loss of ammonium from the holobiont.

149 **Denitrification**

150 Depending on environmental conditions, reef substrates can show high denitrification rates, and
151 denitrification has been detected in marine invertebrates [67,68]. Although denitrifying
152 microorganisms are present in coral microbiomes [10,62], no studies have investigated the
153 denitrifying potential of corals so far. Siboni *et al.* [59] suggested a coupling of nitrification and
154 denitrification (i.e. the reduction of nitrate) ultimately resulting in the production of dinitrogen to
155 remove nitrogen from the coral holobiont. Nitrogen cycling processes are highly dependent on
156 oxygen availability [48,69]. Since oxygen concentrations in coral tissue show strong diel fluctuations,
157 the linkage of aerobic (e.g. nitrification) and anaerobic (e.g. nitrogen fixation and denitrification)
158 processes within the coral holobiont may be possible [70]. Additional evolutionary adaptations by

159 the coral host and associated microbes may provide a spatial or temporal separation of anaerobic
160 processes from oxygen evolution [71].

161 Corals can live successfully at a wide range of nutrient concentrations, ranging from highly
162 oligotrophic to eutrophic conditions [31]. In addition to regulated nutrient transport within the
163 holobiont, denitrification in combination with nitrification may thus help corals to survive elevated
164 nutrient concentrations and to maintain internal nitrogen-limitation of *Symbiodinium* at the same
165 time (Figure 1). Future research should therefore aim to identify the acclimation capacity of corals to
166 anthropogenic nutrient enrichment by nitrification and denitrification processes.

167 **Other pathways in the nitrogen cycle**

168 Additional nitrogen cycling pathways exist in the holobiont. Wegley *et al.* [9] suggested that
169 endolithic fungi may exert an important functional role by reducing nitrate into ammonium. These
170 processes enable a highly efficient internal nutrient cycling and thereby may help to prevent the loss
171 of nitrogen from the holobiont. Current knowledge of coral-associated eukaryotic diversity is mostly
172 limited to insights from metagenomic surveys [9,72]. New studies, e.g. using via 18S amplicon based
173 approaches, may provide further clues to eukaryotic organisms associated with the coral holobiont
174 and the services they may provide.

175 Another pathway of the nitrogen cycle is anaerobic ammonium oxidation (ANAMMOX), transforming
176 fixed nitrogen into elemental dinitrogen. Particularly in the marine nitrogen cycle, the ANNAMOX
177 pathway is of high importance [73]. Its presence has been previously confirmed in sponges [68] and
178 has been suggested in corals, but direct evidence still needs to be provided. The presence of
179 ANAMMOX may constitute another critical mechanism besides denitrification in removing excess
180 nitrogen from the coral holobiont, e.g. during eutrophication events.

181 **Environmental constraints and anthropogenic impact**

182 Although nitrogen cycling in corals appears to be of high relevance for holobiont functioning, little is
183 known about the effects of anthropogenic environmental change on this cycle on the holobiont
184 level. Nitrogen cycling may mitigate or exacerbate the impact on corals by channeling nitrogen
185 through different pathways depending on the type of alteration (e.g. global warming or ocean
186 acidification).

187 Coral reefs have seen an unprecedented global decline over the past decades [74]. Particularly coral
188 bleaching and disease are among the main drivers of the loss of coral reef cover [75]. Both,
189 bleaching and coral diseases involve the disruption of the coral–algae symbiosis [76]. Interestingly,
190 shifts in coral-associated microbial communities, including nitrogen cycling microbes, have been
191 shown to precede the visual symptoms of bleaching and diseases [15,72,77–79]. Although this
192 correlation does not imply causality and alternative explanations are possible, we investigate a
193 potential mechanism, by which environmental effects on coral associated nitrogen cycling capacity
194 may be linked to the disruption of the coral-algae symbiosis in the following paragraph/section.

195 **A potential link between nitrogen cycling and reef degradation?**

196 Several studies revealed that nitrogen fixation activity in corals strongly depends on environmental
197 conditions [45,46,78]. Elevated temperature and dissolved organic carbon (DOC) availability can
198 potentially increase nitrogen fixation activity in corals (Figure 1) [45,78]. Coincidentally, these same
199 two stress factors are considered strong drivers of bleaching and diseases in corals [40, 80-82].

200 An imbalanced nutrient availability, i.e. elevated inorganic nitrogen concentrations in combination
201 with phosphate depletion, rather than enrichment of both nitrogen and phosphate, can destabilize
202 the coral–algae symbiosis [39]. Among other processes, nitrogen fixation can potentially increase the
203 N:P ratio in corals [31]. Hence, environmental impacts that increase nitrogen fixation may ultimately
204 disrupt the nitrogen limitation of *Symbiodinium* in corals. The resulting elevated nitrogen availability
205 would stimulate cell division of *Symbiodinium*, thereby shifting *Symbiodinium* from nitrogen towards
206 phosphate limitation/starvation.

