

Nitrogen fixation rates in algal turf communities of a degraded versus less degraded coral reef

Joost den Haan · Petra M. Visser · Anjani E. Ganase ·
Elfi E. Gooren · Lucas J. Stal · Fleur C. van Duyl ·
Mark J. A. Vermeij · Jef Huisman

Received: 9 January 2014 / Accepted: 1 September 2014 / Published online: 10 September 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Algal turf communities are ubiquitous on coral reefs in the Caribbean and are often dominated by N₂-fixing cyanobacteria. However, it is largely unknown (1) how much N₂ is actually fixed by turf communities and (2) which factors affect their N₂ fixation rates. Therefore, we compared N₂ fixation activity by turf communities at different depths and during day and night-time on a degraded versus a less degraded coral reef site on the island of Curaçao. N₂ fixation rates measured with the acetylene reduction assay were slightly higher in shallow (5–10-m depth) than in deep turf communities (30-m depth), and N₂ fixation rates during the daytime significantly exceeded those during the night. N₂ fixation rates by the turf communities did not differ between the degraded and less degraded reef. Both our study and a literature survey of earlier studies indicated that turf communities tend to have

lower N₂ fixation rates than cyanobacterial mats. However, at least in our study area, turf communities were more abundant than cyanobacterial mats. Our results therefore suggest that turf communities play an important role in the nitrogen cycle of coral reefs. N₂ fixation by turfs may contribute to an undesirable positive feedback that promotes the proliferation of algal turf communities while accelerating coral reef degradation.

Keywords Algal turf · Benthic cyanobacteria · Coral reefs · Nitrogen cycle · N₂ fixation

Introduction

During recent decades, many coral reefs have shifted from coral to algal dominance (Done 1992; McManus and Polsenberg 2004; Hughes et al. 2007; Cheal et al. 2010). The algal-dominated state often consists of macroalgae, but small turf algae have also increased (Barott et al. 2009; Vermeij et al. 2010). Algal turf communities (henceforth called ‘turfs’) are heterogeneous assemblages of

Communicated by Ecology Editor Dr. Stuart Sandin

Electronic supplementary material The online version of this article (doi:10.1007/s00338-014-1207-5) contains supplementary material, which is available to authorized users.

J. den Haan · P. M. Visser (✉) · A. E. Ganase ·
L. J. Stal · M. J. A. Vermeij · J. Huisman
Department of Aquatic Microbiology, Institute for Biodiversity
and Ecosystem Dynamics, University of Amsterdam,
PO Box 94248, 1090 GE Amsterdam, The Netherlands
e-mail: p.m.visser@uva.nl

Present Address:
A. E. Ganase
Coral Reef Ecosystems Lab, School of Biological Sciences, The
University of Queensland, St Lucia, QLD 4072, Australia

E. E. Gooren
Department of Microbiology, Radboud University,
Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

L. J. Stal
Department of Marine Microbiology, Royal Netherlands
Institute for Sea Research (NIOZ), PO Box 140,
4400 AC Yerseke, The Netherlands

F. C. van Duyl
Department of Biological Oceanography, Royal Netherlands
Institute for Sea Research (NIOZ), PO Box 59,
1790 AB Den Burg, The Netherlands

M. J. A. Vermeij
Carmabi Foundation, Piscaderabaai z/n,
PO Box 2090, Willemstad, Curaçao

Chlorophyta, Phaeophyta, Rhodophyta, and filamentous Cyanobacteria (Steneck and Dethier 1994; Diaz-Pulido and McCook 2002; Fricke et al. 2011). Turfs are important primary producers on reefs that are intensively grazed (Adey and Goertemiller 1987; Carpenter and Williams 2007), compete for space against corals (Barott et al. 2009; Vermeij et al. 2010), provide shelter for various meiofauna (Zeller 1988; Logan et al. 2008), and may play a key role in the recycling of nutrients (Charpy-Roubaud et al. 2001; Charpy et al. 2007; Fricke et al. 2011; Charpy et al. 2012). Turfs currently represent the single most dominant benthic component on many coral reefs in the Caribbean (Kramer 2003; Vermeij et al. 2010), Central Pacific (Barott et al. 2009, 2012), Red Sea (Haas et al. 2010), and Indonesia (Wangpraseurt et al. 2012).

Many of the cyanobacteria in turfs are capable of nitrogen (N_2) fixation (Charpy et al. 2012). Nitrogen is one of the key elements often limiting primary productivity on coral reefs (Littler et al. 1991; Delgado and Lapointe 1994; Larned 1998; Den Haan et al. 2013), and hence, N_2 fixation provides access to an important additional N source. N_2 fixation is very sensitive to oxygen, and diazotrophic cyanobacteria evolved various strategies to accommodate the incompatible processes of oxygenic photosynthesis and N_2 fixation (e.g., Bergman et al. 1997; Zehr 2011). Some cyanobacteria developed specialized cells known as heterocysts to separate N_2 fixation from oxygen production, and these heterocystous species often show higher N_2 fixation activity during daytime than at night (Mullineaux et al. 1981; Staal et al. 2002). In contrast, many non-heterocystous diazotrophic cyanobacteria fix N_2 during the night, fuelled by respiration of the carbohydrates built up by photosynthesis during the preceding daytime (Schneegurt et al. 1994; Dron et al. 2012; Brauer et al. 2013). The N_2 fixed by cyanobacteria may be released as dissolved inorganic and organic N through leakage from the cells, cell death, or lysis (Berman and Bronk 2003; Benavides et al. 2013) and thereby becomes available for other organisms in the community (Mulholland et al. 2006; Agawin et al. 2007).

Eutrophication of coastal waters is a major problem in many densely populated (sub)tropical regions (Nyström et al. 2000) and is often associated with a phase shift from coral to algal dominance (Done 1992; Hughes 1994; Cheal et al. 2010). To what extent eutrophication may affect the relative importance of N_2 fixation on coral reefs is still an open question. Generally speaking, N enrichment may alleviate ecosystems from N limitation, which would diminish the need for N_2 fixation, and hence decrease the abundance of N_2 -fixing cyanobacteria (Smith 1983; Conley et al. 2009). However, if N continues to be limiting (e.g., if externally supplied N is utilized as quickly as it is supplied), then N_2 fixation will not be suppressed and N_2 -

fixing organisms may still prevail. Indeed, lake studies have shown that N_2 fixation rates can be high in eutrophic waters, especially during episodes with a relative shortage of N (Howarth et al. 1988; Scott et al. 2009; Paerl 2009).

N_2 fixation on coral reefs has been studied for almost 40 yrs (Webb et al. 1975; Wiebe et al. 1975). Most studies have focused on N_2 fixed by solitary or mat-forming benthic cyanobacteria (e.g., O'Neil and Capone 1989; Charpy-Roubaud et al. 2001; Charpy et al. 2007, 2010), whereas only a few studies have investigated N_2 fixation in turfs (Wilkinson and Sammarco 1983; Wilkinson et al. 1984; Larkum et al. 1988; Williams and Carpenter 1997, 1998; Davey et al. 2008). Little is known about the major environmental determinants of N_2 fixation by turfs. For instance, it is not known to what extent depth-dependent variation in cyanobacterial composition and light conditions affect the N_2 fixation activity of the turfs. Furthermore, it is not known whether turfs fix N_2 mainly during the day or night, or whether N_2 fixation by turfs is affected by the degradation of coral reefs associated with coastal eutrophication. The main goals of this study were therefore (1) to quantify N_2 fixation by turfs in relation to water depth and the day–night cycle and (2) to compare N_2 fixation by turfs on a degraded reef near a densely inhabited area versus a less degraded coral reef at a more remote site. We compared our results with published data from solitary cyanobacteria, mat-forming cyanobacteria, and turfs on reefs elsewhere in the world.

Materials and methods

Research sites and composition of turfs

This study was conducted on the leeward side of the island of Curaçao, Southern Caribbean, during June–August of 2011. We investigated two sites: ‘Buoy 0’ (12°7′29.07″N, 68°58′22.92″W) and ‘Playa Kalki’ (12°22′30.9″N, 69°9′31.49″W; Fig. 1). Buoy 0 is a degraded reef only a few kilometres downstream of the capital Willemstad with its industrial harbour and near the outlet of the eutrophied Piscadera Bay. Over the past 40 yr, coral cover of the shallow reefs at Buoy 0 has decreased dramatically (Bak et al. 2005). Playa Kalki is located ~35 km from the major population centre of Willemstad. It is a less degraded reef with higher hard coral cover, and lower algal and cyanobacterial cover, and lower dissolved nutrient concentrations than at Buoy 0 (see Results).

