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# Erect macroalgae influence epilithic bacterial assemblages and reduce coral recruitment

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ABSTRACT: Macroalgal competition can indirectly influence the health of corals and their response to changing environmental conditions by altering their associated bacterial community. However, the effect of macroalgae on the composition of epilithic microbial biofilms, an important determinant of coral recruitment, is poorly known. In the back-reefs of Moorea (French Polynesia), we evaluated how the experimental removal of either the canopy of the seaweed *Turbinaria ornata* or that of the entire macroalgal assemblage influenced the composition of the bacterial biofilm and coral recruitment on macroalga-free substrata. The number of bacterial colonies on culture plates inoculated with dilutions of 9 d old biofilm from canopy removal sites was smaller compared with control sites. After 3.5 mo, the diversity of bacterial operational taxonomic units (OTUs) was lower at both canopy and total macroalgal removal sites. Total macroalgal removal sites had a lower relative abundance of several bacterial families, including *Rhodobacteraceae*, *Erythrobacteraceae*, *Cyanobacteria* Family IV and Family VIII, *Flavobacteriaceae* and *Verrucomicrobiaceae*. After 8 mo, coral recruitment was generally low, but greater at total macroalgal removal sites. The relative abundance of *Cyanobacteria*, *Sphingobacteria* and *Verrucomicrobia* was negatively correlated with coral recruitment and explained ~70% of variation in coral recruit density. Our study shows that the removal of *T. ornata* and understory macroalgae influences the composition of epilithic bacterial assemblages and coral recruitment. Thus, eradication campaigns are unlikely to sustain long-term reductions in the abundance of *T. ornata* and, hence, increase coral recruitment, when plant holdfasts and understory macroalgae are left in place.

KEY WORDS: Coral reefs · Macroalgae · *Turbinaria ornata* · Epilithic bacterial biofilms · Coral recruitment · French Polynesia

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## INTRODUCTION

Shifts in dominance between corals and macroalgae have been documented on tropical reefs worldwide (Pandolfi et al. 2003, Hughes et al. 2007, 2017). Free space made available by coral mass mortalities due to extreme climatic events (e.g. heatwaves and cyclones), disease or predator outbreaks can be readily exploited by opportunistic macroalgae. Although coral recovery can take decades, the shift from coral

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to macroalgal dominance caused by acute perturbations can be transitory and reversible when biotic and abiotic conditions are not altered by human activities (Dudgeon et al. 2010, Graham et al. 2015). Coral resilience to disturbance can, however, be impaired by chronic alterations of environmental (e.g. temperature, acidification), top-down (e.g. reductions of herbivore pressure due to over-exploitation or disease) and bottom-up (e.g. enhanced nutrient loading) drivers that regulate the interaction between corals and macroalgae (Burkepile & Hay 2006, Vermeij et al. 2010, Gil et al. 2016). Thus, human alteration of biotic and abiotic conditions can sustain the ability of macroalgae to monopolize space through the modification of extant feedback mechanisms or through the generation of novel ones, locking the system into an undesired, less productive state.

Macroalgae can reduce coral abundance or recovery through allelopathy, abrasion, shading, overgrowth and the transfer of algal-associated microbes to corals (Nugues et al. 2004, Rasher & Hay 2010, Barott et al. 2012, Barott & Rohwer 2012, Vega Thurber et al. 2012). Bacteria play a key role in regulating coral health and response to changing environmental conditions, as well as their interactions with competitors, including macroalgae (Rosenberg et al. 2007, Barott & Rohwer 2012). For instance, contact with macroalgae, which act as a reservoir of pathogens (Sweet et al. 2013), can induce the onset of virulent diseases in corals (Nugues et al. 2004, Bender et al. 2012). However, direct contact might not be necessary for macroalgae to induce coral mortality or morbidity (Smith et al. 2006, Barott & Rohwer 2012, Jorissen et al. 2016). Exudation of labile dissolved organic carbon (DOC) by macroalgae can stimulate bacterial respiration both in the planktonic community (Wild et al. 2010, Haas et al. 2011) and at coral–macroalgal interfaces, ultimately reducing local $O_2$ levels (Smith et al. 2006). Likewise, free-living microbes, exosomes and allelochemicals could be released by macroalgae into the diffusive boundary layer (DBL) and transferred to neighboring corals (Barott & Rohwer 2012, Jorissen et al. 2016, Morrow et al. 2017).

Bacterial biofilms are common natural inducers of coral larval settlement (Webster et al. 2004, Tran & Hadfield 2011, Sneed et al. 2014). However, the effect of macroalgae on the microbial community of epilithic biofilms is poorly known (Vermeij et al. 2010). For example, variations in coral settlement on the surface of different species of crustose coralline algae (CCA) have been associated with variations in the bacterial community composition present on their surface (Sneed et al. 2015). Likewise, bacteria isolated from different reef surfaces have different abilities to induce coral settlement (Tran & Hadfield 2011). However, to the best of our knowledge, no field study has experimentally assessed how macroalgal canopies influence the bacterial community on natural surfaces suitable for coral recruitment, such as CCA or dead coral skeletons.

In the last 2 decades, the seaweed *Turbinaria ornata* has progressively expanded its distribution in French Polynesia (Stiger & Payri 1999). *T. ornata* reproduces sexually all year and is characterized by a high morphological plasticity (Stiger & Payri 1999, Stewart 2008). Detached thalli, either as a consequence of natural senescence or dislodgment by hydrodynamic forces, are buoyant due to the presence of airbladders (Stewart 2008) and can form large drifting rafts (see Fig. 1a). Drifting thalli can be reproductive and are likely to underpin the high dispersal potential of this species (Stewart 2008). In addition, *T. ornata* displays both mechanical (spines, thallus toughness) and chemical (phenolic compounds) defenses against herbivores (Stewart 2008) and is a major competitor of corals and an indicator of degraded reefs (Bittick et al. 2010, Bulleri et al. 2013). This species exudes large amounts of DOC (Haas et al. 2011) and could thus be expected to influence bacterial communities on reef surfaces suitable for coral settlement. Thalli of *T. ornata* can be dislodged naturally, through the breakage of senescent stipes by hydrodynamic forces (Stewart 2008), or they can be removed through human intervention within eradication programs. In Moorea, the progressive spreading of *T. ornata* recently prompted eradication attempts by voluntary associations of citizens (Pa'e Pa'e no te Ora; Te Mana O Te Moana; Tamari'i de la Pointe des Pêcheurs) and secondary school students (Lycée agricole de Opunohu). In these cases, the removal of thalli generally leaves the holdfasts, as well as the understory macroalgal assemblage, intact (Bittick et al. 2010). By contrast, intense grazing, such as that by the sea urchin *Diadema savignyi* (Han 2016) is likely to generate areas completely devoid of erect macroalgae. These 2 intensities of macroalgal removal are expected to have different direct and indirect effects on the epilithic bacterial biofilm and on coral recruitment.

