

# The contribution of microbial biotechnology to mitigating coral reef degradation

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Reef-forming scleractinian corals are foundation species of coral reefs, as they build the three-dimensional structure of the reef, provide habitat to possibly millions of marine species and represent the most important primary producers through their association with microbial photosymbionts (*Symbiodinium* spp.) (Harrison and Booth, 2007). In addition to being extremely rich reservoirs of biodiversity, coral reefs greatly contribute to coastal protection, tourism and fisheries and their economic value is estimated in the range of billions of US dollars (Costanza *et al.*, 1997; Burke *et al.*, 2011). Coral reefs have suffered major declines over the past four decades, mainly due to anthropogenic disturbances acting on both local (e.g. overharvesting, pollution) and global (e.g. effects of climate change) scales (Bruno and Selig, 2007; Hoegh-Guldberg, 2011; De'ath *et al.*, 2012). Elevated sea surface temperature (SST) is a major driver of coral bleaching and mortality because it disrupts the critical relationship between corals and their endosymbiotic *Symbiodinium* (Hoegh-Guldberg, 1999; Lesser, 2011). The recent global mass bleaching event that lasted from 2014 to 2016 and which caused unprecedented coral bleaching and mortality (Normile,

2016; Hughes *et al.*, 2017) is reported to be the longest and most widespread on record (Cresset, 2016; NOAA Coral Reef Watch 2017).

Combatting the impacts of climate change and conserving marine resources are among the United Nations' sustainable development goals (United Nations 2017). Despite increasing awareness of the threats of climate change to biodiversity and the establishment of guidelines to preserve marine ecosystems, environmental degradation is occurring faster than the pace of coral adaptation through natural selection on standing genetic variation (i.e. genetic adaptation) (Hoegh-Guldberg, 2004; Hoegh-Guldberg *et al.*, 2007). Active interventions to help corals survive by augmenting their tolerance to and ability to recover from stress are therefore urgently required. Recently, the concept of assisted evolution has been proposed as a possible strategy for accelerating the rate of naturally occurring evolutionary processes and to develop corals capable of coping with current climate change trajectories (van Oppen *et al.*, 2015). Assisted evolution includes selective breeding of coral, preconditioning of coral to sublethal stress, laboratory evolution of *Symbiodinium* followed by inoculation of the coral with tolerant algal symbionts, and manipulation of various members of the coral microbiome (van Oppen *et al.*, 2015, 2017). This commentary focuses on the potential to mitigate coral reef degradation through the manipulation of coral-associated prokaryotes.

Corals are colonized by a huge diversity of prokaryotes (Rohwer *et al.*, 2002; Blackall *et al.*, 2015), with distinct communities occupying various microhabitats within the host, including coral tissues, the surface mucus layer, the gastric cavity and the skeleton (Sweet *et al.*, 2010; Bourne *et al.*, 2016). Bacteria scavenge limiting nutrients (Knowlton and Rohwer, 2003; Zhang *et al.*, 2015), deliver essential products to their host following carbon and nitrogen fixation (Lesser *et al.*, 2007; Kimes *et al.*, 2010) and participate in sulfur and phosphorus cycling (Raina *et al.*, 2009; Zhang *et al.*, 2015). Further, bacteria contribute to coral immune defences by occupying entry niches and by secreting antimicrobial peptides (Ritchie, 2006; Nissimov *et al.*, 2009; Shnit-Orland and Kushmaro, 2009). The composition of the coral microbiome can change with coral life stage, host health state, water temperature and acidity, nutrient levels, pollution, the presence of macroalgae, light intensity, depth or seasonal variation (Hernandez-Agreda *et al.*, 2016a; Glasl *et al.*,

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2017; Sweet and Bulling, 2017). Maintaining a healthy microbiome is thought to be essential for the well-being of corals, as destabilization in the composition and function of their associated microbial communities has been shown to take place in diseased states (Frias-Lopez *et al.*, 2002; Jones *et al.*, 2004; Gil-Agudelo *et al.*, 2006; Sato *et al.*, 2013) and under stressful environmental conditions (Zaneveld *et al.*, 2016). Elevated seawater temperatures and coral bleaching are typically associated with a shift towards opportunistic and/or pathogenic bacteria, with concomitant declines in coral health (Ritchie, 2006; Bourne *et al.*, 2008; Littman *et al.*, 2011; Lins-de-Barros *et al.*, 2013; Tout *et al.*, 2015). Conversely, in some instances coral-associated microbes remain stable despite different host phenotypes (Hadaidi *et al.*, 2017), changing environmental conditions (Teplitski *et al.*, 2016) or in the presence of stressors (such as increased  $p\text{CO}_2$ ) (Webster *et al.*, 2016; Zhou *et al.*, 2016).