Turfs on the reefs at Buoy 0 consist of a mixed community of algae belonging to the Chlorophyta, Rhodophyta, Phaeophyta, and Cyanobacteria (Fricke et al. 2011). This study found that at 5-m depth, the turf community at Buoy 0 was dominated by Cyanobacteria, notably the heterocystous species *Dichothrix utahensis* (Fricke et al.

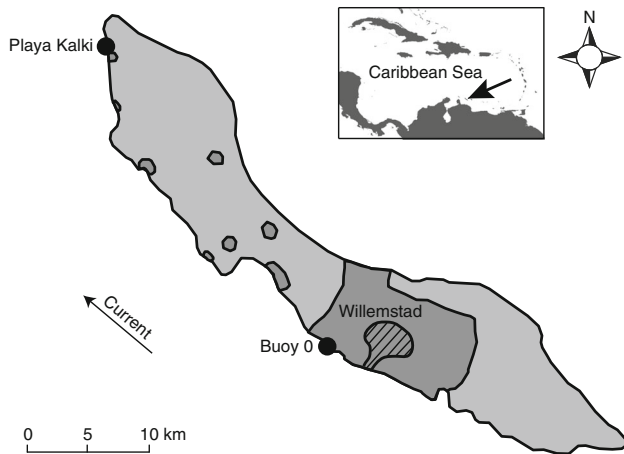


Fig. 1 Map indicating research sites Buoy 0 and Playa Kalki on Curaçao, Southern Caribbean (12°10'N, 68°58'W). Dark grey zones are urbanized areas, including the capital Willemstad and its industrial harbour (hatched area)

2011). At 15-m depth, the relative abundances of Cyanobacteria, Chlorophyta, Rhodophyta, and Phaeophyta were more evenly distributed (Fricke et al. 2011). At 25-m depth, Cyanobacteria represented ~40 % of the turf community, dominated by the non-heterocystous cyanobacteria *Lyngbya* spp. and *Oscillatoria* spp., whereas the heterocystous species *D. utahensis* was rare (Fricke et al. 2011). At Playa Kalki, we observed the same N₂-fixing cyanobacteria as at Buoy 0 (i.e., with *Dichothrix* spp. near the surface, and *Lyngbya* spp. and *Oscillatoria* spp. in deeper parts of the reef).

Reef characteristics

Surveys comparing the dominant benthic communities at both Buoy 0 and Playa Kalki were conducted along 100-m transects, placed alongside the 5, 10, 20, and 30-m isobath. Benthic cover of hard corals, macroalgae, turfs, and cyanobacterial mats was determined from photographs of 60 quadrats (1.5 m²) randomly placed along both sides of the transects. The photographs were analysed using the program Coral Point Count with Excel Extensions (CPCe; Kohler and Gill 2006).

Light profiles were measured with a Hydrolab DS5 Sonde (OTT Messtechnik GmbH & Co., Kempten, Germany; $n = 5$ at both Buoy 0 and Playa Kalki). Furthermore, water samples for nutrient analysis were taken along each horizontal transect at 10 cm above the reef using a 50-ml Terumo syringe (Terumo Europe, Leuven, Belgium; $n = 9$ at both Buoy 0 and Playa Kalki). Water samples were immediately filtered at the diving site using 0.22- μ m Acrodisc filters and stored in 6-ml polyethylene vials (PerkinElmer, MA, USA) at -20 °C until further analysis. Concentrations of NO₃⁻ (Grasshoff et al. 1983), NH₄⁺

(Helder and De Vries 1979), and PO₄³⁻ (Murphy and Riley 1962) were analysed at the Royal Netherlands Institute for Sea Research (NIOZ), Texel, the Netherlands, using continuous flow analysis via a Quatro auto-analyzer (Seal Analytical, UK).

Sampling and incubation of turfs

The N₂ fixation measurements required controlled incubation of turf samples. For this purpose, turfs were not collected directly off the reef, because scraping turfs off the rocks would damage the algal tissue and the rock underneath. Instead, turfs were grown on the exterior of 1.5-L square plastic bottles (FIJI Water Company, CA, USA), which were placed inside 1-m³ chicken-wired cages (mesh \varnothing 2.5 cm). Growing turfs on plastic bottles ensured that the turfs were of approximately similar size and age and resulted in minimal damage to the live tissue of the turfs. Encaging prevented grazing by large herbivores (i.e., >2.5 cm in diameter) to ensure sufficient turf growth on the bottles. In May 2011, the encaged bottles were positioned at depths of 5, 10, 20, and 30 m using SCUBA diving. The bottles were placed 0.5 m above the reef to avoid overgrowth by benthic cyanobacterial mats or macroalgae and remained on the reefs of Buoy 0 and Playa Kalki for 63–79 d and 49–77 d, respectively. This growth period provided ample time for the establishment of turf communities with benthic cyanobacteria.

Plastic bottles were retrieved from each depth (5, 10, 20, and 30 m) between 23 June and 3 August 2011. Small strips (1.5 × 2.0 cm; six strips per depth) containing turfs were cut out of the plastic bottles using scissors. In the laboratory, each strip was placed inside a 10-ml headspace crimp vial (VWR, the Netherlands) containing 7 ml of 0.22 μ m filtered ambient seawater (Whatman cellulose acetate membrane filters, \varnothing 25 mm). Vials were then sealed with a gastight liner and an aluminium cap (Agilent Technologies, Santa Clara, CA, USA) using a vial crimper and stored in a flow-through aquarium to keep the turfs at the same temperature as on the reef (27–29 °C). The average light intensity inside the aquarium, measured with a Hydrolab DS5 Sonde (OTT Messtechnik GmbH & Co., Kempten, Germany), was ~100 μ mol photons m⁻² s⁻¹ during the daytime. This light level is comparable to light intensities measured on the reefs at 20–30-m depth during sunny days. However, it is below the saturation light intensity of 200 μ mol photons m⁻² s⁻¹ of the in situ photosynthetic activity of the turfs, measured according to Ralph and Gademann (2005) with a Diving-PAM/B Underwater Fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany; J. Den Haan, unpublished results). The vials were acclimated inside the flow-through aquarium until the acetylene reduction assay (ARA, see below) commenced at 1900 hrs on the day of collection.

We incubated six strips per depth and per research site, each in a separate vial. In addition, we used one clean strip (without turfs) per depth and per research site as a negative control. Furthermore, the mat-forming cyanobacterium *Oscillatoria* spp. ($n = 6$) was collected off sandy sediments at Buoy 0 and solitary tufts of *Symploca* spp. ($n = 6$) were collected from rocks at Playa Kalki, both at 5-m depth. *Oscillatoria* and *Symploca* spp. are known to be active N_2 fixers (Charpy et al. 2010), and their N_2 fixation rates were compared to the turfs.

Acetylene reduction assay

The ARA was used to measure the nitrogenase activity of the N_2 -fixing organisms residing in the turfs (Stewart et al. 1967; Charpy et al. 2007). Briefly, 2.5 ml of acetylene gas was injected into the vials using a 2.5 MR-GT gastight syringe (SGE Analytical Science, UK) giving an acetylene concentration in the headspace of 33 % (Stal 1988; Zuckermann et al. 1997). Directly after injecting the acetylene, which was always at 1900 hrs (sunset), a 1-ml gas sample was taken from the vial using the same syringe to correct for background ethylene. Subsequent samples were taken after 12 h (at 0700 hrs; sunrise) and after 24 h (at 1900 hrs the next day) to quantify N_2 fixation during night-time and the full 24-h period, respectively. Daytime N_2 fixation was calculated by subtracting the night-time N_2 fixation from the total N_2 fixation after 24 h. Once a gas sample was taken from a vial, the sample was immediately transferred into a 6-ml Vacuette (Greiner Bio-One, The Netherlands), which was completely filled with a saturated salt (NaCl) solution, to properly conserve the gas samples until further analysis.

The ethylene content of the gas samples was analysed with a gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan), using acetylene as an internal standard (Stal 1988). The gas chromatograph was equipped with a flame ionization detector and a 3-m Porapak R column (2 mm inner diameter; Chrompack). The temperatures of the injector and detector were set to 70 and 90 °C, respectively. From the headspace of each Vacuette, 0.1 ml was injected into the gas chromatograph using a gastight disposable syringe. The retention time for ethylene (C_2H_4) and acetylene (C_2H_2) were 1.9 and 2.7 min, respectively. Ethylene production was converted to N_2 fixation using a conversion factor of four (Stal 1988; Mulholland et al. 2004).

Chlorophyll *a* analysis

After 24 h of incubation, the plastic strips were removed from the vials to determine the amount of chlorophyll *a* as a measure of turf biomass. Chlorophyll *a* was extracted by grinding the samples in 10 ml of 100 % methanol using a mortar and pestle in an ice bath and under dim light.