207 Wiedenmann *et al.* (39) found that phosphate starvation associated with increased cell division rates
208 resulted in an increase of sulfo- to phospholipid ratios in *Symbiodinium*, which may cause a
209 destabilization of their thylakoid membranes and explain the lower bleaching threshold of
210 phosphate-starved coral species. Furthermore, Wooldridge [40] proposed a mechanism by which
211 enhanced retention of photosynthates by *Symbiodinium* due to excess nitrogen availability may
212 cause carbon dioxide (CO₂) limitation ultimately resulting in coral bleaching. Therefore,
213 environmental stressors such as heat stress and elevated DOC concentrations may potentially lower
214 the threshold at which a disruption of the coral–algae symbiosis occurs by increasing coral-
215 associated nitrogen fixation rates.

216 Indirect empirical support in favor of this theory is provided by Godinot *et al.* [83], who reported
217 increased phosphate uptake and net inorganic nitrogen release by the coral *Stylophora pistillata*
218 during heat stressed conditions. This suggests that shifts in the N:P ratio can occur at elevated
219 temperatures. Additionally, increased cell division rates (mitotic index) of *Symbiodinium* during heat
220 stress and bleaching events haven been reported, implying a disruption of internal nitrogen
221 limitation of *Symbiodinium* growth [84,85].

222 Therefore the proposed pathway may be of importance, not only to better understand and predict
223 coral bleaching events, but also to reveal the underlying mechanisms of coral diseases, which
224 commonly involve the breakdown of the coral–algae symbiosis.

225 At the same time, breakdown of the coral–algae symbiosis and potential subsequent demise of the
226 coral host may trigger a positive feedback loop of coral degradation (Figure 2), which may act on
227 different scales from colony to ecosystem level (Figure 3). Epilithic algae communities on freshly
228 killed corals show increased nitrogen fixation rates and at the same time release DOC-rich exudates
229 [86,87]. These exudates may stimulate microbial activity (including nitrogen fixation) in nearby
230 corals. Thus, algae overgrowth on the dead coral skeleton may stimulate further bleaching and
231 eventually cause mortality in adjacent corals.

232 **Future perspectives**

233 Although direct evidence is still scarce, the ubiquitous distribution of nitrogen cycling microbes in
234 reef-building corals suggests a high functional relevance of this group for the holobiont. Future
235 research directions should aim to increase our understanding of the possible implications of these
236 symbiotic interactions within the holobiont in order to shed light on the underlying mechanisms of
237 the responses of tropical reef-building corals to present and future anthropogenic changes (Box 2).

238 Recent technological advances in the biological sciences may help to answer these questions (for a
239 detailed review see Pernice & Levy [88]). For instance, the development and application of culture-
240 independent methods to characterize microbial communities has changed our understanding of the
241 coral microbiome. In particular, the application of metatranscriptomics to disentangle expressed
242 functions in different holobiont compartments [89] will help to decipher which microbial partners
243 are important drivers of nitrogen cycling in corals. In this context, the growing number of available
244 (meta-)genomes and (meta-)transcriptomes of corals, *Symbiodinium* and bacterial partners, will
245 allow elucidation of key genes involved in nitrogen cycling and their distribution across holobiont
246 compartments. For example, DMSP biosynthesis was only recently discovered in corals, a process
247 that was believed to be restricted to marine algae and plants [90]. It is thus important to test the
248 established understanding of the distribution of genes related to nitrogen cycling between the coral
249 host and its symbiotic partners through incorporation of data from multiple species and under
250 different conditions.

251 Furthermore, the emerging use of stable isotope analysis and isotopic labeling provides an excellent
252 tool to identify the fate of metabolic products in corals [47,48,91]. In combination with NanoSIMS
253 technology [27,29,53,92], such approaches will enable us to understand how nitrogen uptake and
254 nitrogen fixation affects the various symbiotic partners within the coral holobiont. However, due to
255 the technical limitations from rinsing and dehydration steps during sample preparation, NanoSIMS
256 measurements appear less suitable for detection of nitrification or denitrification. These new

257 technological advances are most valuable when integrated into classical ecological approaches such
258 as the indirect measurement of nitrogen fixation rates via the acetylene reduction assay [93]. Such
259 integrated applications will allow for the holistic understanding of nitrogen cycling in the coral
260 holobiont, which is urgently needed to address the consequences of anthropogenically-driven
261 environmental change in coral reefs.