Here, by means of a field manipulative experiment, we investigated how 2 different intensities of macroalgal removal, i.e. simulating pulse events of disturbance, due to either sea-storms, grazing or human intervention, influence the bacterial community of epilithic biofilms and coral recruitment. We hypothesized that total macroalgal removal (*T. ornata* canopy and holdfasts plus understory macroalgae) would pro-

duce more drastic changes in the composition of epilithic bacterial biofilms and would have greater positive effects on coral recruitment than the removal of macroalgal canopies alone (i.e. leaving holdfasts intact and understory macroalgal assemblage untouched).

## MATERIALS AND METHODS

### Study site

This study was carried out in the lagoon of Taareu (17° 29′ 15.21″ S, 149° 51′ 21.25″ W), on the north coast of Moorea, French Polynesia, from November 2014 to July 2015. Between 1982 and 2016, reefs on this island were exposed to severe disturbances, including hurricanes, outbreaks of the predatory seastar *Acanthaster planci* and coral bleaching (Adjeroud et al. 2009, Kayal et al. 2012, Leray et al. 2012, Lamy et al. 2015, 2016, Beldade et al. 2017).

In Moorea's back-reefs, *Turbinaria ornata* forms patches extending from 10s of $cm^2$ to several $m^2$, with a density varying between 10s and several 100s of plants $m^{-2}$ (Stiger & Payri 1999, Bulleri et al. 2013; Fig. 1b). The seabed is characterized by a mosaic of substrates, including patches of sand, coral rubble and rock, with scattered coral colonies ranging from a few mm to 4 m in size (Lenihan et al. 2011). The largest massive coral colonies, also referred to as 'bommies', belong to the genus *Porites* and provide habitat for branching corals, fish, invertebrates and macroalgae (Lenihan et al. 2011). Assemblages on the top of bommies are often dominated by lush *T. ornata* (Fig. 1c) and, to a lesser extent, *Sargassum* sp. canopies. These bommies represent an ideal system for testing the effects of macroalgae on the epilithic biofilm and coral recruitment since they are isolated one from another, minimizing the potential non-independence of treatment outcomes.

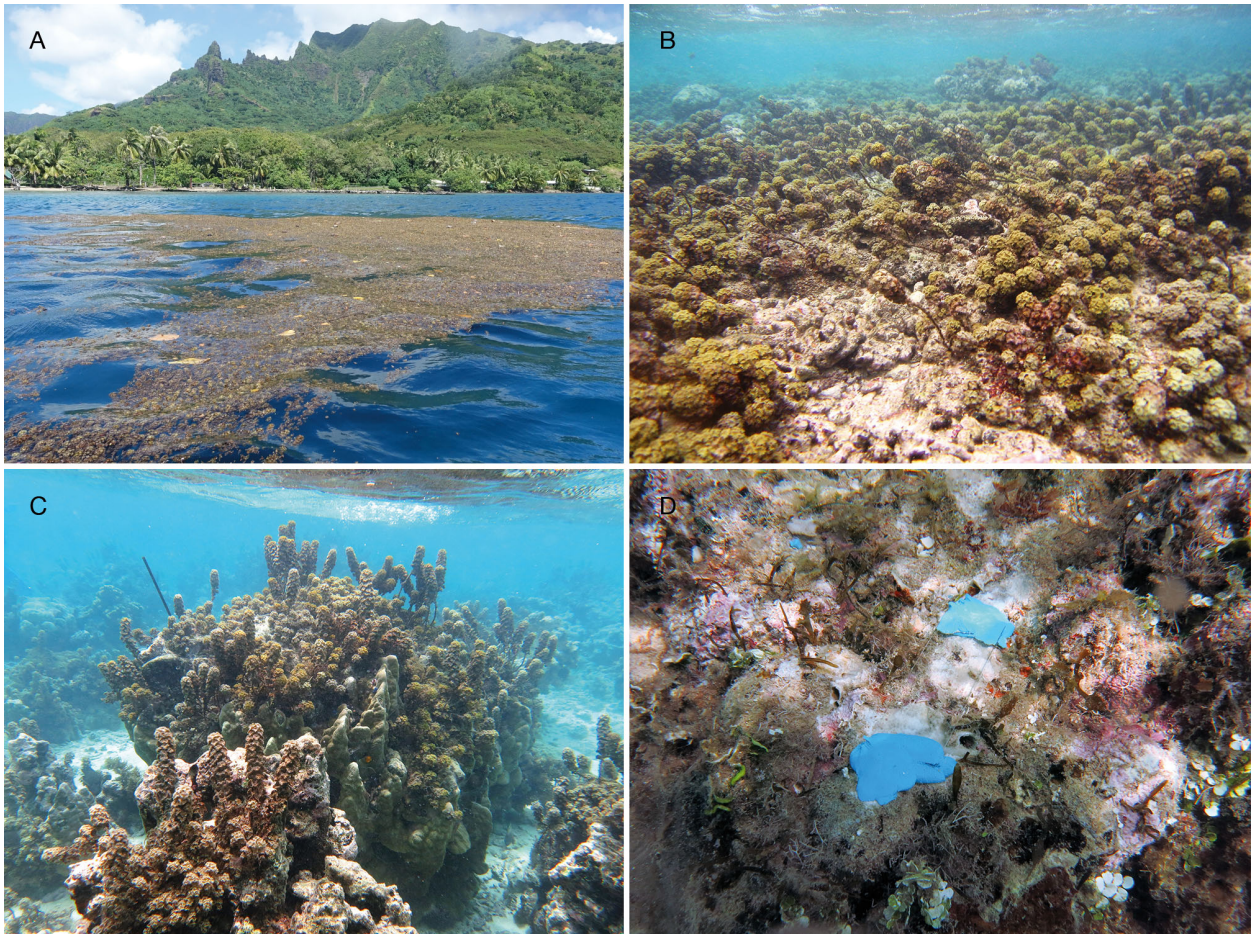


Fig. 1. Photographs of (a) drifting mass formed by floating thalli of *Turbinaria ornata*; (b) stands of *T. ornata* in the back-reef areas on the north coast of Moorea; (c) coral bommies in the lagoon of Taareu colonized by *T. ornata*; (d) glass slide fixed onto a *T. ornata* removal bommie for colonization by the bacterial biofilm