Extraction was continued for 24 h in a refrigerator (4 °C) inside test tubes covered with aluminium foil. Subsequently, the extracts were centrifuged for 15 min at 3,500 rpm to separate the plastic particles from the extraction solvent. The chlorophyll *a* concentration of the extracts was measured on a T60 UV/Vis spectrophotometer (PG Instruments Ltd, Wibtsoft, UK) according to Porra et al. (1989).

Statistical analysis

We used a two-way analysis of variance to test whether the measured nutrient concentrations, turf cover and turf biomass varied significantly with depth (5, 10, 20, 30 m) and research site (Buoy 0, Playa Kalki). A three-way analysis of variance was applied to test whether N_2 fixation rates varied significantly with depth, research site, and time of day (day vs. night). We used type III sum of squares to account for unbalanced data, because some strips were damaged or lost during the N_2 fixation analysis. The dependent variables were log-transformed if this improved the homogeneity of variance, as tested by Levene's test. Post hoc comparisons of the means were based on Tukey's HSD test using a significance level (α) of 0.05.

Results

Reef characteristics

The coral reef ecosystem at Buoy 0 had a significantly lower cover of corals and the macroalga *Dictyota* spp. than Playa Kalki and a significantly higher cover of the macroalga *Lobophora variegata* and cyanobacterial mats (Table 1). The water at Buoy 0 was slightly but significantly more turbid than at Playa Kalki, as revealed by comparison of the slopes of the log-transformed light profiles (ANCOVA, $F_{1,59} = 20.176$, $p < 0.001$; Fig. 2a). NO_3^- concentrations were significantly higher at Buoy 0 than at Playa Kalki at 5- and 10-m depth, but not at 20- and 30-m depth (Figs. 2b, d; Table 2). PO_4^{3-} concentrations were significantly higher at Buoy 0 than at Playa Kalki at 5-m depth only (Fig. 2d; Table 2). NH_4^+ concentrations were similar at both sites (Fig. 2c; Table 2). The ambient N/P ratio of 21.2 ± 1.7 (mean \pm SE) at Buoy 0 was significantly higher than the N/P ratio of 15.3 ± 1.0 at Playa Kalki (Table 2).

Algal turfs

Turf cover on the reef decreased significantly with depth at both Buoy 0 and Playa Kalki (Fig. 3a; Table 3). We did not find systematic differences in turf cover between the two research sites. Turf cover at 5-m and 30-m depth was

Table 1 Comparison of percentage cover by hard corals, macroalgae (dominated by *Lobophora variegata* and *Dictyota* spp.), turfs and cyanobacterial mats at research sites Buoy 0 and Playa Kalki (mean ± SE)

Research sites	Depth (m)	Corals (%)	Macroalgae		Turfs (%)	Cyanobacterial mats (%)
			<i>Lobophora</i> (%)	<i>Dictyota</i> (%)		
Buoy 0 (<i>degraded</i>)	5	7.4 ± 1.7	0.0	6.3 ± 0.9	43.5 ± 3.3	10.6 ± 2.3
	10	5.5 ± 1.3	0.0	16.6 ± 1.4	27.0 ± 2.1	21.7 ± 2.2
	20	16.5 ± 1.5	28.5 ± 1.9	14.0 ± 1.0	10.3 ± 0.8	9.3 ± 1.2
	30	14.0 ± 1.5	24.2 ± 2.2	22.3 ± 1.5	13.8 ± 1.0	1.2 ± 0.5
Playa Kalki (<i>less degraded</i>)	5	4.1 ± 0.8	0.0	38.3 ± 2.1	26.2 ± 1.6	1.6 ± 0.5
	10	17.0 ± 1.9	0.0	23.8 ± 1.9	38.8 ± 2.1	6.8 ± 1.6
	20	30.5 ± 2.3	3.7 ± 0.8	32.1 ± 1.4	13.2 ± 1.5	5.5 ± 0.8
	30	28.3 ± 2.2	18.7 ± 1.6	27.4 ± 1.5	6.6 ± 0.7	2.0 ± 0.6
Significance test						
<i>Depth</i>		<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> = 0.014	<i>p</i> < 0.001	<i>p</i> < 0.001
<i>Location</i>		<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> = 0.014	<i>p</i> < 0.001

Differences between the two research sites are tested with two-way analysis of variance, with depth and research site (location) as the independent variables. Significant *p* values (*p* < 0.05) are indicated in bold

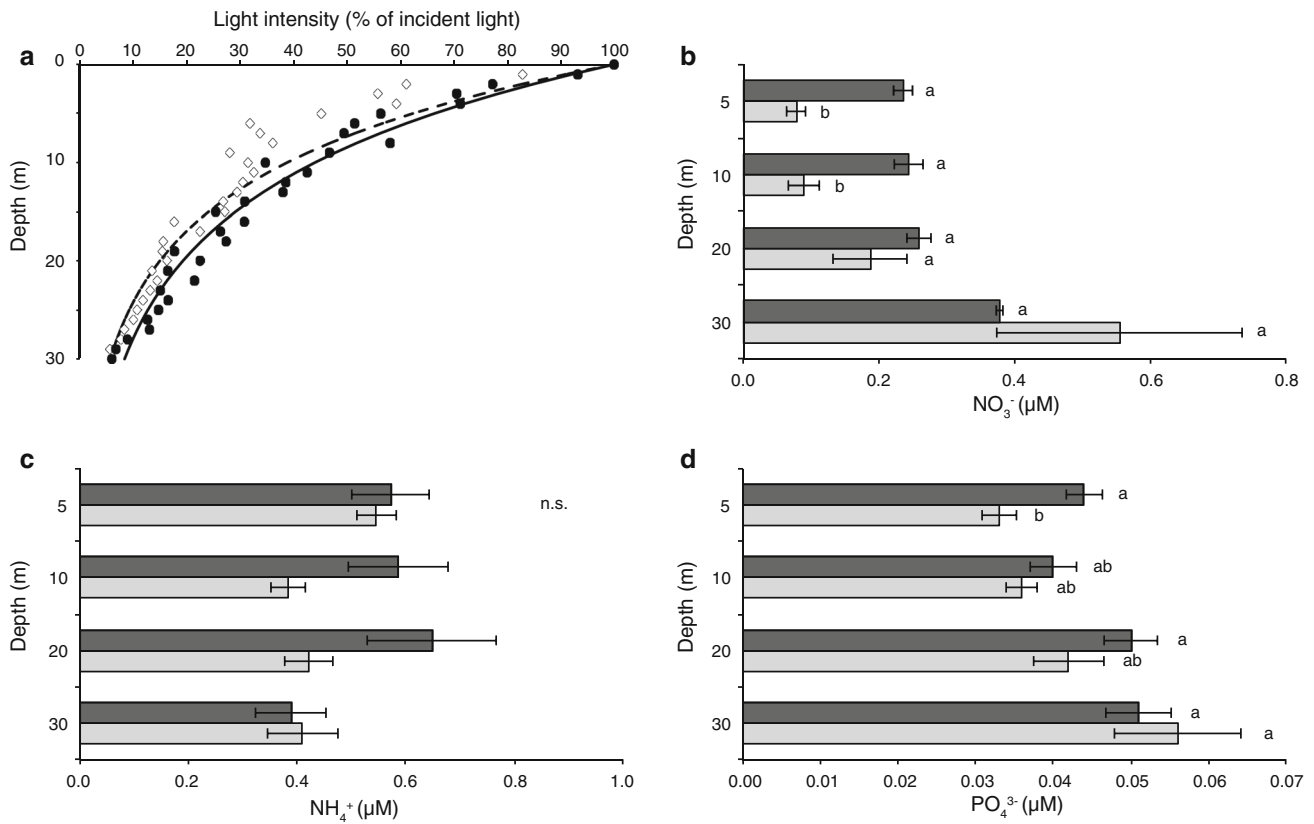


Fig. 2 Light and nutrient conditions. **a** Light profiles at Buoy 0 (*open diamonds with dashed trend line*) and Playa Kalki (*circles with solid trend line*). **b** Nitrate, **c** ammonium, and **d** phosphate concentrations in water samples from different depths at Buoy 0 (*dark grey bars*) and

Playa Kalki (*light grey bars*). Error bars represent SE of the means. Bars that do not share the same letter are significantly different, as tested by two-way analysis of variance followed by post hoc comparison of the means (Table 2; ESM Tables 1 and 2)

significantly higher at Buoy 0, whereas turf cover at 10 m was significantly higher at Playa Kalki. A similar pattern was found for turfs collected from the plastic bottles incubated on

the reef, which showed a significant decrease in turf biomass (expressed as mg Chl *a* m⁻²) with depth that was comparable at both research sites (Fig. 3b; Table 3).