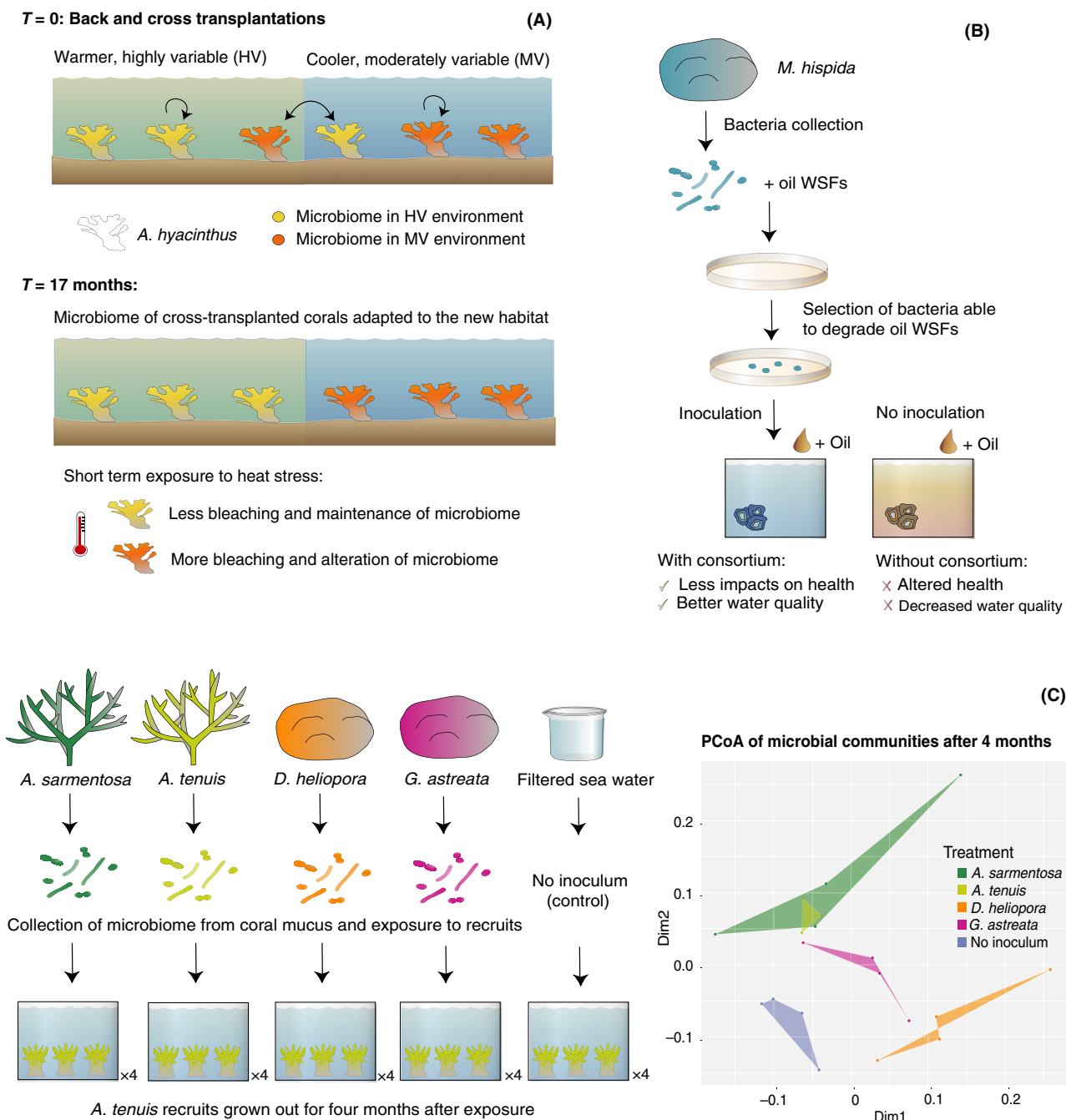
While an optimal microbiome may help protect the host from environmental pressures or compromised health by preserving beneficial functions, its dynamic nature may also confer an adaptive potential (Webster and Reusch, 2017). Whether by alteration in the relative abundance of certain species, acquisition of new species or variants from the environment or by mutations in the genomes of the existing community, modification of the microbiome is hypothesized to provide physiological flexibility to respond to environmental disturbances (Reshef *et al.*, 2006; Rosenberg *et al.*, 2007). For instance, reciprocal transplantation of *Acropora hyacinthus* fragments between thermally distinct environments on the same reef resulted in an adjustment of the microbial communities to the new conditions (Ziegler *et al.*, 2017). While the microbiome of cross-transplanted corals was indistinguishable from the microbiome of native corals in the same pool, it changed compared to the microbiome of the back-transplanted counterparts (Fig. 1A). Moreover, when subjected to short-term heat stress, corals that had spent the preceding 17 months in the more variable and warmer thermal regime were found to bleach less and retained a more stable microbiome. These results suggest that microbial community composition influences the response to heat stress, although host genetic adaptation and acclimatization are known to play additional roles (Barshis *et al.*, 2013; Bay and Palumbi, 2014).

