Review

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

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

Corals in an oligotrophic environment

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

Distinct microbial assemblages are associated with the surface mucus layer, coral tissue, gastro-vascular cavity, and within the coral skeleton [8]. This variety of habitats results in a highly diverse coral microbiome where associated microbes can potentially perform a multitude of services to the functioning of the coral holobiont including carbon fixation, nitrogen metabolism, sulfur cycling, and antimicrobial defense among many others [6,9–11]. Owing to the high importance of different microbial groups for the

Glossary

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

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

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

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

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

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

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

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

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

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

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

Oligotrophic: the deficit of essential nutrients, particularly nitrogen and phosphorus, that are required for primary production.

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

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Box 1. The coral probiotic hypothesis

Corals harbor a variety of symbiotic archaea and bacteria. Changing environmental conditions can alter the composition and abundance of coral-associated microorganisms rapidly. Reshef and colleagues [19] proposed in their 'coral probiotic hypothesis' that a dynamic relationship between symbiotic microorganisms and the coral host selects for the most advantageous composition of the coral holobiont under varying environmental conditions. Therefore, shifts in the microbiome may facilitate coral adjustment to changing environmental conditions considerably faster than by mutation and selection of the host alone. This implies that the combined holobiont rather than its individual members represents the unit of natural selection, and has led to the development of the 'hologenome theory of evolution' [8].

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

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

functioning of the coral holobiont, a variety of mechanisms have evolved to enable an effective intergenerational transfer of specific microbes to ensure coral offspring fitness [12,13]. This vertical transfer presumably facilitated the evolution of host-specific and persistent microbial communities in many corals [14–18]. In this context, Reshef *et al.* [19] proposed the coral probiotic hypothesis – stating that, despite the presence of consistent microbial communities, alterations in the microbiome may help corals to rapidly adjust to environmental conditions (Box 1).

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

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

Nitrogen uptake and symbiont control in the coral holobiont

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

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

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

In this context, adjustment of internal nitrogen availability may help to control algal growth. The ability of the coral host to assimilate ammonium has been suggested to allow more efficient uptake of ammonium from seawater by maintaining the diffusion gradient into the tissue, while simultaneously enabling an internal limitation of nitrogen availability to *Symbiodinium* [27,36,40]. At the same time, regulation of symbiosome pH surrounding *Symbiodinium* may help to control the diffusion of ammonium ions into the symbiosome [41]. Furthermore, nitrogen cycling by coral-associated microbes may be an additional mechanism contributing to the stabilization, or conversely destabilization, of the coral–algae symbiosis.

Nitrogen fixation

Coral reefs are net sources of fixed nitrogen [42]. Nitrogen fixation, in other words the conversion of elemental dinitrogen (N_2) into ammonium, is associated with many substrates (e.g., sand, coral rock, and rubble) and benthic organisms (e.g., corals, macroalgae, and sponges) [21,43]. This input of new fixed nitrogen into the reef ecosystem helps to sustain net productivity under oligotrophic conditions and to compensate for net nitrogen export from the system, for instance by currents [44]. Nitrogen fixation in hermatypic corals has been reported for several different species [45–48], suggesting a high relevance of this process for the coral holobiont. However, reported nitrogen fixation rates in corals are about a magnitude lower than those found in reef sediments and bare rock [43]. Hence, corals appear to be minor contributors to the overall nitrogen budget of coral reefs.

For a long time, cyanobacteria were believed to be the main drivers of nitrogen fixation in corals [49–51], but recent studies revealed that corals harbor ubiquitous diverse communities of diazotrophs (i.e., nitrogen-fixing bacteria and archaea) consisting of mostly heterotrophic bacteria [16,17,20,52]. These communities are coral species-specific and highly persistent over space and time [16,17]. Moreover, Lema *et al.* [18] found a vertical transfer of diazotrophs from parental colonies of the coral *Acropora millepora* to their larvae, mostly Alphaproteobacteria of the group Rhizobiales. This vertical transfer of diazotrophs further suggests a beneficial role of this group for holobiont functioning. Although the magnitude of transfer of fixed nitrogen from diazotrophs into other compartments of the coral holobiont (e.g., *Symbiodinium*) has not been quantified yet, recent studies show that bacterial symbionts contribute to the nitrogen supply of the holobiont [48,53]. However, a recent stable isotope ($^{15}N_2$ gas) tracing application in Red Sea corals revealed no direct incorporation of fixed nitrogen in the coral tissue within the first 24 h of measurement, suggesting coral–diazotroph interactions may be more complex than previously thought [47].