### Experimental setup

In early November 2014, 15 coral bommies, with a diameter between 2 and 3 m and colonized by *T. ornata*, were randomly selected within an area of about 2500 $m^2$. The top of the bommies (see Fig. 1c) had a cover of *T. ornata* varying between 50 and 90% (visually estimated within each central 1 × 1 $m^2$: mean ± SE = 69.7 ± 3.7; n = 15). Five bommies were randomly assigned to each of the following treatments: (1) total removal of erect macroalgae, including the canopy-forming holdfasts and the understory species (hereafter referred to as total macroalgal removal, TMR), (2) removal of stipes and fronds of canopy-forming macroalgae (*T. ornata* and *Sargassum* sp.), leaving the holdfasts and the understory macroalgal community untouched (hereafter referred to as macroalgal canopy removal, MCR) and (3) no manipulation of macroalgal assemblages (control, CTRL). Thalli of canopy-forming species were removed from the substratum manually, whilst in the total removal, holdfasts and erect macroalgae were removed using a paint scraper. Attention was paid not to damage underlying encrusting corallines or to alter the substrate topography through the generation of cracks. After 5 mo, canopy covers were quantified on each bommie by taking pictures of 4 randomly selected, 24 × 18 cm plots. On a PC screen, a grid of 25 sub-quadrats was superimposed onto each image, a score from 0 to 4 was given to each sub-quadrat according to the relative macroalgal cover (0 = absent; 1 = occupation of 1/4 of the space; 2 = occupation of 2/4 of the space; 3 = occupation of 3/4 of the space; 4 = total occupation of the space) and the total percentage cover was obtained by summing over the entire set of sub-quadrats (Dethier et al. 1993). Mean (±SE) percentage canopy covers were 87.33 ± 4.33, 53.06 ± 5.95 and 10.15 ± 2.80 in CTRL, MCR and TMR, respectively, suggesting that the effects of stipe and frond removal due to an intense storm are less long-lasting than those of a heavy grazing event.

### Coral recruit survey

Coral recruitment in Moorea peaks between December and March (Adjeroud et al. 2007). Coral recruits (colonies ≤ 1 cm in diameter) on experimental bommies were sampled in early July 2015 (8 mo into the start of the experiment) in order to encompass the 2014–2015 annual recruitment event and to allow established colonies to reach a minimum size for detection in the field. Artificial substrates are widely used to assess coral recruitment rates as they allow standardization of settling surfaces and identification of recruits to species or genus. However, recruitment rates on tiles are not always indicative of rates on natural surfaces (Edmunds et al. 2004). For instance, larvae often settle around the edges and underneath surfaces of tiles, potentially resulting in an overestimation of recruitment rates on natural open surfaces (Adjeroud et al. 2007). Thus, we investigated coral recruitment on natural surfaces.

Coral colonies were counted by means of nighttime UV census (Piniak et al. 2005, Baird et al. 2006). The use of UV filters at night is a valid technique for the early sampling of coral recruits in the field, allowing the detection of recruits as small as 1 mm, especially in regions, such as the Indo-Pacific, where fluorescent taxa are dominant (Baird et al. 2006). On the other hand, juvenile colonies cannot be identified to a high taxonomic resolution with this technique (Piniak et al. 2005, Baird et al. 2006). For this reason, we did not attempt to distinguish among different taxa, but simply quantified the total number of coral recruits. On the top half of each bommie, coral recruits were counted in three 0.28 $m^2$ plots (circles with a 60 cm diameter) haphazardly selected, at least 0.5 m apart from one another on surfaces devoid of erect algae, in order to minimize biases due to differences in the extent of surfaces without canopies among treatments. Counts were performed by 2 operators on 2 consecutive nights using an Ikelite DS161 torch equipped with a Nightsea barrier filter. Before proceeding with sampling, the operators went through a series of trials to standardize the application of the technique and to minimize false positives between them (Piniak et al. 2005).

### Bacterial community survey

On each bommie, 6 glass slides were haphazardly positioned about 0.5 m apart from one another within areas occupied by either encrusting corallines or dead coral skeletons, and fixed by means of epoxy putty (Veneziani S Subcoat) at the start of the experiment (Fig. 1d). Glass microscope slides have been shown to develop a biofilm highly similar to that on coral skeletons (Witt et al. 2011). In order to promote biofilm formation, slides were roughened using sandpaper before deployment. A total of 3 slides per bommie were retrieved after 9 d to investigate how experimental treatments influenced the early establishment of a bacterial biofilm. Remaining slides on experimental bommies were retrieved in February

2015 (3.5 mo after deployment) in order to assess the composition of the epilithic bacterial community at a time when coral larvae are more likely to be available (Adjeroud et al. 2007).

Due to financial constraints, analysis of biofilm community composition using DNA extraction and sequencing was restricted to 3.5 mo old biofilm, whilst 9 d old biofilm was cultured for aerobic bacterial growth. Slides were immediately transported to the lab in separate plastic containers filled with 0.2 µm filtered seawater. After rinsing with sterile seawater, the biofilm that had formed on the upper surface in 9 d was sampled from the central 3 × 2 cm area with a sterile cotton swab that was inserted into a polypropylene tube with 10 ml of sterile seawater. After 1 min shaking, a 100 µl sample aliquot was plated on aerobic count plates (3M Petrifilm™ ACP) for the total count of aerobic bacteria. Petrifilm ACP represents a suitable alternative to marine agar for counting marine microorganisms (Kudaka et al. 2010). Plates were stored at a constant temperature of 30°C and bacterial colonies were counted 48 h after inoculation.