262 **Concluding remarks**

263 The status of coral health is traditionally largely interpreted as the consequence of the interactions
264 of corals and their endosymbiotic dinoflagellates. With an emerging characterization of the structure
265 and function of the coral microbiome, this simplification may no longer be adequate. Rather, we are
266 provided with a more holistic understanding of functional partitioning within the coral holobiont and
267 the importance of associated microbes. Particularly microbial nitrogen cycling may play a crucial role
268 in stabilizing or destabilizing the holobiont assemblage and function depending on environmental
269 conditions. Shifts in the nitrogen cycling capacity may provide corals with a potential mechanism to
270 persist in variable environments, such as those occurring through anthropogenic eutrophication. At
271 the same time, stressors such as global warming and DOC enrichment may alter interactions of coral
272 hosts and diazotrophs from a mutualistic to an opportunistic pathogenic association. Loss of control
273 over this important microbial functional group by the host may have important consequences for
274 the stability of the coral–algae symbiosis. In order to address this further, the mechanisms by which
275 coral-associated microbes are involved in coral bleaching or diseases remain yet to be investigated.

276

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287 **References**

- 288 1 Darwin, C. (1842) *The structure and distribution of coral reefs*, Smith, Elder and Co.
- 289 2 Sammarco, P.W. *et al.* (1999) Cross-continental shelf trends in coral $\delta^{15}\text{N}$ on the Great
290 Barrier Reef: further consideration of the reef nutrient paradox. *Mar. Ecol. Prog. Ser.* 180,
291 131–138
- 292 3 De Goeij, J.M. *et al.* (2013) Surviving in a marine desert: the sponge loop retains resources
293 within coral reefs. *Science* 342, 108–111
- 294 4 Wild, C. *et al.* (2004) Coral mucus functions as an energy carrier and particle trap in the reef
295 ecosystem. *Nature* 428, 66–70
- 296 5 Muscatine, L. and Porter, J.W. (1977) Reef corals: mutualistic symbioses adapted to nutrient-
297 poor environments. *Bioscience* 27, 454–460
- 298 6 Ainsworth, T.D. *et al.* (2010) The future of coral reefs: a microbial perspective. *Trends Ecol.*
299 *Evol.* 25, 233–40
- 300 7 Rohwer, F. *et al.* (2002) Diversity and distribution of coral-associated bacteria. *Mar. Ecol.*
301 *Prog. Ser.* 243, 1–10
- 302 8 Rosenberg, E. *et al.* (2007) The role of microorganisms in coral health, disease and evolution.
303 *Nat. Rev. Microbiol.* 5, 355–362
- 304 9 Wegley, L. *et al.* (2007) Metagenomic analysis of the microbial community associated with
305 the coral *Porites astreoides*. *Environ. Microbiol.* 9, 2707–19
- 306 10 Kimes, N.E. *et al.* (2010) Microbial functional structure of *Montastraea faveolata*, an
307 important Caribbean reef-building coral, differs between healthy and yellow-band diseased
308 colonies. *Environ. Microbiol.* 12, 541–556
- 309 11 Krediet, C.J. *et al.* (2013) Coral-associated micro-organisms and their roles in promoting coral
310 health and thwarting diseases. *Proc. R. Soc. B Biol. Sci.* 280, 20122328