As the composition and function of microbial communities seem to impact the fitness of their host, manipulation of resident prokaryotes could serve as a powerful tool to increase coral tolerance to stress and assist their adaptation to a changing environment. Such approaches are increasingly used in other biological systems. For instance, in humans, faecal microbiome transplantation is now accepted as an effective treatment for *Clostridium difficile* infections and is also gaining momentum for the

treatment of other bowel conditions (Borody and Khoruts, 2012; Gupta *et al.*, 2016). In agriculture, inoculation of rice plants with microbes collected from other plant species growing in extreme environments can enhance the rice plants' tolerance to drought, salinity and low temperatures (Redman *et al.*, 2011). In contrast to an entire microbiome transplant approach, bacterial species can also be selected and administered to the host to promote health. Probiotics are, for example, widely used in the aquaculture industry to stimulate growth, inhibit pathogens, improve water quality or augment tolerance to stress (Verschuere *et al.*, 2000; Martinez Cruz *et al.*, 2012; Boutin *et al.*, 2013). While these approaches have not been readily applied in open marine systems, the biological control of coral diseases using phage therapy has already shown some promising outcomes in confined areas and in the laboratory for preventing and treating specific infections (Atad *et al.*, 2012; Cohen *et al.*, 2013).

Preliminary studies indicate that coral-associated prokaryotes can be manipulated through inoculations with specific taxa. Bacteria collected from the coral *Mussismilia hartii* were cultured on a selective medium to isolate strains capable of degrading water-soluble oil fractions (WSFs) (dos Santos *et al.*, 2015). When subjected to conditions simulating an oil spill, polyps of *M. hartii* inoculated with a WSF-degrading bacterial consortium (i.e. probiotic bacteria) were less negatively affected compared to the non-inoculated polyps, as assessed by higher photosynthetic efficiencies of photosystem II of *Symbiodinium* (Fig. 1B). In this experiment, exposure to a specific microbial mixture therefore conferred health benefits to corals under environmental stress. Our preliminary experiments further support that the coral microbiome can be artificially influenced through microbiome inoculation (Data S1, S2, S3). Larvae of *Acropora tenuis* were exposed to the mucus collected from either *Acropora sarmentosa*, *Acropora tenuis*, *Diploastrea heliophora* or *Galaxea astreata*, and a no-mucus treatment was included as a control (Fig. 1C). Mucus was chosen as the inoculum as it contains a high density of coral prokaryotes (Thompson *et al.*, 2014) and can be easily collected after briefly exposing coral colonies to air (Brown and Bythell, 2005). Once settled, *A. tenuis* recruits were reared in filter-sterilized and flow-through sea water for 4 months, before being sampled to assess prokaryote microbiome composition. PERMANOVA of Bray–Curtis dissimilarities indicated that the microbiomes differed significantly across treatments suggesting that a single dosage drove the microbiome of experimental corals to develop in distinct directions (Fig. 1C).

Despite these recent encouraging outcomes from artificial microbial manipulations, important challenges for



**Fig. 1.** Examples of studies involving coral microbiome manipulation. A. Transplantation of *A. hyacinthus* fragments between regions of different thermal regimes induced a change in their microbiome (Ziegler *et al.*, 2017). After 17 months, corals that inhabited the highly variable, warmer environment (HV) harboured a microbiome that was distinct from corals located in the cooler and more stable environment (MV). When exposed to short-term heat stress, fragments from the HV environment bleached less, which could reflect a protective effect of their microbiome. B. Bacteria isolated from *M. hartii* were selected for their ability to degrade oil WSFs (dos Santos *et al.*, 2015). Replicate coral colonies were inoculated with the selected bacterial consortium, while others were not exposed to these bacteria (controls). After subjecting the colonies to a treatment simulating an oil spill, the presence of bacteria able to degrade oil WSFs helped to preserve better water quality in the oil treatment and reduced negative effects on coral health. C. *A. tenuis* larvae from a common pool were distributed across 20 experimental tanks. Filtered sea water or 5- $\mu$ m filtered mucus collected from four different coral species were then introduced into four replicate tanks per treatment. Water flow was turned off overnight, and recruits were subsequently reared in flow-through filtered sea water. After four months, recruits were sampled for 16S rRNA gene amplicon sequencing. PERMANOVA of the Bray–Curtis dissimilarities at the OTU level based on 97% sequence identity detected significant differences in the prokaryotic communities associated with recruits that were exposed to distinct inocula (pseudo  $F_{4,14} = 1.7015$ ,  $P < 0.01$ ).