Table 2 Two-way analysis of variance of nutrient concentrations, with depth and location as independent variables

Effect	<i>df</i> ₁ , <i>df</i> ₂	<i>F</i>	<i>p</i>
NO₃⁻			
Location	1, 156	42.901	<0.001
Depth	3, 156	10.340	<0.001
Location × Depth	3, 156	4.634	0.004
NH₄⁺			
Location	1, 158	0.245	0.622
Depth	3, 158	1.286	0.281
Location × Depth	3, 158	1.194	0.314
Dissolved inorganic nitrogen			
Location	1, 156	34.482	<0.001
Depth	3, 156	6.576	<0.001
Location × Depth	3, 156	3.800	0.012
PO₄³⁻			
Location	1, 156	5.300	0.023
Depth	3, 156	6.356	<0.001
Location × Depth	3, 156	1.586	0.195
N:P ratio			
Location	1, 168	4.785	0.030
Depth	3, 168	0.221	0.882
Location × Depth	3, 168	1.646	0.181

Columns indicate the main and interaction effects, the degrees of freedom (*df*₁ and *df*₂), the *F*-statistic (*Fdf*₁, *df*₂) and the corresponding probability (*p*). Significant results (*p* < 0.05) are indicated in bold

N₂ fixation by turfs

N₂ fixation by turfs did not differ significantly between Buoy 0 and Playa Kalki (Fig. 4; Table 4). However, we found significant main effects of depth and time (day vs. night) on N₂ fixation by turfs (Table 4). More specifically, post hoc comparison of the means showed that daytime N₂ fixation was significantly higher than night-time N₂ fixation at each of the four depths (Fig. 4). Furthermore, during daytime, the post hoc comparison showed a tendency for higher N₂ fixation activity at 5- and 10-m depth than at 30-m depth at a significance level of *p* < 0.10, but did not reveal significant differences at *p* < 0.05 between depths (Fig. 4; for details of the post hoc comparison, see Electronic Supplementary Material, ESM, Table 5). Averaged across all four depths and both research sites, the mean N₂ fixation by turfs was 17.4 ± 2.8 (mean ± SE) mg N₂ m⁻² 12 h⁻¹ during daytime and 2.9 ± 0.4 mg N₂ m⁻² 12 h⁻¹ at night. Combined, this resulted in a total daily N₂ fixation of 20.3 ± 2.9 mg N₂ m⁻² d⁻¹.

For comparison, we also measured N₂ fixation by two benthic non-heterocystous cyanobacteria collected at 5 m. Mat-forming *Oscillatoria* spp. collected off sandy sediments at Buoy 0 fixed substantially more N₂ than the turfs, with a N₂ fixation of 153.0 ± 60.3 mg N₂ m⁻² 12 h⁻¹

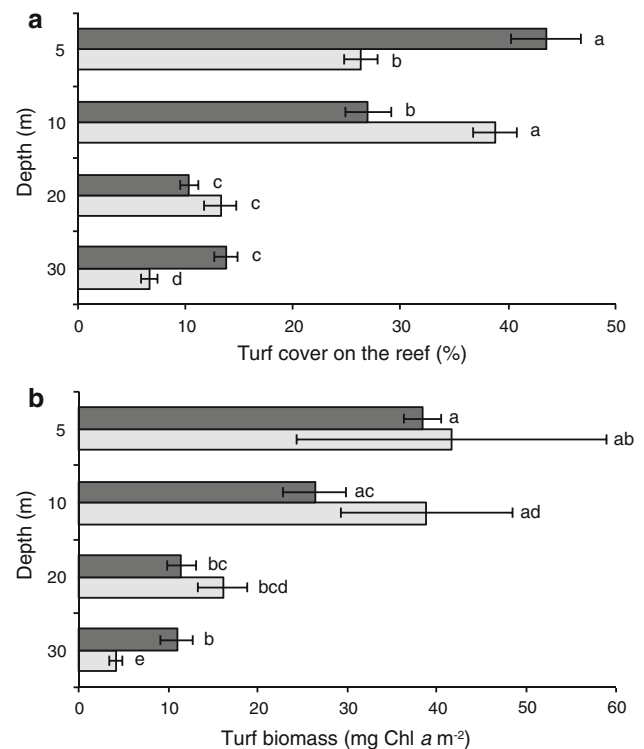


Fig. 3 **a** Percentage cover by algal turfs on the reefs (based on *n* = 60 quadrates per depth and research site). **b** Biomass of algal turfs growing on incubated plastic bottles (*n* = 6 bottles per depth and research site). Data are from the research sites Buoy 0 (dark grey bars) and Playa Kalki (light grey bars). Error bars represent SE of the means. Bars that do not share the same letter are significantly different, as tested by two-way analysis of variance followed by post hoc comparison of the means (Table 3; ESM Tables 3 and 4)

during daytime and 13.7 ± 2.8 mg N₂ m⁻² 12 h⁻¹ at night. Solitary-growing *Symploca* spp. collected from rocks at Playa Kalki also showed high N₂ fixation activity, of 66.9 ± 45.7 mg N₂ m⁻² 12 h⁻¹ during daytime and 4.9 ± 2.7 mg N₂ m⁻² 12 h⁻¹ at night.

We calculated the total N₂ fixation of turfs on a reef-wide scale as the product of their daily N₂ fixation and the cover of turfs on the coral reef. This revealed that total N₂ fixation at the reef scale was higher in the top 10 m than at greater depths, but did not differ between the degraded reef at Buoy 0 and the less degraded reef at Playa Kalki (Fig. 5).

Discussion

N₂ fixation in algal turf communities

Our results show substantial N₂-fixation activity in algal turf communities on the reefs of Curaçao, extending to a depth of at least 30 m. At the reef scale, the highest N₂ fixation activity by turfs was in the upper 10 m, which was mainly due to the higher cover of turfs in these shallow

Table 3 Two-way analysis of variance of the turf cover on the reef and turf biomass on the plastic bottles, with depth and location as independent variables

Effect	<i>df</i> ₁ , <i>df</i> ₂	<i>F</i>	<i>p</i>
Turf cover on the reef			
Location	1, 463	6.035	0.014
Depth	3, 463	110.064	<0.001
Location × Depth	3, 463	18.606	<0.001
Turf biomass on the plastic bottles			
Location	1, 38	3.417	0.072
Depth	3, 38	29.227	<0.001
Location × Depth	3, 38	5.580	0.003

Columns indicate the investigated effects, degrees of freedom (*df*₁ and *df*₂), the *F*-statistic (*F**df*₁,*df*₂) and the corresponding probability (*p*). Significant results (*p* < 0.05) are indicated in bold

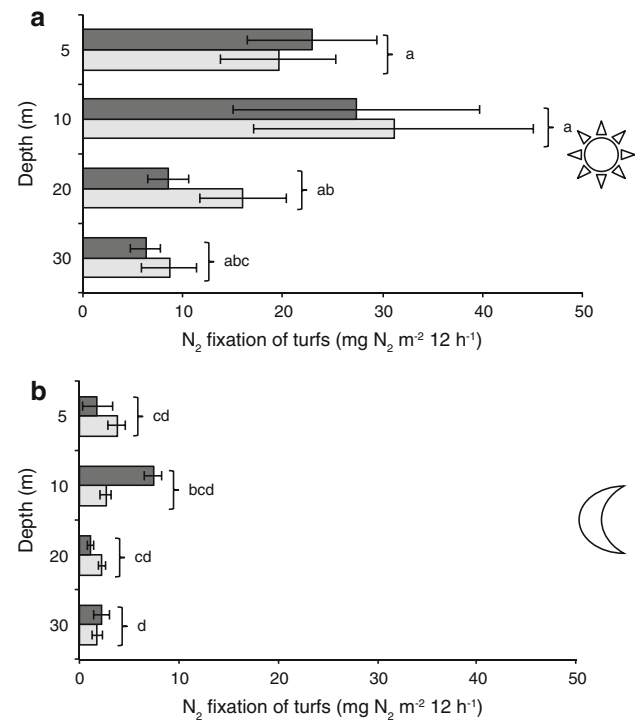


Fig. 4 N₂ fixation by turfs at Buoy 0 (dark grey bars) and Playa Kalki (light grey bars) as function of depth. **a** Daytime N₂ fixation (0700–1900 hrs); **b** night-time N₂ fixation (1900–0700 hrs). Error bars represent SE of the means. Bars that do not share the same letter are significantly different, as tested by three-way analysis of variance followed by post hoc comparison of the means (Table 4; ESM Table 5). Because the rate of N₂ fixation was not significantly different between Buoy 0 and Playa Kalki, both research sites are aggregated in the post hoc comparison as indicated by the braces

parts of the reef. The strong proliferation of turfs in shallow waters was confirmed by the high turf biomass that developed on the plastic bottles incubated within the upper 10 m of the water column. The incubated bottles were

