Little Things Become Big

Drivers and impacts of benthic cyanobacterial blooms on coral reefs

Hannah J Brocke

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Dissertation zur Erlangung des akademischen Grades eines
Doktors der Naturwissenschaften
- Dr. rer. nat. -

Am Fachbereich 2 (Biologie/ Chemie)
der Universität Bremen

Vorgelegt von
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Bremen 2014

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Dedicated to all optimists who search for solutions and don't give up trying.

"In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed."

Charles Darwin

"Every [coral reef] ecosystem I studied is unrecognizably different from when I started. I have a son who is 30, and I used to take him snorkeling on the reefs of Jamaica to show him all the beautiful corals there. I have a daughter who is 17. I can't show her anything but seaweed."

Jeremy Jackson

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Declaration

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Erklärung gemäss §6 Abs. 5 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche.

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel: Little Things Become Big – Drivers and impacts of benthic cyanobacterial blooms on coral reefs –

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Acknowledgements

Vielen herzlichen Dank Kai Bischof for being my Doktorvater and 1st Gutachter. Thank you for always taking time, listening carefully and giving friendly advises. Those were very important to me.

Merci beaucoup Maggy Nugues for being my 2nd Gutachter and more importantly my project leader, giving me this PhD position and putting me on track to unravel the mystery of benthic cyanobacteria.

Heel hartelijk bedankt Dirk de Beer for being my examiner and having me in your group. Thank you for your helpful advises, idea contributions and giving me all this nice equipment for this project.

Vielen Dank Claudio Richter for being my examiner and finding great interest in my work. I am glad I have you in my committee.

Thank you very much Peter Mumby and Rosanna Griffith-Mumby for helping us in turbulent times; and you, Peter, for all the great project discussions. Also a big thank you to all FORCE members. It was a great pleasure to meet you all and be part of your group.

Heel hartelijk bedankt Petra Visser for all the great discussions and your support. You are definitively a paragon of a great scientist to me.

Hartstikke bedankt Mark Vermeij for last minute advises, ideas, support and great discussions.

Mascha danki Carlos and Oscar for spending many hours fixing the boat motor with me, giving me advise on broken cars or the latest self-build equipment. I feel that during my PhD project I also received half a mechanics education from you two.

A big thank you also to Kristin Marhaver as well as to Ashwin Engelen, Forest Rohwer, Stuart Sandin for fruitful discussions and motivational feedback.

Heel erg bedankt Paul Stokkermans for finding solutions for all the administrative problems I was facing on Curaçao. Visa, boat papers, licenses, shipments, sample export… even calling ministers was no problem for you.

Vielen Dank Benjamin Müller for a helpful hand and discussions in the field.

I also thanks all other people from Carmabi, Art & Nature Inn, especially Gerardine Kuijpers and Shirah, as well as Diveversity who made my stay a great experience on Curaçao.

D`akujem moc Lubos for all the great discussions, especially in the first month of my PhD. Thank you also for having a great trust in me that I can understand your mathematics and programs.

הברה הדות Danny Ionescu for all your help. I really appreciated that you always took time and gave me quick, fast and great advices!

لكــم الشـــكر Raeid Abed for teaching me a lot of writing skills, sharing the same fascination towards cyanobacteria, and discussing world politics. I very much enjoyed working with you and going for lunch.

Vielen Dank Miriam Weber for your great motivation and taking all the emergency calls from the field.

Thank you very much Timothy G. Ferdelman for your time and very helpful advises.

X

Abstract

Cyanobacteria are an inherent part of coral reefs and play an important role in nutrient cycling and reef functioning. Coral reefs are nutrient poor ecosystems, hence the ability of cyanobacteria to fix and provide nitrogen is proposed to at least drive part of the reefs' overall primary productivity. For the past couple of decades, many reefs around the world have shifted from coral-dominated to algae-dominated ecosystems. As live coral cover declines on the reefs, cyanobacteria form dense and widespread mats on the seafloor. This is, unfortunately, a worldwide phenomenon. Anthropogenic impacts (i.e., notably eutrophication and global climate change) are thought to augment the proliferation of the benthic cyanobacterial mats on many reefs, yet there are no studies that in detail explain benthic cyanobacterial growth on a mechanistic scale, i.e., sources and transport mode of nutrients. Furthermore, benthic cyanobacterial mats are already known to have negative impacts on coral reefs, for example they hamper coral larval settlement, cause local hypoxia via black band disease, and produce toxins that will lead to minimal grazing of the mats by herbivores. As benthic cyanobacterial mats continue to proliferate on the reef, additional research is vital to better understand their functioning and capabilities as well as their influence on the reef ecosystem as a whole.

The overall objective of this thesis is to better understand the recent successfulness of benthic cyanobacteria mats on coral reefs. Specific goals were to investigate (1) the mechanisms driving the proliferation of cyanobacterial mats, (2) their impact and functioning on the coral reef ecosystem, especially when they reach high abundances and, (3) to implement the findings into coral reef management programs. A variety of methods were used throughout this thesis including (i) monitoring surveys, (ii) examination of environmental parameters (e.g., nutrients, hydrodynamics, seawater temperature, organic matter) at reefs with high and low cyanobacterial abundances, and (iii) the investigation of the species composition inside the mats as well as (iv) its physiological functions using, for example, microsensors. Furthermore, different experimental approaches were used to study the growth response of the benthic cyanobacterial mats, nitrogen fixation capability, net fluxes (O_2, DIC, nutrients, DOC) between mats and water column, nutrient uptake rates and primary productivity.

In **Chapter 2** organic matter degradation was identified to fuel the growth of benthic cyanobacterial mats on the reef and act as a mediator to transport nutrients from land to the reef. Coastal urbanization and reduced hydrodynamics can mediate the accumulation of particulate organic matter on the seafloor, which subsequently fuels the growth of these mats, even without elevated nutrients in the seawater (MS I).

In **Chapter 3-6** the cyanobacterial species present within the mats were found to already be part of the ecosystem, as they were, but also still are harbored by the turf communities (MS II). Furthermore, large-sized mats fixed vast amounts of nitrogen in comparison to other benthic and pelagic diazotrophic organisms on the reef (MS III). *In situ* sediment chamber experiments further revealed that cyanobacterial

mats, in comparison to other reef phototrophic organisms, excreted high amounts of dissolved organic carbon into the water column during both the day and especially the nighttime (MS IV). Results from our N_2 fixation and dissolved organic matter release studies indicate that benthic cyanobacterial mats play a prominent role in active coral reef degradation. In MS IV benthic cyanobacterial mats were found to rapidly take up newly available nutrients in comparison to other reef phototrophic organisms (benthic algae, corals and phytoplankton). Hence, the benthic cyanobacterial mats probably have a competitive advantage over these other organisms during episodic nutrient enrichment (e.g., land runoff after heavy rainfall). In MS V various reef phototrophic organisms were compared (i.e., benthic algae, cyanobacteria, corals and phytoplankton) and highest individual primary productivity rates were recorded for benthic cyanobacterial mats. Finally, in MS VII Beggiatoaceae were observed to migrate in cyanobacterial mats. Beggiatoaceae are bacteria known to grow on sites enriched with organic matter and hydrogen sulfide when anoxic conditions are present. They are considered as indicator species for organic matter enrichment in aquatic environments, for example, close to sewage outlets (MS VI).

Chapter 7 summarizes the main findings of this thesis and explains how these results can be implemented into active coral reef management programs. The increasing human population, accompanied by (nutrient) pollution, the rise in seawater temperatures and the continuous degradation of coral reef ecosystems will contribute to the (continued) proliferation of benthic cyanobacterial mats in the future. Management approaches that prevent the input of nutrients/organic matter generated on land and the restoration of food webs (increase of herbivores) all aid in the reduction of benthic cyanobacterial proliferation on the reef (MS VII). Notably the input of organic matter into the ecosystems poses an immediate threat to the ecosystem and should therefore be included in future monitoring and management programs.

In conclusion, this research presents a missing link explaining - via a mechanistic approach - the recent proliferation of benthic cyanobacterial mats on coral reefs. This thesis indicates that benthic cyanobacterial mats likely have a large impact on the functioning of the reef, especially due to their vast N_2 fixation capabilities and the release of dissolved organic carbon into the water column. Further, benthic cyanobacterial mats are quick in taking up newly available nutrients and have high primary productivity rates, which all contribute to their recent successfulness on the reef. Results from this thesis suggest that nutrient and organic matter reduction is essential to prevent benthic cyanobacterial blooms from occurring on the reef.

Zusammenfassung

Cyanobakterien sind ein fester Bestandteil von Korallenriffen und spielen eine wichtige Rolle in dem Nährstoffkreislauf und der Funktionalität der Riffe. Korallenriffe sind nährstoffarme Ökosyteme, daher hat die Stickstofffixierung der Cyanobakterien und der damit eingetragenen neuen Stickstoffverbindungen im Riffsystem große Bedeutung für deren Primärproduktion. Viele Korallenriffe haben sich in den letzten Jahrzehnten durch menschliche Einflüsse von Korallen- zu Algen-dominierten Ökosystemen gewandelt. Einhergehend mit dem weltweiten Verlust der Korallen haben sich großflächig Cyanobakterienmatten gebildet. Diese sollen von anthropogenen Einflüssen, wie Nährstoffeinträge und Klimawandel, angetrieben werden, doch detaillierte Erklärungen, wie die Herkunft der Nährstoffe und Transportwege, sind noch unklar. Es ist bekannt, dass Cyanobakterienmatten negative Auswirkungen auf die Korallenriffe haben. Die Matten können das Einnisten von Korallenlarven verhindern, bei der Black-Band-Krankheit lokale Hypoxie erzeugen und Toxine produzieren, die die Matten zum Beispiel als Nahrung für Herbivore ungenießbar machen. Die Ausbreitung der Matten erfordert dringend weitere Erkenntnisse darüber, wie die Matten das Ökosystem beeinflussen und verändern.

Das grundlegende Ziel dieser Doktorarbeit ist, den jetzigen Erfolg von Cyanobakterienmatten in den Korallenriffen zu verstehen. Die Forschungsziele waren (1) den Mechanismus der Ausbreitung von den Matten herauszufinden, (2) deren Einflüsse auf das Korallenökosystem zu charakterisieren, vor allem bei großflächigem Vorkommen und (3) diese Forschungsergebnisse zu nutzen um eine Stellungnahme zum Schutz und Management der Korallenriffe zu verfassen. Ein Vielzahl von Methoden wurde angewandt, u.a. (i) verschiedene Monitoringprogramme, (ii) Ermittlungen von Umweltparametern (z.B. Nährstoffe, Wasserbewegungen, Temperatur, organisches Material) an Riffen mit geringer und hoher Verbreitung von Matten, (iii) Untersuchungen von Cyanobakterienarten in den Matten und (iv) Messung der physiologischen Funktionen der Matten, z.B. durch Mikrosensoren. Darüber hinaus wurden verschiedene experimentelle Ansätze verwendet, um die Ursachen für das Wachstum der Matten zu untersuchen, sowie Stickstofffixierungsraten, Stoffflüsse zwischen Wassersäule und Matten, Nährstoffaufnahme, und die Primärproduktion zu charakterisieren.

Im **2. Kapitel** wird dargestellt wie der Abbau von organischem Material das Wachstum von Cyanobakterienmatten im Riff fördert und als Transportmechanismus für Nährstoffe vom Land zum Riff dient. Außerdem wird gezeigt, dass die Bevölkerung der Küste und geringe Hydrodynamiken im Wasser die Anreicherung von organischem Material auf dem Meeresboden verstärken, welche wiederum die Verbreitung der Matten beeinflusst.

In den **Kapiteln 3-6** wird illustriert, dass mattenformende Cyanobakterien in Riffen schon immer ein Teil des Ökosystems waren, indem sie früher wie heute in den Turfalgengesellschaften existieren (MS II). Darüber hinaus zeigt die Studie, dass großflächige Matten relativ hohe Stickstofffixierungsraten im Vergleich zu anderen benthischen und pelagischen Stickstofffixierern im Riff haben (MS II). Unter

Verwendung von *in situ* Sedimentkammern konnte außerdem gezeigt werden, dass Cyanobakterienmatten im Vergleich zu anderen phototrophen Organismen (Korallen, Phytoplankton, Algen) eine große Menge an gelöstem organischen Kohlenstoff (DOC) in die Wassersäule abgeben, sowohl tags als auch nachts (MS III). Die Ergebnisse der Stickstofffixierung und der DOC Absonderung zeigen, dass die Matten, vor allem bei großflächigem Vorkommen, den Zerfall der Riffe weiter fördern könnten. In den Experimenten von MS IV wiesen Cyanobakterienmatten eine der höchsten Nährstoffaufnahmen im Vergleich zu anderen Primärproduzenten (Korallen, Phytoplankton, Algen) auf. Dies kann bei kurzzeitig verfügbaren Nährstoffeinträgen ins Riffsystem einen kompetitiven Vorteil haben besonders während Zeiten episodischer Nährstoffspeicherung (z.B. nach schweren Regenfällen und Abfluss vom Land). Auch im Vergleich der individuellen Primärproduktivität zwischen verschiedenen Rifforganismen zeigen die Matten hohe Produktivitätsraten (MS V). MS VI beschreibt eine Beobachtung horizontaler Wanderbewegungen von Beggiatoa. Diese Bakterien werden auch als Indikatoren für Umweltverschmutzungen verwendet, da sie zum Leben hohe Konzentrationen von organischem Material und Schwefelwasserstoff benötigen, und daher in verschmutzten Gebieten, z. B. bei Abwasserausflüssen, wachsen.

Kapitel 7 fasst die grundlegenden Erkenntnisse dieser Doktorarbeit zusammen und formuliert Empfehlungen für den Schutz und dem Management von Korallenriffen im Bezug auf Cyanobakterienmatten. Die wachsende Bevölkerung und der damit einhergehende Eintrag von Nährstoffen, der Anstieg der Wassertemperatur sowie der fortschreitende Zerfall der Korallenriffe tragen u.a. dazu bei, dass sich Cyanobakterienmatten wahrscheinlich auch in Zukunft weiter verbreiten werden. Wichtige Management- Ansätze wären u.a. den Eintrag von Nährstoffen/organischem Material in das Meer zu reduzieren und die natürlichen Nahrungsnetze in dem Ökosystem wieder aufzubauen.

Zusammenfassend enthüllt diese Doktorarbeit ein bis jetzt fehlendes Bindeglied zum Verständnis der Verbreitung der Cyanobakterienmatten in Korallenriffen. Außerdem weisen verschiedene Studien dieser Arbeit darauf hin, dass die Cyanobakterienmatten ein großes Potential besitzen den weiteren Zerfall der Korallenriffe zu fördern, besonders hinsichtlich der hohen Stickstofffixierungsraten und der Absonderung von gelöstem organischen Kohlenstoff in die Wassersäule. Des Weiteren können die Matten schnell Nährstoffe aufnehmen und besitzen eine hohe Primärproduktion gegenüber anderen Rifforganismen. Dies alles trägt zu dem Erfolg und der vermutlich fortlaufenden Verbreitung der Matten bei. Die Reduzierung von Nährstoffeinträgen und organischem Material ist erforderlich, um Cyanobakterienblüten -und damit einer Ausbreitung der Matten- vorzubeugen.

1. Introduction

1.1. Overview of cyanobacteria and their life in microbial mats

1.1.1. Cyanobacteria

Cyanobacteria are colloquially known as blue-green algae, yet this naming is incorrect as they belong to the domain Bacteria. Cyanobacteria are quantitatively among the most dominant groups of organisms on earth (Whitton & Potts 2012). They occur in all kinds of freshwater, marine and terrestrial ecosystems across all latitudes, and are one of the oldest life-forms on earth, dating back to 3.5 billion years (Awramik et al. 1983). Fig. 1 shows how similar microfossils of cyanobacteria are to present-day cyanobacteria (Schopf 2000). A major reason why cyanobacteria have been so successful is their capability to fix dinitrogen (N_2), an element that is highly abundant across both aquatic and terrestrial ecosystems. Other organisms, such as algae and corals, are dependent on other forms of nitrogen supply (e.g. NO_3^+, NO_2^+, NH_4^+, amino acids), which in most environments limits their growth and abundance.

Fig. 1: Filamentous microfossils of the Neoproterozoic ('850-million-year-old) Bitter Springs chart of central Australia (Schopf 2000)

Cyanobacteria were the first oxygenic phototrophs on earth and released oxygen in the atmosphere. Cyanobacteria are responsible for the transition from an anaerobic to aerobic life, and therefore had the largest impact on the biotic evolution on earth, more than any other group of organism (Cloud 1976). Paradoxically, oxygen inhibits parts of the cyanobacterial metabolism, including photosynthesis and nitrogen fixation. Therefore, cyanobacteria have developed a variety of strategies to cope with oxic environments to ensure optimal growth and proliferation (Paerl 1996). Cyanobacteria further have a large variety of secondary metabolites (Gademann & Portmann 2008). The most commonly known secondary metabolites are toxins, that for example can inhibit grazing pressure. Other metabolites, such as iron chelators, provide bioavailable nutrients when cyanobacteria are growth limited. This allows them to survive in extreme habitats, such as the open ocean, hypersaline lakes and alkaline hot springs (Gademann & Portmann 2008). Apart

from developing biochemical and structural adaptations, cyanobacteria have developed consortial and symbiotic associations with other microorganisms, plants and animals (Paerl 1996). One of the most successful unions are consortia with different taxa of bacteria forming microbial mats.

> Cyanobacteria are oxygenic phototrophs capable of dinitrogen fixation and the production of secondary metabolites - thereby optimizing their survival and growth in the most extreme environments.

1.1.2. Microbial mats - benthic cyanobacteria and their associates

Benthic cyanobacterial mats are consortia of microbes dominated by cyanobacteria, yet also consist of other microorganisms, such as aerobic heterotrophic bacteria, sulfate reducing bacteria and sulfur bacteria. The composition of the mat varies strongly with the type of environment, hence mats are often taxonomically complex. Interestingly, cyanobacterial mats have similar functional groups as shown in Fig. 2 (Paerl et al. 2000). Within the mats, cyanobacteria are the

Fig. 2: Biogeochemistry of cyanobacterial mats. Boxes represent functional groups of bacteria; arrows indicate the cycling of carbon, oxygen and sulfur. On the left-hand side, oxygen and sulfide micro profiles illustrate concentration changes within the mats during day and nighttime. Schematic is modified from Fenchel and Finlay (1995) and Jones and Marais (1995).

main primary producers and consequently are the driving force of the mat. They can provide oxygen, organic carbon and fixed nitrogen via excretion, lysis and decomposition to the rest of the microbial community (Paerl 1996, Paerl & Pinckney 1996). Stal (1995) proposed that CO_2 depletion with subsequent photorespiration by the cyanobacteria provides the major source of organic carbon to the mat community. Most released organic carbons are simple compounds including glycolate, acetate, ethanol and lactate. Aerobic heterotrophic bacteria subsequently
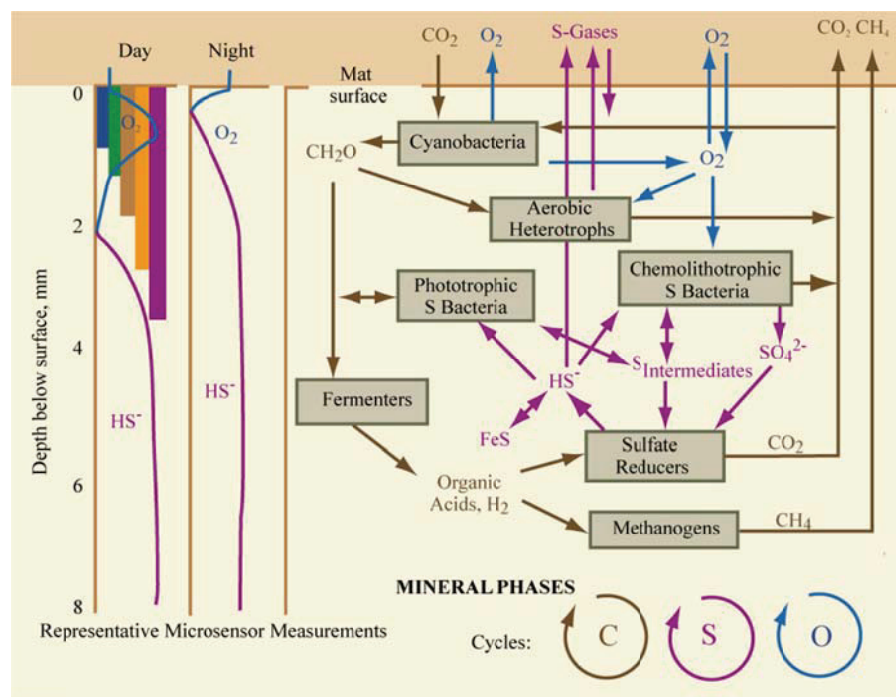
respire the organic carbon, which results in oxygen depletion within the mat and the regeneration of CO_2. In the deeper layer of the mat, organic carbon is degraded anaerobically via dissimilatory sulfate reduction by sulfate reducing bacteria (Jorgensen et al. 1992, Canfield & Des Marais 1993). As a result, sulfate-reducing bacteria provide sulfide to chemolithotrophic sulfur bacteria and phototrophic sulfur bacteria which can oxidize sulfide back to sulfate or sulfur intermediates (i.e., Van Gemerden (1993)). Additionally, organic carbons can be anaerobically fermented by anaerobic fermenting bacteria. The resulting organic compounds can be utilized by sulfate reduction bacteria or methanogenic bacteria (Jorgensen et al. 1992). All processes depicted in Figure 2 are tightly connected with one another, resulting in a complex cycling of elements within the mats. All metabolic processes result in steep microgradients of, for example, oxygen, sulfide and pH that change rapidly within the depth of the mat, but also during the day and nighttime (Revsbech et al. 1983, Paerl & Pinckney 1996) (for details, see Figure 2).

Since cyanobacterial mats have a steep biochemical gradient (e.g., aerobic to anaerobic conditions), multiple bacterial functional groups can coexist and interact metabolically, which can result into an almost self-sustaining microhabitat (Paerl et al. 2000). However, most cyanobacterial mats are still dependent on external nutrients (e.g., carbon, nitrogen, phosphorus) which can be derived by trapping particles (i.e., detritus and mineral particles) (Watkinson et al. 2005), and in the case of nitrogen via N_2 fixation (Charpy et al. 2010, Charpy et al. 2012a). Nevertheless, cyanobacterial mats are often found in physically and chemically stressed environments including nutrient-depleted, hypersaline, calcified, desiccated and high irradiance environments, but they are also pioneers in rapidly changing ecosystems (Paerl et al. 2000, Paul 2008). Interestingly, during the last two decades, the abundance of cyanobacterial mats has increased dramatically on many coral reefs around the world (Kuffner & Paul 2001, Albert et al. 2005, Paul et al. 2005).

> Benthic cyanobacterial mats are consortia of bacteria with combined metabolic activities resulting in steep microgradients of e.g. oxygen and sulfide. The driving force for the bacterial community is the primary production or photorespiration of cyanobacteria via production of organic carbon, fixed nitrogen and oxygen. The metabolic interactivity in the mat can lead to almost self-sustaining communities and makes them pioneers in physically and chemically stressed environments.

1.2. Coral reefs and the phase shift to benthic algae and cyanobacteria dominated ecosystems

1.2.1. Coral reefs - a highly productive ecosystem in a marine desert

On a geological time scale, present-day coral reefs are relatively recent phenomena. Where cyanobacterial mats date back to 3500 million years ago (Awramik et al. 1983), corals emerged only during the last 230 million years (Veron 1995). During this period, coral reefs have succeeded in dominating the benthic seafloor. Today, coral reefs have – together with rainforests – the highest biodiversity in comparison to any other aquatic or terrestrial ecosystem (Roberts et al. 2002). Even though coral reefs cover only 0.1% of the total ocean surface, they provide food and shelter to more than 25% of all marine species (Spalding & Grenfell 1997).

In addition, millions of people worldwide are highly dependent on reefs, as they provide seafood, coastal protection, building materials (i.e.,

Fig. 3: Coral reef located at the east end of Curacao away from anthropogenic disturbances.

houses, streets), new biochemical compounds (e.g., medicines, cosmetics) and tourism (Moberg & Folke 1999). The total economic value of for example the Caribbean reefs is annually approximately $3.1-4.6 billion (Mumby et al. (2014); based on ecosystem service values from tourism, fishing and shoreline protection).

Tropical coral reefs are most commonly found in the upper photic zone of (sub-)tropical waters. The waters overlying the reef are typically low in nutrients, yet have a remarkably high productivity (Crossland et al. 1991, De Goeij et al. 2013). Dissolved inorganic nitrogen, phosphorus and iron often limit the growth of reef phototrophic organisms (e.g., phytoplankton, corals, benthic algae and cyanobacteria) (Lapointe 1997, Larned 1998, Den Haan et al. 2013). To ensure continued productivity – even in highly oligotrophic environments – both benthic and pelagic reef organisms developed various strategies to efficiency retain and recycle nutrients (Hatcher 1997). Especially the recycling and transformation of organic matter, including dissolved organic carbon in sediment by microbes (Wild et al. 2004, Werner et al. 2006) and

sponges (Richter et al. 2001, De Goeij et al. 2013) are considered as key players for nutrient recycling on the reef. Biological nitrogen fixation by diazotrophic organisms is an additional pathway to acquire bioavailable nitrogen and is essential for the reef's primary productivity (Casareto et al. 2008, Charpy et al. 2012a). N_2 fixing benthic cyanobacteria in this perspective are thought to be the major contributor to the total nitrogen availability within the reef ecosystem (Wiebe et al. 1975, Larkum et al. 1988, Charpy-Roubaud et al. 2001, Charpy et al. 2010).