broad-scale application in corals need to be addressed and overcome. First, the functions of the vast majority of coral-associated prokaryotes are yet to be deciphered. We are still at a stage of correlating the presence of specific microbes with coral features as exemplified by *Endozoicomonas* (frequently reported to be present on healthy corals) (Kvennefors *et al.*, 2010; Morrow *et al.*, 2012; Meyer *et al.*, 2014; Glasl *et al.*, 2016; Neave *et al.*, 2017), *Roseobacter* (particularly common in juvenile corals) (Aprill *et al.*, 2012; Ceh *et al.*, 2012, 2013; Sharp *et al.*, 2012; Lema *et al.*, 2014) and diazotrophs (nitrogen provisioning to corals) (Lema *et al.*, 2012, 2015; dos Santos *et al.*, 2014). A deeper knowledge of functions exerted by particular taxa will help designing optimal microbial inocula. Recently, ~200 distinct bacterial OTUs were able to be obtained in pure culture from the coral model *Exaiptasia pallida* using conventional methods (Röthig *et al.*, 2016). These pure cultures represented *E. pallida*'s 'key microbial associates', and given the functional redundancy among members of the coral microbiome (Bell *et al.*, 2005; Blackall *et al.*, 2015; Sunagawa, 2015), these cultures provide opportunities for formulating a bacterial cocktail to evaluate their benefit to the host.

Genetic engineering should also be considered as an avenue to generate coral microbial inocula that possess desired characteristics. Such technologies have been applied to a wide range of organisms including bacteria, plants and mammals to study gene function or enhance phenotypic traits. In marine microbes, genetic engineering has been successfully employed to express high-value bioactive compounds in the eukaryotic microalga, *Chlorella* (Yang *et al.*, 2016), and for aquatic bioremediation and source of fuel in cyanobacteria (Lau *et al.*, 2015). A framework has recently been proposed for creating transgenic *Symbiodinium*, which could ultimately lead to more stress-tolerant variants (Levin *et al.*, 2017). Likewise, coral-associated bacteria could be transformed with genes of interest to produce strains that enhance the performance of the host under climate change. Genomes could for instance be edited at specific sites with the CRISPR-Cas9 system (Hsu *et al.*, 2014) or with mini-Tn7 transposons (Lambertsen *et al.*, 2004). The latter approach has already been used to label *Vibrio coralliilyticus* in corals and visualize host-pathogen interactions (Pollock *et al.*, 2015). Developing genetically engineered symbionts could thus allow seeding vulnerable corals with organisms possessing proven beneficial properties.

Another challenge is the potential difficulty of manipulating microorganisms in open marine systems. While targeted microbiome transplants are performed in relatively closed systems by inoculating animal gut or soil, respectively, such precise interventions may prove less

effective in the marine environment where the inoculum would dilute in sea water. Aquarium rearing would overcome this limitation by ensuring that a sufficiently high density of microorganisms reach corals. Propagation techniques of coral fragments and sexually derived propagules have considerably progressed over the last two decades for the purpose of coral reef restoration (Barton *et al.*, 2015) and the *ex situ* rearing phase could theoretically be combined with microbiome inoculations.

A key uncertainty about the feasibility of manipulating microbes to enhance coral tolerance is whether the taxa in the inoculum will remain associated with the coral over time. The inherent variability of the microbiome (Escalante *et al.*, 2015) may limit the utility of microbiome manipulation for sustainable development. Engineered microbiomes that are acquired environmentally, such as via the rhizosphere in the case of plants, are likely to require continuous selection for retention of the beneficial properties that are conferred to the host. However, members of the core microbiome may be more stably associated with their coral host, and this is a knowledge gap that needs to be urgently addressed. The coral core microbiome comprises bacterial taxa consistently associated with given coral species at a global scale despite contrasting environments (Ainsworth *et al.*, 2015; Chu and Vollmer, 2016; Hernandez-Agreda *et al.*, 2016b). Such ubiquitous interactions across spatiotemporal boundaries suggest that these bacteria might perform critical functions for the host (reviewed in Hernandez-Agreda *et al.* (2016a,b)). It has been reported that some members of the core microbiome are endosymbionts, residing both within host tissue (Ainsworth *et al.*, 2006, 2015; Ainsworth and Hoegh-Guldberg, 2009) and *Symbiodinium* cells (Ainsworth *et al.*, 2015). We postulate that vertically inherited prokaryotes are more stably associated with the coral host compared to those that are horizontally acquired, as observed in other species such as the honeybee gut microbiome (Powell *et al.*, 2014; Mueller and Sachs, 2015). The existence of conserved and intimate relationships between corals and microbes could open new avenues of research, where these stable communities would be targeted for manipulation.