- 311 12 Ceh, J. *et al.* (2013) Intergenerational transfer of specific bacteria in corals and possible
312 implications for offspring fitness. *Microb. Ecol.* 65, 227–231
- 313 13 Hirose, M. *et al.* (2001) Timing and process of entry of zooxanthellae into oocytes of
314 hermatypic corals. *Coral Reefs* 20, 273–280
- 315 14 Bayer, T. *et al.* (2013) The microbiome of the red sea coral *Stylophora pistillata* is dominated
316 by tissue-associated endozoicomonas bacteria. *Appl. Environ. Microbiol.* 79, 4759–4762
- 317 15 Roder, C. *et al.* (2014) Bacterial profiling of White Plague Disease in a comparative coral
318 species framework. *ISME J.* 8, 31–39
- 319 16 Lema, K.A. *et al.* (2012) Corals form characteristic associations with symbiotic nitrogen-fixing
320 bacteria. *Appl. Environ. Microbiol.* 78, 3136–3144
- 321 17 Lema, K.A. *et al.* (2014) Amplicon pyrosequencing reveals spatial and temporal consistency in
322 diazotroph assemblages of the *Acropora millepora* microbiome. *Environ. Microbiol.* DOI:
323 10.1111/1462-2920.12366
- 324 18 Lema, K. a *et al.* (2014) Onset and establishment of diazotrophs and other bacterial associates
325 in the early life history stages of the coral *Acropora millepora*. *Mol. Ecol.* 23, 4682–4695
- 326 19 Reshef, L. *et al.* (2006) The coral probiotic hypothesis. *Environ. Microbiol.* 8, 2068–2073
- 327 20 Olson, N.D. and „, M.P. (2013) Diazotrophic diversity in the Caribbean coral, *Montastraea*
328 *cavernosa*. *Arch. Microbiol.* 195, 853–859
- 329 21 Fiore, C.L. *et al.* (2010) Nitrogen fixation and nitrogen transformations in marine symbioses.
330 *Trends Microbiol.* 18, 455–463
- 331 22 Falkowski, P.G. *et al.* (1993) Population control in symbiotic corals. *Bioscience* 43, 606–611
- 332 23 Béraud, E. *et al.* (2013) The response of the scleractinian coral *Turbinaria reniformis* to
333 thermal stress depends on the nitrogen status of the coral holobiont. *J. Exp. Biol.* 216, 2665–
334 74
- 335 24 Houlbrèque, F. and Ferrier-Pagès, C. (2009) Heterotrophy in tropical scleractinian corals. *Biol.*
336 *Rev. Camb. Philos. Soc.* 84, 1–17
- 337 25 Falkowski, P. *et al.* (1984) Light and bioenergetics of a symbiotic coral. *Bioscience* 34, 705–709
- 338 26 Rahav, O. *et al.* (1989) Ammonium Metabolism in the Zooxanthellate Coral, *Stylophora*
339 *pistillata*. *Proc. R. Soc. B Biol. Sci.* 236, 325–337
- 340 27 Pernice, M. *et al.* (2012) A single-cell view of ammonium assimilation in coral-dinoflagellate
341 symbiosis. *ISME J.* 6, 1314–1324
- 342 28 Wang, J.T. and Douglas, a. E. (1999) Essential amino acid synthesis and nitrogen recycling in
343 an alga-invertebrate symbiosis. *Mar. Biol.* 135, 219–222

- 344 29 Kopp, C. *et al.* (2013) Highly dynamic cellular-level response of symbiotic coral to a sudden
345 increase in environmental nitrogen. *MBio* 4, e00052–13
- 346 30 Reynaud, S. *et al.* (2009) Effect of light and feeding on the nitrogen isotopic composition of a
347 zooxanthellate coral: Role of nitrogen recycling. *Mar. Ecol. Prog. Ser.* 392, 103–110
- 348 31 D’Angelo, C. and Wiedenmann, J. (2014) Impacts of nutrient enrichment on coral reefs: new
349 perspectives and implications for coastal management and reef survival. *Curr. Opin. Environ.*
350 *Sustain.* 7, 82–93
- 351 32 Gates, R.D. *et al.* (1995) Free amino acids exhibit anthozoan “host factor” activity: they
352 induce the release of photosynthate from symbiotic dinoflagellates in vitro. *Proc. Natl. Acad.*
353 *Sci. U. S. A.* 92, 7430–7434
- 354 33 Wang, J. and Douglas, A.E. (1997) Nutrients, signals, and photosynthate release by symbiotic
355 algae. *Plant Physiol.* 114, 631–636
- 356 34 Cook, C.B. and Davy, S.K. (2001) Are free amino acids responsible for the “host factor” effects
357 on symbiotic zooxanthellae in extracts of host tissue? *Hydrobiologia* 461, 71–78
- 358 35 Titlyanov, E.A. *et al.* (1996) Degradation of zooxanthellae and regulation of their density in
359 hermatypic corals. *Mar. Ecol. Prog. Ser.* 139, 167–178
- 360 36 Yellowlees, D. *et al.* (2008) Metabolic interactions between algal symbionts and invertebrate
361 hosts. *Plant. Cell Environ.* 31, 679–694
- 362 37 Muscatine, L. *et al.* (1989) The effect of external nutrient resources on the population
363 dynamics of zooxanthellae in a reef coral. *Proc. R. Soc. London. Ser. B. Biol. Sci.* 236, 311–324
- 364 38 Dubinsky, Z. and Jokiel, P.L. (1994) Ratio of energy and nutrient fluxes regulates symbiosis
365 between zooxanthellae and corals. *Pacific Sci.* 48, 313–324
- 366 39 Wiedenmann, J. *et al.* (2012) Nutrient enrichment can increase the susceptibility of reef
367 corals to bleaching. *Nat. Clim. Chang.* 2, 1–5
- 368 40 Wooldridge, S.A. (2013) Breakdown of the coral-algae symbiosis: Towards formalising a
369 linkage between warm-water bleaching thresholds and the growth rate of the intracellular
370 zooxanthellae. *Biogeosciences* 10, 1647–1658
- 371 41 Barott, K.L. *et al.* (2014) Coral host cells acidify symbiotic algal microenvironment to promote
372 photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* DOI: 10.1073/pnas.1413483112
- 373 42 Webb, K.L. *et al.* (1975) Enewetak (Eniwetok) Atoll: Aspects of the nitrogen cycle on a coral
374 reef. *Limnol. Oceanogr.* 20, 198–210
- 375 43 Cardini, U. *et al.* (2014) Benthic N₂ fixation in coral reefs and the potential effects of human-
376 induced environmental change. *Ecol. Evol.* DOI: 10.1002/ece3.1050
- 377 44 Wiebe, W.J. *et al.* (1975) Nitrogen fixation in a coral reef community. *Science* 188, 257–259