> Coral reefs are important ecosystems to both marine life and humans. Interestingly, they are highly productive ecosystems, despite living in oligotrophic waters. Recycling processes by both benthic and pelagic reef organisms provide nutrients for growth on a reef-wide scale. Important key players in this perspective are the transformation of organic matter in the sediments by microbes and sponges, as well as the fixation of nitrogen by benthic cyanobacteria.

1.2.2. Degradation of coral reefs and the revolution of microbes

The World Resources Institute estimated 60% of the coral reef under immediate threat, and when considering other future prognoses, such as thermal stress, this can go up to 75% (Burke & Spalding 2011). Human impacts on coral reefs have increased in scale and frequency. Overfishing, nutrient enrichment via sewage discharge, but also rising seawater temperatures due to climate change and ocean acidification are causes for reef degradation (Jackson et al. 2001, Hughes et al. 2003, Pandolfi et al. 2003, Hoegh-Guldberg et al. 2007) (see as an example Fig. 4). Especially in the Caribbean region, many coral reefs have undergone a sharp loss of live coral cover (Jackson et al. 2014). Gardner et al. (2003) reported on a 60% decline of live coral cover in the Caribbean in only three decades. Roff and Mumby (2012) argued that the resilience of Caribbean reefs are much lower than other regions (i.e., Pacific, Indian Ocean) due to the century-long deposits of iron via

Fig. 4: Degraded coral reef close to Willemstad (Curacao) that experience regular land run-off (brown cloud upper left corner).

Saharan dust, the relatively faster growth of macroalgae, fewer herbivores, and the lack of acroporid corals (i.e., Acroporidae).

The decline of herbivores and coral cover, in combination with other stressors such as eutrophication and the rise in seawater temperature, have caused many reefs to shift from coral dominated to benthic cyanobacteria and algae dominated ecosystems (Hughes 1994, Jackson et al. 2001, Paerl & Paul 2012). These shifts created a change in the ecosystem functioning by forming increasingly detritus-based over grazing-based food webs (McDole et al. 2012). An algae dominated ecosystem will have more algae exudates (e.g., organic carbons). Several studies indicated that such excretions can lead to enhanced bacterial growth and a change in the bacterial composition of the water column (Haas et al. 2011, Nelson et al. 2011, Haas et al. 2013, Nelson et al. 2013). Consequently, this can lead to an increase in coral diseases (Kuntz et al. 2005, Dinsdale et al. 2008, Barott et al. 2012, Nelson et al. 2013). Also bacterial communities living within the coral mucus layer can become more pathogenic due to the increased availability of organic carbons and lead to increased coral mortality (Kline et al. 2006, Smith et al. 2006, Morrow et al. 2012). Even though cyanobacteria increased in abundance on many reefs, little is known about their role in coral reef recovery.

> Coral reefs are under increasing pressure from human impacts. Notably in the Caribbean region, many reefs have lost the majority of their live coral cover. The shift in community compositions change the ecosystem functioning by forming increasingly detritus-based over grazing-based food webs leading to an increase of organic carbons and a change in the microbial community.

1.3. State of the art - benthic cyanobacterial mats on coral reefs

1.3.1. Expansions and causes of cyanobacterial mats

Especially during the last decade, the dominance of cyanobacteria has increased on many coral reefs around the world (Ibrahim & Peter 2004, Albert et al. 2005, Paerl & Paul 2012). Unfortunately, in coral reef monitoring studies, cyanobacteria have often been included in the category 'algae'. As a consequence, cyanobacterial proliferation on coral reefs is not well documented, notably prior to the 1990's. Since that time, the presence of cyanobacterial mats have more often been documented, including reefs from Australia (Albert et al. 2005), California

(Armitage & Fong 2004), Florida (Paul et al. 2005), Guam (Mariana Islands) (Kuffner & Paul 2001), Hawaii (Dailer et al. 2012), La Reunion (Charpy et al. 2010), New Caledonia (Pringault et al. 2005), Taiwan (Lin & Hung 2004) and the Tuamotu Archipelago (Palinska et al. 2012).

The ability of cyanobacterial mats to tolerate environmental changes caused by anthropogenic impacts have been suggested to explain their increasing abundance on coral reefs (Hallock 2005). Unfortunately, no study explicitly showed - from a mechanistic perspective - what in fact drives cyanobacterial proliferation. Several *in vivo* studies confirmed that the cyanobacterium *Lyngbya majuscula* increased its growth rate upon nutrient enrichment by nitrogen and phosphorus (Kuffner & Paul 2001, Elmetri & Bell 2004, Ahern et al. 2006a, Bell & Elmetri 2007, Arthur et al. 2009). This suggests that nutrient enrichment (i.e., eutrophication) might be one of the drivers for the occurrence of cyanobacterial blooms on the reef (Paul et al. 2005). Interestingly, dissolved inorganic nitrogen and phosphorus concentrations in the water column overlying the cyanobacterial mats (i.e., NH_4^+, NO_3^- and PO_4^{3-}) have not been reported to be elevated (Richardson 1998, Thacker & Paul 2001), even on eutrophied reefs (Koop et al. 2001). The reason why nutrient concentrations in the reef's water column are not elevated when cyanobacterial mats are present might be the quick uptake of these nutrients by the organisms Unfortunately, little is known about the uptake of nutrients by benthic cyanobacteria, but also benthic algae, corals and phytoplankton.

Since cyanobacteria and other bacteria in mats are capable of fixing atmospheric N_2 (Wiebe et al. 1975, Charpy-Roubaud et al. 2001, Elmetri & Bell 2004, Charpy et al. 2010), their growth is unlikely to be limited by nitrogen. Manipulative studies on cyanobacterial mats present on coral reefs are few, but suggest that they may be limited by phosphorus (P) (Fong et al. 1993, Kuffner & Paul 2001), chelated iron (Fe) (Arthur et al. 2009) or mixed N and P (Miller et al. 1999, Paerl 2008). However, it remains unclear whether such manipulative studies are representative of what actually drives cyanobacterial growth on the reef.

In Moreton Bay, Australia, a large-scale *L. majuscula* bloom occurred after increased nutrient loading into the bay (i.e., P, Fe and organic carbon) that was caused by long periods of heavy rainfall and subsequent augmented land runoff (Watkinson et al. 2005). Additionally, after the heavy rainfall events, periods of high incident light, elevated temperatures and relatively calm water ensured the proliferation of *L. majuscula* (Watkinson et al. 2005). Albert et al. (2005) confirmed using bioassays *in vivo* that land runoff indeed increased the productivity of *L. majuscula* and therefore suggested that land runoff was a key driver of the cyanobacterial bloom. Increased dissolved organics in the water column that is enriched with bioavailable Fe and P may facilitate the transport of these nutrients to *L. majuscula* (Ahern et al. 2006a,b, 2008). Recently, Fe released

from corroding shipwrecks was suggested to stimulate cyanobacterial assemblages in central Pacific reefs (Kelly et al. 2012).

Given that cyanobacterial mats bloom on the seafloor, they will probably also acquire nutrients released from the benthos. Macrofaunal excretions (e.g., polysaccharides), groundwater seeps (e.g., nitrogen) and remineralized organic matter (OM) in the sediment were found to release bioavailable nutrients (Larned 1998, Schaffelke 1999, Rasheed et al. 2003, Watkinson et al. 2005, Ahern et al. 2006b). It remains unclear which mechanisms in fact drive the proliferation of benthic cyanobacterial mats on the reef.

> The abundance of cyanobacterial mats on coral reefs has increased worldwide. Eutrophication is proposed to be the key driver of their proliferation. Some results seem controversial (for example the low nutrient concentrations in the water column of eutrophied reefs that are dominated by cyanobacterial mats), and details of factors and mechanisms that drive their proliferation remain largely unknown.

1.3.2. Ecological relevance of benthic cyanobacteria dominating the reef

The proliferation of cyanobacterial mats poses a new threat to the survival of many coral reefs around the world. Multiple characteristics attribute to their recent successfulness on these reefs. Benthic cyanobacterial mats have fast growth rates and as a consequence are often the first to occupy newly available substrate, such as recently died corals (Littler & Littler 1997, Diaz-Pulido & McCook 2002). Benthic cyanobacteria that are in the direct vicinity of live corals can induce local hypoxia ultimately causing coral tissue death, i.e. black band disease (Dow & Swoboda 2000). Cyanobacteria can also excrete toxins making them less palatable to herbivores (Nagle & Paul 1998, 1999). Additionally, cyanobacteria can inhibit coral larvae from settling by creating an unfavorable substrate (Kuffner et al. 2006). These characteristics will increase the ability of cyanobacterial mats to persist, especially in degrading coral reef ecosystems (see paragraphs above). However, little is known about the actual role benthic cyanobacterial mats play within the functioning of the reef ecosystem, especially when they start dominating the system.

As stated before in paragraph 1.2.2, when reefs become increasingly dominated by benthic algae, their exudates are suggested to increase the dissolved organic carbon content of the water column, which in turn affects their bacterial composition. This will result into coral mortality but also diminish coral recovery. Up to date, no study has investigated the release of

organic carbon by cyanobacterial mats on coral reefs, yet several studies from other environments have shown that these cyanobacterial mats too can release dissolved organic compounds that are easily degraded by other bacteria (Bateson & Ward 1988, Jonkers et al. 2003).

As mentioned in paragraph 1.2.1, benthic cyanobacteria are major contributors of nitrogen in coral-dominated ecosystem via N_2 fixation. However, it remains unclear how this contribution changes when cyanobacterial mats start proliferating on degraded coral reefs. Few studies indicated that cyanobacteria in so-called 'mini-blooms' fixed large amounts of nitrogen (Charpy-Roubaud et al. 2001, Charpy et al. 2010, Charpy et al. 2012a, Charpy et al. 2012b). To date, no study has investigated the total N_2 fixation potential of large-scale cyanobacterial mats on degraded reefs. In the Caribbean, no studies have been conducted on N_2 fixation by large sized benthic cyanobacterial mats, and to date there are only three other studies on (cyano-)bacteria and turf algae in this region (Carpenter & Price 1977, Williams & Carpenter 1997, Den Haan et al. 2014). This is surprising, as the Caribbean is known as a hotspot for pelagic N_2 fixation (Luo et al. 2012), especially due to its exceptional nutrient conditions, i.e. iron input via the Saharan dust (Roff & Mumby 2012).

On a competition level, it remains unknown how productive benthic cyanobacterial mats are in comparison to other reef phototrophic organisms (corals, benthic algae, phytoplankton), and whether they in fact are capable of taking up newly available nutrients the quickest upon availability (e.g. during land runoff events or direct discharge of sewage onto the reef). These characteristics may highlight their ability to further proliferate on the already degrading reefs.

Cyanobacterial mats are fast colonizers, excrete substances harmful to corals, lead to selective browsing of herbivores, and inhibit coral larvae from settlement. Unfortunately, not much is known about their direct effect on the ecosystem functioning when they are dominating the reefs, especially with regard to dissolved organic carbon release, nitrogen fixation, primary productivity and nutrient uptake.

1.4. Aims and objectives of PhD thesis

The aim of this PhD thesis is **(1)** to improve our understanding to the factors and mechanisms driving the proliferation of cyanobacterial mats on coral reefs. Further, **(2)** it investigates the possible impacts of such proliferation on the functioning of the reef ecosystem. Here, the focus lies on nitrogen fixation, release of DOC, nutrient uptake and primary production of cyanobacterial mats in relation to other primary producers (i.e., corals, phytoplankton and benthic algae). Finally, **(3)** the thesis discusses how the results can contribute to the effective implementation of coral reef protection.

Fig 5: Large-scale patch of cyanobacterial mat (red) on Curacao growing on different substrata.

All studies were conducted on the island of Curaçao, Southern Caribbean. Many reefs on Curaçao have seen a sharp decline in live coral cover over the past three decades (Bak et al. 2005). Many of these reefs are currently dominated by benthic cyanobacterial mats (see for example Fig. 5). Curaçao was the ideal place to study the proliferation of benthic cyanobacteria, as it has both very healthy (i.e., coral dominated, Fig. 3) and highly degraded reefs (i.e., dominated by benthic algae and cyanobacteria, Fig. 4).

Outline of my thesis:

(1) Factors driving the proliferation of benthic cyanobacteria on the reef

In the first manuscript (**MS I**), we tested the hypothesis that nutrients originating from human waste discharge drive the proliferation of benthic cyanobacterial mats. The study is based on (1) an extensive monitoring survey at 63 dive sites along the leeward coast of Curaçao over a two-year period, (2) the investigation of environmental parameters (e.g. nutrients, temperature, water movement, organic matter content of sediment) at random selected sites, and (3) an *in situ* organic matter enrichment experiment measuring the growth-respond of cyanobacterial mats. Additionally, we conducted sediment analyses and *in situ* microsensor measurements to estimate the microbial activity of the mats. From these results, we constructed the possible sources and transport modes of nutrients stimulating the growth of cyanobacterial mats.

(2) Impacts of cyanobacteria proliferation on the functioning of the reef ecosystem

The second manuscript (**MS II**) tests the hypothesis that large patches (>20 cm^2) of cyanobacterial mats fix vast amounts of nitrogen and discusses their contribution to the nitrogen cycle of the reef. This study is based on (1) a diversity survey performed at four sites (two sites with high and two sites with low mat abundance) to identify the species composition of benthic cyanobacterial mats at different depths (3-30 m) and target cyanobacteria species dominating large-sized patches, and (2) incubations of the six ecologically most relevant cyanobacterial mats. Cyanobacteria were identified based on direct microscopy and 16S rRNA gene sequencing. Nitrogen fixation was measured via acetylene reduction assays.

The third manuscript (**MSIII**) tests the hypothesis that cyanobacterial mats can release dissolved organic matter (DOC) into the water column. DOC released by algae has been shown to augment the occurrence and severity of coral diseases and reef degradation (see paragraph 1.2.2). Therefore, we determined the net fluxes of DOC, dissolved inorganic carbon and oxygen of cyanobacterial mats *in situ* using benthic chambers. Additionally, we measured the nutrient fluxes associated with the mats and the distribution of oxygen within the sediment-water interface during 24 hours using *in situ* oxygen microprofiles.

The next two manuscripts are part of a collaboration with the University of Amsterdam, The Netherlands. The fourth manuscript (**MS IV**) tests the hypothesis that cyanobacterial mats take up nutrients (NH_4^+, NO_3^- and PO_4^{3-}) faster than other reef phototrophic organisms. In this study, we determined the nutrient uptake rates of the most abundant reef phototrophic organisms (corals, macroalgae, turf algae, benthic cyanobacteria and phytoplankton) *in vitro*.

In the fifth manuscript (**MS V**) we determined which benthic organism (i.e., similar as in MS IV) had the highest primary productivity on the reef using the ^{13}C method. Further we determined whether the overall primary productivity of our studied reef, which is now dominated by benthic algae and cyanobacteria, decreased by comparing our findings to a historic baseline.

The sixth manuscript (**MS VI**) is an observation of Beggiatoaceae migrating in cyanobacterial mats. Beggiatoaceae are bacteria known to grow on sites enriched with organic matter and hydrogen sulphide when anoxic conditions are present. They are considered as indicator species for organic matter enrichment in aquatic environments especially close to sewage outlets.

(3) Implementation of my results towards coral reef protection

This PhD thesis is part of a large EU-funded project called the Future of Reefs in a Changing Environment (FORCE) (http://www.force-project.eu/). The FORCE project has

assembled a book for coral reef managers to further protect Caribbean coral reefs in the future. For this book we wrote a policy brief, which in my thesis is presented as the last manuscript (**MS VII**).

The PhD thesis address following research questions:

1. What are the causes and mechanisms driving the proliferation and growth of cyanobacterial mats? (Chapter 2)
2. What is the impact of cyanobacterial proliferation on the reef's functioning? What advantages do cyanobacterial mats have in comparison to other benthic groups, including corals, macroalgae and turf algae? (Chapters 3-6)
3. What can we do to prevent cyanobacterial blooms? (Chapter 7)

1.5. Overview of manuscripts and authors contributions

The amount of crosses ('x') signifies the contribution of the authors to the manuscript. First author wrote the entire manuscript, co-authors that helped with the writing are marked identified in the column 'writing'.

(Co-) Author contributions:	Ideas	Conduction of experiments	Sample analyses	Data analyses	Contribution of reagents/materials/ analyses/ tools	Writing
Chapter 2: MS I: Organic matter degradation drives benthic cyanobacterial mat abundance on Caribbean coral reefs (accepted in PLoS ONE)						
1. **Brocke HJ**	xxx	x	x	xxx		x
2. Polerecky L	x			x		
3. De Beer D	x				x	
4. Weber M	x					
5. Claudet J				x		
6. Nugues MM	x			xx	x	x
Chapter 3: MS II: Nitrogen fixation and diversity of expanding benthic cyanobacterial mats in coral reefs of Curaçao (in preparation)						
1. **Brocke HJ**	x	x	x	xxx		x
2. Piltz B	x	x	x	x		
3. Herz N			xx			
4. Abed RMM						x
5. Palinska KA			x			
6. John U			x			
7. Nugues MM	x				x	
Chapter 4: MS III: High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef community (published in Scientific Reports)						
1. **Brocke HJ**	xx	x	x	x		x
2. Wenzhoefer F				x	x	
3. De Beer D	x				x	
4. Mueller B			x			
5. Van Duyl F					x	
6. Nugues MM	x				x	x

(Co-) Author contributions:	Ideas	Conduction of experiments	Sample analyses	Data analyses	Contribution of reagents/materials/ analyses/ tools	Writing
Chapter 5: **MS IV:** Nutrient uptake rates of Caribbean corals, phytoplankton, benthic algae and cyanobacteria (in review)						
1. Den Haan J	x	x	x	x		x
2. Huisman J				x		x
3. Brocke HJ	x	x				
4. Göhlich H		x	x			
5. Pander J		x	x			
6. Latijnshouwers KRW		x	x			
7. Van Heeringen S		x	x			
8. Honcoop SAS		x	x			
9. Bleyenberg TE		x	x			
10. Nugues MM						x
11. Schouten S					x	
12. Cerli C					x	
13. Hoitinga L					x	
14. Vermeij MJA	x				x	x
15. Visser PM	x			x	x	x
Chapter 6: **MS V:** Changes in primary productivity of a degraded coral reef (in review)						
1. Den Haan J	x	x	x	x		x
2. Visser PM	x				x	x
3. Brocke HJ	x	x				
4. Pander J		x	x			
5. Brunner R		x	x			
6. De Wit M		x	x			
7. Mes D		x	x	x		
8. De Baat ML		x	x			
9. Nugues MM						x
10. Mueller B		x				
11. Schouten S					x	
12. Cerli C					x	
13. Vermeij MJA	x				x	x
14. Huisman J					x	x

(Co-) Author contributions:	Ideas	Conduction of experiments	Sample analyses	Data analyses	Contribution of reagents/materials/ analyses/ tools	Writing
Chapter 7: MS VI: Diel vertical migration of Beggiatoaceae in benthic cyanobacterial mats (in preparation)						
1. **Brocke HJ**	x	xxx	x	x		x
2. De Beer D		x				
3. Kamp A			x			
4. Nugues MM						x
Chapter 8: MS VII: Policy brief - Preventing blooms of cyanobacterial mats (printed June 2014)						
1. **Brocke HJ**	x					x
2. Nugues MM						x

2. Manuscript I

MS I: Organic matter degradation drives benthic cyanobacterial mat abundance on Caribbean coral reefs

Running head: Drivers of benthic cyanobacterial mats

Organic matter degradation drives benthic cyanobacterial mat abundance on Caribbean coral reefs

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Abstract

Benthic cyanobacterial mats (BCMs) are impacting coral reefs worldwide. However, the factors and mechanisms driving their proliferation are unclear. We conducted a multi-year survey around the Caribbean island of Curaçao, which revealed highest BCM abundance on sheltered reefs close to urbanised areas. Reefs with high BCM abundance were also characterised by high benthic cover of macroalgae and low cover of corals. Nutrient concentrations in the water-column were consistently low, but markedly increased just above substrata (both sandy and hard) covered with BCMs. This was true for sites with both high and low BCM coverage, suggesting that BCM growth is stimulated by a localised, substrate-linked release of nutrients from the microbial degradation of organic matter. This hypothesis was supported by a higher organic content in sediments on reefs with high BCM coverage, and by an *in situ* experiment which showed that BCMs grew within days on sediments enriched with organic matter. We propose that nutrient runoff from urbanised areas stimulates phototrophic blooms and enhances organic matter concentrations on the reef. This organic matter is transported by currents and settles on the seabed at sites with low hydrodynamics. Subsequently, nutrients released from the organic matter degradation fuel the growth of BCMs.

1. Introduction

Cyanobacteria are ubiquitous on coral reefs and play an important role in reef formation and nutrient cycling [1]. However, on declining reefs, they can form dense and widespread benthic cyanobacterial mats (BCMs), with negative consequences for reef health [1, 2]. Since the early 1990s, BCMs have become increasingly prominent on many reefs worldwide, including Australia [3], California [4] , Florida [5], Guam [6], Hawaii [7], La Reunion [8], New Caledonia [9], Taiwan [10] and Tuamotu Archipelago [11]. The mats reduce coral settlement and recruitment [12], alter coral-associated microbial community [13], act as coral pathogens [14], and produce chemicals which have been linked to mass reef fish die-offs and deter grazing [15, 16]. As many cyanobacteria are able to fix nitrogen (N) [17], their proliferation could also increase fixed nitrogen in the system, which enhance the growth of coral competitors, such as macroalgae [18].

The ability of BCMs to tolerate environmental conditions associated with anthropogenic impacts and global climate change has been suggested to explain their increasing abundance on degraded reefs [2]. However, the links between potential anthropogenic and climate drivers and their proliferation on coral reefs are often tenuous and not supported by a mechanistic explanation. Water column measurements in coral reefs with naturally growing BCMs have not shown elevated inorganic nutrient concentrations [19, 20], including in reefs considered under eutrophic exposure [21], probably because these inorganic nutrients are rapidly converted into biomass. Since many BCMs are diazotrophs [22], their growth is unlikely to be limited by N. Manipulative studies on BCMs present on coral reefs are few, but suggest that they may be limited by phosphorus (P) [6, 23], chelated iron (Fe) [24] or mixed N and P [25, 26]. However, the sources and transport routes of nutrients which enhance BCMs are unclear.

In Moreton Bay, Australia, a bloom of the benthic cyanobacterium *Lyngbya majuscula*, which also proliferate on coral reefs [5, 6], was preceded by a pulse of rainfall and initiated by a period of high incident light, elevated temperature and calm weather [27]. Soil extracts rich in P, Fe and organic carbon enhanced the productivity of *L. majuscula* in bioassays, suggesting land runoff as a key driver of the bloom [3]. Increased dissolved organics in the water column may facilitate the transport of bio-available Fe and P to *L. majuscula* via the formation of Fe-organic complexes [28, 29]. Recently, Fe released from corroding shipwrecks was suggested to stimulate algal/cyanobacterial assemblages in central Pacific reefs through a similar mechanism [30]. Given that BCMs bloom on the benthos, they could also acquire benthic nutrients released from macrofaunal excretions, groundwater seeps and remineralised organic matter (OM) [18, 31]. High effluxes of both P and N have been measured in benthic cores containing *L. majuscula*, seagrasses and sediments, highlighting the potential of sediments to act as local supply of nutrients for uptake [27].

This study aimed to improve our understanding of the factors and mechanisms driving the proliferation of BCMs on coral reefs in Curaçao, Southern Caribbean. Over the last three decades, many reefs on the island have exhibited signs of degradation and increasing BCM dominance [32]. We hypothesised that organic matter degradation acts as a mechanism of nutrient supply and growth impulse for benthic cyanobacterial mats in nutrient-poor coral reefs and that coastal urbanisation and hydrodynamics combine to mediate the accumulation of particulate organic matter on the seafloor, which subsequently stimulates the growth of the mats. This study is based on (i) large-scale surveys of BCM, coral and algal abundance around the island, (ii) local surveys of potential environmental drivers, including inorganic nutrient concentrations, temperature, water movement and OM content in sediments, and (iii) an *in situ* organic enrichment experiment of the sediments. In addition, we used microsensors to estimate microbial activity and degradation of OM across BCM patches. From these results, we deduced the possible sources and transport mode of nutrients stimulating BCM growth.

2. Material and methods

(a) Study area

The research was conducted on the south leeward coast of the southern Caribbean island of Curaçao (12°10'N, 68°58'W, ca. 60 x 11km, Fig. 1a), where well-developed fringing reefs border the landward shore [33]. The island is exposed to all-year-around trade winds running from east to west [34]. In 2012, 150,563 inhabitants lived on the island (Central Bureau of Statistics, Curaçao). Curaçao does not have large scale agriculture, but heavy oil industry and mining activity are present. In the east and central parts of the island, waste water treatments are installed, but receptive basins have leaks and overflow regularly, which makes household wastewater runoff a significant source of nutrients into the ocean [35]. In the west part of the island, most houses have sewage cesspits that leak slowly into the groundwater. The island is surrounded by a belt of Quaternary and Neogene limestone [36], which is a porous material that allows fast groundwater transport.