Once in the lab, slides collected 3.5 mo after deployment were rinsed with sterile seawater and stored at −20°C. The biofilm was scraped off from the central 3 × 2 cm area with a sterile lancet. DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen), and its quality and quantity in the obtained solution was checked using a NanoDrop 2000C. PCRs for sequencing library preparation and high throughput sequencing (HTS) of the 16S rDNA via a 300 bp paired-end Illumina sequencing approach on a MiSeq platform V3 were conducted at BMR genomics (Padua, Italy). The hypervariable regions V3–V4 were chosen as the target for prokaryotic identification using the universal bacterial primer pair S-D-Bact-0341-b-S-17 (5′-CCT ACG GGN GGC WGC AG-3′) /S-D-Bact-0785-a-A-21 (5′-GAC TAC HVG GGT ATC TAA TCC-3′) (Herlemann et al. 2011). FastQC v.0.11.4 was used to evaluate sequence quality and identify index and adaptor sequences (Babraham Bioinformatics; Andrews 2010) which were removed using cutadapt v.1.9.1 (Martin 2011). Raw sequencing data are publicly accessible at NCBI Sequence Read Archive (SRA) with accession number SRP103680. Paired-end reads were merged and quality trimmed, and clustered into operational taxonomic units (OTUs) using UParse and USearch, respectively (Edgar 2010, 2013). The pipeline used was as proposed in the pipeline's online tutorial (http://drive5.com/usearch/manual/uparse_pipeline.html) with the following modifications or sequence related particularities: while merging, the max-diff parameter was set to 8, sequences were filtered to a min. and max. length of 344 and 366 respectively, with a max. error of 0.5 and clustered at 97% sequence identity for the generation of the OTU table. Taxonomic groups were assigned to the OTUs using the Greengenes database v.13.5 (DeSantis et al. 2006). Before data analysis, we rarefied to the read number of the sample with the lowest counts (namely 7700) using the package 'GUnifrac' v.1.0 (Chen et al. 2012) in R (R Development Core Team 2016). OTUs with the taxonomic affiliation 'chloroplast', 'mitochondria' or 'archaea' were removed, since the primers used were not designed to target them (Klindworth et al. 2013).

### Data analysis

The number of bacterial colonies on plates (i.e. from 9 d old biofilm) was analyzed using a linear mixed model, including treatment as a fixed effect and bommie as a random effect, using the function 'lmer' in the R package 'lme4' (Bates et al. 2015). An ANOVA method was used to calculate *F*-values for the fixed-effect term (Bates et al. 2015) and the function 'lsmeans' in the R package 'lsmeans' (Lenth 2016) was used for post hoc comparisons among treatment levels.

After 3.5 mo, 5 glass slides could not be recovered from the field; in addition, DNA readings could not be obtained from 3 slides, resulting in a total of 37 samples: 12 controls, 13 canopy removals and 12 total removals. Variation among treatments in the bacterial community was assessed by means of permutational analysis of variance (PERMANOVA; Anderson 2001), a technique that allows handling unbalanced designs. The analysis was performed on Bray-Curtis similarity coefficients calculated using untransformed data and included the factors treatment (fixed) and bommie (random and nested within treatment). Pair-wise *t*-tests were used for *a posteriori* ranking of the means. Non-metric multidimensional scaling (nMDS) was used to visualize multivariate patterns. The same design was used to analyze data at the levels of OTU and family. Since these yielded identical results (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m597p065_supp.pdf), we only refer to family level in the following sections. The percentage contributions of each family to dissimilarity patterns were calculated using SIMPER. Families contributing at least 2% of dissimilarity for any pair-wise comparison were considered important differentiators.

Variation among treatments in the number of bacterial OTUs and families and in the abundance of each of the bacterial families was assessed by means of the linear mixed model previously described. Assumptions of linearity and homogeneity of variance were checked by means of residual plots and, when necessary, data were log transformed.

In order to take into account potential non-independence in the data and avoid bias introduced by a high number of zeros, coral recruitment was analyzed by means of a zero-inflated negative binomial generalized linear mixed model (GLMM) (Zuur et al. 2010), using the 'glmmadmb' function in the R package 'glmmADMB' (Bolker et al. 2012). The factor treatment (CTRL versus MCR versus TMR) was included in the model as a fixed effect and bommie as the grouping factor.

The relationship between coral recruitment and the composition of the bacterial community was assessed using multiple regression. In order to reduce the number of covariates in the analysis, the density of coral larvae was regressed against the relative abundance of bacterial orders most differentiating experimental treatments (SIMPER analysis). More specifically, values of covariates measured 3.5 mo after the start of the experiment were used as predictors of coral recruitment after 8 mo. Collinearity among covariates was assessed using variance inflation factor (VIF) procedures. Covariates with the highest VIF values, calculated using the R package 'car' (Fox & Weisberg 2011), were sequentially dropped from the model, until all VIF values were smaller than 5 (Zuur et al. 2010). The best-fit model was identified through a step-wise procedure (forward and backward) by Akaike's information criterion (AIC), using the 'stepAIC' function from the R package 'MASS' (Venables & Ripley 2002). Linearity and homogeneity of variances were visually checked by means of residual plots. The relative importance (as a percentage) and bootstrap confidence intervals of the explanatory variables retained in the best-fit models were assessed using the Lindemann-Merenda-Gold (lmg) method for calculating sequentially weighted partial $R^2$, using the R package 'relaimpo' (Gromping 2006). This method calculates an average coefficient of partial determination for each model permutation using the individual contribution of each explanatory variable.

## RESULTS

After 9 d, a significantly smaller number of bacterial colonies were recovered from the MCR than from the CTRL biofilm (Table 1, Fig. 2a). Although differences were not significant, the number of bacterial colonies from the TMR biofilm was smaller than that from the CTRL biofilm (Fig. 2a).

After 3.5 mo, significantly fewer bacterial OTUs and lower family richness were found in the epilithic biofilm from both MCR and TMR bommies compared with that from CTRL (Table 1, Fig. 2b,c).