378 45 Shashar, N. *et al.* (1994) Nitrogen fixation (acetylene reduction) in stony corals: evidence for
379 coral-bacteria interactions. *Mar. Ecol. Prog. Ser.* 111, 259–264

380 46 Rådecker, N. *et al.* (2014) Ocean acidification rapidly reduces dinitrogen fixation associated
381 with the hermatypic coral *Seriatopora hystrix*. *Mar. Ecol. Prog. Ser.* 511, 297–302

382 47 Grover, R. *et al.* (2014) Nitrogen fixation in the mucus of Red Sea corals. *J. Exp. Biol.* DOI:
383 10.1242/jeb.111591

384 48 Lesser, M.P. *et al.* (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of
385 nitrogen for the scleractinian coral *Montastraea cavernosa*. *Mar. Ecol. Prog. Ser.* 346, 143–
386 152

387 49 Lesser, M.P. *et al.* (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals.
388 *Science* 305, 997–1000

389 50 Williams, W.M. *et al.* (1987) Nitrogen fixation (acetylene reduction) associated with the living
390 coral *Acropora variabilis*. *Mar. Biol.* 94, 531–535

391 51 Crossland, C.J. and Barnes, D.J. (1976) Acetylene reduction by coral skeletons. *Limnol.*
392 *Oceanogr.* 21, 153–156

393 52 Olson, N.D. *et al.* (2009) Diazotrophic bacteria associated with Hawaiian *Montipora* corals:
394 diversity and abundance in correlation with symbiotic dinoflagellates. *J. Exp. Mar. Bio. Ecol.*
395 371, 140–146

396 53 Ceh, J. *et al.* (2013) Nutrient cycling in early coral life stages: Pocillopora damicornis larvae
397 provide their algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates.
398 *Ecol. Evol.* DOI: 10.1002/ece3.642

399 54 Mortenson, L.E. (1964) Ferredoxin and atp, requirements for nitrogen fixation in cell-free
400 extracts of *Clostridium pasteurianum*. *Proc. Natl. Acad. Sci. U. S. A.* 52, 272–279

401 55 Knapp, A.N. (2012) The sensitivity of marine N₂ fixation to dissolved inorganic nitrogen.
402 *Front. Microbiol.* 3,

403 56 Scheffers, S.R. *et al.* (2004) Removal of bacteria and nutrient dynamics within the coral reef
404 framework of Curaçao (Netherlands Antilles). *Coral Reefs* 23, 413–422

405 57 Risk, M.J. and Muller, H.R. (1983) Porewater in coral heads: evidence for nutrient
406 regeneration. *Limnol. Oceanogr.* 28, 1004–1008

407 58 Wafar, M. *et al.* (1990) Nitrification in reef corals. *Limnol. Oceanogr.* 35, 725–730

408 59 Siboni, N. *et al.* (2008) Global distribution and diversity of coral-associated archaea and their
409 possible role in the coral holobiont nitrogen cycle. *Environ. Microbiol.* 10, 2979–2990

410 60 Schiller, C. and Herndl, G.J. (1989) Coral reefs evidence of enhanced microbial activity in the
411 interstitial space of branched corals: possible implications for coral metabolism. *Coral Reefs* 7,
412 179–184