Despite the recognition that coral reefs are threatened by human activities, measures to reduce negative impacts are insufficient. The United Nations have put forward targets that ought to be reached in the coming decade to protect marine ecosystems and avoid further adverse impacts (United Nations 2017). These goals include the sustainable management of marine zones by reducing pollution and destructive fishing, as well as the transfer of scientific knowledge to improve ocean health and support developing countries that rely on

coral reefs. Intervention methods such as manipulation of the microbiome and genetic engineering have been successfully applied to terrestrial organisms to increase their tolerance to stress. Numerous lines of evidence suggest that a translation of these technologies to corals and their symbionts might effectively enhance coral resilience and contribute to the success of coral reef restoration efforts.

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### Conflict of interest

None declared.

### References

- Ainsworth, T.D., and Hoegh-Guldberg, O. (2009) Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. *Aquat Biol* **4**: 289–296.
- Ainsworth, T.D., Fine, M., Blackall, L.L., and Hoegh-Guldberg, O. (2006) Fluorescence in situ hybridization and spectral imaging of coral-associated bacterial communities. *Appl Environ Microbiol* **72**: 3016–3020.
- Ainsworth, T.D., Krause, L., Bridge, T., Torda, G., Raina, J.B., Zakrzewski, M., *et al.* (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J* **9**: 2261–2274.
- Apprill, A., Marlow, H.Q., Martindale, M.Q., and Rappe, M.S. (2012) Specificity of associations between bacteria and the coral *Pocillopora meandrina* during early development. *Appl Environ Microbiol* **78**: 7467–7475.
- Atad, I., Zvuloni, A., Loya, Y., and Rosenberg, E. (2012) Phage therapy of the white plague-like disease of *Favia fava* in the Red Sea. *Coral Reefs* **31**: 665–670.
- Barshis, D.J., Ladner, J.T., Oliver, T.A., Seneca, F.O., Traylor-Knowles, N., and Palumbi, S.R. (2013) Genomic basis for coral resilience to climate change. *PNAS* **110**: 1387–1392.
- Barton, J.A., Willis, B.L. and Hutson, K.S. (2015) Coral propagation: a review of techniques for ornamental trade and reef restoration. *Rev Aquacult* [In press] doi: 10.1111/raq.12135.
- Bay, R.A., and Palumbi, S.R. (2014) Multilocus adaptation associated with heat resistance in reef-building corals. *Curr Biol* **24**: 2952–2956.
- Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., and Lilley, A.K. (2005) The contribution of species richness and composition to bacterial services. *Nature* **436**: 1157–1160.
- Blackall, L.L., B., W. and van Oppen, M.J. (2015) Coral – The world's most diverse symbiotic ecosystem. *Mol Ecol* **24**: 5330–5347.
- Borody, T.J., and Khoruts, A. (2012) Fecal microbiota transplantation and emerging applications. *Nat Rev Gastroenterol Hepatol* **9**: 88–96.
- Bourne, D., Iida, Y., Uthicke, S., and Smith-Keune, C. (2008) Changes in coral-associated microbial communities during a bleaching event. *ISME J* **2**: 350–363.
- Bourne, D.G., Morrow, K.M., and Webster, N.S. (2016) Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* **70**: 317–340.
- Boutin, S., Audet, C., and Derome, N. (2013) Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of *Salvelinus fontinalis*. *Can J Microbiol* **59**: 662–670.
- Brown, B.E., and Bythell, J.C. (2005) Perspectives on mucus secretion in reef corals. *Mar Ecol Prog Ser* **296**: 291–309.
- Bruno, J.F., and Selig, E.R. (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS ONE* **2**: 1–8.
- Burke, L., Reyter, K. and Spaulding, M.P.A. (2011). *Reefs at Risk Revisited*. Washington: World Resources Institute.
- Ceh, J., Raina, J.B., Soo, R.M., van Keulen, M., and Bourne, D.G. (2012) Coral-bacterial communities before and after a coral mass spawning event on Ningaloo Reef. *PLoS ONE* **7**: e36920.
- Ceh, J., van Keulen, M., and Bourne, D.G. (2013) Intergenerational transfer of specific bacteria in corals and possible implications for offspring fitness. *Microb Ecol* **65**: 227–231.
- Chu, N.D. and Vollmer, S.V. (2016) Caribbean corals house shared and host-specific microbial symbionts over time and space. *Environ Microbiol Rep* **8**: 493–500.
- Cohen, Y., Joseph Pollock, F., Rosenberg, E., and Bourne, D.G. (2013) Phage therapy treatment of the coral pathogen *Vibrio coralliilyticus*. *Microbiologyopen* **2**: 64–74.
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., *et al.* (1997) The value of the world's ecosystem services and natural capital. *Nature* **387**: 253–260.
- Cresset, D. (2016) Coral crisis: Great Barrier Reef bleaching is “the worst we've ever seen. *Nat News* 1–2.
- De'ath, G., Fabricius, K.E., Sweatman, H. and Puotinen, M. (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci* **109**, 17995–17999.
- Escalante, A.E., Rebolledo-Gomez, M., Benitez, M., and Travisano, M. (2015) Ecological perspectives on synthetic biology: insights from microbial population biology. *Front Microbiol* **6**: 143.