Table 4 Three-way analysis of variance of the N₂ fixation rate by turfs (mg N₂ m⁻² turf 12 h⁻¹), with depth, location (Buoy 0 vs. Playa Kalki) and time of day (day vs. night) as independent variables

Effect	<i>df</i> ₁ , <i>df</i> ₂	<i>F</i>	<i>p</i>
Location	1, 68	0.006	0.940
Depth	3, 68	5.979	0.001
Time (day vs. night)	1, 68	66.089	<0.001
Location × Depth	3, 68	1.527	0.215
Location × Time	1, 68	0.659	0.420
Depth × Time	3, 68	0.678	0.568
Location × Depth × Time	3, 68	0.972	0.411

Columns indicate the investigated effects, degrees of freedom (*df*₁ and *df*₂), the *F*-statistic (*F**df*₁,*df*₂) and the corresponding probability (*p*). Significant results (*p* < 0.05) are indicated in bold

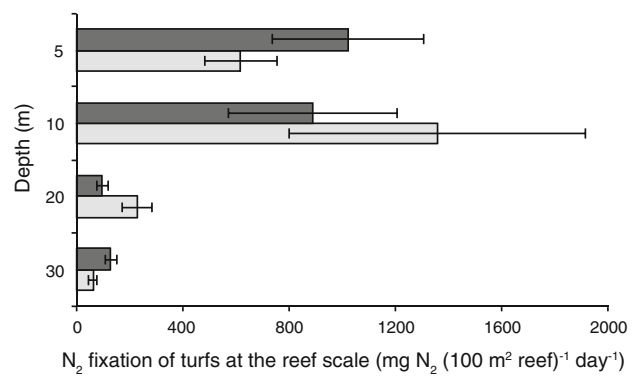


Fig. 5 Total daily N₂ fixation on 100 m² of reef at Buoy 0 (dark grey bars) and Playa Kalki (light grey bars). Error bars represent SE of the means

protected from grazing by herbivores larger than 2.5 cm, and therefore, the prevalence of turfs in shallow waters was probably not due to depth-dependent variation in grazing rates of mid- and large-sized herbivores. Several studies have pointed out that turfs have high light requirements (e.g., Carpenter 1985; Klumpp et al. 1987), and therefore at greater depth, they have difficulty competing with other algae (Steneck and Dethier 1994). This would offer a plausible explanation for the observed depth distribution of turfs and is also consistent with the higher abundances of turfs in the shallower parts of other reefs (Van den Hoek et al. 1975; Adey and Goertemiller 1987; Steneck and Dethier 1994).

We observed a much higher N₂ fixation activity during the day than at night. This contrasts with several studies of microbial mats in the temperate zone, which typically show higher night-time N₂ fixation activity (Villbrandt et al. 1990; Severin and Stal 2008). However, cyanobacterial mats dominated by heterocystous species showed higher levels of N₂ fixation during daytime on coral reefs in St. Croix and New Caledonia (Williams and Carpenter 1997; Charpy-Roubaud et al. 2001; Charpy et al. 2007).

Table 5 Nitrogen fixation rates by turfs and benthic (cyano-) bacteria from other coral reefs around the world

Benthic community	Region	Location	Depth (m)	Substrate	Total N ₂ fixation (mg N ₂ m ⁻² d ⁻¹)	Reference
Turf	Caribbean	Buoy 0, Curaçao	5	Plastic discs	24.8 ± 6.9	This study
Turf	Caribbean	Buoy 0, Curaçao	10	Plastic discs	34.8 ± 12.4	This study
Turf	Caribbean	Buoy 0, Curaçao	20	Plastic discs	9.7 ± 2.1	This study
Turf	Caribbean	Buoy 0, Curaçao	30	Plastic discs	8.7 ± 1.9	This study
Turf	Caribbean	Playa Kalki, Curaçao	5	Plastic discs	23.4 ± 5.2	This study
Turf	Caribbean	Playa Kalki, Curaçao	10	Plastic discs	33.8 ± 15.3	This study
Turf	Caribbean	Playa Kalki, Curaçao	20	Plastic discs	18.4 ± 5.3	This study
Turf	Caribbean	Playa Kalki, Curaçao	30	Plastic discs	10.5 ± 3.4	This study
Turf	Caribbean	Tague Bay, US Virgin Islands	3	Dead coral	6.6 ± 1.1	Williams and Carpenter (1997)
Turf	Caribbean	Smithsonian Institute		Microcosm	5.6– 80.6*	Carpenter et al. (1991)
Turf	Great Barrier Reef	Heron Island– exp. tanks		Dead coral	1.0– 19.2*	Davey et al. (2008)
Turf	Great Barrier Reef	Britomart	10	Dead coral	13.9– 61.7*	Wilkinson and Sammarco (1983)
Turf	Great Barrier Reef	Myrmidon & Dip Reef	1–9	Rock	2.7– 3.2*	Wilkinson et al. (1984)
Turf	Great Barrier Reef	One Tree Island	0–5	Dead coral	8.3*	Larkum et al. (1988)
Turf	Hawaiian Islands	Kaneohe Bay, Oahu	≤2	Dead coral	22.7 ± 9.2*	Williams and Carpenter (1998)
<i>Oscillatoria</i> spp.	Caribbean	Buoy 0, Curaçao	5	Sand	166.8 ± 60.3	This study
<i>Symploca</i> spp.	Caribbean	Playa Kalki, Curaçao	5	Rock	71.8 ± 47.9	This study
(cyano)bacteria	Red Sea	Gulf of Eilat	0.5–10	Sand	32.8 ± 29.4	Shashar et al. (1994)
(cyano)bacteria	Red Sea	Gulf of Eilat	4–6	Dead coral	93.2 ± 47.9	Shashar et al. (1994)
(cyano)bacteria	SW Indian Ocean	La Saline, La Réunion	0–2	Dead coral	2.1 ± 1.2	Casareto et al. (2008)
Cyanobacteria	SW Indian Ocean	La Saline, La Réunion	0–2	Mats	97.0 ± 2.3	Casareto et al. (2008)
Cyanobacteria	Great Barrier Reef	One Tree Island	0–5	Sand	0.2– 0.5	Larkum et al. (1988)
(cyano)bacteria	French Polynesia	Tikehau (58 sites)	0–4	Sand	0.4– 3.9	Charpy-Roubaud et al. (2001)
(cyano)bacteria	French Polynesia	Tikehau (58 sites)	0–4	Sock	2.1	Charpy-Roubaud et al. (2001)
Cyanobacteria	Marshall Islands	Enewetak Atoll	intertidal	Mats	42.9– 71.7	Wiebe et al. (1975)
Cyanobacteria	Marshall Islands	Enewetak Atoll	3–6	Algae	5.7*	Wiebe et al. (1975)
<i>Calothrix</i> spp.	Marshall Islands	Enewetak Atoll	9	Dead coral	5.4*	Mague and Holm-Hansen (1975)
<i>Nostoc</i> spp.	Marshall Islands	Enewetak Atoll	9	Mats	100.8*	Mague and Holm-Hansen (1975)
Cyanobacteria	SW Pacific	New Caledonia	21	Mats	16.4 ± 5.4	Charpy et al. (2007)

Table 5 continued

Benthic community	Region	Location	Depth (m)	Substrate	Total N ₂ fixation (mg N ₂ m ⁻² d ⁻¹)	Reference
(cyano)bacteria	NW Pacific	Sesoko, Ryukya Islands	1–3	Dead coral	2.4 ± 1.9	Casareto et al. (2008)
(cyano)bacteria	NW Pacific	Sesoko, Ryukya Islands	1–3	Sand	3.1 ± 1.8	Casareto et al. (2008)
Cyanobacteria	NW Pacific	Sesoko, Ryukya Islands	1–3	Mats	94.8 ± 7.4	Casareto et al. (2008)
Bacteria	Caribbean	San Salvador Island, Bahamas	1–25	Sand	0.1– 0.7	O’Neil and Capone (1989)
Bacteria	Caribbean	SW coast, Puerto Rico	1–25	Sand	0.7– 5.17	O’Neil and Capone (1989)
Bacteria	NW Atlantic	Bermuda (7 sites)	1–25	Sand	0.1– 4.0	O’Neil and Capone (1989)
Bacteria	Great Barrier Reef	5 islands near Townsville	1–25	Sand	0.2–1.4	O’Neil and Capone (1989)
Bacteria	Caribbean	St. Croix, US Virgin Islands	15–35	Diatom mat	3.4	King et al. (1990)
Bacteria	Caribbean	St. Croix, US Virgin Islands	15–35	Detritus	1.4	King et al. (1990)

All data (mean ± SE) in this table are based on acetylene reduction assays; we used a conversion factor of 4 to convert acetylene reduction to N₂ fixation

* Based on daytime N₂ fixation measurements only

Heterocystous cyanobacteria were also the main diazotrophs at our sites, especially in turfs growing in the upper 10 m of the reef, where *Dichothrix utahensis* dominated the turf communities (Fricke et al. 2011). Although most heterocystous cyanobacteria can fix N₂ in the dark, they usually exhibit higher N₂ fixation activity during daytime (Mullineaux et al. 1981; Staal et al. 2002). Hence, the dominance of heterocystous cyanobacteria in the turfs likely explains the observed higher daytime N₂ fixation.