- 413 61 Beman, J.M. *et al.* (2007) Distribution and diversity of archaeal ammonia monooxygenase
414 genes associated with corals. *Appl. Environ. Microbiol.* 73, 5642–5647
- 415 62 Yang, S. *et al.* (2013) Phylogenetically diverse denitrifying and ammonia-oxidizing bacteria in
416 corals *Alcyonium gracillimum* and *Tubastraea coccinea*. *Mar. Biotechnol.* 15, 540–551
- 417 63 Siboni, N. *et al.* (2012) Geographic specific coral-associated ammonia-oxidizing archaea in the
418 Northern Gulf of Eilat (Red Sea). *Microb. Ecol.* 64, 18–24
- 419 64 Taguchi, S. and Kinzie III, R.A. (2001) Growth of zooxanthellae in culture with two nitrogen
420 sources. *Mar. Biol.* 138, 149–155
- 421 65 D’Elia, C.F. *et al.* (1983) Nutrient uptake kinetics of freshly isolated zooxanthellae. *Mar. Biol.*
422 167, 157–167
- 423 66 Domotor, S.L. and D’Elia, C.F. (1984) Nutrient uptake kinetics and growth of zooxanthellae
424 maintained in laboratory culture. *Mar. Biol.* 101, 93–101
- 425 67 Koop, K. *et al.* (2001) ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of
426 results and conclusions. *Mar. Pollut. Bull.* 42, 91–120
- 427 68 Hoffmann, F. *et al.* (2009) Complex nitrogen cycling in the sponge *Geodia barretti*. *Environ.*
428 *Microbiol.* 11, 2228–2243
- 429 69 Compaoré, J. and Stal, L.J. (2010) Effect of temperature on the sensitivity of nitrogenase to
430 oxygen in two heterocystous cyanobacteria. *J. Phycol.* 46, 1172–1179
- 431 70 Shashar, N. *et al.* (1993) Extremediel fluctuations of oxygen in diffusive boundary layers
432 surrounding stony corals. *Biol. Bull.* 185, 455–461
- 433 71 Lloyd, D. *et al.* (1987) Persistence of bacterial denitrification capacity under aerobic
434 conditions: the rule rather than the exception. *FEMS Microbiol. Lett.* 45, 185–190
- 435 72 Thurber, R.V. *et al.* (2009) Metagenomic analysis of stressed coral holobionts. *Environ.*
436 *Microbiol.* 11, 2148–2163
- 437 73 Dalsgaard, T. *et al.* (2005) Anaerobic ammonium oxidation (anammox) in the marine
438 environment. *Res. Microbiol.* 156, 457–464
- 439 74 Bellwood, D.R. *et al.* (2004) Confronting the coral reef crisis. *Nature* 429, 827–833
- 440 75 Baker, A.C. *et al.* (2008) Climate change and coral reef bleaching: an ecological assessment of
441 long-term impacts, recovery trends and future outlook. *Estuar. Coast. Shelf Sci.* 80, 435–471
- 442 76 Cervino, J.M. *et al.* (2004) Zooxanthellae regulation in yellow blotch/band and other coral
443 diseases contrasted with temperature related bleaching: in situ destruction vs expulsion.
444 *Symbiosis* 37, 63–85
- 445 77 Bourne, D. *et al.* (2008) Changes in coral-associated microbial communities during a bleaching
446 event. *ISME J.* 2, 350–363

- 447 78 Santos, H.F. *et al.* (2014) Climate change affects key nitrogen-fixing bacterial populations on
448 coral reefs. *ISME J.* DOI: 10.1038/ismej.2014.70
- 449 79 Roder, C. *et al.* (2014) Bacterial profiling of white plague disease across corals and oceans
450 indicates a conserved and distinct disease microbiome. *Mol. Ecol.* 23, 965–974
- 451 80 Kline, D.I. *et al.* (2006) Role of elevated organic carbon levels and microbial activity in coral
452 mortality. *Mar. Ecol. Prog. Ser.* 314, 119–125
- 453 81 Brown, B.E. (1997) Coral bleaching: causes and consequences. *Coral Reefs* 16, 129–138
- 454 82 Randall, C.J. and van Woesik, R. (2015) Contemporary white-band disease in Caribbean corals
455 driven by climate change. *Nat. Clim. Chang.* DOI: 10.1038/nclimate2530
- 456 83 Godinot, C. *et al.* (2011) Coral uptake of inorganic phosphorus and nitrogen negatively
457 affected by simultaneous changes in temperature and pH. *PLoS One* 6, 1–10
- 458 84 Strychar, K. and Coates, M. (2004) Loss of Symbiodinium from bleached Australian
459 scleractinian corals (*Acropora hyacinthus*, *Favites complanata* and *Porites solida*). *Mar.*
460 *Freshw. Res.* 55, 135–144
- 461 85 Bhagooli, R. and Hidaka, M. (2002) Physiological responses of the coral *Galaxea fascicularis*
462 and its algal symbiont to elevated temperatures. *Galaxea* 4, 33–42
- 463 86 Davey, M. *et al.* (2008) High rates of nitrogen fixation (acetylene reduction) on coral skeletons
464 following bleaching mortality. *Coral reefs* 27, 227–236
- 465 87 Smith, J.E. *et al.* (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced
466 coral mortality. *Ecol. Lett.* 9, 835–845
- 467 88 Pernice, M. and Levy, O. (2014) Novel tools integrating metabolic and gene function to study
468 the impact of the environment on coral symbiosis. *Front. Microbiol.* DOI:
469 10.3389/fmicb.2014.00448
- 470 89 Closek, C.J. *et al.* (2014) Coral transcriptome and bacterial community profiles reveal distinct
471 yellow band disease states in *Orbicella faveolata*. *ISME J.* DOI: 10.1038/ismej.2014.85
- 472 90 Raina, J.-B. *et al.* (2013) DMSP biosynthesis by an animal and its role in coral thermal stress
473 response. *Nature* 502, 677–80
- 474 91 Tremblay, P. *et al.* (2013) Photosynthate translocation increases in response to low seawater
475 pH in a coral–dinoflagellate symbiosis. *Biogeosciences* 10, 3997–4007
- 476 92 Pernice, M. *et al.* (2014) A nanoscale secondary ion mass spectrometry study of dinoflagellate
477 functional diversity in reef-building corals. *Environ. Microbiol.* DOI: 10.1111/1462-2920.12518
- 478 93 Wilson, S.T. *et al.* (2012) Comparative assessment of nitrogen fixation methodologies ,
479 conducted in the oligotrophic North Pacific Ocean. *Appl. Environ. Microbiol.* 78, 6516–6523