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

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

Nitrification

In contrast to nitrogen fixation, other pathways of the nitrogen cycle in corals have received little attention to date. Nonetheless, particularly nitrification and denitrification may potentially be of importance for holobiont functioning. High rates of nitrification, in other words

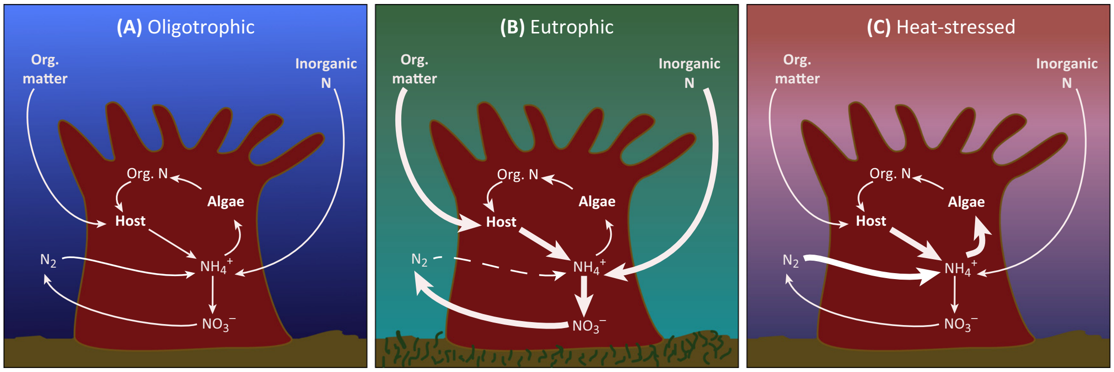

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Figure 1. Hypothesized nitrogen cycling in the coral holobiont. Representation of major nitrogen-cycling pathways in the coral holobiont under **(A)** oligotrophic, **(B)** eutrophic, and **(C)** an elevated temperature scenario. Bold arrows indicate potential relative amplification of pathways compared to an oligotrophic scenario. Broken arrows indicate potential relative diminution of pathways compared to an oligotrophic scenario. Microbial nitrogen cycling may help to regulate algal growth under oligotrophic and eutrophic conditions, but may lead to elevated nitrogen availability under heat-stressed conditions. A representation of the localization of individual process within the holobiont is omitted because sufficient knowledge is lacking to date. Abbreviation: Org., organic.

the oxidation of ammonium into nitrite (NO_2^-) and nitrate (NO_3^-), have been measured in coral reef environments [42,56]. Nitrification associated with corals occurs in their skeletons [57], living tissues [58], mucus [59], and the interstitial waters of coral branches [60], likely rendering nitrification a ubiquitous coral-associated process. Consequently, recent studies revealed that nitrifying bacteria and archaea are highly abundant in the microbiomes of many coral species [10,59,61,62]. Among these, at least the mucus-associated ammonia-oxidizing archaeal communities appear to depend more on location and environmental conditions than on the coral host [59,63]. The metabolic activity of these communities, however, remains yet to be determined and the role of nitrification in the holobiont is largely unknown. *Symbiodinium* prefer uptake of ammonium over other forms of DIN [64,65], and ammonium may inhibit the uptake of nitrate [66]. Nitrification in corals may thus ultimately reduce the amount of nitrogen available for *Symbiodinium* growth. Wafar *et al.* [58] reported that bacterial nitrification almost exclusively utilized ammonium derived from coral metabolism, and that this process occurred at rates effectively competing with the autotrophic uptake of ammonium. Consequently, nitrogen cycling may serve as a way to retain nitrogen within the coral holobiont, with nitrification preventing loss of ammonium from the holobiont.

Denitrification

Depending on environmental conditions, reef substrates can show high denitrification rates, and denitrification has been detected in marine invertebrates [67,68]. Although denitrifying microorganisms are present in coral microbiomes [10,62], no studies have investigated the denitrifying potential of corals so far. Siboni *et al.* [59] suggested a coupling of nitrification and denitrification (i.e., the reduction of nitrate) ultimately resulting in the production of dinitrogen to remove nitrogen from the coral holobiont. Nitrogen-cycling processes are highly dependent on oxygen availability [48,69]. Because oxygen concentrations in coral tissue show strong diel fluctuations, the linkage of aerobic (e.g., nitrification) and anaerobic (e.g., nitrogen fixation and denitrification) processes within the coral holobiont may be possible [70]. Additional evolutionary adaptations by the coral host and associated microbes may provide a spatial or temporal separation of anaerobic processes from oxygen evolution [71].