(b) Large-scale survey

To provide an island-wide view of BCM abundance and potential drivers, we conducted semi-quantitative multi-season surveys along the entire south-west coast of Curaçao and linked our observations to coastal urbanisation, wave action and seasonality. These large-scale surveys were coupled with local surveys of selected environmental parameters at haphazardly chosen sites with high and low BCM abundance.

The semi-quantitative surveys of BCM abundance were conducted at 64 sites over four periods: twice during the warmer and rainier season (Sept 2010 and November 2011) and twice during the colder and drier season (May 2011 and June 2012). At each site and period, the observer first dove to 20 m depth and then slowly swam upwards to 5 m depth at a distance of ca. 2 m from the reef while watching attentively the seabed (observation time: ~3 min per site). The abundance of BCMs was ranked on a scale of 1 to 5 where 1 corresponds to a reef where cyanobacteria are rare (i.e. a small patch could occasionally be seen) and 5 represents a reef where most sand and hard bottom surfaces are covered by BCMs. During the last survey (June 2012), the abundance of corals and macroalgae (defined as all algae > 1 cm above the reef substratum) was similarly ranked at each site to investigate their spatial relationships with BCMs. The lead observer (HJB) led all surveys and the ranking was cross-validated between the different divers at the beginning of each survey period. In addition, representative mats from two different depths (5 and 15 m) were collected at several study sites and analysed microscopically to identify dominant species.

Each site was assigned two individual scores: one score for the level of urbanisation of the adjacent shore and one for the wave height. For urbanisation, each site was plotted in Google Earth Pro and urbanisation level was defined as follows: 1, absence of urbanisation (i.e. absence of houses, industry, dumping areas) in a 500 m radius; 2, presence of urbanisation in a 500 m radius; 3, presence of a drainage outlet with an urbanised watershed in the 500 m radius; and 4, both 2 and 3. For wave heights, the semi-quantitative estimates of van Duyl [33] were used, where 1 represents low wave energy environments (waves 0-30 cm high) and 5, high wave energy environments (waves 1.5-2 m high).

(c) Local surveys of environmental parameters

Based on the results of the island-scale surveys, four sites with high (rank 4-5) and four sites with low (rank 1) BCM abundance were randomly selected in subsequent, medium-scale surveys of selected environmental parameters. If the site characteristic (i.e., high vs. low mat abundance) changed during the multi-season survey, another site that fulfilled the required classification criterion was selected. The parameters monitored during these medium-scale surveys included temperature, water movement, nutrients (NO_x, PO_4^{3-}), particulate organic matter in the water column and OM content in sediments. Temperature was recorded in 30 min intervals from September 2010 to June 2012 at a water depth of 10 m using temperature loggers (Hobbo Pendant, Onset). Water movement was estimated in September 2010 and May 2011 at a water depth of 10 m based on the dissolution of clodcards (i.e. plaster of paris blocks; [37]). These data were used as an additional support for the above wave height data.

(i)   Nutrients

Nutrients were analysed in water samples collected 3-4 times during a warmer and rainier season (October-November 2010) and 3-4 times during a colder and drier season (April-May 2011). To identify possible differences that could be relevant at the medium scale, samples were collected at each site in 8 locations (Fig. 2a): (1) above the reef slope just below the water surface (surface water); (2) 15 m away from the reef slope at a water depth of 15 m (open ocean water); above the reef slope, 1 m above the seabed at water depths of 5 m (3) and 15 m (4) (intermediate water); directly above BCMs (within 1 cm of the seabed) at water depths of 5 m (5) and 15 m (6) (bottom water), and directly above BCM-free substrate at water depths of 5 m (7) and 15 m (8) (control bottom water). Control bottom water samples at 5 and 15 m depths were taken above sand and hard substrate, respectively. Since mats differed in substrate preference and species composition across depths, the effects of depth, substrate and mat type were unavoidably confounded. Immediately upon collection, samples were filtered with 0.22 μm pore-size syringe filters (Minisart® NML Syringe Filters 16534), transported on ice and in the dark to local laboratory (CARMABI), and stored and transported at -20 C° until analysis at MPI, Germany. PO_4^{3-} was analysed using the molybdenum blue method [38] and NO_x (nitrate + nitrite) was analysed with a NO_x analyser (CLD 86; Eco- Physics).

(ii)   Particulate organic matter content in water column

Water samples (2 l) were collected 4 times 1 m above the seabed at a depth of 10 m during a warmer and rainier season (October-November 2010) and filtered immediately after returning to the laboratory through a precombusted GFF filter. Each filter was separately packed in individual acid washed filter box and dried at 40°C. The filters were steamed with smoking hydrochloric acid for 24 h, dried again, packed in tin cups and analysed with a CNS element analyser.

(iii)   Organic matter content in sediments.

Sediment cores (6 cm^2 area x 3 cm deep) were collected at a water depth of 7-8 m in April 2012 (n = 3 per site). For this parameter, 5 sites with high and 4 sites with low BCM abundance were sampled. In all sites, sampling was conducted on sand patches far away (> 5 m) from BCMs to minimize their potential influence. To study small-scale variations at the mat level, sediment cores were collected in the centre, at the edge, next to (ca. 10 cm away) and far away (> 5 m) from brown-coloured BCM patches (n = 6 patches per location) at one BCM dominant site (Pest Bay, 12°09'53.77" N 69°00'39.66"W , Fig. 1a). Mats were removed by hand picking before sampling. Each sample was dried at 40°C, homogenised and analysed for organic carbon content with a Delta Plus mass spectrometer.

(d) In situ organic enrichment experiment

To test the hypothesis that a substrate-bound degradation of OM stimulates the growth of the BCMs, an *in situ* organic enrichment experiment was conducted in May 2012 at a depth of 6-9 m using brown-coloured mats in sandy areas in the patched reef at Pest Bay (Fig. 1a). A total of 48 buckets (14 L) were installed with a minimum gap of 1 m between buckets (total experimental area was ca. 1000 m^2). Half of them had the base removed and the other half were intact to test for the possible effects of seepage. Buckets were pushed approximately 20 cm into the sand, with the same sand level inside and outside the bucket. Each set of 24 buckets was subject to four treatments differing in the OM content of the sediment and the presence of an initial "seed" of brown-coloured BCM: (i) the experimental control, without addition of OM and BCM seed; (ii) the seeding treatment, with addition of BCM seed, but without OM enrichment; (iii) the OM enrichment treatment, with OM enrichment, but no addition of BCM seed; and (iv) the combined treatment with additions of both OM and BCM seed.

To realize these treatments, sediment from the upper 15-20 cm layer of all buckets was discarded and replaced by sediment that was collected at the same site, repeatedly flushed *in situ* with water and well mixed. The untreated sediment contained 2.5 ± 0.1 μg C_{org} mg^{-1} DW (0.27 ± 0.02 SEM % C_{org} of sediment DW) and was enriched with *Spirulina* tablets (1 per bucket, 18.2 ± 0.7 mg C_{org}), resulting in an additional organic carbon content of $+0.7 \pm 0.2$ % C_{org} in the upper 1 cm of the sediment (n = 6 for all measurements). OM content was sampled and analysed with the same method mentioned above. To enrich the sediment with OM, each tablet was dissolved in filtered (0.22 μm pore-size) seawater in a 60 ml syringe and slowly injected into the first 0.5-1 cm surface sediment in a spiral movement from the centre of the bucket at day 0 and again at day 7. Initial BCM "seeds" were scooped out of a ca. 6 cm^2 surface of BCMs growing at the experimental site and placed at the centre of the bucket 4 h after the first OM enrichment. To compare BCM growth, buckets were photographed daily for 11 days and once after 18 days. BCM cover was estimated using the free software Vidana which allowed BCMs to be delineated by eye in each bucket and their surface to be determined.

(e) In situ oxygen measurements and other sediment analyses

To document microbial activity and degradation of OM across BCM patches, vertical profiles of dissolved oxygen around the sediment-water interface were measured with a diver-operated microsensor system [39] at a depth of 6-9 m at Pest Bay (Fig. 1a). Over 350 profiles were acquired during several 24 h cycles in the centre, at the edge and next to brown-coloured BCM patches. Analysis of the profiles was done using custom-made programs MPR-plotter and

L@MP (www.microsen-wiki.net). To verify that differences in O_2 concentration were solely due to variations in microbial activity within the mats and not due to differences in sediment structure, particle size distribution, porosity and permeability were investigated in carbonate sediments collected in sand patches with and without BCMs. Particle size distribution was determined in 9 replicate sediment cores (6 cm diameter x 18 cm high) at each location (i.e. centre, edge, next to BCM). Porosity was measured by weight loss after drying 7 replicate sediment cores (6 cm diameter x 10 cm high) sliced at 2.5 cm depth intervals. Permeability was measured in 7 replicate sediment cores (36 mm diameter) with the falling-head method [40].

(f) Statistical analysis

Most environmental data violated parametric assumptions, so we evaluated them using non-parametric univariate analyses. BCM abundance scores were analysed using PERMANOVA with season (colder vs. warmer) as a fixed factor, time periods nested within season as a random factor and scores of urbanisation and wave height as covariate. Spearman rank-order correlation analyses were conducted to examine relationships between BCM, corals and macroalgae. Clodcard dissolution was analysed by PERMANOVA with BCM site abundance (low vs. high) and time (colder vs. warmer) as crossed fixed factor and site nested within BCM site abundance as a random factor. Ocean, surface, intermediate and bottom water nutrients and particular organic matter in the water column were separately analysed using temporal replicates by PERMANOVA with, as appropriate for each variable, season, BCM site abundance, depth (5 vs. 15 m) and BCM presence (above BCM vs. above BCM-free substrate) as crossed fixed factors and site as a random factor nested within BCM abundance. Temperature time-series (n = 38000 per site) were averaged for each site and analysed with ANOVA with BCM site abundance as fixed factor. Particle size distribution was analysed by PERMANOVA with size fractions and BCM presence as fixed factors, porosity by ANOVA with sediment depth interval and BCM presence as fixed factors, and permeability by ANOVA with BCM presence as fixed factor only. Other parameters were tested as described in the figures. All PERMANOVA tests used Euclidian distances and 9999 permutations of raw data from residuals under a reduced model [41].

3. Results

(a) Surveys of BCM abundance and environmental parameters

BCMs were rare at the southern-most part of the island and increased in abundance when moving in the north-west direction along the coast, particularly in the sheltered and densely populated areas close to Willemstad (Fig. 1a). On the west part of the island away from Willemstad, BCMs were also abundant. This part did not exhibit large populated or industrialised

areas, but second homes and tourism development was frequent along a narrow (ca. 500 m) strip of coast (i.e. not visible on the island map). Urbanisation and wave height interacted significantly to explain BCM abundance (Table S1). Areas with high cyanobacterial mat abundance were associated with high urbanisation and low wave energy, while sites with lower coverage were related to high wave energy and low urbanisation (Fig. 1b). Examples of high-BCM sites include sheltered bays such as Santa Martha Bay and Piscadera Bay; examples of low-BCM sites include eastward facing promontories capes of St. Marie and Lÿhoek (Fig. 1a). The negative influence of wave height was further supported by clodcard measurements, which revealed higher water movement at sites with low BCM abundance than at sites with high BCM abundance (mean ± SEM: 64 ± 3 % vs. 35 ± 2 % weight loss; Table S2). BCMs were more abundant during the warm/rainy season than during the cold/dry season (abundance score of 2.9 ± 0.1 vs. 2.6 ± 0.1; Table S1). Seasonal fluctuations in water temperature were ~3°C (~26-29°C), while daily fluctuations were ~0.5°C (Fig. S1). Average temperatures did not differ between sites with high and low BCM abundance (27.94 ± 1.02°C vs. 27.91 ± 1.01°C; $F = 0.15$, $df = 1$, $P = 0.71$), indicating that temperature influenced seasonal, but not spatial, variation in BCM abundance. In June 2012, BCM abundance was negatively correlated with coral abundance ($r_s = -0.691$, $P < 0.001$) and positively correlated with macroalgal abundance ($r_s = 0.555$, $P < 0.001$).

Across depths, dominant mats differed in substrate preference and species composition. BCMs at 5 m depth occurred largely on sand and were mostly brown-coloured. Morphological microscopic identification indicated that they consisted primarily of *Oscillatoria bonnemaisonii* Crouan ex Gomont and secondarily of *Blennothrix glutinosa* Gomont ex Gomont. BCMs at 15 m depth occurred predominantly on hard substrate and were red-coloured. They consisted of different species of the genus *Oscillatoria* which could not be identified to species level.

Local surveys of inorganic nutrients at eight locations within each site (Fig. 2a) showed that nutrient concentrations in the open ocean and surface waters did not differ with season or between sites with low and high BCM abundance (Fig. 2b; Table S3). Intermediate water (i.e. 1 m above the reef substrate) had higher NO_x concentration at 5 m depth at sites with high BCM abundance than at sites with low BCM abundance. Most noticeable were the elevated PO_4^{3-} water concentrations above BCM compared with BCM-free substrate, especially during the cold/dry season. This trend occurred regardless of depth and BCM site abundance. NO_x bottom water concentrations above BCM and BCM-free substrates showed similar, but less marked, differences. NO_x concentrations above BCMs were higher than above BCM-free substrate at sites of high BCM abundance at 15 m depth, regardless of season. No such trend was found for PO_4^{3-}.

Average concentration of particulate organic matter in the water column did not differ between sites with low and high BCM abundance (28.91 ± 1.48 μg/l vs. 25.82 ± 1.22 μg/l; Table S4). However, OM content in BCM-free sediments at sites with high BCM abundance was significantly higher than at sites with low BCM abundance (Fig. 3a). It was also highest in the middle of BCMs and decreased with distance away from the mats, supporting an increased OM accumulation underneath the mats (Fig. 3b).

(b) In situ organic enrichment experiment

Cyanobacterial growth occurred only on sediments that were seeded with a piece of BCM (Fig. S2; row 2 and 3). Starting from day 4 onwards, the cover of BCM seeded on OM-enriched sediments was significantly higher than on non-enriched sediments (Fig. 4). Differences between the buckets with open and closed bottoms were mostly not significant, although at certain time-points the open bottom buckets with the OM-enriched sediment had a significantly larger BCM coverage than the closed bottom buckets (e.g., day 9 or 18). BCM cover peaked on days 5 and 6 in the OM enriched open and closed bottom treatments after the first OM addition. Both also showed a large increase in cover after the second OM addition on day 7, with a peak in abundance on days 9 and 10 and a decline afterwards. The open bottom buckets showed a slower decline in BCM cover than the closed bottom buckets.

(c) In situ oxygen measurements and other sediment analyses

During daytime, maximum surface O_2 concentrations in the middle of the natural BCM patches were 2-8 fold higher than at the edge and next to BCMs (Fig. 5a), indicating high photosynthetic productivity in the mats. During night time, the mats were fully anoxic, whereas O_2 penetrated 3-4 mm into the sediments in the absence of BCMs (compare Figs. 5b and 5c). No differences in grain sizes (regardless of size fraction), porosity and permeability were found (PERMANOVA/ANOVA, all factors and interactions, $P > 0.05$). Permeability averaged $2.4 \pm 0.2 \times 10^{-10}$ m^2, indicating very permeable sediments.

4. Discussion

BCM growth on coral reefs is typically limited by nutrients [6, 23-26]. In our study, BCMs were congruent with high PO_4^{3-} and NO_x in their close vicinity (millimetre-scale) in an otherwise oligotrophic water column (NO_x ~0.5 µM; PO_4^{3-} ~0.1 µM), regardless of depth and BCM dominance on an island scale. Such locally enhanced nutrient concentrations could originate from the underlying benthic surface via direct groundwater seepage. In Australia, *L. majuscula* showed increased growth by exposure to groundwater [42]. However, in our case, several observations are inconsistent with this mechanism of nutrient supply. First, whereas seepage will be less in hard substratum which is much less permeable than sand, BCMs were prominent over hard substratum at 15 m depth where they were also associated with locally enhanced nutrient concentrations. Second, our nutrient sampling periods were in September 2010 and May 2011 which experienced 195 mm/month and 53mm/month of rain, respectively (Meteorological Dept, Curaçao). Yet, higher concentrations of nutrients were found in the dryer sampling period when any possible groundwater seepage would have been reduced. Third, differences between the buckets with open and closed bottoms in the organic enrichment experiment were mostly not significant. Finally, while NO_x benefits from a high mobility in sediments, PO_4^{3-} in groundwater tends to get absorbed by calcium present in limestone and should thus be low in groundwater outflow [43], which contrasts with our data.

Sediments associated with mat-forming and rhizophytic benthic algae on coral reefs have been shown to function as localised nutrient sources, making sustained growth possible despite the oligotrophic water column [18]. Similarly, *L. majuscula* growth may be maintained by additional inputs of nutrients through sediment efflux in seagrass beds in Moreton Bay [27]. Given that (i) BCMs were prominent on reefs with more organically rich sediments, and (ii) the mat types growing on sediments developed within days when OM was added to the sediments, our results suggest that the nutrient source for BCMs originates from OM that has settled on the seafloor and is decomposed by microbial degradation as proposed in Fig. 6b. Due to the degradation, an anoxic zone develops at the sediment surface. The ensuing Fe^{3+} reduction leads to release of Fe^{3+}-bound phosphate to the water column, and possibly also of Fe^{2+} [44]. This local nutrient release from the benthos subsequently stimulates the growth of BCMs. Cyanobacterial mats produce OM via photosynthesis, excretion and cell decomposition. Aerobic heterotrophs and anaerobic sulphate-reducing bacteria mainly respire the produced OM [45]. An indication of these photosynthetic and remineralising processes within the BCMs is the elevated O_2 concentrations and the anoxia at the mat surface during day and night, respectively. Overtime, OM accumulates under the mat, leading to further BCM growth and patch expansion. To enhance this process, many cyanobacteria excrete extracellular polymeric substances mainly

composed of polysaccharides, which form sticky structures capable of catching detritus from the water column [46].

While elevated nutrient concentrations were found above BCMs growing over both hard substratum and sediments, we did not conduct an organic enrichment experiment on BCMs occurring on hard substrates. However, similar mechanisms of BCM growth enhancement by OM may operate here too. In Curaçao, most BCMs covering hard substratum grew over turfs and macroalgae. Turfs and macroalgae can act as sediment traps, accumulate particulate OM on or within their canopies [31, 47] and provide dissolved OM by photosynthesis and degradation [48, 49]. Schaffelke [31] demonstrated that the layer of particulate matter deposited on the thalli of *Sargassum* was sufficient to supplement their nutrient supply. She postulated that a nutrient-rich diffusive boundary layer was created on the thallus surface by an epiphytic microbial community that remineralised the bound nutrients. Likewise, the OM accumulating on hard substratum could provide a nutrient base for BCMs in this study.

The results of this study also show that BCMs (i) were prominent in sheltered reefs close to the urbanised areas, highlighting the importance of urban and industrial development and wave energy, and (ii) were more abundant in the warm/rainy season than in cold/dry season. Elevated temperature and rainfall are known to favour benthic cyanobacteria, as shown in Moreton Bay [3, 27, 42]. While high wave energy can physically remove BCMs growing loosely on the seabed [20], it could also prevent the deposition and accumulation of OM on the seafloor. Wave-exposed habitats are commonly composed of coarse carbonate sand, with low organic content and low pore-water nutrient concentrations [18]. In such habitats, advection from the water column has been considered as the primary source of nutrients for benthic organisms, while, at protected sites, the contribution of nutrients from benthic sources increases [18]. Schaffelke [31] found that organic content of particulate matter on *Sargassum* was negatively correlated with water flow. Strong currents will create thinner boundary layers, enhance oxidative remineralisation processes within the sediment, rapidly dilute nutrients released from the seabed, and decrease the exposure time to nutrients and consequently BCM growth.

Very few studies have connected BCM abundance to urbanisation on coral reefs. Only in Moreton Bay was land use directly linked to mat abundances [3, 27, 42]. Together our data suggest that the distribution and abundance of BCMs in oligotrophic coral reef systems depend on the interplay between the input and production of organics and mineral particles to and in the system (which is influenced by coastal urbanisation and reef degradation), the rate of settling on the surface (which is determined by local hydrodynamics), and the subsequent cascade of heterotrophic microbial processes following the settling. We have integrated these processes into the proposed model in Fig. 6a. Both runoff and seepage are known to occur in Curaçao. Gast et

al. [50] found NO_x and PO_4^{3-} values to reach up to 0.92 µM and 0.29 µM, respectively, at the entrance of the harbour of the capital city Willemstad. NO_3^- concentrations up to 1612 µM have been measured in ground water [35]. Since the Caribbean has been exposed to Fe-rich influxes of African dust for 40,000 years, water column and sediments may act as reservoirs of Fe in the region [51]. In a Fe-loaded system, N and P may be the limiting nutrients controlling benthic and planktonic phototrophic blooms, which, upon decay, convert nutrients into particulate OM. Thus, nutrients may be transported from land to the reef bound in particulate organic matter, while water column concentrations remain low.

The quantity of nutrients and organics in the water column did not differ between sites with high and low BCM abundance. Nevertheless, the high OM concentrations in BCM-free sediments at sites with high BCM abundance are indicative of periods of high OM loads. Water column nutrients, chlorophyll and organics vary greatly depending on wave action, tides, storms and rainfall [52], making it difficult to accurately assess nutrification status by water column measurements alone. The quantity of organics and nutrients in sediments, however, are thought to be a good proxy for long-term nutrient pollution [53]. In coral reefs, the majority of the productivity occurs on the benthos and nutrients are rapidly converted into biomass by benthic algae or macrophytes [54], even before phytoplankton can accumulate. The produced OM subsequently settles, buries and accumulates in sediments, which serve as repository for both water column and benthic production, as well as detrital materials originating from external sources [53, 55-57]. Finally, reef degradation is likely to enhance this OM accumulation by forming increasingly detritus-based over grazing-based food webs, with a switch in energy allocation from fish to microbes [58].

Many studies underscore the role of nutrients regenerated from OM degradation in supporting primary producers on coral reefs and other marine coastal systems [57, 59-62]. Future studies need to include the origins of the OM, the role of other potentially limiting nutrients (e.g. Fe, molybdenum) and microbial processes. The combined influences of urbanisation, temperature, rainfall, hydrodynamics and reef degradation in mediating BCM abundance suggest that BCMs will be more common under environmental conditions associated with anthropogenic impacts and global change. Management approaches that prevent the input of nutrients generated on land and restore food webs should reduce BCM proliferation on coral reefs. Because OM also kills coral tissue when abundant in dissolved form in the water column [63] or within sediments encroaching corals [64], our results add on to the multiple detrimental effects of increasing OM on coral reefs and suggest that organic loading of sediments and other benthos should be routinely monitored.