The PERMANOVA using Bray-Curtis dissimilarities calculated on untransformed data indicated that the bacterial community differed significantly among the 3 macroalgal treatments (Table 2). The nMDS suggested that, although significantly different, the bacterial communities from MCR and TMR were more similar to each other than to CTRL (Fig. 3). In addition, the dissimilarity of bacterial communities between TMR and CTRL was greater than that between MCR and CTRL. Also, the PERMANOVA on presence–absence-transformed data showed significant effects of the macroalgal treatment (see Table S2 in the Supplement); however, there were significant differences between CTRL and either MCR and TMR, but not between MCR and TMR (pair-wise tests; Table S2).

In total, 18 bacterial families contributed most to differences in the bacterial community among treatments (SIMPER analysis; Table S3 in the Supplement). Of these, 9 families differed in relative abundance among treatments (Fig. 4, Table S4 in the Supplement). Both MCR and TMR caused a significant decrease in the relative abundance of *Rhodobacteraceae*, *Cyanobacteria* Family IV and Family VIII, and *Verrucomicrobiaceae* (Fig. 4). The decrease of *Cyanobacteria* Family VIII was greater in TMR than MCR. Only the TMR caused a significant decrease in the relative abundance of *Flavobacteri-*

Table 1. Linear mixed model assessing the effects of treatment (control versus macroalgal canopy removal versus total macroalgal removal) for number of bacterial colonies from glass slides incubated in the field for 9 d, number of bacterial OTUs and number of bacterial families from glass slides incubated in the field for 3.5 mo. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

| Source of variation | Number of bacterial colonies | | Number of OTUs | | Number of families | |
|---|---|---|---|---|---|---|
| | MS | *F* | MS | *F* | MS | *F* |
| Treatment | 1.072 | 7.045** | 12807663 | 9.420*** | 2917.9 | 11.171*** |

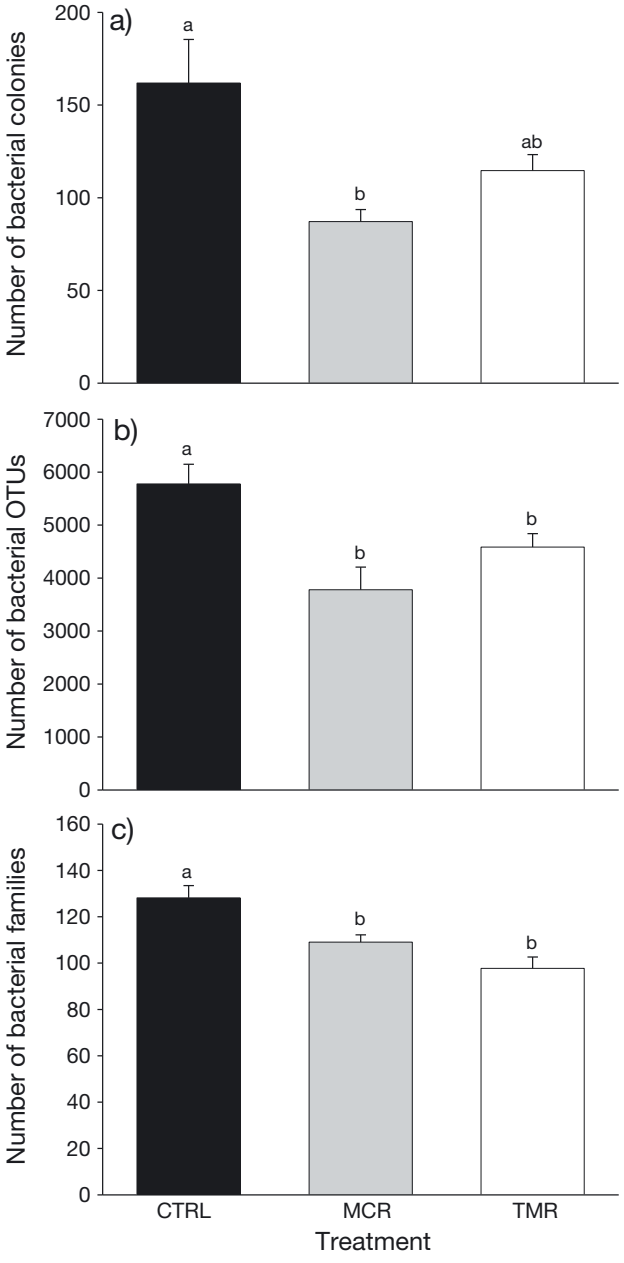


Fig. 2. Mean (±SE) (a) number of bacterial colonies in the different treatments from 9 d field-incubated glass slides; n = 15; (b) number of bacterial operational taxonomic units (OTUs) and (c) number of bacterial families in different treatments from 3.5 mo field-incubated glass slides; n = 12 for control (CTRL) and total macroalgal removal (TMR); n = 13 for macroalgal canopy removal (MCR). Different letters above bars indicate significant differences from post hoc tests

*aceae* and *Erythrobacteraceae* in comparison with CTRL (Fig. 4). In contrast, TMR caused a significant increase in the relative abundance of *Kilionellaceae*. Finally, MCR caused a significant increase in the relative abundance of *Cyanobacteria* Family I, in comparison with CTRL (Fig. 4).

Table 2. PERMANOVA on Bray-Curtis similarity coefficients calculated using untransformed data assessing the effects of treatment (3 levels: control [CTRL], macroalgal canopy removal [MCR], total macroalgal removal [TMR]; fixed) and bommie (random and nested within treatment) on the structure of the bacterial community (family level), 3.5 mo after the start of the experiment

| Sources of variation | df | MS | Pseudo-*F* | p |
|---|---|---|---|---|
| Treatment | 2 | 2987.2 | 3.69 | 0.001 |
| Bommie (treatment) | 12 | 818.8 | 1.21 | 0.118 |
| Residual | 22 | 675.9 | | |
| Pairwise tests | | *t* | p | |
| CTRL – TMR | | 2.010 | 0.015 | |
| CTRL – MCR | | 2.026 | 0.004 | |
| TMR – MCR | | 1.632 | 0.047 | |

After 8 mo, only 54 coral recruits (colony diameter ≤ 1 cm) were found on experimental bommies. Of these, 7 recruits were observed on both CTRL and MCR bommies, while 40 recruits were sampled on TMR bommies. Despite these small numbers, coral recruit density was significantly greater on TMR bommies compared with those assigned to the other treatments (Table 3, Fig. 5).