The data were obtained by growing turf algae on artificial substrates incubated at different depths. Main advantages of this approach are (1) that the plastic bottles offered controlled and replicated conditions, as the turfs were grown on an identical substrate and were of similar size and age, (2) damage to algal tissues during sampling was minimal, and (3) the plastic strips could be easily handled during subsequent laboratory analyses. However, the use of an artificial substrate might have selected turf communities different from those found on the surrounding reef. We observed a similar composition of algae and cyanobacteria on the bottles as described by Fricke et al. (2011) for colonized turf algae on ceramic tiles in the same study area and after similar incubation times. The bottles were incubated for more than 7 weeks prior to sampling, which ensured the establishment of turf communities comprising all major taxa that were also observed in natural turfs, including Chlorophyta, Rhodophyta, Phaeophyceae, and Cyanobacteria (J. den Haan, pers. obs.). Hence, although future studies may consider a more detailed taxonomic analysis, the turf communities that developed on the bottles seemed representative of the natural turf assemblages on the reefs.

Non-heterocystous diazotrophic cyanobacteria, including *Lyngbya* spp. and *Oscillatoria* spp., prevailed in the turfs at 20–30-m depth (Fricke et al. 2011). Most non-heterocystous cyanobacteria avoid the problem of oxygen inhibition of nitrogenase by fixing nitrogen during the night (Gallon 1992; Bergman et al. 1997). Interestingly, however, in our study, the non-heterocystous cyanobacteria *Oscillatoria* spp. and *Symploca* spp. showed highest rates of N₂ fixation during the day. In a laboratory study, Fredriksson et al. (1998) demonstrated that the nitrogenase activity of *Symploca* PCC 8002 was indeed highest during daytime, consistent with our field observations.

In addition to cyanobacteria, many other Bacteria and Archaea are also capable of N₂ fixation and diazotrophic microbial communities are known to be highly diverse (Zehr et al. 1995; Olson et al. 1999; Severin and Stal 2010). Hence, it is possible that not only cyanobacteria but also other bacterial groups contributed to the N₂ fixation activity that we observed in algal turfs at Curaçao. However, the observation that the N₂ fixation activity was much higher during daytime than at night suggests that phototrophic organisms played a major role. Given the high abundance of diazotrophic cyanobacteria, it is therefore likely that most N₂ fixation activity in the turfs can be attributed to cyanobacteria.

Comparison with coral reefs around the world

The rates of N₂ fixation in the benthic cyanobacterial mats of *Oscillatoria* spp. and the solitary tufts of *Symploca* spp. on the reefs of Curaçao were higher than those of the turfs

and are also high compared to those of turfs, cyanobacteria, and bacteria on coral reefs in other parts of the world (Table 5). Similarly high N_2 fixation rates by tropical benthic cyanobacteria have been reported from the Marshall Islands (Wiebe et al. 1975), the Red Sea (Shashar et al. 1994), La Réunion (Casareto et al. 2008), and the Ryukyu Islands (Casareto et al. 2008). These findings are consistent with a recent review of benthic N_2 fixation on coral reefs, which revealed that microbial (cyanobacterial) mats tend to have higher rates of N_2 fixation than other benthic reef components (Cardini et al. 2014).

Algal turf communities comprise a mixture of diazotrophic and non-diazotrophic organisms, and it is therefore not surprising that their rates of N_2 fixation were lower than those of the cyanobacteria *Oscillatoria* spp. and *Symploca* spp. (Table 5). The N_2 fixation rates of the turfs in our study are in a similar range as those reported for other algal turf communities (Table 5). We note that the high N_2 fixation rates of turfs reported by Carpenter et al. (1991) and Williams and Carpenter (1998) were associated with high flow rates, which tend to enhance N_2 fixation activity, whereas our study was performed under stagnant conditions. Although turfs tend to have lower N_2 fixation rates than cyanobacterial mats, algal turfs are likely to play an important role in the nitrogen budget of coral reefs. In particular, cyanobacterial mats and tufts are of a relatively ephemeral nature (Nagle and Paul 1998; Kuffner and Paul 2001; Albert et al. 2005; Paul et al. 2005), whereas algal turfs are present throughout the year. Moreover, at least in our study area, algal turfs cover a much larger area of the reef than cyanobacterial mats (Table 1).

Relationship with coastal eutrophication

Several decades ago turfs were already recognized as important primary producers and potential N_2 fixers on coral reefs of the Caribbean (e.g., Adey and Goertemiller 1987). Since then, many Caribbean reefs have shown a decrease in coral cover (Gardner et al. 2003; Burke et al. 2011) and are now often dominated by turfs (Kramer 2003; Vermeij et al. 2010). Our results show that the coastal waters of Buoy 0 are more eutrophic than those of Playa Kalki. This conclusion is based on the fact that Buoy 0 shows significantly higher concentrations of nitrate and phosphate in the upper layers of the water column, a significantly higher N/P ratio, and its water is significantly more turbid in comparison with the more remote site of Playa Kalki. Moreover, Buoy 0 has a lower cover of hard corals and higher cover of the macroalga *L. variegata* and cyanobacterial mats than Playa Kalki. Over the past 40 yr, the live coral cover of shallow reefs in the vicinity of Buoy 0 has decreased by more than 50 % (Bak et al. 2005).

One might hypothesize that eutrophication would result in lower rates of N_2 fixation. High N availability (especially in the form of ammonium) usually decreases N_2 fixation, because the assimilation of combined N is energetically cheaper than acquiring N via the energy-costly N_2 fixation process (Zevenboom et al. 1981; Holl and Montoya 2005; Brauer et al. 2013). N enrichment indeed decreased N_2 fixation of benthic cyanobacteria in a field study on One Tree Island, Great Barrier Reef (Koop et al. 2001). Yet, in our study, we did not find differences in the rate of N_2 fixation between the turfs of Buoy 0 and Playa Kalki, despite the different trophic status of the two reef systems. Similarly, no relationship was found between N_2 fixation by benthic cyanobacteria and N enrichment from sewage discharge in the Kaneohe Bay, Hawaii (Hanson and Gundersen 1976). An explanation for the high N_2 fixation activity in these degraded reefs is that the utilization of N might be close to its supply rate, thus maintaining low concentrations of dissolved inorganic N in the overlying water column. The dense cover by turfs, macroalgae, and cyanobacterial mats may enable effective removal of externally supplied N. In addition, phytoplankton above the reefs may also rapidly assimilate N (Van Duyl et al. 2002; Furnas et al. 2005), and denitrification may contribute to N losses from the system (Gruber and Sarmiento 1997; Deutsch et al. 2007). Even at Buoy 0, near the city of Willemstad and its industrial harbour, the concentrations of ammonium and nitrate remained below $1 \mu\text{mol L}^{-1}$, which is sufficiently low for continued N_2 fixation by marine diazotrophic cyanobacteria (Mulholland et al. 2001; Agawin et al. 2007). These results mirror observations in eutrophic lakes, which also show high N_2 fixation rates by cyanobacteria during periods of low N availability, despite the high nutrient loads to these systems (Howarth et al. 1988; Scott et al. 2009).

Previously, we showed that the photosynthetic activity of the macroalga *L. variegata* is co-limited by N and P at both Buoy 0 and Playa Kalki (Den Haan et al. 2013), which is in agreement with the low concentrations of dissolved inorganic N and P at a N/P ratio close to the Redfield ratio of 16:1. Co-limitation by N and P has also been reported for primary producers in other marine ecosystems (Elser et al. 2007), including macroalgae on the Great Barrier Reef (Schaffelke and Klumpp 1998; Schaffelke 1999). The high N_2 fixation activity in combination with low ambient N and P concentrations and co-limitation by N and P indicates that, even at Buoy 0, the N input from N_2 fixation and land-derived sources does not yet meet ecosystem N demands. Hence, enhanced inputs of N and P will most likely lead to further expansion of cyanobacterial and algal communities. Dual reduction in both N and P loads from terrestrial sources may offer a valuable management strategy (Paerl 2009; Lewis et al. 2011) that may help to

curtail further degradation of these valuable reef ecosystems.