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Figures
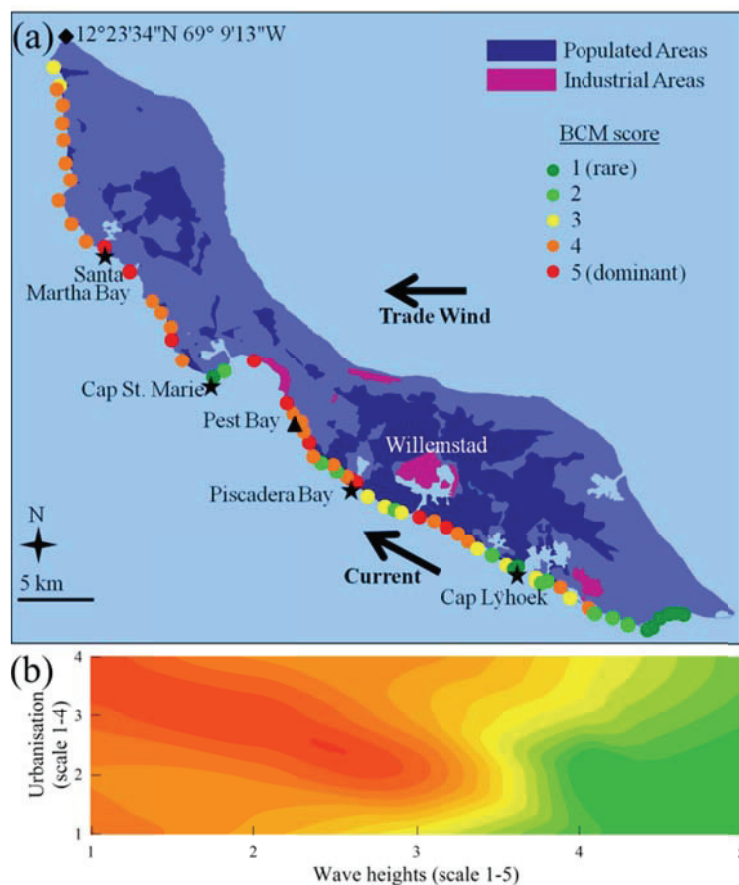
Figure 1. (*a*) BCM abundance score (scale 1 to 5) averaged across the 4 surveys along the south coast of Curaçao. Populated and industrial areas are shown in dark blue and pink, respectively. Trade wind and water current are indicated by arrows. (*b*) Contour plot showing the relationships between BCM abundance, wave heights and urbanisation. Colours represent BCM score as shown in (*a*). Note that populated areas and BCM abundance are not obviously related in the large-scale map (*a*), especially in the West part of the island, but populated areas were often present along a narrow (ca. 500 m wide) strip of coast, which cannot be visualised in the map. This small-scale pattern was taken into account to score urbanisation levels used in the contour plot (*b*) (see Materials and Methods for details).
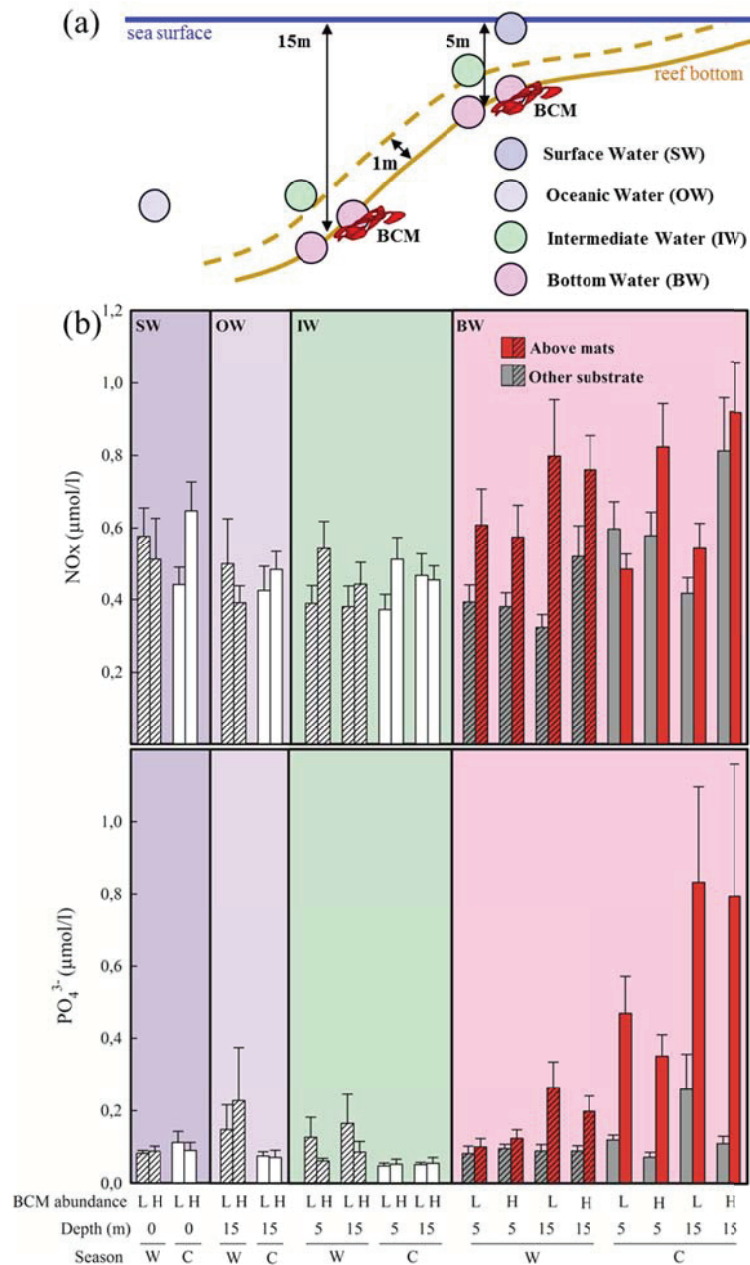
Figure 2. (*a*) Overview of different nutrient sampling locations at each site. (*b*) NO_x and PO_4^{3-} concentrations (mean ± SEM, n = 3-4 temporal replicates) as a function of season, ie. (W) warm/rainy (hatched) and (C) cold/dry (plain), depth, BCM abundance, ie. low (L) vs. high (H) abundance sites, and substrate type (as applicable) for surface, open ocean, intermediate and bottom waters.
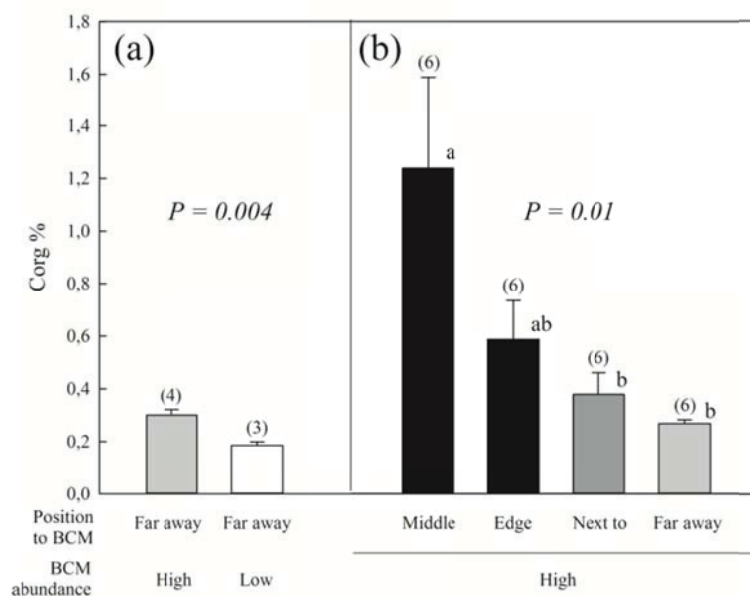
Figure 3. Percent organic carbon (C_{org}) (mean ± SEM) of (*a*) sediment cores collected far away (> 5 m) from any BCM patch at sites of low and high BCM abundance, and (*b*) sediment cores collected at Pestbay (site of high BCM abundance) in the middle, edge, next to (ca. 10cm away) and far away (> 5 m) from BCM patches. Analysed by ANOVA. Letters indicate homogeneous subgroups by posthoc Scheffe tests. Numbers of replicates are in parenthesis.
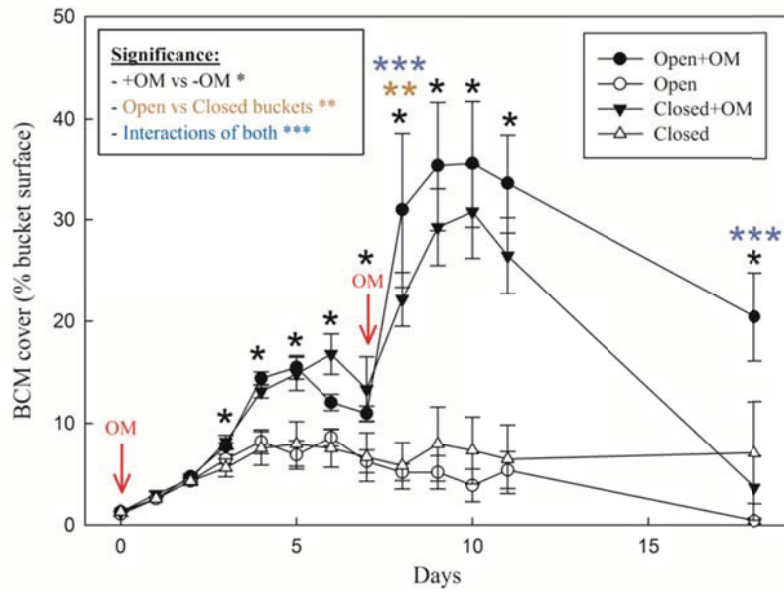
Figure 4. BCM coverage (mean ± SEM, n = 6) in open bottom and closed bottom buckets with initial BCM seed with and without OM enrichment over the duration of the experiment. Red arrows indicate when OM was added. Analysed by two-way ANOVA. Stars indicate significant differences.
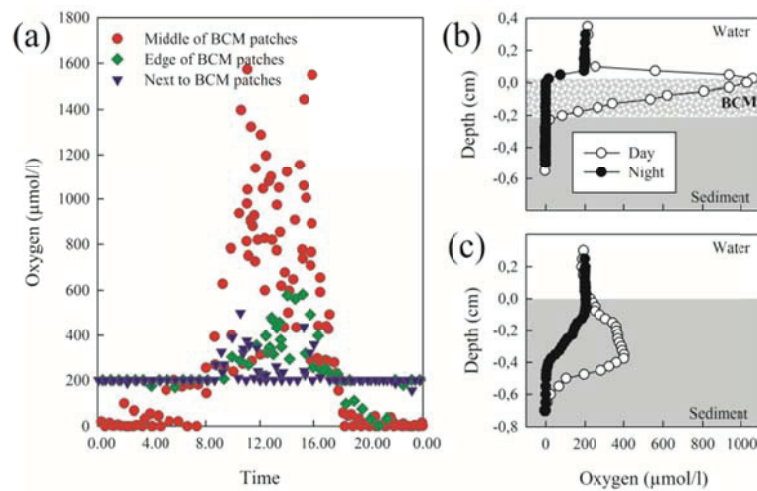
Figure 5. (*a*) O_2 concentration across BCM patches. During photosynthesis, O_2 peak concentrations per profile are plotted; otherwise surface concentrations are given (n = 350 profiles). (*b* and *c*) Examples of *in situ* O_2 profile across the water-sediment interface during day and night with BCM (*b*) and without BCM (*c*).
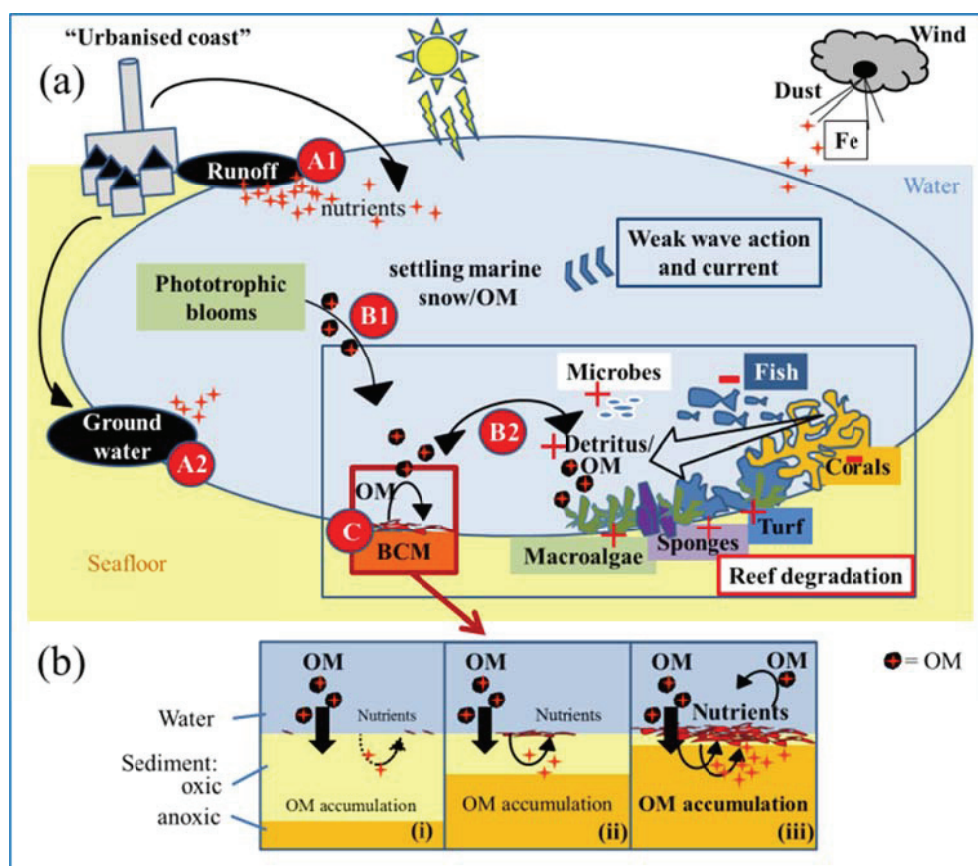
Figure 6. (*a*) Proposed model of sources and cycle of nutrients stimulating BCM growth. Nutrient inputs from land runoff (A1) or groundwater seepage (A2) cause benthic and planktonic phototrophic blooms. Fe is largely available as a result of long-term Fe addition by the African dust [51]. The blooms decay and produce particulate OM (B1) which settles on the seafloor as a function of wave action and current. The coral reef community takes up, produces and releases OM (B2) [18, 31, 48, 65]. Reef degradation leads to a detritus-based food web and enhances OM accumulation on the seafloor. Increased OM loading leads to BCM growth (C). (*b*) Schematic drawing of the water/sediment interface. Drawings from far away from (i), near to (ii), and middle of (iii), BCM patches are used as analogs for what we anticipate as OM accumulates over time. Increased OM concentration in the sediments results in a thinner oxygenated surface layer and an increased nutrient release, which triggers BCM growth. When BCMs develop, they produce OM and trap OM from the water column to sustain their growth and expand.

Supplements
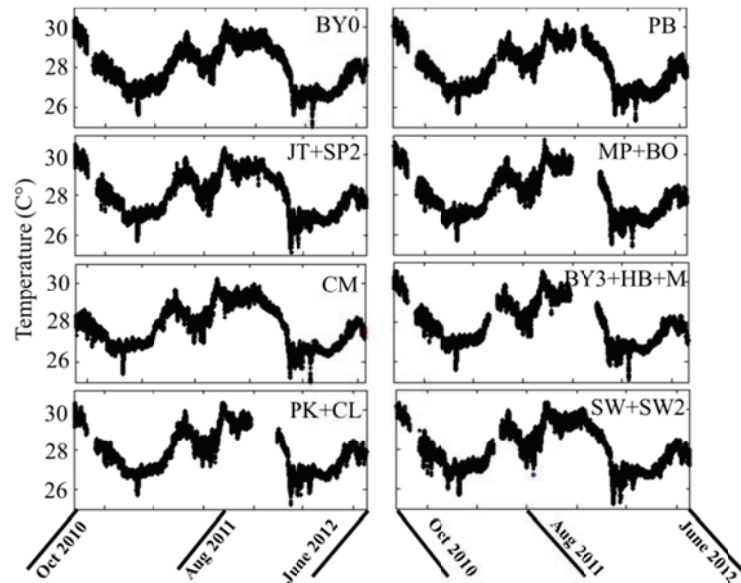
Figure S1. Seawater temperatures on the reef slope at 10 m depth at the 4 low BCM abundance (left column) and the 4 high BCM abundance (right column) sites from September 2010 to June 2012. Site abbreviations: BY0 = Carmabi buoy 0, PB = Pest Bay, JT = Jan Thiel, SP2 = South Port station 2, MP = Marie Pompon, BO = Boca (Bullen Bay), CM = Cap Malmeeuw, BY3 = Carmabi buoy 3; HB = Holiday Beach; M = Santa Martha Bay; PK = Playa Kalki; Cap Lÿhoek; SW = Spanish Water station 1+2.
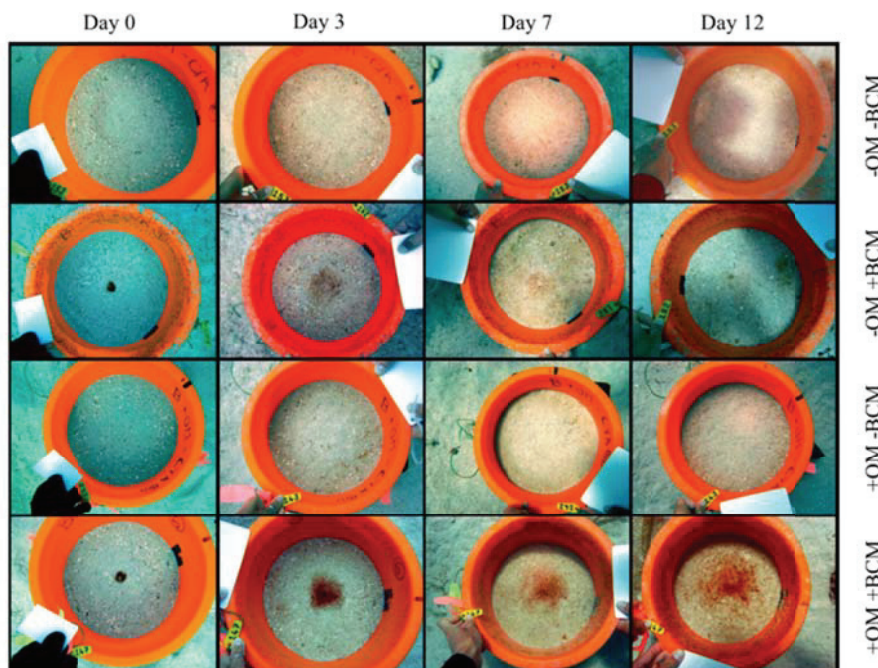
Figure S2. Representative photographs of the sediment surface for BCM seeded and non-seeded treatments with and without OM enrichment on days 0, 3, 7 and 12 (closed bucket).

Table S1. PERMANOVA results of the effects of season (fixed), time periods nested within season (random), urbanisation and wave height (covariates) on BCM abundance scores.

Source in relation to cyanobacterial abundance	df	Pseudo-F	P(perm)
season (se)	1	3,06	0,001***
time points (nested in season) (ti(se))	2	1,04	0,374
urbanisation status (ur)	1	53,98	0,001***
wave heights (wa)	1	102,57	0,001***
ur x wa	1	3,99	0,037*
ur x se	1	0,03	0,844
wa x se	1	0,10	0,763
ur x ti(se)	2	1,05	0,357
wa x ti(se)	2	2,14	0,12
ur x wa x se	1	3,02	0,099
ur x wa x ti(se)	2	0,58	0,536

Table S2. PERMANOVA results of the effects of season (fixed), BCM site abundance (fixed) and site nested within BCM site abundance (random) on Clodcard dissolution rates.

Source in relation to clodcard dissolution rates	df	Pseudo-F	P(perm)
season (se)	1	4,06	0,091
BCM abundance (BCM)	1	104,95	0,026*
site nested in BCM (si(BCM))	6	9,75	0,001***
BCM x se	1	0,02	0,89
se x si(BCM)	6	18,13	0,001***

Table S3. PERMANOVA results of the effects of season, BCM site abundance, depth, BCM patch (i.e. above BCM vs. above BCM-free substrate) and site nested within BCM abundance (as appropriate) on NOx and PO_4^{3-} concentrations in the surface water (SW), open ocean water (OW), intermediate water (IW) and bottom water (BW).

	Source in relation to nutrient concentrations	df	Pseudo-F[a]	P(perm)	df	Pseudo-F[a]	P(perm)
			NOx			**PO_4^{3-}**	
SW	season (se)	1	0,00	0,987	1	0,85	0,437
	BCM abundance (BC)	1	2,68	0,161	1	1,11	0,258
	site nested in BCM (si(BC))	6	0,24	0,962	6	0,88	0,615
	se x BC	1	2,17	0,188	1	0,86	0,448
	se x si(BC)	6	1,06	0,396	6	0,97	0,518
OW	season (se)	1	0,18	0,687	1	1,54	0,294
	BCM abundance (BC)	1	0,05	0,81	1	0,32	0,686
	site nested in BCM (si(BC))	6	0,41	0,873	6	1,42	0,233
	se x BC	1	2,02	0,21	1	0,30	0,667
	se x si(BC)	6	0,34	0,899	6	1,84	0,121
IW	season (se)	1	0,02	0,882	1	2,36	0,179
	BCM abundance (BC)	1	4,35	0,071	1	0,68	0,553
	Site nested in BCM (si(BC))	6	1,05	0,424	6	2,15	0,051
	depth (de)	1	0,23	0,651	1	2,77	0,128
	se x BC	1	0,09	0,776	1	0,95	0,405
	se x de	1	0,37	0,548	1	2,25	0,182
	BC x de	1	7,60	0,04*	1	0,08	0,769
	se x si(BC)	6	2,67	0,028*	6	2,02	0,055
	si(BC) x de	6	0,39	0,895	6	0,16	0,991
	se x BC x de	1	0,03	0,886	1	0,08	0,774
	se x si(BC) x de	6	1,67	0,15	6	0,12	0,997
BW	season (se)	1	6,96	0,041*	1	6,88	0,05
	BCM abundance (BC)	1	8,80	0,005**	1	0,20	0,632
	Site nested in BCM (si(BC))	6	1,18	0,308	6	3,34	0,004**
	depth (de)[b]	1	6,22	0,047*	1	4,34	0,068
	patch (pa)	1	11,26	0,011*	1	13,76	0,01**
	se x BC	1	8,16	0,043*	1	0,21	0,634
	se x de[b]	1	1,19	0,32	1	1,74	0,219
	se x pa	1	2,98	0,137	1	8,10	0,029*
	BC x de[b]	1	7,87	0,032*	1	0,04	0,837
	BC x pa	1	0,15	0,701	1	0,00	0,991
	de[b] x pa	1	4,25	0,102	1	3,40	0,113
	se x si(BC)	6	0,71	0,624	6	2,91	0,009**
	si(BC) x de[b]	6	0,40	0,886	6	1,73	0,102
	si(BC) x pa	6	1,18	0,331	6	1,81	0,098
	se x BC x de[b]	1	1,34	0,315	1	0,02	0,911
	se x BC x pa	1	1,71	0,241	1	0,02	0,863
	se x de[b] x pa	1	0,62	0,467	1	0,65	0,463
	BC x de[b] x pa	1	8,76	0,03*	1	0,06	0,827
	se x si(BC) x de[b]	6	0,22	0,975	6	1,66	0,112
	se x si(BC) x pa	6	1,04	0,413	6	1,53	0,159
	si(BC) x de[b] x pa	6	0,26	0,957	6	1,11	0,363
	se x BC x de[b] x pa	1	0,34	0,602	1	0,26	0,647

| se x si(BC) x de[b] x pa | 6 | 0,48 | 0,825 | 6 | 1,25 | 0,287 |

[a] * = $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; [b] The effect of depth is confounded with the effects of substratum and mat types since brown-colored mats over sand and red-colored mat over hard substrates were sampled at 5 and 15 m depths, respectively.

Table S4. PERMANOVA results of the effects of BCM site abundance (fixed) and site nested within BCM site abundance (random) on concentration of particulate organic matter in the water column.

Source in relation to of particulate organic matter	df	Pseudo-F	P(perm)
BCM abundance (BCM)	1	1,56	0,245
site nested in BCM (si(BCM))	6	2,19	0,074

3. Manuscript II

MS II: Nitrogen fixation and diversity of expanding benthic cyanobacterial mats on Curaçaoan coral reefs, Southern Caribbean

(In preparation)

Nitrogen fixation and diversity of expanding benthic cyanobacterial mats in coral reefs of Curaçao, Southern Caribbean

Short Title: Cyanobacterial mats on Curaçaoan coral reefs

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Abstract

The abundance of benthic cyanobacterial mats has increased drastically on many coral reefs worldwide, however little is known about the ecological and functional roles of these mats on Caribbean coral reefs. We investigated the abundance and cyanobacterial diversity of microbial mats at four coral reef sites on Curaçao, Southern Caribbean: two sites with low total mat cover (< 1% cover of the reef benthic substrate) and two sites with high total mat cover (5-40% cover). Nitrogen fixation rates were measured only in large-sized mats (>20 cm^2). In total, we found 22 different cyanobacterial species from the order *Oscillatoriales* present within the mats. More specifically, the large-sized mats were dominated by *Hydrocoleum glutinosum*, *Oscillatoria bonnemaisonii* or *Lyngbya majuscula*. 16S rRNA-based phylogeny revealed similar cyanobacterial species to those 22 species identified by microscopy, and additionally we foundsequences belonging to unicellular (*Xenococcus* and *Chroococcidiopsis*) and heterocystous (*Rivularia* and *Calothrix*) cyanobacteria. The detection of similar cyanobacterial types on the same sites as 40 years ago indicates that changes in environmental conditions over these years favored indigenous species to bloom, rather than introducing invasive species into the ecosystem. Nitrogen fixation rates of the large-sized mats were 3-10 times higher in the light than in the dark. The highest areal nitrogen fixation rate was recorded in the mat dominated by *O. bonnemaisonii* (i.e., 169.1 mg N m^{-2} d^{-1}), found at a reef site with high mat cover. A scale up of nitrogen fixation at this site yielded an areal rate of 13 mg N m^{-2} reef d^{-1}, which exceeds rates reported in open ocean blooms of *Trichodesmium* in the Caribbean. Our results suggest that the Caribbean basin is not only a hotspot for planktonic nitrogen fixation, but also for benthic nitrogen fixation. Because the mats fix vast amounts of nitrogen, they likely have a strong influence on the nitrogen budget of the reef ecosystem as a whole. (311/300 words)

Introduction

Coral reefs are highly productive ecosystems yet thrive in highly nutrient-poor environments (Crossland et al. 1991). Dissolved inorganic nitrogen, next to phosphorus and iron, often limits the growth of reef phototrophic organisms (Lapointe 1997; Larned 1998; Den Haan et al. 2013). Biological nitrogen fixation (diazotrophy) by cyanobacteria has been considered as an important source of nitrogen, sustaining a substantial part of the reef's primary production (Wiebe et al. 1975; Larkum et al. 1988; Charpy-Roubaud et al. 2001). Therefore, cyanobacteria are suggested to play a key role within the reef's nitrogen cycle (Wiebe et al. 1975; Hallock 2005; Charpy et al. 2012). On current-day coral reefs with a high loss of live coral, this might especially be apparent, as many have become increasingly dominated by benthic cyanobacterial mats (Thacker and Paul 2001; Albert et al. 2005; Paul et al. 2005; Paerl and Paul 2012). Future predictions signify that

cyanobacterial mats will continue to increase in cover on many reefs around the world, as they favor changes in environmental conditions that are associated with direct and indirect anthropogenic impacts (e.g., eutrophication and overfishing), and climate change (e.g., increase in water temperature) (Hughes 1994; Jackson et al. 2001; Hallock 2005; Diaz-Pulido et al. 2009; Paerl and Paul 2012). Benthic cyanobacterial mats inhibit coral larvae settlement (Kuffner et al. 2006), acts as coral pathogens (Carlton and Richardson 1995), and can produce toxins that lead to selective browsing by herbivorous fish (Nagle and Paul 1998, 1999).