The best-fit regression model retained 3 bacterial classes, namely *Cyanobacteria*, *Sphingobacteria* and *Verrucomicrobiae*, and explained 70.3% of the variability in coral recruit density (Table 4). The relationship between the density of coral recruits and the relative abundance of each of the 3 bacterial classes was negative. *Cyanobacteria* accounted for a

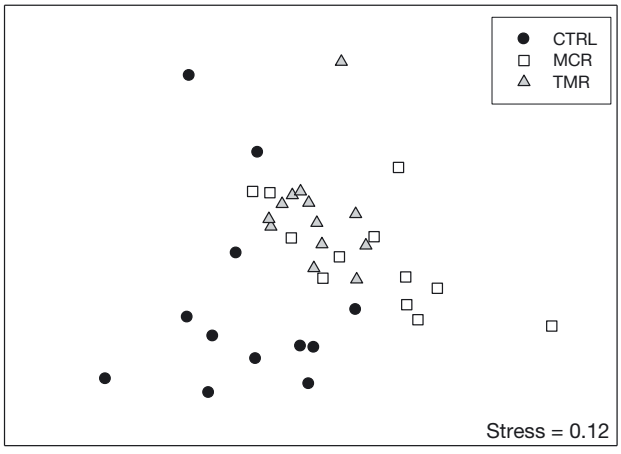


Fig. 3. Two-dimensional non-metric multidimensional scaling (nMDS) ordination on untransformed data, comparing the bacterial community among treatments 3.5 mo after the start of the experiment. Each point represents 1 glass slide. Black-filled circles: control (CTRL); grey-filled triangles: macroalgal canopy removal (MCR); white-filled squares: total macroalgal removal (TMR)

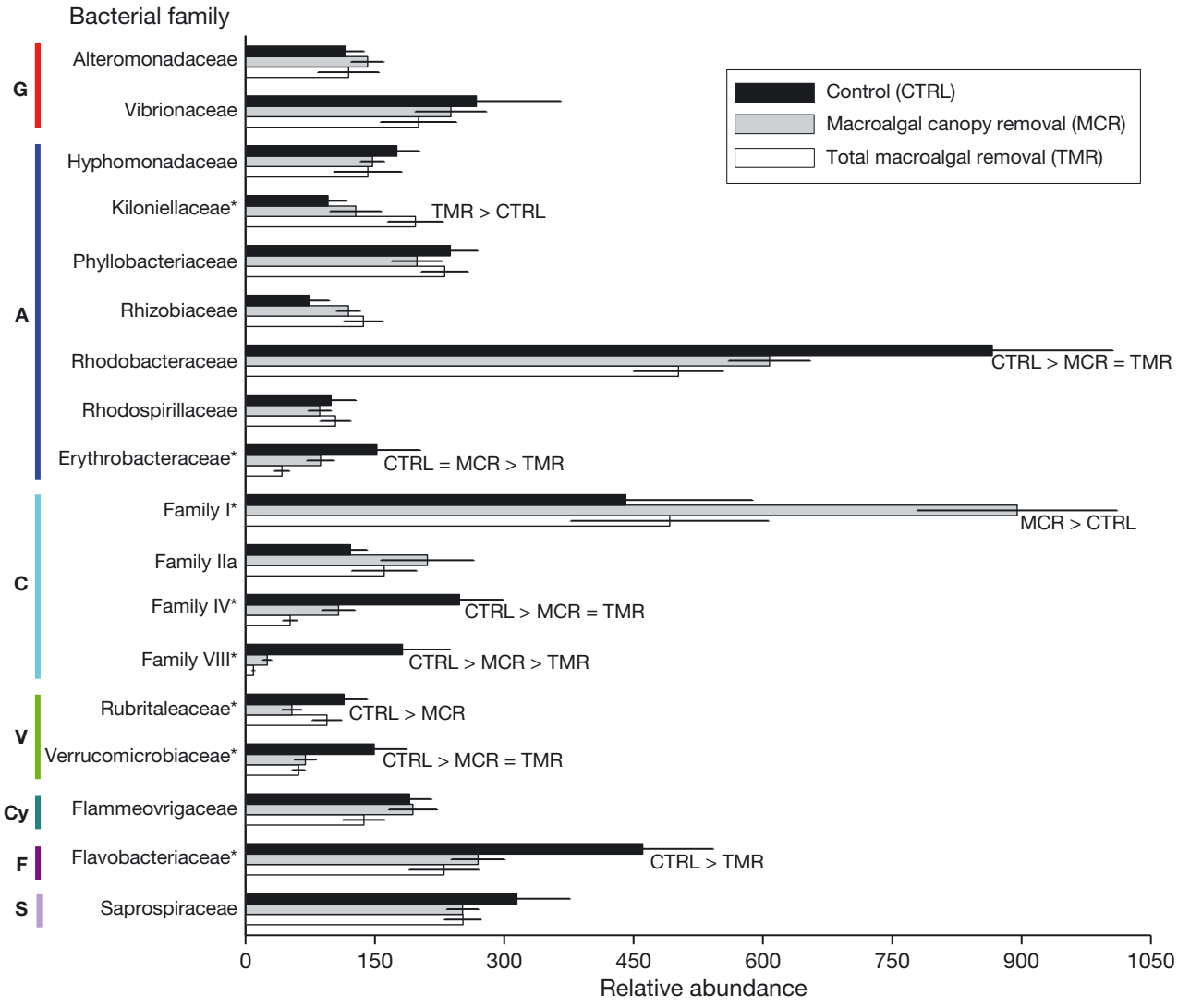


Fig. 4. Relative abundance of bacterial families that contributed most to multivariate patterns in the different experimental treatments (CTRL: control; MCR: macroalgal canopy removal; TMR: total macroalgal removal). Families for which significant differences were shown by the analysis are indicated by an asterisk and results of post-hoc comparisons are reported. A: *Alphaproteobacteria*; G: *Gammaproteobacteria*; C: *Cyanobacteria*; F: *Flavobacteriia*; V: *Verrucomicrobiae*; S: *Sphingobacteria*; Cy: *Cytophagales*

Table 3. Zero-inflated negative binomial GLMM on coral recruitment, 8 mo after the start of the experiment. Total macroalgal removal (TMR) is used as the baseline level in the analysis. ***$p < 0.001$

| | | |
|---|---|---|
| **Fixed effects (coefficients + SE)** | | |
| Intercept | 0.786 | (0.404) |
| Macroalgal canopy removal (MCR) | −1.769 | (0.499)*** |
| Control (CTRL) | −1.851 | (0.508)*** |
| **Random effect (variance)** | | |
| Bommie | 0.476 | |

greater proportion (~60%) of the variability in coral recruit density than *Sphingobacteria* or *Verrucomicrobiae* (~20% each).