- Frias-Lopez, J., Zerkle, A.L., Bonheyo, G.T., and Fouke, B.W. (2002) Partitioning of Bacterial Communities between Seawater and Healthy, Black Band Diseased, and Dead Coral Surfaces. *Appl Environ Microbiol* **68**: 2214–2228.
- Gil-Agudelo, D.L., Myers, C., Smith, G.W., and Kim, K. (2006) Changes in the microbial communities associated with *Gorgonia ventalina* during aspergillosis infection. *Dis Aquat Organ* **69**: 89–94.
- Glasl, B., Herndl, G.J. and Frade, P.R. (2016) The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *ISME J* **10**: 2280–2292.
- Glasl, B., Webster, N.S., and Bourne, D.G. (2017) Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. *Mar Biol* **164**: 1–18.
- Gupta, S., Allen-Vercoe, E., and Petrof, E.O. (2016) Fecal microbiota transplantation: in perspective. *Ther Adv Gastroenterol* **9**: 229–239.
- Hadaidi, G., Rothig, T., Yum, L.K., Ziegler, M., Arif, C., Roder, C., et al. (2017) Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* **7**: 45362.
- Harrison, P. L. and Booth, D. J. (2007). Coral reefs: naturally dynamic and increasingly disturbed ecosystems. In *Marine Ecology*. Connell, S. D., Gillanders, B. M. (eds.). Melbourne: Oxford University Press, pp. 316–377.
- Hernandez-Agreda, A., Gates, R.D. and Ainsworth, T.D. (2016a) Defining the core microbiome in corals' microbial soup. *Trends Microbiol* **25**: 125–140.
- Hernandez-Agreda, A., Leggat, W., Bongaerts, P., and Ainsworth, T.D. (2016b) The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *MBio* **7**: e00560–16.
- Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshwater Res* **50**: 839–866.
- Hoegh-Guldberg, O. (2004) Coral reefs in a century of rapid environmental change. *Symbiosis* **37**: 1–31.
- Hoegh-Guldberg, O. (2011). The Impact of Climate Change on Coral Reef Ecosystems. In *Coral Reefs: An Ecosystem in Transition*. Dubinsky, Z. and Stambler, N. (eds.). Netherlands: Springer, pp. 391–403.
- Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science* **318**: 1737–1742.
- Hsu, P.D., Lander, E.S., and Zhang, F. (2014) Development and applications of CRISPR-Cas9 for genome engineering. *Cell* **157**: 1262–1278.
- Hughes, T.P., Kerry, J.T., Alvarez-Noriega, M., Alvarez-Romero, J.G., Anderson, K.D., Baird, A.H., et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature* **543**: 373–377.
- Jones, R.J., Browyer, J., Hoegh-Guldberg, O., and Blackall, L.L. (2004) Dynamics of a temperature-related coral disease outbreak. *Mar Ecol Prog Ser* **281**: 63–77.
- Kimes, N.E., Van Nostrand, J.D., Weil, E., Zhou, J., and Morris, P.J. (2010) Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environ Microbiol* **12**: 541–556.
- Knowlton, N., and Rohwer, F. (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. *Am Nat* **162**: 51–62.
- Kvennefors, E.C.E., Sampayo, E., Ridgway, T., Barnes, A.C., and Hoegh-Guldberg, O. (2010) Bacterial Communities of Two Ubiquitous Great Barrier Reef Corals Reveals Both Site- and Species-Specificity of Common Bacterial Associates. *PLoS ONE* **5**: 1–14.
- Lambertsen, L., Sternberg, C., and Molin, S. (2004) Mini-Tn7 transposons for site-specific tagging of bacteria with fluorescent proteins. *Environ Microbiol* **6**: 726–732.
- Lau, N.S., Matsui, M., and Abdullah, A.A. (2015) Cyanobacteria: photoautotrophic microbial factories for the sustainable synthesis of industrial products. *Biomed Res Int* **2015**: 754934.
- Lema, K.A., Willis, B.L., and Bourne, D.G. (2012) Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Appl Environ Microbiol* **78**: 3136–3144.
- Lema, K.A., Bourne, D.G., and Willis, B.L. (2014) Onset and establishment of diazotrophs and other bacterial associates in the early life history stages of the coral *Acropora millepora*. *Mol Ecol* **23**: 4682–4695.
- Lema, K.A., Clode, P.L., Kilburn, M.R., Thornton, R., Willis, B.L. and Bourne, D.G. (2015) Imaging the uptake of nitrogen-fixing bacteria into larvae of the coral *Acropora millepora*. *ISME J* **10**: 1804–1808.
- Lesser, M.P. (2011). Coral Bleaching: Causes and Mechanisms. In *Coral Reefs: An Ecosystem in Transition*. Dubinsky, Z. and Stambler, N. (eds.). Netherlands: Springer, pp. 405–419.
- Lesser, M.P., Falcón, L.I., Rodríguez-Román, A., Enríquez, S., Hoegh-Guldberg, O., and Iglesias-Prieto, R. (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. *Mar Ecol Prog Ser* **346**: 143–152.
- Levin, R.A., Voolstra, C.R., Agrawal, S., Steinberg, P.D., Suggett, D. and van Oppen, M. (2017) Engineering strategies to decode and enhance coral symbiont biology. *Front Microbiol* [In press] doi: 10.3389/fmicb.2017.01220.
- Lins-de-Barros, M.M., Cardoso, A.M., Silveira, C.B., Lima, J.L., Clementino, M.M., Martins, O.B., et al. (2013) Microbial community compositional shifts in bleached colonies of the Brazilian reef-building coral *Siderastrea stellata*. *Microb Ecol* **65**: 205–213.
- Littman, R., Willis, B.L., and Bourne, D.G. (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environ Microbiol Rep* **3**: 651–660.
- Martinez Cruz, P., Ibanez, A.L., Monroy Hermosillo, O.A., and Ramirez Saad, H.C. (2012) Use of probiotics in aquaculture. *ISRN Microbiol* **2012**: 916845.
- Meyer, J.L., Paul, V.J., and Teplitski, M. (2014) Community shifts in the surface microbiomes of the coral *Porites astreoides* with unusual lesions. *PLoS ONE* **9**: e100316.
- Morrow, K.M., Moss, A.G., Chadwick, N.E., and Liles, M.R. (2012) Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Appl Environ Microbiol* **78**: 6438–6449.