The diversity of dense cyanobacterial mats and their nitrogen fixation potential has been investigated on coral reef ecosystems in the North and South Pacific Ocean, as well as in the Indian Ocean (Larkum et al. 1988; Kayanne et al. 2005; Abed et al. 2006; Charpy et al. 2007; Bauer et al. 2008; Charpy et al. 2010). The Caribbean region however remains largely understudied, except in the Florida Keys (Paul et al. 2005), even though it is known as a hotspot for pelagic nitrogen fixation (Luo et al. 2012). Here, iron is likely not a limiting nutrient due to the periodic input of iron via aeolian Saharan dust (Roff and Mumby 2012). Therefore, the aims of this study were (1) to estimate the abundance of cyanobacterial mats and their cyanobacterial diversity, (2) to identify the major cyanobacterial species forming these mats, (3) to investigate the nitrogen fixation potential of the most dominant mats, and (4) to upscale nitrogen fixation of the mats to the reef-wide scale.

Materials and Methods

Mat distribution and sample collection

This study was conducted in the fall (October-December) of 2010 at four different reef sites on the island of Curaçao, Southern Caribbean (Fig. 1). We included two sites with high cyanobacterial mat abundance on the reef (i.e., Pest Bay (PB) and Buoy 0 (BY)), and two sites with low mat abundance (i.e., Cap Malmeeuw (CM) and Spanish Waters (SW)). The percentage cover of the most dominant cyanobacterial mats at all four sites was determined from 25 quadrates (1 m^2), which were randomly placed alongside the 3, 7, 10, 20 and 30 m isobaths. The coverage was assessed visually with the help of cross intersects inside the quadrate and photos which were processed using the software CPCe (Kohler and Gill 2006). Additionally, all cyanobacterial mats within the first 12-15 quadrates from each depth and site were sampled by hand picking (1-2 cm^2) (total amount of mats sampled see Electronic Supplementary Material (ESM) Table 1), placed inside individual Falcon tubes (60 ml), shaded, and were immediately transported back to the laboratory inside a small, cool box filled with ambient seawater (~29°C). In the laboratory, each mat sample was (1) fixed with 4% formaldehyde (w:v) solution for morphological analysis, and (2) frozen at -20°C for molecular analysis (see below). Also, we

categorized each sampled mat into 11 visually distinguishable mat types, and determined their species composition accordingly (Table 1).

Morphological analyses

Genus or species of each cyanobacterial mat sampled were identified using direct microscopy. The phenotypic identification was performed using a Zeiss Axioskop 40 microscope on which a Zeiss camera (AxicCam ICc1) was mounted. Using the computer program Axiovision (Carl Zeiss, Jena, Germany), we determined morphological features such as sheath, calyptras, end cell morphology, cell sizes (n=50 per sample) and proportions and degree of constriction at cross-walls. Subsequent taxonomic identification of the cyanobacterial species was based on the identification keys of Komárek and Anagnostidis (2005) and Komárek and Hauer (2009). The dominance of each genus or species within each cyanobacterial mat was recorded after screening multiple samples (n=3-5). The distribution of the dominant morphotypes was compared at the different depths of the four studied sites.

Molecular analyses

Extraction of DNA was performed from 62 mat samples collected at different depths and sites according to Zhou et al. (1996). PCR amplification was done by using the cyanobacteria-specific primers CYA359F and CYA781R (Nuebel et al. 1997), with 40 nucleotide GC-clamp. DGGE was carried out on 16S rRNA gene amplicons according to the protocol as described by Muyzer and Smalla (1998). Amplified PCR products were loaded on a Dcode DGGE system (Bio-rad, Hercules, USA) with a linear gradient of 30-65% of urea and formamide. Electrophoresis was performed for 4 h at 200 V and 60 °C. Staining of nucleic acids was performed in 1% SYBRGold Nucleic Acid Stain (Molecular Probes, Eugene, USA) and dominant bands were excised and sequenced. DNA re-amplification for sequencing was performed using the initial primers without GC-clamp at the same cycling conditions. PCR products were purified from contaminants using the MiniElute PCR purification kit (Quiagen, Hamburg, Gemany). Sequencing was performed using the BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Carlsbad, USA). Products from the sequencing reactions were purified using the Agencourt CleanSEQ kit (Beckman Coulter Genomics, Danvers, USA). Sequences were retrieved using a 3130x Genetic Analyzer (Applied Biosystems, Calsbad, USA). Sequence data was processed using the CLC main workbench software version 6.0.2 (CLCbio, Aarhus, Denmark). Ambiguous bases in all sequences were resolved manually.

The obtained sequences were aligned and analyzed using the ARB software version 071207 (Ludwig et al. 2004) and the official SILVA database (http://www.arb-silva.de) for small subunit

RNA sequences (SSURef_NR99_115_SILVA_20_07_13_opt.arb) (Pruesse et al. 2007). Complete cyanobacterial 16S rRNA gene sequences available from GenBank were imported and aligned to the sequences in the ARB database. The obtained cyanobacterial forward and the reverse complementary of the reverse sequences obtained were aligned against each other in order to obtain consensus sequences. These sequences were then aligned with the sequences in the ARB database using the alignment ARB tool. The alignment was corrected manually. The phylogenetic trees were calculated by maximum likelihood (ML) method, based on long 16S rRNA gene sequences (>1300 bp). The partial sequences obtained in this study were inserted into the ML tree using the parsimony ARB tool, while maintaining the overall tree topology without changes.

Nitrogen fixation experiments

At the study sites with high cyanobacterial mat abundance (i.e., PB and BY), we manually collected six large-scale mats from five different depths using SCUBA diving, which we called Mats A - F (see Fig. 2A-F). The mats were directly placed inside shaded 5 ml Falcon tubes and immediate transported back to the laboratory. Furthermore, we collected *in situ* seawater using Plexiglas tubes (5.3 L). In the laboratory, the *in situ* seawater was filtered using 0.22 μm Whatman cellulose acetate membrane filters. Nitrogen fixation rates of large-scale cyanobacterial mats (>20 cm^2) were assessed using the acetylene reduction assay (ARA). Incubation experiments were simulated under natural conditions over a diel cycle according to Montoya et al. (1996). Incubations were performed in 38 ml gas-tight serum bottles that were placed inside a flow-through aquarium. This aquarium was connected to a water pump that provided continuous water flow to ensure a similar temperature in the aquarium as on the reef (27-29°C). Furthermore, the maximum light intensities in the laboratory were 201.6 ± 27.9 μmol photons m^{-2} s^{-1} for the incubated cyanobacterial mats originating from 7 m depth, and 90.9 ± 4.3 μmol photons m^{-2} s^{-1} for the mats originating from 20 m depth. These light intensities were similar to those reported at 7 and 20 m depth at Buoy 0 by Den Haan et al. (2014). For all the incubations we used 1 cm^2 of mat. Incubations lasted for 24 h, starting at 1830 hrs (sunset) and ending at 1830 hrs the next day. Light intensity and temperature during the incubations were recorded with a UA-002-08 data logger (Onset Computer Corporation, Pocasset, USA). Each incubation-set for each mat consisted of triplicate mat samples and one control, with no biological material added to the seawater. 4.5 ml of high purity acetylene gas (Linde Gas, Willemstad, Curaçao) was added to the headspace (13 ml) at 1830 hrs with a gas-tight syringe. Due to constraints in the volume of gas that could be withdrawn from each bottle, several sets were used. One set (three replicates plus one control) was sampled during the night (1830 hrs to 0630 hrs), while another was

sampled during the day (0930 hrs to 1800 hrs). During the daytime a separate set was kept dark. A 1 ml gas sample was taken from each replicate and each control every 3 h with a gas-tight syringe. The gas then was injected into 6 ml Vaccuetes® (Greiner Bio-One, Frickenhausen, Germany) filled with a saturated sodium chloride solution in order to properly conserve the sample until further analysis. Ethylene production was measured by injecting 0.2 ml of headspace gas into a gas chromatograph with flame ionization detector at the University of Bremen (HRGC-4000 A, Konik, Sant Cugat del Vallès, Spain) (Stewart et al. 1967). After the incubation experiment, chlorophyll a was extracted from the incubated mats with pure methanol and concentrations were subsequently measured spectrophotometrically according to Porra et al. (1989). Nitrogenase activity was calculated according to Capone (1993) and results are presented as rates normalized to chlorophyll a content (phototrophic biomass) as well as surface area (m^2). Nitrogenase has a 3-4 fold higher affinity towards acetylene in comparison to dinitrogen (Montoya et al. 1996), therefore we used a conversion factor of 4 as described previously by Peterson and Burris (1976).

Statistical analysis

To determine how dominant each cyanobacterial species was within the cyanobacterial mats (from all four sites), we calculated the species' frequency distribution and subsequently used the Pearson correlation to determine the similarity between the mats. The Shannon Index was used to determine whether the diversity of the cyanobacterial species was comparable between all studied sites. We used the analysis of covariance (ANCOVA) to determine whether species richness differed between sites with low and high mat cover, and whether species richness within the mats was affected by depth.

A Bray-Curtis similarity matrix, with a dummy variable to account for partly denuded assemblages in some samples (Clarke et al. 2006), was used to look for differences in species composition between mat types. Differences were formally tested across sites using a one-way permutation-based analysis of variance (PERMANOVA, Anderson 2001) based on unrestricted permutations of the raw untransformed abundance score data and mat type as a fixed factor. Given the low number of possible permutations in the individual pair-wise tests, we used the Monte Carlo asymptotic p values. Principal coordinates analysis (PCO) was used to visualize the data in two dimensions. Species contributing to differences between mat types were identified based on the strength of their Spearman correlations with the PCO axes. Analyses were performed in PRIMER 6 and PERMANOVA+ statistical package (Anderson et al. 2008).

Results

Cyanobacterial mat and coral coverage

At all four sites, dense cyanobacterial mats were detected on both hard substrates (incl. coral skeleton, turfs and macroalgae) and on the sediment. Mat structures ranged from soft gelatinous masses (ca. 3-6 mm thick) to firmer tufts (ca. 0.5-20 cm thick). The mats exhibited a range of different colors, including brown and violet mainly in the upper 10 m of the reef, to orange or red deeper down (Fig. 2). The sites PB and BY had the highest total cyanobacterial mat cover (15.7% and 10.8% of the quantified area, respectively, averaged over all five depths), whereas benthic mats at the sites CM and SW remained largely inconspicuous (<1% at all five depths) (ESM Fig. 1). Conversely, the total coral cover, averaged over all depths, was higher at the CM and SW sites (52.4% and 36.2%, respectively) compared to the PB and BY sites (13.5% and 12.4%, respectively). At PB and BY, cyanobacterial mats were most dominant in the upper 10 m of the reef as opposed to in the deeper parts of the reef (i.e., 20 and 30 m depth).

Microscopic and phylogenetic diversity of cyanobacteria

A total of 22 different cyanobacteria were identified to genus or species level based on their microscopic features (ESM Fig. 2). Cyanobacterial mats were dominated by filamentous non-heterocystous cyanobacteria of the order Oscillatoriales. The diversity of dominant cyanobacterial species was comparable at all sites (Shannon Index: CM = 2.6, SW = 2.5, PB = 2.7, BY = 2.4) and no significant differences in species richness could be found between the sites with low and high mat cover (ANCOVA: $F_{1,17}$ = 0.591, p = 0.452). Species richness within the mats was also unaffected by depth (ANCOVA: $F_{1,14}$ = 0.947, p = 0.466).

Each mat was primarily dominated (60-98%) by a single cyanobacterial species, although other minor species were also observed. The major mat-building species belonged to the genera *Hydrocoleum*, *Lyngbya*, *Phormidium*, *Symploca*, *Oscillatoria*, *Tychonema*, *Schizothrix*, *Pseudanabaena* and *Dichothrix* (Fig. 3). Across all sites and depths, the species *Lyngbya majuscula* was most frequently observed within the mats (15.5%), followed by *Hydrocoleum glutinosum* (12.7%) and *Symploca hydnoides* (11.2%) (Fig. 3). At the sites PB and BY, *L. majuscula* exhibited its highest abundance at 20 m and 30 m whereas *H. glutinosum* was more abundant at 3 m and 7 m depths. The abundance of the cyanobacterium *S. hydnoides*, averaged over all depths, was slightly higher at the CM and SW sites compared to PB and BY sites (16% and 15% vs. 9% and 5%, respectively). The cyanobacteria *Oscillatoria* spp., *O. bonnemaisonii* and *Dichothrix utahensis* could be detected at all sites, except the SW site. *O. bonnemaisonii* exhibited its highest abundance of 38% of the total area at the PB site at 7 m depth. Indeed, this cyanobacterium formed large dark brown and light brown mats on sand patches at PB and BY sites at 3 and 7 m depths (used for nitrogen fixation measurements, see below). The color and appearance of these mats could always be linked to the

cyanobacterial species composition therein, where the dark brown mats contained ~100% *O. bonnemaisonii,* while the light brown mats consisted mainly of *H. glutinosum* (~70%) and *O. bonnemaisonii* (~30%). *Symploca atlantica*, *Lyngbya sordida* and *Lyngbya aestuarii* could not be found at any depth of the PB site but were detected at least at one depth of the remaining sites. The cyanobacteria *Schizothrix* spp. and *Pseudanabaena* were not encountered at any depth of the CM site. The remaining cyanobacterial species were found at least at one depth of all the four sites. DGGE in most cases displayed multiple bands (2-5), indicating the co-dominance of more than one cyanobacterial species in each mat (ESM Fig. 3). A total of 137 good quality sequences were obtained from DGGE bands and these sequences were phylogenetically affiliated to different species of unicellular and filamentous cyanobacteria (Fig. 4). A large fraction of the sequences (total 33) was related to thin filamentous cyanobacteria of the genera *Phormidium*, *Leptolyngbya* and *Pseudanabaena*. These sequences were encountered in mats from all four sites. Sequences related to *Symploca* spp. constituted 13% of retrieved sequences and were only detected at BY and CM sites. Sequences belonging to unicellular cyanobacteria were detected at PB, BY and SW sites and were affiliated to the genera *Xenococcus* and *Chroococcidiopsis*. A single sequence closely related to *Spirulina subsalsa* was exclusively found at the BY site. Sequences related to known heterocystous cyanobacteria of the genera *Rivularia* and *Calothrix* were only encountered at the PB and BY but not at the SW and CM sites. While 4 sequences obtained from the PB site fell phylogenetically close to *Oscillatoria spongeliae*, another 14 obtained from the other sites (i.e. BY, CM and SW) fell close to the sequence of *Oscillatoria margaritifera*. *Hydrocoleum*-related sequences were also retrieved from all sites, except the CM site, and were closely affiliated to sequences of the species *H. lyngbyaceum* and *H. glutinosum*. Mats from all sites generated sequences related to the genus *Lyngbya*. PERMANOVA indicated that there were significant differences in species composition among the 11 visual mat types (Pseudo-F = 11,63, P_{perm} < 0.001) (see Table 1 and ESM Tables 1 and 2). However, the species composition of a mat type could not be predicted solely on the appearance of that mat type on the reef. However, four mat types, (i.e., 'brown mat', 'brown shade', 'red hairy' and 'red veil on red hairy') were always dominated by the same species: *O. bonnemaisonii* (brown mat), *H. glutinosum* (brown shade) and *L. majuscula* (red hairy and red veil on red hairy). Species number versus number of samples were constructed and each time showed a plateau (ESM Fig. 4), indicating that the number of samples was appropriate to document the presence/absence of each species within the mat type.

Nitrogen fixation

All incubated mats fixed nitrogen with rates 3-10 times higher during light periods than in the dark (Table 2). The highest rates were measured in the morning between 0930 hrs and 1230 hrs,

except in Mat C (i.e., cyanobacterial mat dominated by *O. bonnemaisonii*, see Fig. 2C), where the highest rate was detected at 1430 hrs (ESM Fig. 5). The calculated areal nitrogen fixation rate was highest in Mat C, which was exclusively found at the PB and BY sites, and was estimated at 169.1 mg N m^{-2} d^{-1}. While the areal nitrogen fixation rates were 9.3 and 14.4 mg N m^{-2} d^{-1} in Mat A (Fig. 2A) and Mat B (Fig. 2B), respectively, it was ≤ 4.3 mg N m^{-2} d^{-1} in the remaining mats. When considering the chlorophyll *a* concentration for the calculation of nitrogen fixation rates, Mat A and B revealed the highest values of 166.8 and 66.6 nmol N$_2$ μg chl *a* d^{-1}. Mat C (Fig. 2C), which had the highest chlorophyll *a* concentration per cm^2, showed a lower nitrogen fixation rate than the other mats. Since the mat types 'brown mats' (n=20) and 'brown shade' (n=6) were homogeneous in species composition, as confirmed by ESM Fig. 4, we were able to up-scale the combined total N$_2$ fixation potential of Mat A and C to the reef-wide scale at 7 m depth at PB, which amounted to 13 mg N m^{-2} reef d^{-1}.

Discussion

Cyanobacterial mats present on Curaçaoan coral reefs harbor a large variety of cyanobacterial species, as revealed by microscopy and molecular tools. An advantage of the morphological analyses is that it enabled us to compare the current cyanobacterial diversity with that observed in the same coral reef more than 40 years ago (Van den Hoek et al. 1975; Vooren 1981). Interestingly, most of the identified cyanobacteria in our microbial mats were also identified in the previous studies (Van den Hoek et al. 1975; Vooren 1981), however as part of turf communities, including *L. majuscula*, *L. sordida*, *Phormidium* sp. and *O. bonnemaisonii*. This might indicate that the changes in environmental conditions over the past 40 years have favored existing cyanobacterial species to bloom rather than introducing invasive species of cyanobacteria into the ecosystem. Most of the cyanobacterial genera encountered by direct microscopy, such as *Lyngbya*, *Oscillatoria*, *Hydrocoleum*, *Phormidium* and *Symploca*, were consistently detected using 16S rRNA-DGGE. However, additional genera such as *Leptolyngbya*, *Xenococcus*, *Chroococcidiopsis*, *Spirulina*, *Rivularia* and *Calothrix* could only be detected using the molecular technique. This underlines the need to combine different techniques to study the cyanobacterial diversity in order to circumvent the limitations associated with each technique (Abed and Garcia-Pichel 2001; Abed et al. 2003).

Most of the detected cyanobacterial species and phylotypes described in this study have also been documented in other coral reefs in the Pacific and Indian Ocean (Abed et al. 2003; Thacker and Paul 2004; Charpy et al. 2010; Charpy et al. 2012). Interestingly, microbial mats from these coral reef systems as well as from Curaçao have often been dominated by a single cyanobacterium (Abed et al. 2003; Thacker and Paul 2004; Charpy et al. 2010; Charpy et al. 2012).

At the four studied sites, the large-sized patches were mainly dominated either by *Hydrocoleum glutinosum*, *Oscillatoria bonnemaisonii* or *Lyngbya majuscula*. In recent years, similar mass occurrences of *Lyngbya*, including *L. majuscula*, have been reported around the world (Thacker and Paul 2001; Albert et al. 2005; Paul et al. 2005; Charpy et al. 2012; Paerl and Paul 2012). *Hydrocoleum* spp. and *O. bonnemaisonii* have also been detected in high abundance in microbial mats from La Reunion and Tikehau Atoll (Charpy et al. 2007; Charpy et al. 2010; Charpy et al. 2012). *O. bonnemaisonii* and *H. glutinosum* are known to occur in shallow tropical coral reefs growing mostly on carbonate sediment (Charpy et al. 2010), as observed in this study. Both species have the ability to form multi-filamentous structures enabling them to be pioneer organisms of unstable sediments (Garcia-Pichel and Wojciechowski 2009). The filamentous cyanobacteria belonging to the genus *Hydrocoleum* are among the most common mat-forming cyanobacteria in tropical oceans (Abed et al. 2003; Abed et al. 2006). These cyanobacteria have been shown to be a major contributor to nitrogen fixation in tropical oceans and to share a common evolutionary origin with the planktonic *Trichodesmium* species (Abed et al. 2006; Charpy et al. 2010). Members of the genus *Lyngbya* and other benthic cyanobacteria have been intensively studied with regard to their production of toxins, which enables them to survive grazing pressure by herbivores (Thacker et al. 1997; Nagle and Paul 1999; Thacker and Paul 2004). This renders benthic cyanobacteria as a successful competitor in their environment.

A major goal of our study was to assess the contribution of expanding cyanobacterial mats to nitrogen fixation on the coral reefs of Curaçao. All studied cyanobacterial mats exhibited an ability to fix nitrogen at higher rates during the day than during the night. This indicates that most of the nitrogen fixation was performed by phototrophs, most likely cyanobacteria (Stal et al. 2010). The cyanobacteria *O. bonnemaisonii*, *H. glutinosum*. and *L. majuscula*, which dominated large-sized mats, have previously been shown to fix nitrogen in cultures or to be associated with a nitrogen-fixing microbial mat community (Elmetri and Bell 2004; Charpy et al. 2010; Charpy et al. 2012). Non-heterocystous *Oscillatoriales* species were generally believed to separate the incompatible reactions of oxygenic photosynthesis and nitrogen fixation on a temporal basis, fixing mainly at night when oxygen concentrations are low (Bergman et al. 1997). On the contrary, studies on marine microbial mats demonstrated that nitrogen fixation was stimulated by light and phototrophic microorganisms were actively involved (Severin and Stal 2008, 2010). Our diurnal nitrogen fixation measurements are consistent with the latter pattern. This pattern has also been observed in cyanobacterial mats and turf communities in other coral reef systems (Wiebe et al. 1975; Charpy-Roubaud et al. 2001; Charpy-Roubaud and Larkum 2005; Charpy et al. 2007; Den Haan et al. 2014).

Our measured nitrogen fixation rates ranked amongst the highest reported so far, when compared to all published rates from other coral reef ecosystems (as a comparison, see Table 5 in Den Haan et al. (2014)). While a maximum nitrogen fixation rate of 169 mg N m^{-2} d^{-1} was measured in Mat C dominated by *O. bonnemaisonii*, most of the reported rates ranged between 0.1 and 5 mg N m^{-2} d^{-1}. In the Caribbean region, there is a lack of recent information on nitrogen fixation rates and those last documented were from the 80s and 90s, except for a recent study by Den Haan et al. (2014). Interestingly, Den Haan et al (2014) studied besides turf algae a brown mat also dominated by *O. bonnemaisonii* at another site in Curaçao in the summer of 2011, and found a similar nitrogen fixation rate: 167 mg N m^{-2} d^{-1}. This confirms the accuracy of our measurements. When the nitrogen fixation rates are scaled up at 7 m depth of the PB site, where cyanobacterial mats reached their highest coverage and were mainly consisting of mats of *O. bonnemaisonii* and *H. glutinosum* (Mat C and A), gross areal nitrogen fixation rates amounted to approximately 13 mg N m^{-2} reef d^{-1}. This value is higher than what was estimated for the shallow lagoon of La Reunion Island heavily colonized by mats (2 mg N m^{-2} lagoon d^{-1}) (Charpy et al. 2010), the reef site One Tree Reef in the southern Great Barrier Reef (2 - 4 mg N m^{-2} reef d^{-1}) (Larkum et al. 1988), the reef site/lagoon in Tikehau atoll, French Polynesia (1 - 8 mg N m^{-2} lagoon d^{-1}) (Charpy-Roubaud et al. 2001; Charpy-Roubaud and Larkum 2005) and the reef of Enewetak Atoll (0.5 mg N m^{-2} reef d^{-1}) (Wiebe et al. 1975). The observed rate was even slightly higher than the areal nitrogen fixation measured in open ocean blooms of *Trichodesmium* in the Caribbean (2.59 - 9.78 mg N m^{-2} d^{-1}) (Carpenter and Price 1977; Karl et al. 1997). In spite of that, it should be kept in mind that nitrogen fixation rates will most likely vary throughout the year due to seasonal changes in light, temperature and nutrient/organic matter availability, thus these estimations should be treated with caution. Nevertheless, our data suggests that the large-sized cyanobacterial mats on the coral reefs of Curaçao can fix vast amounts of nitrogen.

The Caribbean basin is known to be a hotspot for pelagic nitrogen fixation (Luo et al. 2012), yet our results signify that benthic nitrogen fixation is also substantial. High nitrogen fixation might be stimulated by the special conditions in the Caribbean basin: iron input by aeolian deposits (Sahara dust) can lead to nitrogen limitation, which favors diazotrophs (Roff and Mumby 2012; Den Haan et al. 2013). Additionally, increasing anthropogenic eutrophication and reef degradation in the Caribbean (Jackson et al. 2001; Gardner et al. 2003; Jackson et al. 2014) might further enhance nitrogen fixation. Previous studies on reefs from Mayotte, Tulear and La Reunion have shown that eutrophication and overfishing favored sudden bloom forming cyanobacterial mats able to fix dinitrogen (Charpy et al. 2012). Furthermore, high nitrogen fixation rates were reported in organic matter enriched reefs and sediments (Hanson and

Gundersen 1976; O'Neil and Capone 1989; King et al. 1990) and, organic matter is thought to increase on degraded reefs (Barott and Rohwer 2012).