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

Other pathways in the nitrogen cycle

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

Another pathway of the nitrogen cycle is anaerobic ammonium oxidation (ANAMMOX), transforming fixed nitrogen into elemental dinitrogen. Particularly in the marine nitrogen cycle, the ANAMMOX pathway is of high importance [73]. Its presence has been previously confirmed in sponges [68] and has been suggested in corals, but direct evidence still needs to be provided. The presence of ANAMMOX may constitute another crucial mechanism in addition to denitrification as a means to remove excess nitrogen from the coral holobiont, for example, during eutrophication events.

Environmental constraints and anthropogenic impact

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

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

A potential link between nitrogen cycling and reef degradation

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

An imbalanced nutrient availability, in other words elevated inorganic nitrogen concentrations in combination with phosphate depletion, rather than enrichment of both nitrogen and phosphate, can destabilize the coral–algae symbiosis [39]. Among other processes, nitrogen fixation can potentially increase the N:P ratio in corals [31]. Hence, environmental impacts that increase nitrogen fixation may

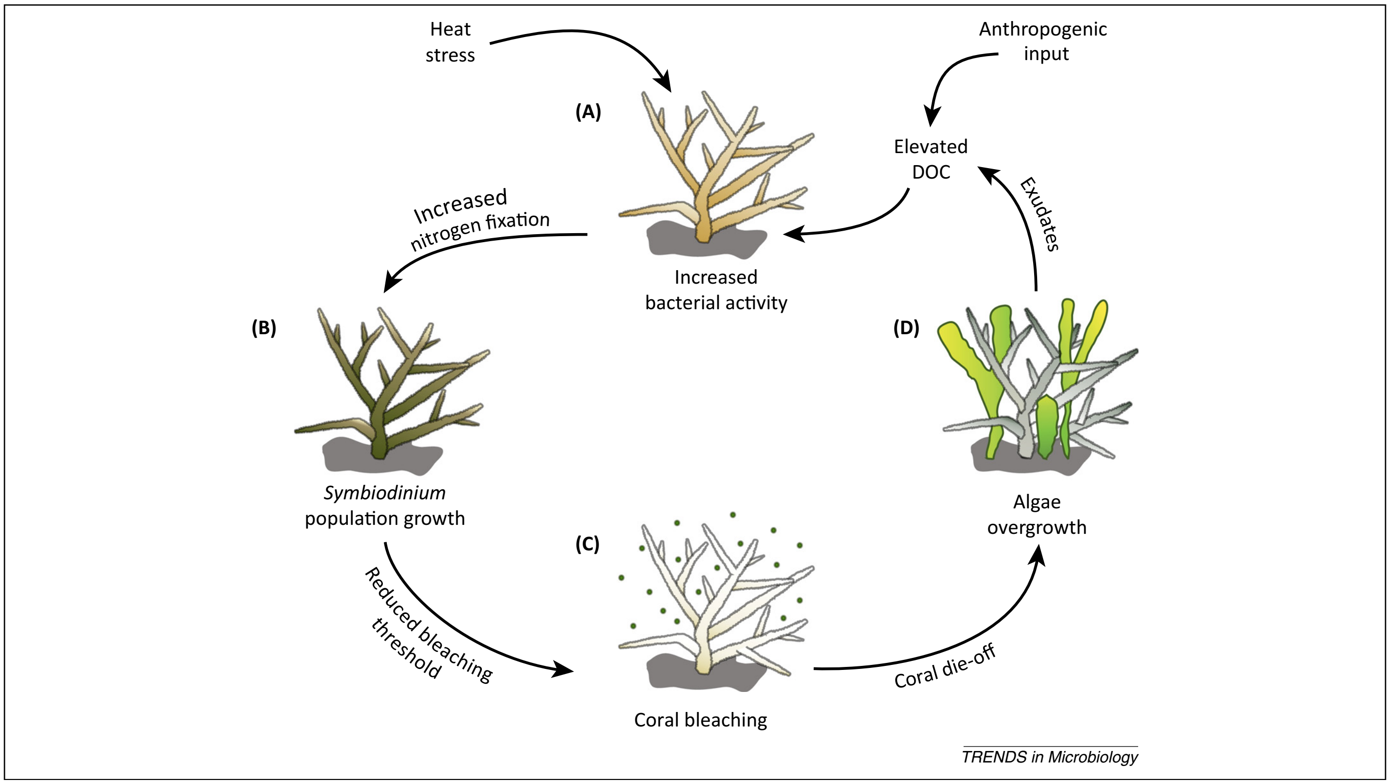

Figure 2. The proposed role of nitrogen fixation in reef degradation. **(A)** Elevated concentrations of dissolved organic carbon (DOC) and heat stress induce increased microbial activity in corals and stimulate nitrogen fixation activity. **(B)** The resulting excess supply of fixed nitrogen triggers rapid population growth of the endosymbiotic dinoflagellate *Symbiodinium.* **(C)** The resulting shift from nitrogen limitation to phosphate starvation in combination with increased *Symbiodinium* population sizes causes a lowered bleaching threshold of corals and eventually coral bleaching. **(D)** Following colony die-off, the remaining coral skeleton may be overgrown by algae, which release DOC-rich exudates. Hence algae may stimulate further microbial activity including nitrogen fixation in adjacent corals, thereby exacerbating the reef degradation process.

ultimately disrupt the nitrogen limitation of *Symbiodinium* in corals. The resulting elevated nitrogen availability would stimulate cell division of *Symbiodinium*, thereby shifting *Symbiodinium* from nitrogen towards phosphate limitation/starvation.