- 480 94 Silverstein, R.N. *et al.* (2014) Change in algal symbiont communities after bleaching, not prior
481 heat exposure, increases heat tolerance of reef corals. *Glob. Chang. Biol.* DOI:
482 10.1111/gcb.12706
- 483 95 Hume, B.C.C. *et al.* (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic
484 alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Sci. Rep.* 5, 8562
- 485 96 Nissimov, J. *et al.* (2009) Antimicrobial properties of resident coral mucus bacteria of *Oculina*
486 *patagonica*. *FEMS Microbiol. Lett.* 292, 210–215
- 487 97 Bednarz, V.N. *et al.* (2014) Seasonal variation of N₂ fixation and O₂ fluxes associated with two
488 dominant zooxanthellate soft corals from the Northern Red Sea. *Mar. Ecol. Prog. Ser.* DOI:
489 doi: 10.3354/meps11091
- 490

491 **Figures & boxes**

492

493 **Glossary**

494 **ANAMMOX:** the relatively recently discovered anaerobic conversion of ammonium (NH_4^+)
495 and nitrate (NO_3^-) into dinitrogen (N_2) by bacteria.

496 **Coral bleaching:** the loss of endosymbiotic *Symbiodinium* or symbiont pigmentation in
497 corals commonly induced by temperature or light stress, thereby resulting in a bleached
498 appearance of affected corals.

499 **Coral holobiont:** the complex meta-organism comprising the coral animal host, its symbiotic
500 algae, and other microorganisms, including protozoans, fungi, bacteria, and archaea.

501 **Coral microbiome:** the community of microorganisms residing within the coral holobiont.
502 These associations include mutualistic, commensalistic, and/or pathogenic interactions.

503 **Denitrification:** the anaerobic reduction of nitrates (NO_3^-) and nitrites (NO_2^-) into dinitrogen
504 (N_2) via a series of oxidised nitrogen intermediates by microorganisms.

505 **Diazotrophs:** the diverse group of bacteria and archaea capable of nitrogen fixation.

506 **Dissolved inorganic nitrogen (DIN):** the sum of all inorganic nitrogen compounds dissolved
507 in water, mostly ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). DIN is the most
508 important nutrient source for aquatic primary production.

509 **Dissolved organic carbon (DOC):** the sum of all organic carbon compounds dissolved in
510 water strongly variable in composition and lability. Dissolved organic carbon compounds
511 include saccharides (e.g. glucose), amino acids, or humic acids. The biodegradable fraction
512 may stimulate microbial activity and has been linked to coral mortality.

513 **Eutrophic:** the surplus of essential nutrients, especially nitrogen and phosphorous, required
514 for primary production. Under eutrophic conditions algae may proliferate causing harmful
515 blooms. The input of nutrients into a water body resulting in eutrophic conditions may thus
516 be referred to as eutrophication.

517 **Nitrification:** the aerobic respiration of ammonium (NH_4^+) into nitrite (NO_2^-) and
518 subsequently into nitrate (NO_3^-) by autotrophic bacteria and archaea.