We conclude that the increase in the abundance of cyanobacterial mats and their associated nitrogen fixation activities may pose a serious threat to coral reefs, particularly in the Caribbean region, which is known to have the lowest resilience of coral reefs worldwide (Roff and Mumby 2012). The measured high rates of nitrogen fixation by these mats may suggest that the Caribbean is not only a hotspot for planktonic but also for benthic nitrogen fixation.

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Figures
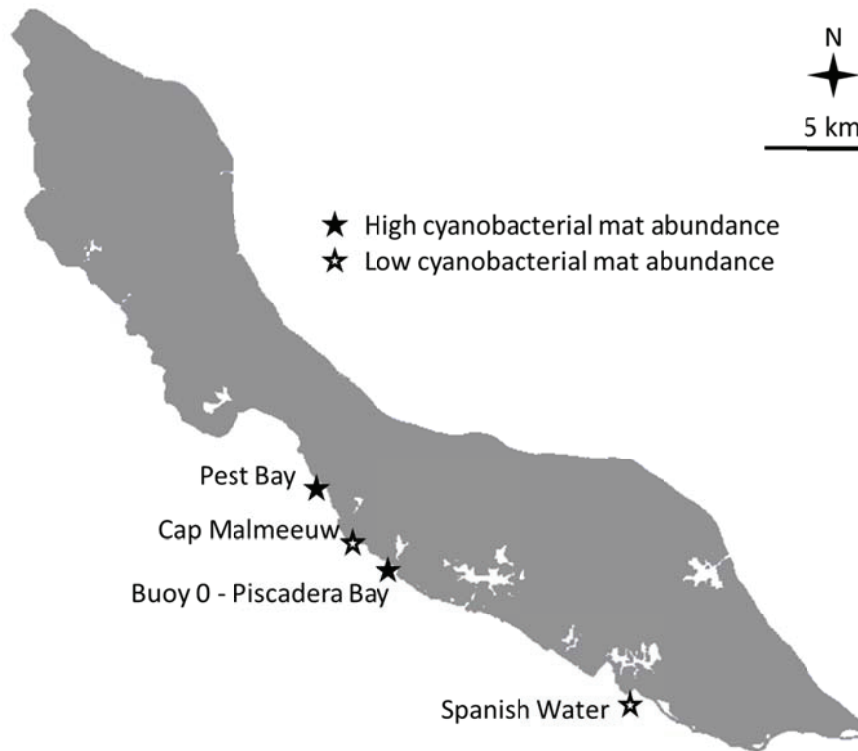
Fig. 1: Map of Curaçao, Southern Caribbean. Stars illustrate study sites.
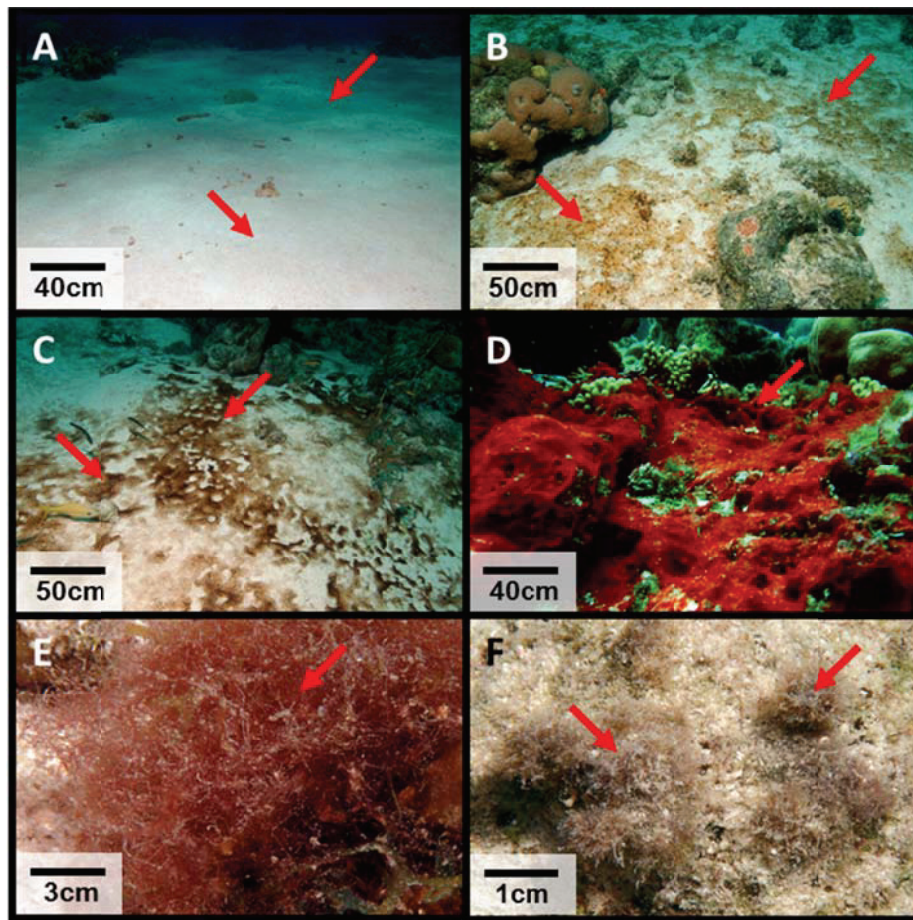
Fig. 2: Large scale patches of different cyanobacterial mats on Curaçao, which were analyzed for nitrogen fixation. Dominant species: (A) *Hydrocoleum glutinosum* & *Oscillatoria bonnemaisonii*, (B) *Hydrocoleum glutinosum* & *Lyngbya majuscula*, (C) *O. bonnemaisonii*, (D) *Oscillatoria* sp., (E) *Lyngbya majuscula* & *Trichocoleus acutissimus*, (F) *Dichothrix* sp.
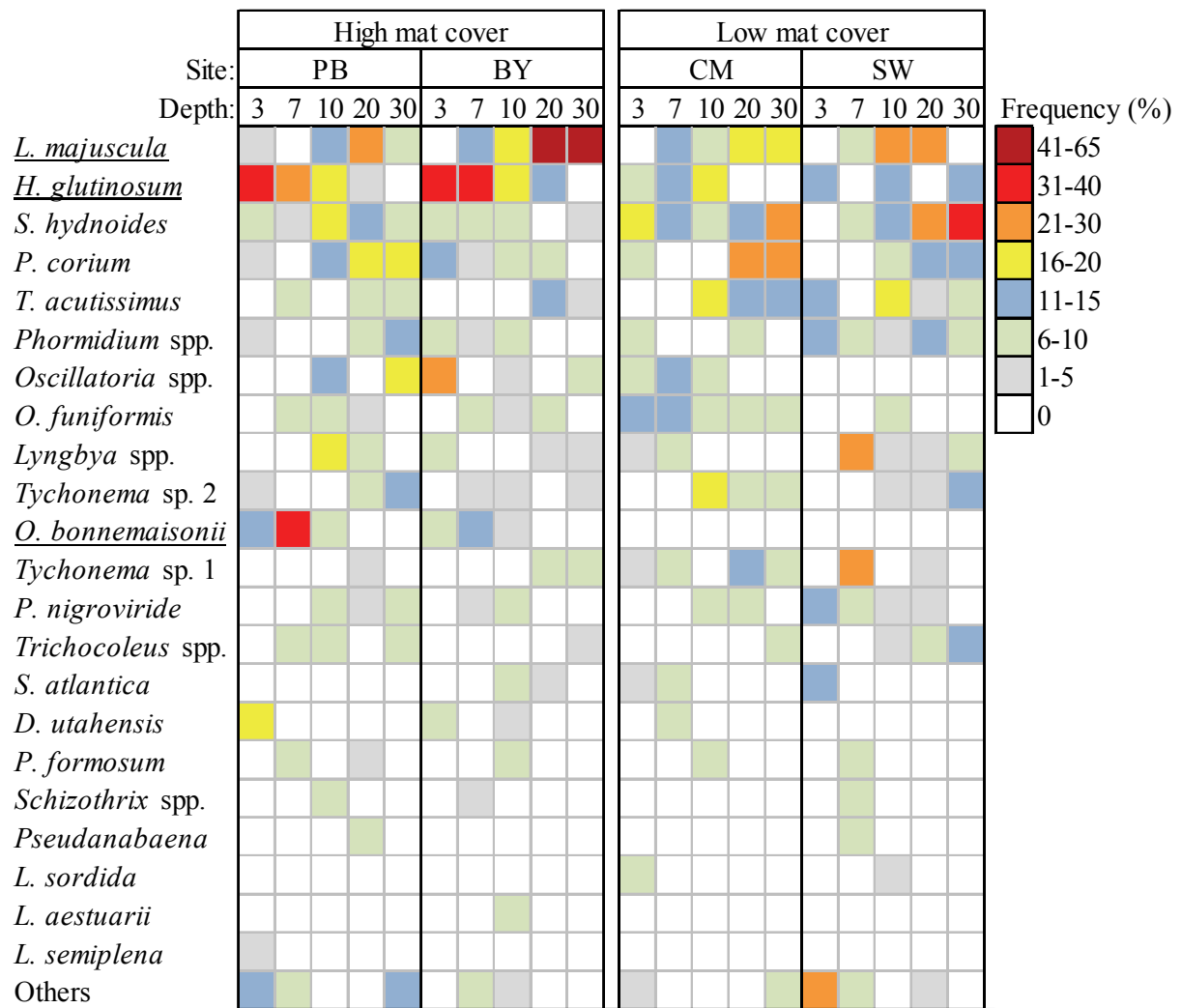
Fig. 3: Frequency of dominant species in sampled mats at four sites (Pest Bay = PB, Buoy 0 = BY, Cap Malmeeuw = CM, Spanish Waters = SW) and five depths (3, 7, 10, 20, 30m). Underlined names belong to most prominent species in cyanobacterial mats with the size class over 20 cm^2.

Fig. 4: (A) Genetic tree of analyzed sequences constructed using maximum likelihood method. (B) Overview of number of sequences successfully sequenced (Pest Bay = PB, Cap Malmeeuw = CM, Buoy 0 = BY, Spanish Waters = SW).

MS II

Table 1: Species list, means scores of abundance and total number of species and samples for the different visual types. Mean scores above 3 (≥ 40%) are highlighted in bold.

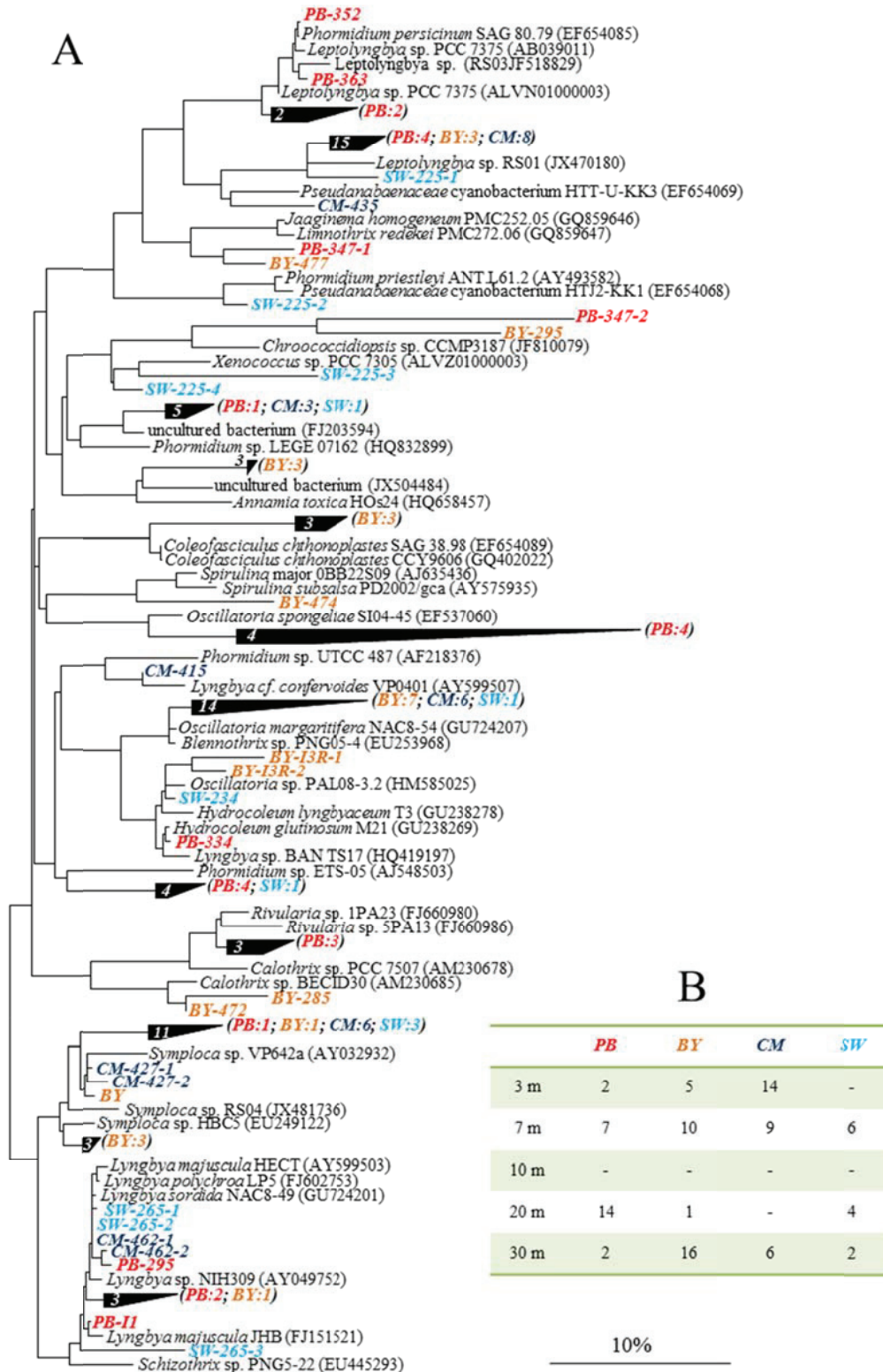| Species/visual type	Brown mat	Brown shade	Grey mass	Orange mat	Purple tuft	Red Hair (RH)	Red Mat	Red tips	Red veil	Red veil on RH	Other
Dichothrix utahensis					0.86±0.36						0.10±0.06
Hydrocolium glutinosum		**3.00±0.00**	2.75±0.72	1.00±0.68	0.21±0.17		0.37±0.11		0.33±0.33	0.73±0.29	0.52±0.13
Lyngbya aestuarii			0.42±0.42								0.02±0.02
Lyngbya majuscula			0.17±0.17		0.52±0.29	**4.73±0.11**	0.30±0.08	0.09±0.09		**3.20±0.35**	0.65±0.14
Lyngbya semiplena											0.04±0.04
Lyngbya sordida					0.34±0.24		0.04±0.04	0.06±0.06	0.33±0.33	0.17±0.17	0.05±0.04
Lyngbya spp.			0.67±0.43	0.50±0.50	0.69±0.33		0.18±0.08				0.20±0.09
Oscillatoria bonnemaisonii	**5.0±0.0**	2.33±0.21		1.13±0.64	0.34±0.24						0.04±0.04
Oscillatoria fumiformis			0.83±0.56				0.06±0.04				0.11±0.06
Oscillatoria spp.				0.50±0.50	0.24±0.18	0.05±0.05	0.41±0.11	0.12±0.09		0.13±0.10	0.21±0.08
Phormidium corium					0.10±0.10		0.40±0.11	2.18±0.39			0.48±0.13
Phormidium formosum							0.15±0.07			0.47±0.26	0.36±012
Phormidium nigroviride							0.27±0.09		1.00±0.53		0.11±0.06
Phormidium spp.							0.17±0.07	0.29±0.20			0.70±0.16
Pseudoanabeana					0.17±0.17	0.05±0.04	0.04±0.04		0.33±0.33		
Schizotrix spp.							0.01±0.01			0.07±0.07	0.13±0.07
Symploca atlantica					0.62±0.30		0.01±0.01	0.44±0.25	0.33±0.33		0.04±0.04
Symploca hydnoides				0.50±0.50	0.90±0.34	0.08±0.08	0.83±0.15	1.68±0.36	0.13±0.13	0.57±0.14	
Trichocoleus acutissimus					0.07±0.07	0.08±0.08	0.67±0.14	0.15±0.15	1.33±0.59	0.27±0.15	
Trichocoleus spp.				0.13±0.13			0.37±0.11		0.13±0.13	0.20±0.17	0.04±0.04
Tychonema sp. 1			0.33±0.33				0.30±0.10				0.12±0.07
Tychonema sp. 2				0.63±0.63		0.05±0.05	0.39±0.11	0.15±0.15	0.33±0.33	0.10±0.10	0.11±0.06
Undetermined				0.88±0.64			0.13		0.33±0.33		0.39±0.12
no. Species	1	2	6	8	12	6	19	9	10	9	21
no. Samples	20	6	12	8	29	37	135	34	15	30	122

Table 2: Nitrogen fixation rates per biomass and area of incubated cyanobacterial mats. Rates are converted from ethylene production to nitrogen fixation using a factor of 4 according to Peterson and Burris (1976).

Mat	Dominant cyanobacteria	Sample site	Depth	Chla concentration	Nitrogen fixation per biomass			Areal nitrogen fixation per m^{-2} mat		
			(m)	(µg Chla cm^{-2} mat)	Dark (12h)	Light (12h)	Day (24h)	Dark (12h)	Light (12h)	Day (24h)
					(nmol N$_2$ µg Chl a^{-1} time^{-1})			(mg N m^{-2} time^{-1})		
A	*H. glutinosum & O. bonnemaisonii*	PB	7	0,7 ± 0,1	46,5 ± 7,8	120,3 ± 18,0	166,8 ± 19,3	2,2 ± 0,3	7,2 ± 0,5	9,3 ± 0,8
B	*H. glutinosum & L. majuscula*	BY	7	0,9 ± 0,1	19,5 ± 3,7	47,1 ± 7,1	66,6 ± 14,8	4,3 ± 1,0	10,1 ± 0,5	14,4 ± 1,5
C	*O. bonnemaisonii*	PB	7	21,3 ± 2,2	1,1 ± 0,1	9,3 ± 1,7	10,5 ± 1,6	14,9 ± 0,1	154,2 ± 25,3	169,1 ± 25,4
D	*Oscillatoria sp.*	BY	20	2,3 ± 0,1	1,6 ± 0,1	29,8 ± 4,5	31,4 ± 4,4	0,3 ± 0,1	2,5 ± 1,4	2,7 ± 1,5
E	*L. majuscula & T. auctissimus*	BY	20	1,9 ± 0,2	2,3 ± 1,3	19,5 ± 1,6	21,8 ± 0,4	0,6 ± 0,3	3,6 ± 0,3	4,3 ± 0,6
F	*Dichothrix sp.*	BY	7	1,7 ± 0,1	2,0 ± 0,2	4,5 ± 1,3	6,5 ± 1,1	0,4 ± 0,1	1,5 ± 0,4	1,9 ± 0,5