Wiedenmann *et al.* [39] found that phosphate starvation associated with increased cell division rates resulted in an increase of sulfo- to phospholipid ratios in *Symbiodinium*, and this may cause a destabilization of their thylakoid membranes and explain the lower bleaching threshold of phosphate-starved coral species. Furthermore, Wooldridge [40] proposed a mechanism by which enhanced retention of photosynthates by *Symbiodinium* due to excess nitrogen availability may cause CO_2 limitation, ultimately resulting in coral bleaching. Therefore, environmental stressors such as heat stress and elevated DOC concentrations may potentially lower the threshold at which a disruption of the coral–algae symbiosis occurs by increasing coral-associated nitrogen fixation rates.

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

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

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

Future perspectives

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

Recent technological advances in the biological sciences may help to answer these questions (reviewed in detail by

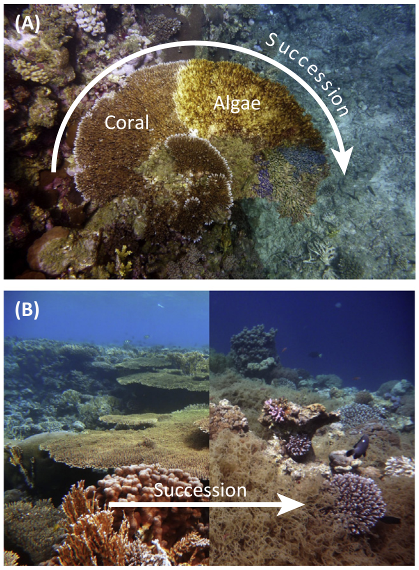

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Figure 3. Reef degradation by coral–algae interactions. Progressing coral mortality by interaction with algae may act on different scales, ranging from **(A)** the coral colony level to **(B)** the community or ecosystem levels. These interactions may involve algae-stimulated nitrogen fixation as a mechanism to interrupt the coral–algae symbiosis. Photo credits: N. Rädecker (A), M. Naumann (B).

Pernice and Levy [88]). For instance, the development and application of culture-independent methods to characterize microbial communities has changed our understanding of the coral microbiome. In particular, the application of metatranscriptomics to disentangle expressed functions in different holobiont compartments [89] will help to decipher which microbial partners are important drivers of nitrogen cycling in corals. In this context, the growing number of available (meta-)genomes and (meta-)transcriptomes of corals, *Symbiodinium*, and bacterial partners will allow the elucidation of key genes involved in nitrogen cycling and their distribution across holobiont compartments. For example, DMSP biosynthesis was only recently discovered in corals, a process that was believed to be restricted to marine algae and plants [90]. It is thus important to test the established understanding of the distribution of genes related to nitrogen cycling between the coral host and its symbiotic partners through incorporation of data from multiple species and under different conditions.

Furthermore, the emerging use of stable isotope analysis and isotopic labeling provides an excellent tool to identify the fate of metabolic products in corals [47,48,91]. In combination with NanoSIMS technology [27,29,53,92], such approaches will enable us to understand how nitrogen uptake and nitrogen fixation affect the various symbiotic partners within the coral holobiont. However, owing to the technical limitations from rinsing and dehydration steps during sample preparation, NanoSIMS measurements appear less suitable for detection of nitrification or denitrification. These new technological advances are most valuable when integrated into classical ecological approaches such as the indirect measurement of nitrogen fixation rates via the acetylene reduction assay [93]. Such integrated applications will allow the holistic understanding of nitrogen cycling in the coral holobiont, and this is urgently needed to address the consequences of anthropogenically-driven environmental change in coral reefs.

Box 2. Outstanding questions

- What are the main functional players in the holobiont microbial community that are 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?
- How is the coral host involved in nitrogen cycling and exerting control over microbial nitrogen-cycling activity?
- To what extend can corals adjust to anthropogenic changes, such as eutrophication, by altering their nitrogen-cycling capacity?
- How is coral-associated nitrogen cycling governed by environmental conditions, and what are the implications for coral bleaching and disease?

Concluding remarks

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tim.2015.03.008.

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