- Mueller, U.G., and Sachs, J.L. (2015) Engineering microbiomes to improve plant and animal health. *Trends Microbiol* **23**: 606–617.
- Neave, M.J., Michell, C.T., Apprill, A., and Voolstra, C.R. (2017) Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Sci Rep* **7**: 40579.
- Nissimov, J., Rosenberg, E., and Munn, C.B. (2009) Antimicrobial properties of resident coral mucus bacteria of *Oculina patagonica*. *FEMS Microbiol Lett* **292**: 210–215.
- NOAA Coral Reef Watch. (2017). "Global coral bleaching 2014-2017: Status and an appeal for observations." URL [https://coralreefwatch.noaa.gov/satellite/analyses\\_guidance/global\\_coral\\_bleaching\\_2014-17\\_status.php](https://coralreefwatch.noaa.gov/satellite/analyses_guidance/global_coral_bleaching_2014-17_status.php). Accessed 01 May 2017
- Normile, D. (2016). "Massive bleaching killed 35% of the coral on the northern end of the Great Barrier Reef." URL <http://www.sciencemag.org/news/2016/05/massive-bleaching-killed-35-coral-northern-end-great-barrier-reef>. Accessed 01 May 2017
- van Oppen, M.J.H., Oliver, J.K., Putnam, H.M. and Gates, R.D. (2015) Building coral reef resilience through assisted evolution. *Proc Natl Acad Sci* **112**: 2307–2313.
- van Oppen, M.J., Gates, R.D., Blackall, L.L., Cantin, N., Chakravarti, L.J., Chan, W.Y., *et al.* (2017) Shifting paradigms in restoration of the world's coral reefs. *Glob Chang Biol* [In press] DOI 10.1111/gcb.13647.
- Pollock, F.J., Krediet, C.J., Garren, M., Stocker, R., Winn, K., Wilson, B., *et al.* (2015) Visualization of coral host-pathogen interactions using a stable GFP-labeled *Vibrio coralliilyticus* strain. *Coral Reefs* **34**: 655–662.
- Powell, J.E., Martinson, V.G., Urban-Mead, K., and Moran, N.A. (2014) Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl Environ Microbiol* **80**: 7378–7387.
- Raina, J.B., Tapiolas, D., Willis, B.L., and Bourne, D.G. (2009) Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Appl Environ Microbiol* **75**: 3492–3501.
- Redman, R.S., Kim, Y.O., Woodward, C.J.D.A., Greer, C., Espino, L., Doty, L.S., and Rodriguez, R.J. (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. *PLoS ONE* **6**: 1–10.
- Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., and Rosenberg, E. (2006) The coral probiotic hypothesis. *Environ Microbiol* **8**: 2068–2073.
- Ritchie, K.B. (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* **322**: 1–14.
- Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N. (2002) Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.
- Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* **5**: 355–362.
- Röthig, T., Costa, R.M., Simona, F., Baumgarten, S., Torres, A.F., Radhakrishnan, A., *et al.* (2016) Distinct bacterial communities associated with the coral model *Aiptasia* in aposymbiotic and symbiotic states with *Symbiodinium*. *Front. Mar. Sci.* **3**: 1–12.
- dos Santos, H.F., Carmo, F.L., Duarte, G., Dini-Andreote, F., Castro, C.B., Rosado, A.S., *et al.* (2014) Climate change affects key nitrogen-fixing bacterial populations on coral reefs. *ISME J* **8**: 2272–2279.
- dos Santos, H.F., Duarte, G.A., Rachid, C.T., Chaloub, R.M., Calderon, E.N., Marangoni, L.F., *et al.* (2015) Impact of oil spills on coral reefs can be reduced by bioremediation using probiotic microbiota. *Sci Rep* **5**, 18268.
- Sato, Y., Willis, B.L. and Bourne, D.G. (2013) Pyrosequencing-based profiling of archaeal and bacterial 16S rRNA genes identifies a novel archaeon associated with black band disease in corals. *Environ Microbiol* **15**: 2994–3007.
- Sharp, K.H., Distel, D., and Paul, V.J. (2012) Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*. *ISME J* **6**: 790–801.
- Shnit-Orland, M., and Kushmaro, A. (2009) Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiol Ecol* **67**: 371–380.
- Sunagawa, S. (2015) Structure and function of the global ocean microbiome. *Science* **348**, 1261359. 1261351-1261359-1261359.
- Sweet, M.J. and Bulling, M.T. (2017) On the importance of the microbiome and pathobiome in coral health and disease. *Front Mar Sci* **4**: 1–11.
- Sweet, M.J., Croquer, A., and Bythell, J.C. (2010) Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* **30**: 39–52.
- Teplitski, M., Krediet, C.J., Meyer, J.L. and Ritchie, K.B. (2016) Microbial interactions on coral surfaces and within the coral holobiont. In *The Cnidaria, Past, Present and Future*. Goffredo, S. and Dubinsky, Z. (Eds.). Springer International Publishing: Switzerland, pp. 331–346.
- Thompson, J.R., Rivera, H.E., Closek, C.J., and Medina, M. (2014) Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Front Cell Infect Microbiol* **4**: 176.
- Tout, J., Siboni, N., Messer, L.F., Garren, M., Stocker, R., Webster, N.S., *et al.* (2015) Increased seawater temperature increases the abundance and alters the structure of natural *Vibrio* populations associated with the coral *Pocillopora damicornis*. *Front Microbiol* **6**: 432.
- United Nations. (2017). "Sustainable Development Goals." URL <https://sustainabledevelopment.un.org/sdgs>. Accessed 15 May 2017.
- Verschuere, L., Rombaut, G., Sorgeloos, P., and Verstraete, W. (2000) Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol Rev* **64**: 655–671.
- Webster, N.S. and Reusch, T. (2017) Microbial contributions to the persistence of coral reefs. *ISME J* [Epub ahead of print] doi: 10.1038/ismej.2017.66.
- Webster, N.S., Negri, A.P., Botté, E.S., Laffy, P.W., Flores, F., Noonan, S., *et al.* (2016) Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. *Sci Rep* **6**: 19324.
- Yang, B., Liu, J., Jiang, Y., and Chen, F. (2016) *Chlorella* species as hosts for genetic engineering and expression