Electronic Supplementary Material
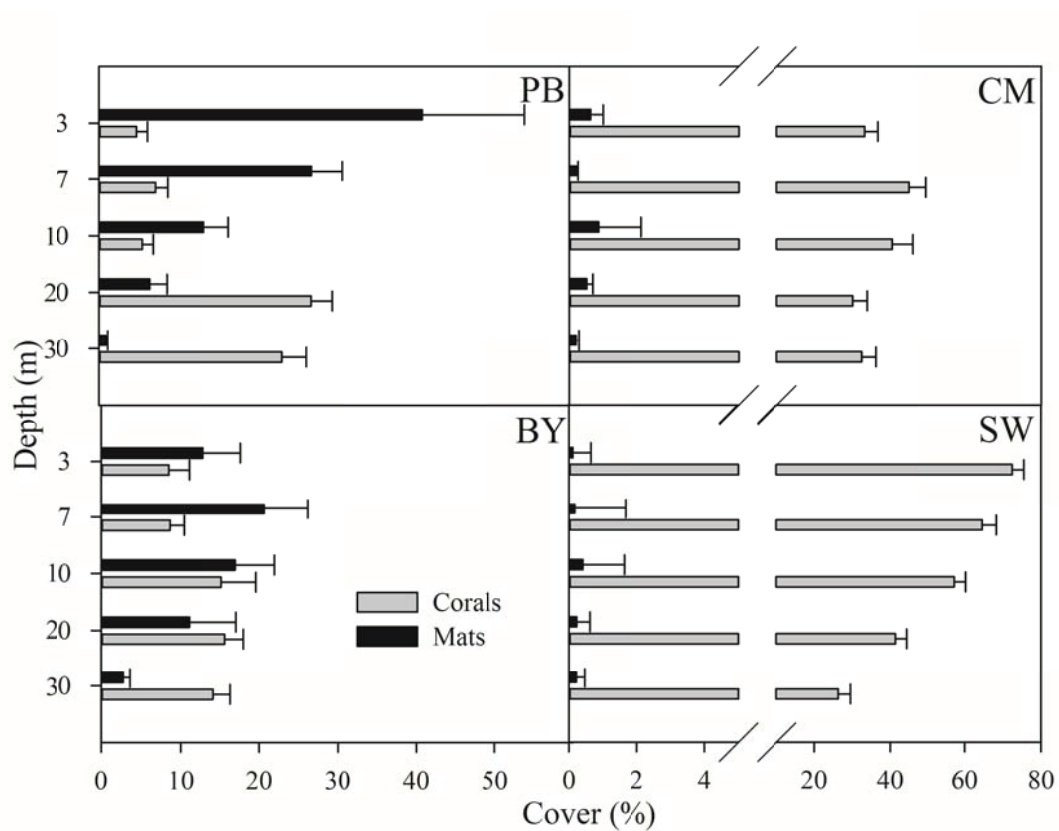
ESM Fig. 1: Cyanobacterial mat and coral abundance on four sites (Pest Bay = PB, Cap Malmeeuw = CM, Buoy 0 = BY, Spanish Waters = SW) at five different depths. Error bars represent standard error of the means (SEM).
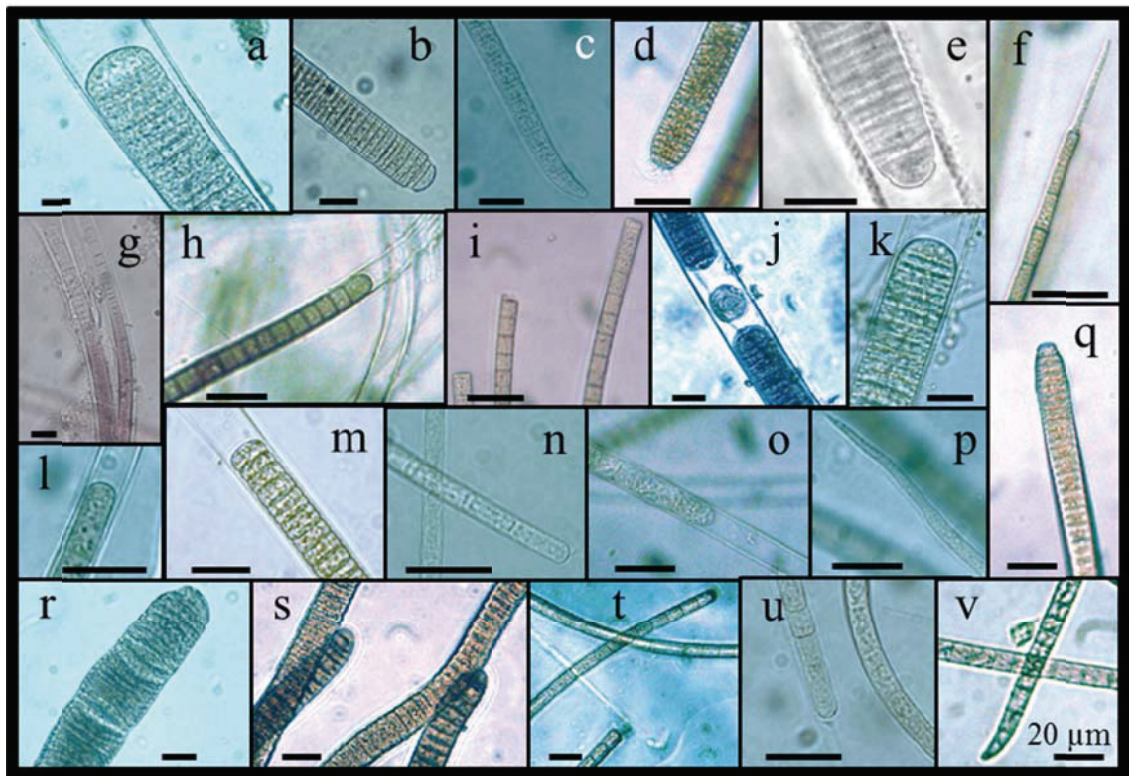
ESM Fig. 2: Microscopic photos of all found morphotypes: a) *L. majuscula* b) *H. glutinosum* c) *P. formosum* d) *P. nigroviride* e) *L. semiplena* f) *T. acutissimus* g) *D. utahensis* h) *Pseudanabaena* spp. i) *Schizothrix* spp. j) *L. aestuarii* k) *Lyngbya* spp. l) *S. hydnoides* m) *L. sordida* n) *Phormidium* spp. o) *S. atlantica* p) *Trichocoleus* spp. q) *Oscillatoria* spp. r) *O. bonnemaisonii* s) *O. funiformis* t) *Tychonema* sp.2 u) *P. corium* v) *Tychonema* sp.1.
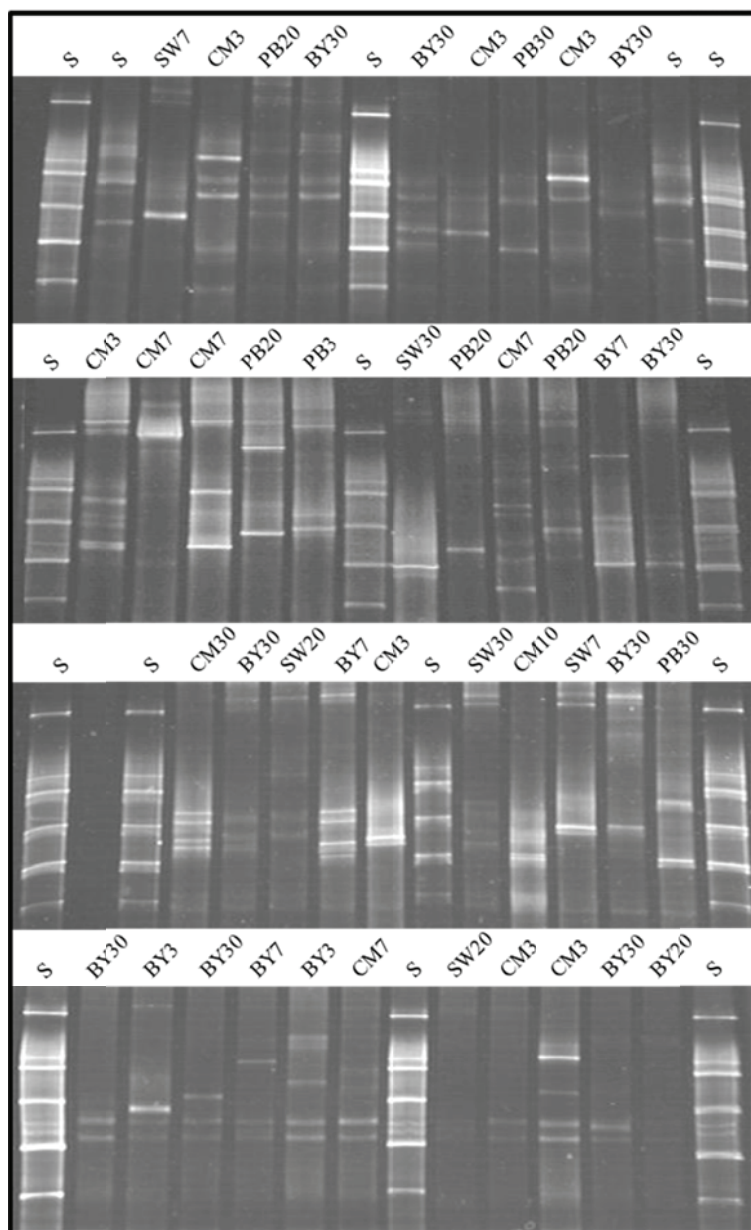
ESM Fig. 3: Example of DGGE gels.
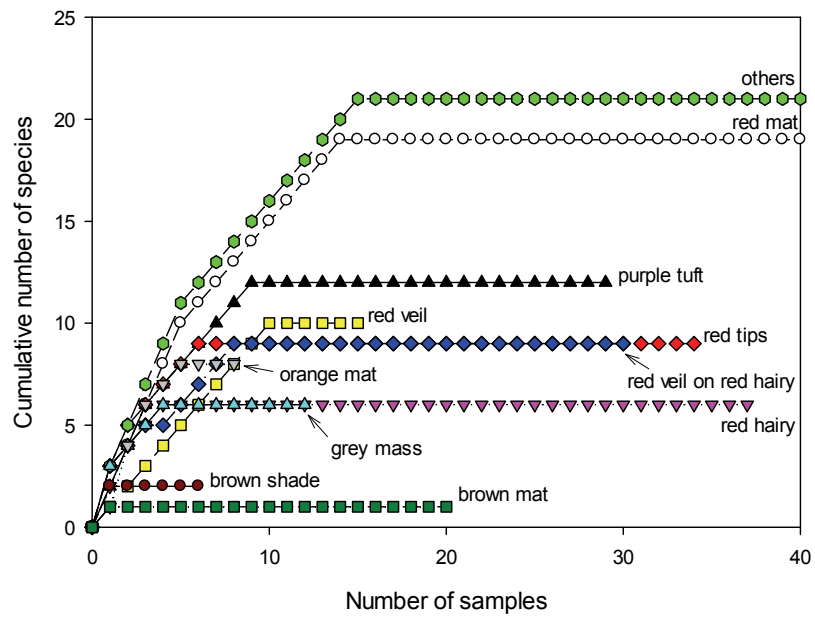
ESM Fig. 4: Average cumulative number of species as a function of the number of samples for each visual mat type. See ESM Table 1 for exact number of species.
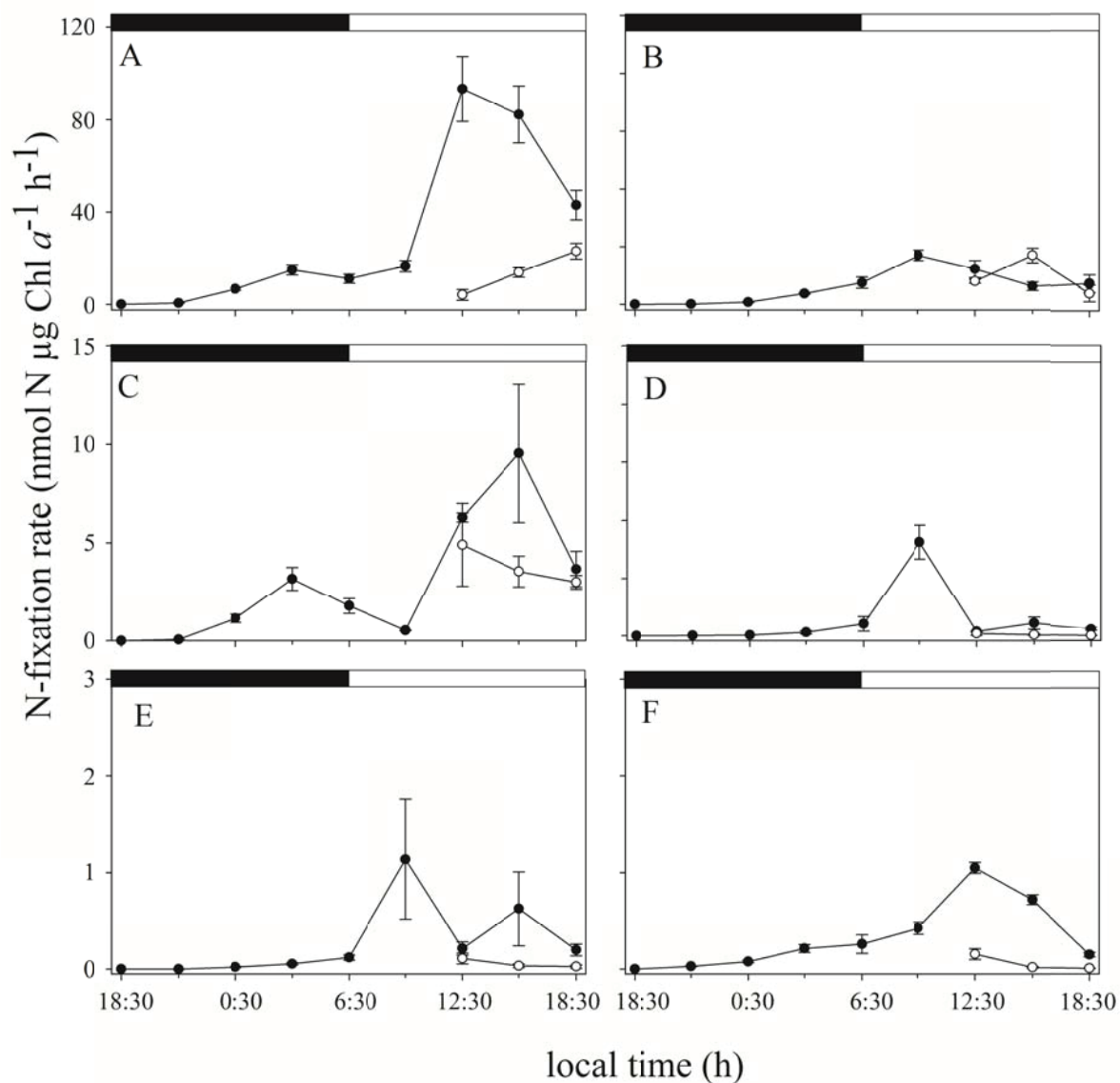
ESM Fig. 5: Nitrogen fixation rates of benthic cyanobacterial mats, calculated from acetylene reduction rates using the conversion factor 4 (Peterson and Burris 1976). Values represent the mean of triplicates. Error bars represent standard error of the mean. White symbols represent dark-incubated samples. Dominant mats – A, B, C, D, E, F. Note the difference in Y-axis scaling between graphs. Brown mat (C) had about 10x higher chlorophyll *a* content than other mat types.

ESM Table 1: Overview of total number of samples per site and number of detected cyanobacterial morphotypes at each depth.

Mat abundance	High		Low	
	PB	BY	CM	SW
Number of mats collected	127	132	100	119
Number of quadrates deployed	62	60	74	63
Cyanobacterial species richness per sample	1-3	1-4	1-4	1-2
Total number of different cyanobacteria at				
3 m	15	9	15	8
7 m	10	14	13	15
10 m	14	18	10	12
20 m	14	16	14	14
30 m	11	12	12	11

ESM Table 2: Pair-wise tests showing Monte Carlo p values P_{mc}, ns = $P_{mc} > 0.05$.

	Brown mat	Brown shade	Grey mass	Orange mat	Purple tuft	Red Hairy	Red Mat	Red tips	Red veil	Red veil on Red Hairy
Brown shade	0.001									
Grey mass	0.001	0.016								
Orange mat	0.001	ns	ns							
Purple tuft	0.001	0.001	0.001	ns						
Red hairy (RH)	0.001	0.001	0.001	0.001	0.001					
Red mat	0.001	0.001	0.001	ns	0.003	0.001				
Red tips	0.001	0.001	0.001	0.002	0.001	0.001	0.001			
Red veil	0.001	0.001	0.002	ns	0.013	0.001	ns	0.001		
Red veil on RH	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
Other	0.001	0.001	0.001	ns	0.003	0.001	0.001	0.001	0.001	0.001

Mat type descriptions:

The common type **Red mat** was observed forming flat, expanded and often dense structures on various hard substrates. The thallus was dark- or light red, and occasionally had protrusive filamentous outgrowths.

A **Brown mat** was observed growing as a loose and expanded dark brown thallus comprised of interwoven filaments, with single filaments visible to the naked eye. It was found exclusively on sandy substrates.

A **Brown shade** was observed (instead of the brown mat) on sandy substrates (after a storm event). It consisted of intertwined brown and red filaments forming an organo-sedimentary structure with the substrate.

A **Red hairy** cyanobacterial type was observed as curled tufts several centimeters high consisting of red filaments, which were visible to the naked eye. It was found on various hard substrates.

A **Red veil** cyanobacterial type formed a thin net-like structure, frequently covering macroalgae and other cyanobacterial colonies. It was frequently found growing on the red hairy cyanobacterial type.

A cyanobacterial mat type designated **Red tips** was observed to form small finger-like thalli. These were comprised of a mucilaginous mass that was whitish at base, rust-red at tips and grew up to 2.5 cm in height. It was found mainly, but not exclusively, on sandy substrates.

The globular cyanobacterial colony, designated **Purple ball**, was characterized by a purple-grey round thallus, 1-2 cm in diameter with protruding filaments, growing attached to various hard substrates. The thallus was comprised of several falsely branching trichomes joined in a common "root" structure.

A **Purple tuft** cyanobacterial type described a discrete upright colony with filamentous structures of dark purple coloration. It was found exclusively on hard substrates.

A **Grey mass** cyanobacterial type was observes as a grey-white undifferentiated gelatinous thallus. It showed between 1-4 cm thickness and was found mostly on hard substrates

4. Manuscript III

MS III: High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef ecosystem

(Published in Scientific Reports)

High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef ecosystem

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Abstract

Benthic cyanobacterial mats (BCMs) are increasing in abundance on coral reefs worldwide. However, their impacts on biogeochemical cycling in the surrounding water and sediment are virtually unknown. By measuring chemical fluxes in benthic chambers placed over sediment covered by BCMs and sediment with BCMs removed on coral reefs in Curaçao, Southern Caribbean, we found that sediment covered by BCMs released 1.4 and 3.5 mmol C m^{-2} h^{-1} of dissolved organic carbon (DOC) during day and night, respectively. Conversely, sediment with BCMs removed took up DOC, with day and night uptake rates of 0.9 and 0.6 mmol C m^{-2} h^{-1}. DOC release by BCMs was higher than reported rates for benthic algae (turf and macroalgae) and was estimated to represent 74% of the total DOC released over a 24h diel cycle at our study site. The high nocturnal release of DOC by BCMs is most likely the result of anaerobic metabolism and degradation processes, as shown by high respiration rates at the mat surface during nighttime. We conclude that BCMs are significant sources of DOC. Their increased abundance on coral reefs will lead to increased DOC release into the water column, which is likely to have negative implications for reef health.

Introduction

Cyanobacteria are a common benthic and planktonic component of coral reef ecosystems[1]. They are important contributors to primary production, nitrogen fixation and reef building[1,2]. In recent decades however, many coral reefs have experienced massive blooms of noxious benthic species forming dense mats over the seabed[3-6]. These BCMs inhibit coral settlement and recruitment, potentially limiting the ability of corals to recover from disturbances[7]. They also act as coral pathogens[8] and disturb coral reef-associated microbial communities[9]. The blooms are difficult to control by grazers as these organisms produce potent allelochemicals that deter feeding[10,11]. They also appear to be facilitated by environmental conditions associated with anthropogenic impacts and global climate change, which are likely to become worse in the near future. Therefore, it is predicted that their abundance will increase in the coming decades[2,12].

Aquatic primary producers, such as cyanobacteria, release part of their photosynthetically fixed carbon as DOC into the water column[e.g.13-16]. Changes in the abundance of primary producers can alter the quantity and chemical composition of organic materials supplied to the reef environment and have long-term impacts on reef communities[17-19]. For example, algal exudates are thought to play a pivotal role in community shifts from coral to algal dominance occurring on many coral reefs worldwide[18]. Corals can retain organic materials by trapping

particles from the water column, which are subsequently remineralized[20] and they can release DOC[16,21,22]. However, benthic algae release higher amounts of, and comparatively more neutral sugar rich, DOC than corals[19,23]. Algal exudates have been shown to induce microbe-induced coral mortality[24], foster faster growth of less diverse and more pathogenic microbes than coral exudates[23] and favor net heterotrophic metabolism[19].

Despite the increasing abundance of BCMs and the recent focus on biogeochemical cycling and microbial processes, hardly anything is known about the DOC release of BCMs and their impact on carbon cycling in coral reefs. BCMs release DOC, either as photosynthates[14] and as products of anaerobic metabolism and degradation processes at night[15]. Their exudates are thought to play an important role in controlling bacterioplankton activity in aquatic systems[25]. However, most studies on carbon cycling in coral reefs have focused on planktonic cyanobacteria[26]. The goal of this study was to investigate the influence of BCMs covering large areas of coral reef sediment on biogeochemical processes in Curaçao, Southern Caribbean. Firstly, we estimated DOC, dissolved inorganic carbon (DIC), oxygen and inorganic nutrient fluxes over diel cycles using benthic chambers placed over sediment covered by BCMs and sediment with BCMs removed. Secondly, to assess the influence of BCMs on sedimentary carbon cycling, we compared the carbon budgets of both experimental treatments. Thirdly, to estimate the contribution of BCMs to the DOC pool at the reef scale, we assessed the cover of the major benthic components at our study site and multiplied their cover with their respective DOC release rates over a diel cycle using the results of this and other studies. Finally, the vertical distribution of oxygen was determined across the sediment-water interface with and without the presence of BCMs using microsensors to investigate photosynthetic and respiration processes.

Results

Fluxes of O_2, DIC and DOC in sediment covered with BCMs (CYA treatment) were higher than in sediment with BCMs experimentally removed (CTRL 1 treatment) (Table 1). During the day, sediment covered with BCMs released O_2 and took up DIC, with 5-6 times higher fluxes than sediment with BCMs removed. During the night, O_2 was respired and DIC was released, with 3-4 times higher fluxes. Sediment covered with BCMs net released 1.4 (SD 1.2) mmol C m^{-2} h^{-1} DOC during the day and doubled this amount during the night [3.5 (SD 2.0) mmol C m^{-2} h^{-1}]. Conversely, sediment from which BCMs were removed took up DOC during both day and night [i.e. -0.9 (SD 0.6) and -0.6 (SD 0.7) mmol C m^{-2} h^{-1}]. Net fluxes of inorganic nutrients over the sediment water interface were very low and often close to detection limits (NH$_4^+$ < 0.07μM, NO$_2^-$/NO$_3^-$ < 0.05, PO$_4^{3-}$ < 0.01; Table 1). No net nutrient release was observed, indicating that

cells from the BCMs were not lysing/dying during the incubations. Benthic chambers were also placed over undisturbed sediment without BCMs (CTRL 2 treatment). This second control did not differ from the first control (sediment from which were BCMs removed) in either DIC, oxygen or nutrient fluxes (Table 1). DOC fluxes were not measured on naturally bare sediments. During the incubations, salinity was consistently 35 PSU in all chambers. Daytime PAR availability did not differ among the experimental treatments (Table 1). Water temperatures were slightly lower during the incubations on naturally bare sediments compared to the other treatments, but differences were minute (≤ 0.4 °C) (Table 1).

Over a 24h diel cycle, the carbon budgets indicated that the presence of BCMs reduced the net organic carbon input into the sediment by 57%, with net uptake rates of 0.6 (SD 2.8) mmol C m^{-2} h^{-1} for sediment with BCMs and 1.4 (SD 1.5) mmol C m^{-2} h^{-1} for sediment with BCMs removed (Fig. 1). Rates of daytime DOC release by BCMs were within the range of rates reported for macroalgae or turf, both of which generally also released DOC (Table 2). No clear trends were recognizable among macroalgal divisions when pooling the different studies. Corals generally showed a net uptake of DOC. Nighttime DOC release by BCMs was higher than rates obtained in *ex situ* dark incubations for most primary producers in coral reefs.

At our study site, the ecosystem compartments that produced DOC (BCMs, macroalgae and turf) covered 24, 17 and 19 % of the seabed, respectively (Table 3a). Averaged over the reef and over a 24 h cycle, the BCMs, macroalgae and turf were estimated to release DOC at rates of 0.59, 0.04 and 0.18 mmol C m^{-2} reef h^{-1}, respectively. The two other ecosystem compartments (corals and bare sediments) did not release DOC over a 24 h cycle. Thus, BCMs contributed to 74 % of the total DOC released. Taking into account the net uptake of DOC by corals (13% cover) and bare sediments (25% cover), the reef yielded a net release of DOC (+0.19 mmol C m^{-2} reef h^{-1}). In a theoretical scenario with all BCMs removed, the reef yielded a net uptake of DOC (-0.6 mmol C m^{-2} reef h^{-1}) (Table 3b).

O$_2$ microprofiles measured over a 24 hour cycle showed that, during the day, maximal O$_2$ concentrations were 2-8 times higher in sediment covered with BCMs than in sediment next to BCMs (Fig. 2). The depth of the oxygenated layer was reduced when BCMs covered the sediment. During the night, the sediment and BCMs became rapidly anoxic up to the surface.

Discussion

The results of this study provide the first rates of DOC release by BCMs on coral reefs. Our comparison between daytime and nighttime rates with previously reported DOC releases by macroalgae, turfs and corals on coral reefs worldwide suggest that BCMs release high quantities

of DOC into the water column, especially at night. DOC release by Caribbean benthic primary producers on coral reefs has been shown to be positively related to light intensity[16,22,27,28]. Similarly, DOC release by hot spring cyanobacterial mats is enhanced under elevated light intensities[14]. Therefore we expected nocturnal DOC release to be lower than during the day. However, our study demonstrates a high nocturnal release of DOC by BCMs. During daytime, DOC is most likely released by the excretion of photosynthates, as supported by the high rates of oxygen production at the mat surface (Fig. 2). During nighttime, the released DOC most likely consists of products from incomplete organic matter degradation and fermentation[29], as supported by the high heterotrophic activities in the mat (Fig. 2). Several species of *Oscillatoria* maintain their metabolism by glycogen-glucose fermentation to survive and grow under dark and anaerobic conditions[30-32].

When roughly estimating the areal DOC release on the reef flat at Pest Bay by combining literature data and results of this study, BCMs, with a coverage of 24 %, provided the largest positive contribution to the DOC pool. Unlike other benthic primary producers on coral reefs, BCMs released large amounts of DOC during both day and night. DOC released by other primary producers, such as algae, typically increases with light intensity and thus decrease with water depth[16,22,33]. However, the dark DOC release by BCMs may not be restricted by depth and seasonal variations in daylight or cloudy weather. The presence of BCMs also annihilated the capacity of sediment to act as a net sink for DOC. This largely affected the DOC pool at reef scale, with the reef switching from being a net sink to a net source of DOC when BCMs covered 24 % of the seabed (Table 3a vs b).

The presence of BCMs reduced the net carbon gain in the sediment by more than half. Sediment with BCMs showed a net primary production, high respiration rates and released large quantities of DOC. In contrast, sediment with BCMs removed revealed a lower net production and also respired less and took up DOC from the water column. Sediments, including carbonate sediments on coral reefs, are a well-known sink for organic matter, such as DOC, through recycling and burial[34-38]. For example, Werner et al.[36] estimated that the total area of Heron Reef occupied by sediments (sediment area = 19.5 km^2) showed annual turnover rates of 3 700 to 13 000 t C. Our results suggest that the presence of BCMs over coral reef sediment may influence sedimentary recycling processes and result in larger DOC pools in the water column.

The impact of the released DOC from BCMs into the surrounding water will depend on its bioavailability. Lactate, glycolate, formate, ethanol and acetate are released during nocturnal fermentation processes in the genus *Oscillatoria*[32]. Such compounds are easily degraded by microbes. They could favor more heterotrophic metabolism, as shown for algal exudates[19] and lead to a system-wide decrease in DOC concentrations via enhanced heterotrophy and co-

metabolism in which a surplus of labile carbon allow bacteria to use refractory carbon sources[39]. Further supporting the assumption that BCMs can provide bioavailable DOC, heterotrophic metabolism was 10 fold higher above cyanobacterial/algal dominated reefs called Black Reefs in Central Pacific[40]. The released DOC could also affect nearby corals. Fermentation products, such as lactate, have been shown to cause coral mortality in aquaria[41]. BCMs produce potent allelochemicals[10,11,42]. Both lipo- and hydrophilic extracts from two species of *Lyngbya* cyanobacteria enhanced the growth of coral reef-associated bacterial taxa[9].

There are some uncertainties in our budget calculation. Firstly, in our reef-scale DOC calculations, we used DOC fluxes from sediment with BCMs experimentally removed, as no DOC data were available for undisturbed BCM-free sediment. As all other fluxes were the same, we assume that the DOC fluxes were representative for a natural situation. Secondly, our budget calculation over whole reefs includes literature data obtained from ex situ incubations (fluxes on corals, turf and macrophytes). Stressfull sampling and maintenance in the artificial laboratory environment can lead to overestimation of the DOC release, due to cutting of tissue and unrealistic hydraulic conditions[16,33]. Indeed we observed a rapid deterioration of the health of the mats upon ex situ incubations (lysis), thus for the BCMs we rely on our in situ data. Possibly due to different nutrients, light and temperature regimes, the reported DOC releases of benthic algae and corals vary strongly between studies. In short, the literature data may provide an overestimation of the DOC release by corals and turf, which further emphasizes the importance of BCMs for carbon cycling in coral reefs.

Although further investigations of DOC release by BCMs and undisturbed sediment are warranted, this study supports that BCMs are significant sources of DOC and can strongly contribute to the DOC pool on coral reefs. Their increased abundance will lead to increased DOC supply to the reef overlying water and have profound consequences for element cycling, microbial processes and coral survival in tropical reefs.

Methods

Study site. The experiments were performed between September and November 2011 at 7 to 8 m water depth on a fringing coral reef at Pest Bay on the leeward side of the island of Curaçao (Fig. S1a; 12°09'894"N 69°00'657"W). At this depth, the reef consisted of coral heads separated by sand patches largely covered by brown-colored BCMs (Fig. S1b). The mats were primarily dominated by *Oscillatoria bonnemaisonii* P.L.Crouan & H.M.Crouan ex Gomont, a common bloom-forming genus on coral reefs[43].