519 **Nitrogen fixation:** the conversion of dinitrogen (N_2) into ammonium (NH_4^+) by diazotrophs.

520 **Oligotrophic:** the deficit of essential nutrients, particularly nitrogen and phosphorous,
521 required for primary production.

522 **Symbiosome:** the multiple-layer host-derived membrane complex that surrounds
523 *Symbiodinium* when in symbiosis with the coral host.

524

525 **Box 1. The coral probiotic hypothesis**

526 Corals harbour a variety of symbiotic archaea and bacteria. Changing environmental
527 conditions can alter the composition and abundance of coral associated microorganisms
528 rapidly. Reshef & colleagues [19] proposed in their “coral probiotic hypothesis” that a
529 dynamic relationship between symbiotic microorganisms and the coral host selects for the
530 most advantageous composition of the coral holobiont under varying environmental
531 conditions. Therefore, shifts in the microbiome may facilitate corals to adjusting to changing
532 environmental conditions considerably faster than by mutation and selection of the host
533 alone. This implies that the combined holobiont rather than its individual members
534 represents the unit of natural selection, and has lead to the development of the
535 “hologenome theory of evolution” [8].

536 Some studies suggest that environmental changes acting on the coral holobiont may select
537 for partners which are beneficial for coral holobiont functioning [94,95]. Particularly the
538 development of anti-microbial resistance in corals, despite the lack of an adaptive immune
539 system, highlights the adaptive potential of microbial interactions within the holobiont [96].

540 It has been shown that environmental conditions can affect nitrogen cycling in corals [46],
541 and that nitrogen fixation in soft corals can supplement reduced nutrient availability in the
542 Red Sea [97]. It is likely that these alterations in nitrogen cycling capacity are the results of
543 shifts within the coral microbiome. Hence, in accordance with the coral probiotic
544 hypothesis, these changes may serve as a way for corals to adapt to changing nutrient
545 availability.

546

547 **Box 2. Outstanding questions**

- 548
- What are the main functional players of the holobiont microbial community involved in coral-associated nitrogen cycling pathways, and how are shifts in diversity and abundance of these functional groups related to changes in the nitrogen cycling capacity of corals?
- 549
- 550
- How is the coral host involved in nitrogen cycling and exerting control over microbial nitrogen cycling activity?
- 551
- 552
- To what extent can corals adjust to anthropogenic changes, such as eutrophication, by altering their nitrogen cycling capacity?
- 553
- 554
- How is coral-associated nitrogen cycling governed by environmental conditions, and what are the implications for coral bleaching and disease?
- 555
- 556

557

558 **Figure Legends**

559 **Figure 1. Hypothesized nitrogen cycling in the coral holobiont.**

560 Representation of major nitrogen cycling pathways in the coral holobiont under (A) oligotrophic, (B)
561 eutrophic, and (C) an elevated temperature scenario. Bold arrows indicate potential relative
562 amplification of pathways compared to an oligotrophic scenario. Dashed arrows indicate potential
563 relative diminution of pathways compared to an oligotrophic scenario. Microbial nitrogen cycling
564 may help to regulate algal growth under oligotrophic and eutrophic conditions, but may lead to
565 elevated nitrogen availability under heat stressed conditions. A representation of the localization of
566 individual process within the holobiont was omitted, as sufficient knowledge is lacking to date.
567 Abbreviation: org., organic.

568

569 **Figure 2. The proposed role of nitrogen fixation in reef degradation.**

570 (A) Elevated concentrations of dissolved organic carbon (DOC) and heat stress induce increased
571 microbial activity in corals and stimulate nitrogen fixation activity. (B) The resulting excess supply of
572 fixed nitrogen triggers rapid population growth of the endosymbiotic dinoflagellate *Symbiodinium*.
573 (C) The resulting shift from nitrogen limitation to phosphate starvation in combination with
574 increased *Symbiodinium* population sizes causes a lowered bleaching threshold of corals and
575 eventually coral bleaching. (D) Following colony die-off, the remaining coral skeleton may be
576 overgrown by algae, which release DOC-rich exudates. Hence algae may stimulate further microbial
577 activity including nitrogen fixation in adjacent corals, thereby exacerbating the reef degradation
578 process.

579

580 **Figure 3. Reef degradation by coral-algae interactions.**

581 Progressing coral mortality by interaction with algae may act on different scales, ranging from (A)
582 coral colony level to (B) community or ecosystem level. These interactions may involve algae-
583 stimulated nitrogen fixation as a mechanism to interrupt the coral–algae symbiosis. Photo credits: N.
584 Rådecker (A), M. Naumann (B).