## DISCUSSION

Our experimental study shows that *Turbinaria ornata*, a species currently expanding its distribution in the South Pacific, influences the composition of epilithic bacterial biofilms and coral recruitment on adjacent surfaces (i.e. dead coral skeleton and CCA).

The biofilm developing on glass slides fixed on coral reef surfaces is an accurate descriptor of benthic bacterial communities when deployed over weeks to months (Witt et al. 2011). Slide deployment for shorter periods of time is unlikely to yield reliable estimates of the surrounding bacterial community, but it can provide insight into the effects of local

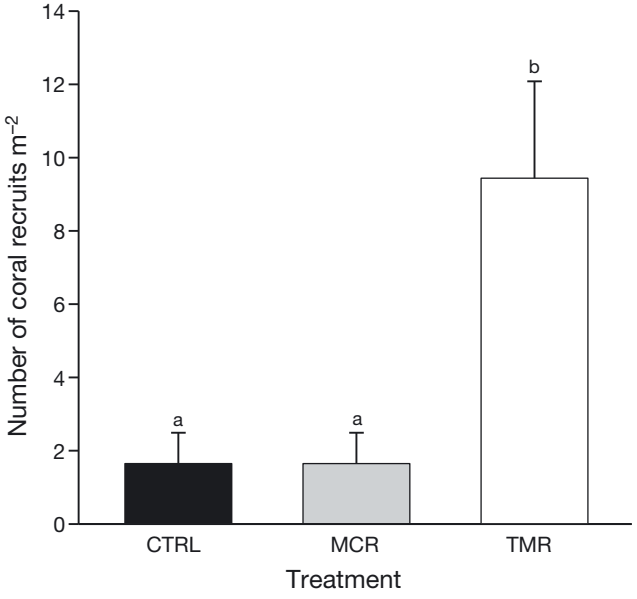


Fig. 5. Mean (±SE) coral recruit density on bommies exposed to different treatments, 8 mo after the start of the experiment (CTRL: control; MCR: macroalgal canopy removal; TMR: total macroalgal removal); 3 plots × 5 bommies: n = 15. Different letters above bars indicate significant differences from the statistical analysis in Table 3

biotic and environmental conditions on the early development of a novel biofilm. Here, the MCR and, to a lesser extent the TMR assemblages, resulted in fewer bacterial colonies on plates inoculated from 9 d old biofilms compared with the CTRL, suggesting that intact canopies stimulated biofilm colonization by readily cultivable bacteria. Since glass slides were positioned onto surfaces devoid of erect algae (i.e. not in contact with erect macroalgae), canopy effects were likely water-mediated. High rates of DOC release by *T. ornata* may have accelerated bacterial growth on slides, although exudates from this species generate cell yields in the bacterioplankton smaller than other macroalgae (Haas et al. 2011). In addition, reduced rates of water flushing beneath canopies could have enhanced the local concentration of dissolved and particulate organic matter (Eckman & Duggins 1991), promoting the growth of different types of bacteria.

Table 4. Multiple regression model examining coral recruit density (log transformed) against the abundance of bacterial orders that contributed most to multivariate patterns. Coefficient estimates, standard errors (SE) and *t*-values are provided for the variables retained in the best-fit model. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

| | Estimate | SE | *t* |
|---|---|---|---|
| Intercept | 3.1004 | 0.4288 | 7.231*** |
| *Cyanobacteria* | −0.0014 | 0.0003 | −5.112*** |
| *Sphingobacteria* | −0.0021 | 0.0008 | −2.627* |
| *Verrucomicrobiae* | −0.0024 | 0.0008 | −3.161** |

$F_{(3,11)} = 12.04$, p = 0.0008
Adjusted $R^2 = 0.703$

Over a longer time period (3.5 mo into the experiment), the magnitude of changes in the microbial community caused by TMR was greater than the removal of the canopy alone (average dissimilarity: 47.19 versus 41.62, for TMR and MCR, respectively; Fig. 3). Bacterial communities of both experimental treatments differed from those of the control treatment using both untransformed data and presence–absence-transformed data, suggesting that differences were likely due to variations in both bacterial family turnover and their relative abundance. By contrast, there was no difference in bacterial communities between MCR and TMR when multivariate analyses were performed on presence–absence-transformed data (see Table S2 in the Supplement), indicating that these 2 treatments did not differ in terms of composition or frequency of occurrence of bacterial families, but rather in their relative abundance.

The richness of bacterial OTUs and families on both MCR and TMR bommies was significantly lower than that of the control treatment. Increased microbial diversity has been associated with coral disease (Sunagawa et al. 2009), exposure to anthropogenic stressors such as increased sedimentation and nutrient loading (Ziegler et al. 2016), seasonal peaks in algal cover (Roik et al. 2016) and proliferation of macroalgae (Zaneveld et al. 2016). Macroalgal exudates (photosynthates/DOC) can promote the growth of pelagic and coral-associated bacteria (Kline et al. 2006, Haas et al. 2011, Morrow et al. 2012, Nelson et al. 2013). In addition, macroalgae host a more diverse bacterial assemblage than corals (Barott et al. 2012). In a similar way to algal-associated metabolites and solutes, free-living microbes could concentrate in the DBL of macroalgae and be transferred to downstream substrata (Barott & Rohwer 2012, Wangpraseurt et al. 2012, Jorissen et al. 2016). A similar mechanism may explain the greater bacterial diversity on glass slides incubated on bommies supporting a greater macroalgal biomass. Also the canopy removal caused a decrease in bacterial OTUs and family diversity compared to controls, suggesting that holdfasts and understory macroalgal assemblages contribute to shaping the composition of bacterial biofilms on adjacent substrata.