of heterologous proteins: progress, challenge and perspective. *Biotechnol J* **11**: 1244–1261.

- Zaneveld, J.R., Burkepile, D.E., Shantz, A.A., Pritchard, C.E., McMinds, R., Payet, J.P., *et al.* (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat Commun* **7**: 11833.
- Zhang, Y., Ling, J., Yang, Q., Wen, C., Yan, Q., Sun, H., *et al.* (2015) The functional gene composition and metabolic potential of coral-associated microbial communities. *Sci Rep* **5**: 16191.
- Zhou, G., Yuan, T., Cai, L., Zhang, W., Tian, R., Tong, H., *et al.* (2016) Changes in microbial communities, photosynthesis and calcification of the coral *Acropora gemmifera* in response to ocean acidification. *Sci Rep* **6**: 1–9.
- Ziegler, M., Seneca, F.O., Yum, L.K., Palumbi, S.R., and Voolstra, C.R. (2017) Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat Commun* **8**: 14213.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Relative abundance of phyla present in water (left) and coral (right) samples.

**Fig. S2.** PCoA plot of the Bray-Curtis dissimilarities calculated at the phylum level depicts a striking difference between microbial communities present in coral and water samples.

**Fig. S3.** Box plots indicating beta diversity values in coral and water samples within each of the five inoculation treatments.

**Data S1.** Methods and results.

**Data S2.** Read counts for each sample at the genus level.

**Data S3.** Read counts for all samples at the phylum level.