Acknowledgments We thank the reviewers for their helpful comments on the manuscript. We are most grateful to Hannah Brocke of the Max Planck Institute for Marine Microbiology for her help with the field incubations, Verena Brauer and Pieter Slot of the University of Amsterdam for their help with the gas chromatographic analyses, and Jan van Ooijen of the Royal Netherlands Institute for Sea Research (NIOZ) for the nutrient analyses. This research was funded by the European Union Seventh Framework Programme (P7/2007–2013) under Grant Agreement No. 244161 (Future of Reefs in a Changing Environment), and the Schure-Beijerinck-Popping Fund (SBK/JK 2011-31 KNAW, the Netherlands).

References

- Adey WH, Goertemiller T (1987) Coral reef algal turfs: master producers in nutrient poor seas. *Phycologia* 26:374–386
- Agawin NSR, Rabouille S, Veldhuis MJW, Servatius L, Hol S, van Overzee HMJ, Huisman J (2007) Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. *Limnol Oceanogr* 52:2233–2248
- Albert S, O’Neil JM, Udy JW, Ahern KS, O’Sullivan CM, Dennison WC (2005) Blooms of the cyanobacterium *Lyngbya majuscula* in coastal Queensland, Australia: disparate sites, common factors. *Mar Pollut Bull* 51:428–437
- Bak RPM, Nieuwland G, Meesters EH (2005) Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curaçao and Bonaire. *Coral Reefs* 24:475–479
- Barott K, Smith J, Dinsdale E, Hatay M, Sandin S, Rohwer F (2009) Hyperspectral and physiological analyses of coral-algal interactions. *PLoS ONE* 4:e8043
- Barott KL, Williams GJ, Vermeij MJA, Harris J, Smith JE, Rohwer FL, Sandin SA (2012) Natural history of coral-algae competition across a gradient of human activity in the Line Islands. *Mar Ecol Prog Ser* 460:1–12
- Benavides M, Bronk DA, Agawin NSR, Pérez-Hernández MD, Hernández-Guerra A, Aristegui J (2013) Longitudinal variability of size-fractionated N₂ fixation and DON release rates along 24.5°N in the subtropical North Atlantic. *J Geophys Res Oceans* 118:3406–3415
- Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N₂ fixation by non-heterocystous cyanobacteria. *FEMS Microbiol Rev* 19:139–185
- Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Mar Ecol Prog Ser* 31:279–305
- Brauer VS, Stomp M, Rosso C, van Beusekom SAM, Emmerich B, Stal LJ, Huisman J (2013) Low temperature delays timing and enhances the cost of nitrogen fixation in the unicellular cyanobacterium *Cyanothece*. *ISME J* 7:2105–2115
- Burke L, Reyntar K, Spalding M, Perry A (2011) Reefs at risk revisited. World Resources Institute, Washington, D.C. 130 pp
- Cardini U, Bednarz VN, Foster RA, Wild C (2014) Benthic N₂ fixation in coral reefs and the potential effects of human-induced environmental change. *Ecol Evol* 4:1706–1727
- Carpenter RC (1985) Relationships between primary production and irradiance in coral reef algal communities. *Limnol Oceanogr* 30:784–793
- Carpenter RC, Williams SL (2007) Mass transfer limitation of photosynthesis of coral reef algal turfs. *Mar Biol* 151:435–450
- Carpenter RC, Hackney JM, Adey WH (1991) Measurements of primary productivity and nitrogenase activity of coral reef algae in a chamber incorporating oscillatory flow. *Limnol Oceanogr* 36:40–49
- Casareto BE, Charpy L, Langlade MJ, Suzuki T, Ohba H, Niraula M, Suzuki Y (2008) Nitrogen fixation in coral reef environments. *Proc 11th Int Coral Reef Symp* 2:890–894
- Charpy L, Alliod R, Rodier M, Golubic S (2007) Benthic nitrogen fixation in the SW New Caledonia lagoon. *Aquat Microb Ecol* 47:73–81
- Charpy L, Casareto BE, Langlade MJ, Suzuki Y (2012) Cyanobacteria in coral reef ecosystems: a review. *J Mar Biol* 2012:1–9
- Charpy L, Palinska KA, Casareto B, Langlade MJ, Suzuki Y, Abed RMM, Golubic S (2010) Dinitrogen-fixing cyanobacteria in microbial mats of two shallow coral reef ecosystems. *Microb Ecol* 59:174–186
- Charpy-Roubaud C, Charpy L, Larkum A (2001) Atmospheric dinitrogen fixation by benthic communities of Tikehau Lagoon (Tuamotu Archipelago, French Polynesia) and its contribution to benthic primary production. *Mar Biol* 139:991–998
- Cheal AJ, MacNeil MA, Cripps E, Emslie MJ, Jonker M, Schaffelke B, Sweatman H (2010) Coral macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. *Coral Reefs* 29:1005–1015
- Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling eutrophication: nitrogen and phosphorus. *Science* 323:1014–1015
- Davey M, Holmes G, Johnstone R (2008) High rates of nitrogen fixation (acetylene reduction) on coral skeletons following bleaching mortality. *Coral Reefs* 27:227–236
- Delgado O, Lapointe BE (1994) Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. *Coral Reefs* 13:151–159
- Den Haan J, Huisman J, Dekker F, ten Brinke JL, Ford AK, van Ooijen J, van Duyl FC, Vermeij MJA, Visser PM (2013) Fast detection of nutrient limitation in macroalgae and seagrass with nutrient-induced fluorescence. *PLoS ONE* 8(7):e68834
- Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP (2007) Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 445:163–167
- Diaz-Pulido G, McCook LJ (2002) The fate of bleached corals: patterns and dynamics of algal recruitment. *Mar Ecol Prog Ser* 232:115–128
- Done TJ (1992) Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia* 247:121–132
- Dron A, Rabouille S, Claquin P, Le Roy B, Talec A, Sciandra A (2012) Light–dark (12:12) cycle of carbon and nitrogen metabolism in *Crocospaera watsonii* WH8501: relation to the cell cycle. *Environ Microbiol* 14:967–981
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142
- Fredriksson C, Malin G, Siddiqui PJ, Bergman B (1998) Aerobic nitrogen fixation is confined to a subset of cells in the non-heterocystous cyanobacterium *Symplaca* PCC 8002. *New Phytol* 140:531–538
- Fricke A, Teichberg M, Beilfuss S, Bischof K (2011) Succession patterns in algal turf vegetation on a Caribbean coral reef. *Botanica Marina* 54:111–126
- Furnas M, Mitchell A, Skuza M, Brodie J (2005) In the other 90%: phytoplankton responses to enhanced nutrient availability in the Great Barrier Reef Lagoon. *Mar Pollut Bull* 51:253–265
- Gallon JR (1992) Reconciling the incompatible: N₂ fixation and O₂. *New Phytol* 122:571–609
- Gardner T, Côté I, Gill J, Grant A, Watkinson A (2003) Long term region-wide declines in Caribbean corals. *Science* 301:958–960
- Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis. *Verslag Chemie* pp 1:419 pp