Experimental treatments caused major shifts in bacterial dominance. In particular, TMR resulted in a decrease of some *Cyanobacteria* (Family IV and Family VIII), *Verrucomicrobia* (*Verrucomicrobiaceae*), *Flavobacteriia* (*Flavobacteriaceae*) and α-*Proteobacteria* (*Rhodobacteraceae* and *Erythrobacteraceae*). Some of the families that responded negatively to experimental treatments, such as *Rhodobacteraceae* and *Flavobacteriaceae*, have been identified as major components of seawater bacterial assemblages at sites impacted by sedimentation and wastewater outfalls (Ziegler et al. 2016). Others, such as *Verrucomicrobiaceae*, increase in the coral microbiome with increasing erect algal cover (Zaneveld et al. 2016). Likewise, cyanobacteria are generally dominant on declining reefs, often forming benthic mats (Brocke et al. 2015). Our results suggest that stands of *T. ornata* might contribute to the enrichment of epilithic biofilms with bacteria generally thriving under altered environmental conditions.

Macroalgae can negatively influence coral recruitment through interference mechanisms, such as space pre-emption (Nugues & Szmant 2006, Vermeij 2006), surface abrasion (Gleason 1996) and enhanced deposition of sediments enriched in organic matter (Eckman & Duggins 1991). In addition, macroalgae can release water-borne compounds that can reduce the survival (Kuffner & Paul 2004, Kuffner et al. 2006) and settlement of larvae (Birrell et al. 2008, Morrow et al. 2017), either directly or through the alteration of bacterial communities on settling surfaces (Vermeij et al. 2009, Tran & Hadfield 2011, Sneed et al. 2015). Here, few juvenile coral colonies were found on experimental bommies (max. 10 recruits per 0.28 $m^2$ plot), in accordance with previous studies showing that recruitment rates in the lagoon of Moorea are very low (Adjeroud et al. 2007). Since coral larvae preferentially recruit in holes and crevices (Nozawa 2008, Edmunds et al. 2014), low recruitment rates could be also a consequence of the low complexity of open surfaces sampled on experimental bommies. Nonetheless, the total removal of macroalgae caused an approximately 4-fold increase in the density of coral larvae, in agreement with previous reports of lower recruitment rates on tiles deployed in areas dominated by *T. ornata* (Gleason 1996).

Our study cannot formally identify the mechanism(s) through which TMR fostered coral recruitment. By virtue of the fact that coral recruitment was quantified on surfaces devoid of erect algae, differences in coral recruit density cannot be inferred to variation in space availability among treatments. Canopy removal alone did not promote coral recruitment, suggesting that abrasion by sweeping fronds (Gleason 1996) was unlikely the main mechanism through which *T. ornata* stands reduced the settlement of larvae and/or the survival of spats and juvenile colonies. Indeed, the presence of holdfasts and associated small-sized erect algae was sufficient to prevent coral recruitment on adjacent free surfaces.

There is compelling evidence that bacteria play a key role in regulating the recruitment of marine invertebrates (Negri et al. 2001, Webster et al. 2004, Freckelton et al. 2017). Although this remains to be experimentally demonstrated, enhanced recruitment following TMR might be connected to the major changes observed in epilithic bacterial biofilms. Lower OTU richness and decreased abundance of *Rhodobacterales*, *Flavobacteriaceae*, *Cyanobacteria* (Family IV and Family VIII), *Verrucomicrobiaceae*, families belonging to orders that generally characterize degraded reefs or are found in diseased coral tissues (Sunagawa et al. 2009, Kelly et al. 2014, Roder et al. 2014, Santos et al. 2016, Zaneveld et al. 2016, Ziegler et al. 2016), may have promoted larval recruitment in the TMR treatment.

There was a negative correlation between coral recruit density and the abundance of *Cyanobacteria*, *Sphingobacteria* and *Verrucomicrobiae*. Variations in the abundance of these 3 bacterial orders explained a high proportion (~70%) of the total variability in coral recruit density. *Cyanobacteria* accounted for most of the variability explained, bringing support to the hypothesis that positive effects of the TMR could be mediated by the response of cyanobacteria. Cyanobacteria can act as coral pathogens (Carlton & Richardson 1995, Gantar et al. 2009, Santos et al. 2016) and lead to increased DOC release into the water column (Brocke et al. 2015). For example, benthic cyanobacteria of the order *Oscillatoriales* produce potent allelopathic compounds and inhibit coral larval settlement (Kuffner & Paul 2004, Kuffner et al. 2006). Here, while *Cyanobacteria* Family IV and Family VIII decreased following both canopy and total macroalgal removal, Family I increased. The increase in *Cyanobacteria* Family I was particularly marked in MCR and might explain the failure of this treatment to induce positive effects on coral recruits. Overall, our results indicate that the response of benthic cyanobacteria to the removal of erect macroalgae and, possibly, their effects on coral recruitment, vary considerably among the families belonging to this phylum.

Although they had a lower importance than *Cyanobacteria*, *Sphingobacteria* and *Verrucomicrobi-*

*aceae* contributed to explaining variability in coral recruitment. Bacteria belonging to these orders were found to increase in the mucus of corals in response to thermal stress or algal contact (Lee et al. 2016, Zaneveld et al. 2016). Negative effects of these bacteria may not be limited to adult corals, but could extend to juvenile stages.

Finally, our results suggest that the dislodgement of *T. ornata* stipes due to natural senescence or during storms does not provide long-lasting windows of opportunity for coral recovery. Likewise, *T. ornata* eradication campaigns operated by citizen associations are unlikely to sustain long-term reductions in the abundance of this seaweed and, hence, coral recovery when plant holdfasts are left in place. By contrast, complete eradication of *T. ornata* and associated erect algal assemblages, even though for relatively short periods, could promote coral recruitment. However, the time and financial efforts necessary for generating and maintaining vast areas of reefs free of this species cast doubts on the viability of this strategy for restoring infested reefs.

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