- Gruber N, Sarmiento JL (1997) Global patterns of marine nitrogen fixation and denitrification. *Global Biogeochem Cycles* 11:235–266
- Haas A, El-Zibdah M, Wild C (2010) Seasonal monitoring of coral–algae interactions in fringing reefs of the Gulf of Aqaba, Northern Red Sea. *Coral Reefs* 29:93–103
- Hanson RB, Gundersen KR (1976) Bacterial nitrogen fixation in a polluted coral reef flat ecosystem, Kaneohe Bay, Oahu, Hawaiian Islands. *Pac Sci* 30:385–393
- Helder W, De Vries RTP (1979) An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters. *Neth J Sea Res* 13:154–160
- Holl CM, Montoya JP (2005) Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (Cyanobacteria). *J Phycol* 41:1178–1183
- Howarth RW, Marino R, Cole JJ (1988) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnol Oceanogr* 33:688–701
- Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265:1547–1551
- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskij N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr Biol* 17:360–365
- King GM, Carlton RG, Sawyer TE (1990) Anaerobic metabolism and oxygen distribution in the carbonate sediments of a submarine canyon. *Mar Ecol Prog Ser* 58:275–285
- Klumpp DW, McKinnon D, Daniel P (1987) Damsel fish territories: zones of high productivity on coral reefs. *Mar Ecol Prog Ser* 40:41–51
- Kohler KE, Gill SM (2006) Coral Point Count with Excel extensions (CPCe): a Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Comput Geosci* 32:1259–1269
- Koop K, Booth D, Broadbent A, Brodie J, Bucher D, Capone D, Coll J, Dennison W, Erdmann M, Harrison P, Hoegh-Guldberg O, Hutchings P, Jones GB, Larkum AWD, O’Neil J, Steven A, Tentori E, Ward S, Williamson J, Yellowlees D (2001) ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. *Mar Pollut Bull* 42:91–120
- Kramer PA (2003) Synthesis of coral reef health indicators for the western Atlantic: results of the AGRRA program (1997–2000). *Atoll Res Bull* 496:1–55
- Kuffner IB, Paul VJ (2001) Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam. *Mar Ecol Prog Ser* 222:63–72
- Larkum AWD, Kennedy IR, Muller WJ (1988) Nitrogen fixation on a coral reef. *Mar Biol* 98:143–155
- Larned ST (1998) Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. *Mar Biol* 132:409–421
- Lewis WM Jr, Wurtsbaugh WA, Paerl HW (2011) Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environ Sci Technol* 45:10300–10305
- Littler MM, Littler DS, Titlyanov EA (1991) Comparisons of N- and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles Archipelago: a test of the relative-dominance paradigm. *Coral Reefs* 10:199–209
- Logan D, Townsend KA, Townsend K, Tibbetts IR (2008) Meiofauna sediment relations in leeward slope turf algae of Heron Island reef. *Hydrobiologia* 610:269–276
- Mague TH, Holm-Hansen O (1975) Nitrogen fixation on a coral reef. *Phycologia* 14:87–92
- McManus JW, Polsenberg JF (2004) Coral-algal phase shifts on coral reefs: ecological and environmental aspects. *Prog Oceanogr* 60:263–279
- Mulholland MR, Ohki K, Capone DG (2001) Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (Cyanobacteria). *J Phycol* 37:1001–1009
- Mulholland MR, Bronk DA, Capone DG (2004) Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101. *Aquat Microb Ecol* 37:85–94
- Mulholland MR, Bernhardt PW, Heil CA, Bronk DA, O’Neil JM (2006) Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. *Limnol Oceanogr* 51:1762–1776
- Mullineaux PM, Gallon JR, Chaplin AE (1981) Acetylene reduction (nitrogen fixation) by cyanobacteria grown under alternating light-dark cycles. *FEMS Microbiol Lett* 10:245–247
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Nagle DG, Paul VJ (1998) Chemical defense of a marine cyanobacterial bloom. *J Exp Mar Bio Ecol* 225:29–38
- Nyström M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment. *Trends Ecol Evol* 15:413–417
- Olson JB, Litaker RW, Paerl HW (1999) Ubiquity of heterotrophic diazotrophs in marine microbial mats. *Aquat Microb Ecol* 19:29–36
- O’Neil JM, Capone DG (1989) Nitrogenase activity in tropical carbonate marine sediments. *Mar Ecol Prog Ser* 56:145–156
- Paerl HW (2009) Controlling eutrophication along the freshwater–marine continuum: dual nutrient (N and P) reductions are essential. *Estuaries Coasts* 32:593–601
- Paul VJ, Thacker RW, Banks K, Golubic S (2005) Benthic cyanobacterial bloom impacts the reefs of South Florida (Broward County, USA). *Coral Reefs* 24:693–697
- Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim Biophys Acta* 975:384–394
- Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquat Bot* 82:222–237
- Schaffelke B (1999) Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. *Mar Ecol Prog Ser* 182:305–310
- Schaffelke B, Klumpp DW (1998) Nutrient-limited growth of the coral reef macroalga *Sargassum baccularia* and experimental growth enhancement by nutrient addition in continuous flow culture. *Mar Ecol Prog Ser* 164:199–211
- Schneegurt MA, Sherman DM, Nayar S, Sherman LA (1994) Oscillating behavior of carbohydrate granule formation and dinitrogen fixation in the cyanobacterium *Cyanothece* sp. strain ATCC 51142. *J Bacteriol* 176:1586–1597
- Scott JT, Stanley JK, Doyle RD, Forbes MG, Brooks BW (2009) River-reservoir transition zones are nitrogen fixation hot spots regardless of ecosystem trophic state. *Hydrobiologia* 625:61–68
- Severin I, Stal LJ (2008) Light dependency of nitrogen fixation in a coastal cyanobacterial mat. *ISME J* 2:1077–1088
- Severin I, Stal LJ (2010) Spatial and temporal variability in nitrogenase activity and diazotrophic community composition in coastal microbial mats. *Mar Ecol Prog Ser* 417:13–25
- Shashar N, Feldstein T, Cohen Y, Loya Y (1994) Nitrogen fixation (acetylene reduction) on a coral reef. *Coral Reefs* 13:171–174

- Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science* 221:669–671
- Staal M, Hekkert STL, Herman P, Stal LJ (2002) Comparison of models describing light dependence of N₂ fixation in heterocystous cyanobacteria. *Appl Environ Microb* 68:4679–4683
- Stal LJ (1988) Nitrogen fixation in cyanobacterial mats. *Methods Enzymol* 167:474–484
- Steneck RS, Dethier MN (1994) A functional group approach to the structure of algal-dominated communities. *Oikos* 69:476–498
- Stewart WD, Fitzgerald GP, Burris RH (1967) In situ studies on N₂ fixation using the acetylene reduction technique. *Proc Natl Acad Sci USA* 58:2071–2078
- Van den Hoek C, Cortel-Breeman A, Wanders J (1975) Algal zonation in the fringing coral reef of Curaçao, Netherlands Antilles, in relation to zonation of corals and gorgonians. *Aquat Bot* 1:269–308
- Van Duyl F, Gast G, Steinhoff W, Kloff S, Veldhuis M, Bak R (2002) Factors influencing the short-term variation in phytoplankton composition and biomass in coral reef waters. *Coral Reefs* 21:293–306
- Vermeij MJA, van Moorselaar I, Engelhard S, Hörnlein C, Vonk SM, Visser PM (2010) The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. *PLoS One* 5:e14312
- Villbrandt M, Stal LJ, Krumbein WE (1990) Interactions between nitrogen fixation and oxygenic photosynthesis in a marine cyanobacterial mat. *FEMS Microbiol Lett* 74:59–71
- Wangpraseurt D, Weber M, Røy H, Polerecky L, de Beer D, Nugues MM (2012) In situ oxygen dynamics in coral-algal interactions. *PLoS One* 7:e31192
- Webb KL, DuPaul WD, Wiebe W, Sottile W, Johannes RE (1975) Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. *Limnol Oceanogr* 20:198–210
- Wiebe WJ, Johannes RE, Webb KL (1975) Nitrogen fixation in a coral reef community. *Science* 188:257–259
- Wilkinson CR, Sammarco PW (1983) Effects of fish grazing and damselfish territoriality on coral reef algae. II. nitrogen fixation. *Mar Ecol Prog Ser* 13:15–19
- Wilkinson CR, Williams DMB, Sammarco PW, Hogg RW, Trott LA (1984) Rates of nitrogen fixation on coral reefs across the continental shelf of the central Great Barrier Reef. *Mar Biol* 80:255–262
- Williams SL, Carpenter RC (1997) Grazing effects on nitrogen fixation in coral reef algal turfs. *Mar Biol* 130:223–231
- Williams SL, Carpenter RC (1998) Effects of unidirectional and oscillatory water flow on nitrogen fixation (acetylene reduction) in coral reef algal turfs, Kaneohe Bay, Hawaii. *J Exp Mar Bio Ecol* 226:293–316
- Zehr JP (2011) Nitrogen fixation by marine cyanobacteria. *Trends Microbiol* 19:162–173
- Zehr JP, Mellon M, Braun S, Litaker W, Steppe T, Paerl HW (1995) Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. *Appl Environ Microbiol* 61:2527–2532
- Zeller DC (1988) Short-term effects of territoriality of a tropical damselfish and experimental exclusion of large fishes on invertebrates in algal turfs. *Mar Ecol Prog Ser* 44:85–93
- Zevenboom WJ, Van der Does J, Bruning K, Mur LR (1981) A non-heterocystous mutant of *Aphanizomenon flos-aquae*, selected by competition in light-limited continuous culture. *FEMS Microbiol Lett* 10:11–16
- Zuckermann H, Staal M, Stal LJ, Reuss J, Te Lintel HS, Harren F, Parker D (1997) On-line monitoring of nitrogenase activity in cyanobacteria by sensitive laser photoacoustic detection of ethylene. *Appl Environ Microb* 63:4243–4251