***In situ* benthic chamber experiment.** To investigate the exchange rates of O_2, DIC, DOC and nutrients (PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+) across the sediment-water interface, benthic chambers were deployed over three types of carbonate sediment: (1) sediment covered with BCMs (CYA), (2) sediment initially covered with BCMs, but experimentally removed (CTRL 1), and (3) sediment without BCMs (CTRL 2).

We used a modified version of the *in situ* benthic chamber used in Cook et al.[44] and Huettel et al.[45]. The benthic chambers consisted of an acrylic cylinder (Ø 190 mm) with a compensator bag for diver-operated time-series sample retrieval (Fig. 1b). The chamber were inserted into the sediment (10-15 cm) and sealed with a lid. Mixing of the overlying water was maintained by a rotating acrylic stirrer disc (10 cm diameter). The stirring speed of the disk was set to a "non-advective mode" at 20 rpm with a reversing rotational direction every 15 s to ensure mixing without creating a pressure gradient[44]. The mixing process was validated by adding a tracer (ink) and following the color visually over time and space prior to the experiments. After addition of the tracer, the stirred chamber was entirely and homogeneously colored within two minutes. The chamber enclosed a seafloor area of 284 cm^2. BCMs covered ≥ 90 % of the surface area of each benthic chamber for the CYA and CTRL 1 (i.e. before experimental removal) treatments (Fig. 1b). The overlaying water column was 4–6 liters (equivalent to chamber height of 10–15 cm).

Incubations were performed day- (start: 10:30 AM ± 30 mins) and night-time (start: 08:30 PM ± 30 min) for a duration of 6 h each. Water samples (180 ml) were slowly withdrawn over a period of 5 min from the overlying water of the chambers through a stopcock at the start (T0), after 3 h (T3) and after 6 h (T6). The replacement of the sample volume was ensured through a volume compensator attached to the chamber (Fig. S1b).

The chamber set up consisted of four individual chambers linked to a single battery by 2 m long cables which prevented placing the chambers simultaneously in sediments with BCMs (CYA) and without BCMs (CTRL 2), but allowed each chamber to be positioned at least 2 m apart. Thus, all 4 chambers were first deployed on sediment with BCMs (CYA), with day and night incubations performed over two consecutive days (i.e. day 1: day incubations, day 2: night incubations) without moving the chambers. Still without moving the chambers, the mats were removed by hand shaking min. 18 h before the start of the incubations for inducing equilibrium of sediment. Another batch of day and night incubations were run on day 4 and 5 (CTRL 1). In between running the CYA and CTRL 1 incubations, the chambers were left opened to allow water exchange. The chambers were then moved to an area free of BCM to run the CTRL 2 day and night incubations. The same procedure was repeated twice using new patches (total deployment area: ca. 500 m^2), followed by an additional batch of CYA chambers, resulting in 12

replicates for CYA, and 8 replicates for both CTRL 1 and CTRL 2 for each day and night incubation. Light and temperature was monitored during the experiment using loggers (Hobbo Pendant, Onset).

Sample processing. Oxygen concentrations were measured after retrieval of samples on land at *in situ* temperature with an oxygen optode (Hach HQ10+LDO). Salinity was measured with a refractometer to check for groundwater seepage which, if present, would be expected to lower salinity. For DIC analyses, 6 ml of each sample was transferred into gas tight exetainers without headspace, fixed with mercury chloride, and stored in the dark at 4 °C. DIC concentrations were measured with the flow injection method (conductivity detector: VWR scientific model 1054) according to Hall & Aller[46].

Samples for DOC (40 ml) were filtered (< 20 kPa Hg suction pressure) over a 0.2 μm polycarbonate filter (Whatman, 25 mm). Prior to filtration, filters, glassware and pipette tips were rinsed three times with acid (10 ml 0.4 M HCl) and twice with sample water (10 ml). Afterwards 20 ml of the sample water was filtered, each filtrate containing DOC was transferred to a pre-combusted (4 h at 450 °C) glass ampoule and sealed immediately after acidification with 6–7 drops of concentrated HCl (38%) to remove inorganic C and stored at 4 °C until analysis. There were not enough glass ampoules to measure DOC in the three experimental treatments so CTRL 2 was excluded. DOC concentrations were measured using a total organic C analyzer (TOC-VCPN; Shimadzu) according to Ogawa et al.[47]. The instrument was calibrated with a standard addition curve of Potassium Phthalate (0; 25; 50; 100; 200 μmol C l^{-1}). A consensus reference materials provided by Hansell and Chen of the University of Miami (Batch 12, 2012; 41-44 μmol C l^{-1}) was used as positive control. Concentrations measured for the entire batch gave an average value of 45 (SD 2) μmol C l^{-1}. Average analytical error of the instrument was < 3 % (5-7 injections per sample).

Samples for nutrients (50 ml) were immediately filtered with 0.22 μm syringe filters (Minisart® NML sterile Syringe Filters 16534, Hydrophilic), stored in 6 ml Pony vials and frozen (-20 °C). Nutrients were also analyzed at NIOZ, Texel, using continuous flow analysis via a Quatro auto-analyzer (Seal Analytical, UK) following the methodologies of Grasshoff et al.[48] for NO_3^- and NO_2^-, Helder & De Vries[49] for NH_4^+ and Murphy & Riley[50] for PO_4^{3-}.

Flux and carbon budget. Fluxes of O_2, DIC, DOC and nutrients were calculated from the linear regression of the respective concentration versus time[51]:

$$Flux = dC/dt * V_{chamber}/A_{chamber}$$

where dC/dt is the change of the concentration over the incubation time, $V_{chamber}$ is the volume of enclosed bottom water, and $A_{chamber}$ is the surface area enclosed by the chamber. Positive fluxes show a release of the solute across the sediment-water interface into the bottom water, while negative fluxes indicate an uptake of the solute. Error estimates caused by water efflux through the sediment were calculated for all chambers using maximal and minimal values for each treatment. Flux data were tested by one-way ANOVA with experimental treatment (i.e. CYA vs CTRL 1 vs CTRL 2) as fixed factor for each day and night period, followed by Scheffe posthoc tests.

Carbon budget calculations were based on the assumption that for each mole of oxygen produced/respired, one mole of carbon is fixed/respired (1:1). Oxygen flux data were thus used as a base for the calculations. Carbon budgets were estimated for sediments with and without BCMs using data from the CYA and CTRL 1 treatments, respectively. O_2 fluxes during the day were used as net production rates (NP). Carbon budgets were then calculated by subtracting from NP all carbon losses by respiration in the night and DOC releases/uptakes. Standard deviation (SD) of each carbon budget was calculated by taking the square root of the sum of all SD each to the power of two used in the calculation.

Reef scale DOC calculations. To compare DOC release rates of BCMs with other reported rates, we compiled data on benthic primary producers of coral reefs from the literature. To estimate the contribution of BCMs to the DOC pool in the water column at the reef scale, percent cover of major benthic groups (BCMs, macroalgae, turf, corals and sediment not covered by BCMs) were determined from 20 quadrats of 4 m² (2 x 2 m), which were haphazardly placed at 7 m depth at Pest Bay. Each quadrat was photographed on November 2011 using a series of four overlapping photographs (ca. 1.5 m² each) which were subsequently assembled to make one overview photograph. Each overview photograph was analysed using the program Coral Point Count with Excel Extensions (CPCe) using 120 points[52]. DOC release/uptake rates for BCM and sediment using data from the CYA and CTRL 1 treatments, respectively. DOC release/uptake rates for macroalgae (*Dictyota*), corals (*Madracis*) and turf (daytime only) were taken from Mueller et al.[16]. Mueller et al.[16] determined DOC release in *ex situ* incubations in April 2011 (daytime incubations) and between May and July 2010 (dark incubations) using samples collected at 8 m depth near the Carmabi biological research station (Fig. S1a; 12°7'18.06"N, 68°58'10.59"W). Night DOC release for turf was not available in their study. Thus data were taken from Haas et al. [19] which conducted nighttime *ex situ* incubations using turf algae collected at 2-2.5 m depth in Moorea, French Polynesia. Individual DOC release/uptake rates over a diel cycle were multiplied

by the cover of the major benthic components at our study site to obtain their respective contribution to the DOC pool at reef scale.

In situ sediment oxygen profiles. To document the mat activity, vertical profiles of dissolved oxygen were measured over a 24 h cycle at 40 min intervals in the center and outside of five BCM patches at Pest Bay using an *in situ* diver operated microsensor system[53]. Profiles were measured in 200 μm steps until anoxic sediments (i.e. consistently low values) were detected. Calibrations were done by assuming the overlying water to be 100% saturated with oxygen and deeper sediment anoxic. Analysis of the profiles was done using custom-made programs MPR-plotter and L@MP.

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Acknowledgements

Author contributions statement

H.J.B., F.W., D.d.B and M.M.N designed the study. H.J.B performed the research and analyzed the data. B.M. and F.C.v.D contributed to data collection. H.J.B and M.M.N. wrote the manuscript. All authors reviewed the manuscript.
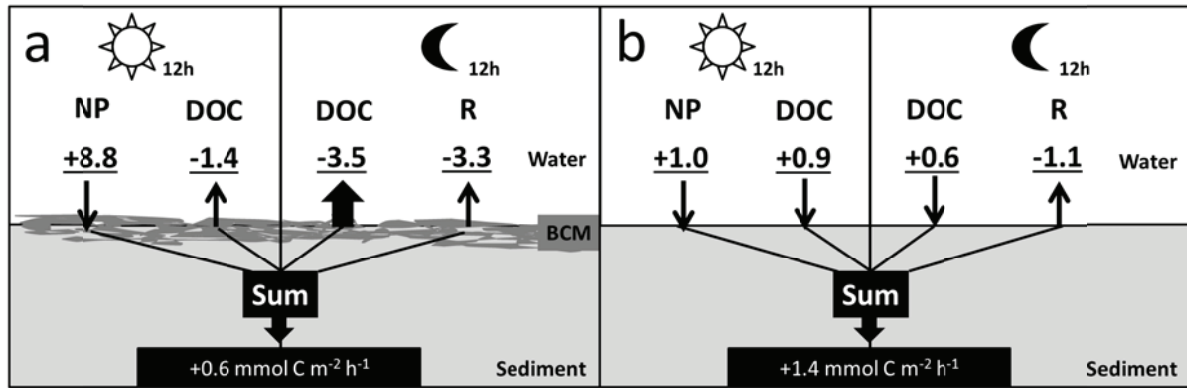
Figures

Figure 1. Day and night carbon budgets for sediment with BCMs (a) and without BCMs (b). (+) indicates uptake and (-) loss of carbon from sediment. NP = Net production (day); R = respiration (night).
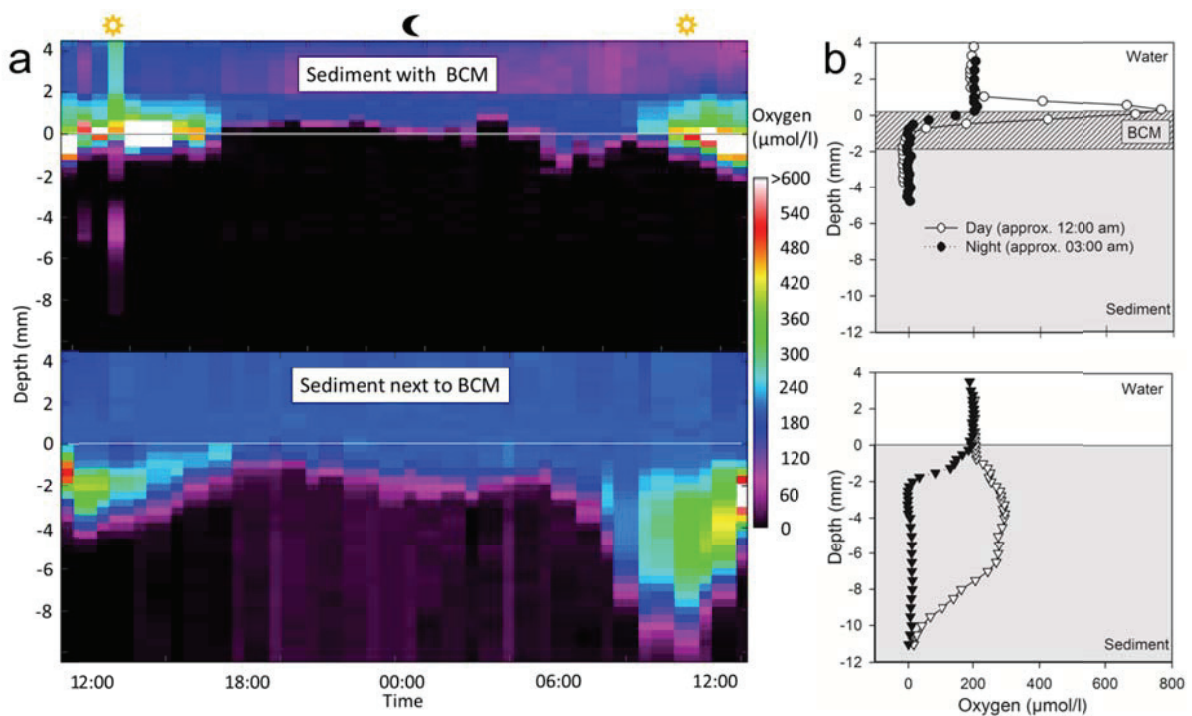

Figure 2. *In situ* oxygen profiles. (a) over a period of 24 hours in sediment covered with BCMs and next to BCMs. Colors indicate oxygen concentration over time and depth. (b) Examples of *in situ* O_2 profile at the sediment-water interface during day and night with BCMs and next to BCMs.

Tables

Table 1. Estimated fluxes (mmol C m^{-2} h^{-1}), light (μmol photons m^{-2} s^{-1}) and temperature (°C) for sediment with BCMs (CYA), after experimental removal (CTRL 1), and for sediment without BCM (CTRL 2). Flux calculations are based on 6 h except for O_2 and DIC due to flux changes in the last hours caused by high concentrations. DOC fluxes are not available for CTRL 2 due to a shortage of sample containers. Differences among treatments from each day and night period were analysed by one-way ANOVA (Significance < 0.05, ns = non significant). Significance column indicates homogeneous subgroups by posthoc Scheffé tests. n.s. = not significant. na = data not available.

Treatment:	CYA		CTRL 1		CTRL 2		
	Mean	SD	Mean	SD	Mean	SD	Significance
DAY							
O_2	8.77 ±	0.86	0.99 ±	0.41	1.29 ±	0.43	CYA > CTRL1 = CTRL2
DIC	-12.10 ±	1.16	-1.94 ±	1.26	-1.88 ±	1.56	CYA > CTRL1 = CTRL2
DOC	1.36 ±	1.21	-0.85 ±	0.67	na ±	na	CYA > CTRL1
PO_4^{3-}	-0.0003 ±	0.0003	-0.0002 ±	0.0002	0.0000 ±	0.0004	n.s.
NO_2^-	0.0005 ±	0.0013	-0.0001 ±	0.0002	-0.0003 ±	0.0001	CYA > CTRL1 = CTRL2
NO_3^-	-0.0028 ±	0.0009	-0.0048 ±	0.0022	-0.0042 ±	0.0021	CYA > CTRL1
NOx	-0.0027 ±	0.0010	-0.0049 ±	0.0023	-0.0044 ±	0.0022	n.s.
NH_4^+	-0.0217 ±	0.0459	0.0073 ±	0.0137	-0.0110 ±	0.0423	n.s.
Light	193.8 ±	49.0	245.6 ±	16.2	163.6 ±	5.7	n.s.
Temperature	29.9 ±	0.3	30.1 ±	0.2	29.7 ±	0.3	CYA = CTRL1 > CTRL2
Error (%)[a]	4.4-8.7 ±	2.9-5.8	5.2-10.5 ±	2.6-5.2	4.5-9.0 ±	3.3-6.6	
NIGHT							
O_2	-3.25 ±	0.81	-1.14 ±	0.73	-1.39 ±	0.36	CYA > CTRL1 = CTRL2
DIC	8.75 ±	2.60	2.24 ±	1.16	2.44 ±	2.30	CYA > CTRL1 = CTRL2
DOC	3.52 ±	2.03	-0.64 ±	0.69	na ±	na	CYA > CTRL1
PO_4^{3-}	0.0003 ±	0.0003	-0.0002 ±	0.0004	0.0003 ±	0.0004	CYA = CTRL2 > CTRL1
NO_2^-	0.0007 ±	0.0022	-0.0004 ±	0.0001	-0.0002 ±	0.0007	CYA = CTRL2 > CTRL2
NO_3^-	-0.0054 ±	0.0031	-0.0065 ±	0.0021	-0.0026 ±	0.0010	n.s.
NOx	-0.0048 ±	0.0039	-0.0069 ±	0.0022	-0.0028 ±	0.0013	n.s.
NH_4^+	0.0032 ±	0.0254	-0.0029 ±	0.0423	0.0142 ±	0.0235	n.s.
Temperature	29.3 ±	0.3	29.3 ±	0.3	28.9 ±	0.2	CYA = CTRL1 > CTRL2
Error (%)[a]	4.5-9.0 ±	3.4-6.7	6.6-13.1 ±	3.3-6.6	4.6-9.2 ±	3.8-7.6	

[a] Error estimates for flux data.

Table 2. Reported DOC releases **(mmol C m^{-2} h^{-1})** of different primary producers on coral reefs.

Group	Division	Species	DOC release[a] Day	Night/Dark	Reference
Macroalgae	Chlorophyta	*Avrainvillea* sp.	-0.50	n.a.	[33]
		Caulerpa sp.	0.56 to 1.11	0.05	[27]
		Cladophora sp.	2.02	0.05	[16]
		Enteromorpha sp.	0.14	n.a.	[27]
		Halimeda opuntia	0.21	n.a.	[28]
		Halimeda opuntia	2.85	n.a.	[16]
		Halimeda sp.	-0.07	n.a.	[33]
		Penicillus sp.	0.25	n.a.	[33]
		Rhipocephalus sp.	1.01	n.a.	[33]
		Ulva sp.	0.28	n.a.	[27]
		Range:	**-0.50 to 1.11**		
	Phaeophyta	*Dictyota ceylanica*	0.48	0.16	[19]
		Dictyota menstrualis	0.49	-0,01	[16]
		Hydroclathrus sp.	0.41	n.a.	[27]
		Lobophora variegata	0.49	-0.01	[16]
		Lobophora sp.	0.40	n.a.	[27]
		Lobophora sp.	0.85	n.a.	[33]
		Sargassum sp.	0.47	n.a.	[27]
		Turbinaria ornata	0.49	n.a.	[28]
		Range:	**0.40 to 0.85**		
	Rhodophyta	*Amansia rhodantha*	0.80	n.a.	[28]
		Hydrolithon reinboldii	0.47	n.a.	[28]
		Hydrolithon reinboldii	0.24	0.04	[19]
		Liagora sp.	0.41	n.a.	[27]
		Lithophyllum congestum	5.35	n.a.	[16]
		Peyssonnelia sp.	0.24 to 2.96	n.a.	[27]
		Range:	**0.24 to 5.35**		
Turf	Consortia	turf algae	0.52 to 5.53	n.a.	[27]
		turf algae	1.40	n.a.	[28]
		turf algae	1.08	n.a.	[16]
		turf algae	0.46	0.11	[19]
		Range:	**0.52 to 5.53**		
Scleractinian corals	Scleractinia	*Acropora formosa*	1.25	n.a.	[54]
		Acropora nobilis	2.22	n.a.	[21]
		Acropora pulchra	0.37	n.a.	[55]
		Acropora sp.	2.56	-0.15	[22]
		Fungia sp.	-1.18	n.a.	[22]
		Goniastrea sp.	1.83	n.a.	[22]
		Madracis mirabilis	-0.87 to 0.91	-0.27	[16]
		Manicina sp.	-13.03	n.a.	[33]
		Millepora sp.	0.77	n.a.	[22]
		Montipora digitata	0.09	n.a.	[55]
		Orbicella annularis	0.15	3.01	[16]
		Pocillopora damicornis	0.07	0.07	[19]
		Pocillopora sp.	-21.93	n.a.	[22]
		Porites lobata	0.18	n.a.	[28]
		Porites sp.	3.17	n.a.	[33]
		Stylophora sp.	-1.17	n.a.	[22]
		Range:	**-21.93 - 3.17**		

[a] DOC release rates are measured under different experimental conditions, such as various light intensities, and do not picture the rates of an entire day, only the release per hour during the short *in vivo* incubations at daytime.

Table 3. a) Estimated DOC release on the reef flat at Pest Bay. b) Scenario without BCMs. BCMs were replaced with BCM-free sediment.

	DOC release (mmol C m^{-2} h^{-1})			Benthic cover (%)	Reef DOC release (mmol C m^{-2} reef h^{-1})	References
	Day	Night	24 hrs[a]		24 hrs[a]	
a)						
BCMs	1.36	3.52	2.44	24	0.59	Present study
Macroalgae (*Dictyota*)	0.49	-0.01	0.24	17	0.04	[16]
Turf	1.08	0.11	0.60	19	0.11	Day[16] Night[19]
Sediment	-0.85	-0.64	-0.75	25	-0.19	Present study
Corals (*Madracis*)	-4.58	-1.42	-3.00	13	-0.39	[16]
Total				98	**0.16**	
b)						
Sediment	-0.85	-0.64	-0.75	24	-0.18	Present study
Macroalgae (*Dictyota*)	0.49	-0.01	0.24	17	0.04	[16]
Turf	1.08	0.11	0.60	19	0.11	Day[16] Night[19]
Sediment	-0.85	-0.64	-0.75	25	-0.19	Present study
Corals (*Madracis*)	-4.58	-1.42	-3.00	13	-0.39	[16]
Total				98	**-0.60**	

[a] assuming 12 h each for both day and night.

Supplementary Information:

High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef ecosystem

Hannah J. Brocke, Frank Wenzhoefer, Dirk de Beer, Benjamin Mueller, Fleur C. van Duyl, Maggy M. Nugues
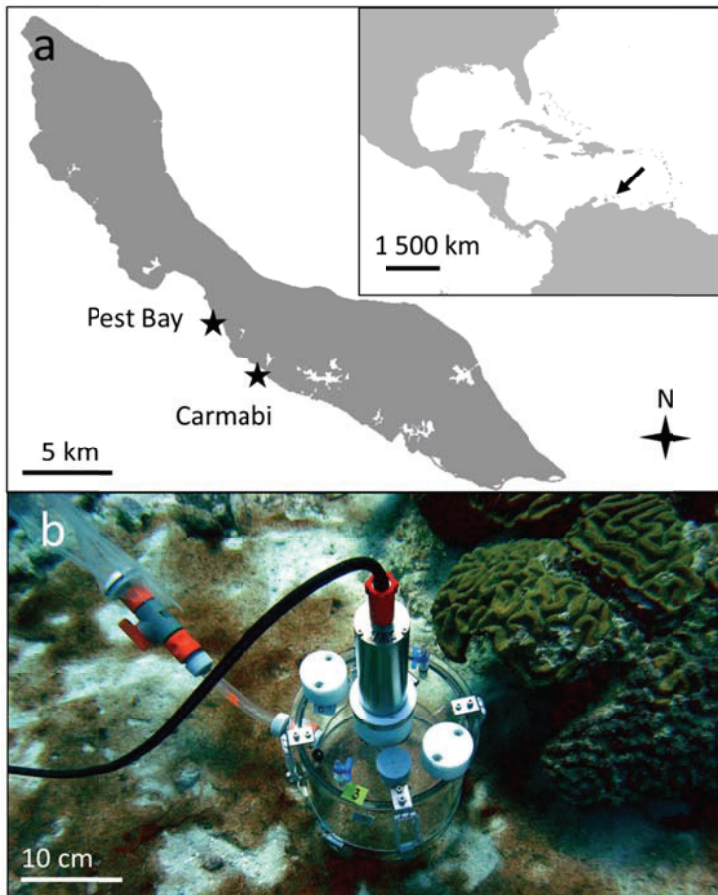

Figure S1. (a) Map of the island Curaçao. Stars display locations of the experimental site Pest Bay and Carmabi. *Inset* shows Central America and the Caribbean Sea. Arrow indicates the location of Curaçao. **(b) Photo of sediment surface and incubation chamber next to a coral head.** Carbonate sediments are covered by brown-colored BCMs.

5. Manuscript IV

MS IV: Nitrogen and phosphorus uptake by the coral reef community in response to enhanced nutrient influxes after rainfall events in the Caribbean

(In review)

Nitrogen and phosphorus uptake by the coral reef community in response to enhanced nutrient influxes after rainfall events in the Caribbean

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KEY WORDS

Benthic cyanobacteria, coral reef, eutrophication, macroalgae, nitrate, phosphate, terrestrial runoff, turf algae

ABSTRACT

Coral reefs are often associated with clear waters with chronically low nutrient levels. However, after rainfall events, terrestrial runoff resulted in a sharp increase of the nitrate (75-fold), phosphate (31-fold) and ammonium concentrations (3-fold) in waters overlying a fringing reef at the island of Curaçao in the Southern Caribbean. It is typically assumed that such high nutrient pulses will be in favor of opportunistic algae and cyanobacteria. However, the ultimate results of nutrient enrichment may depend on the type of nutrient involved, its concentration and the period it is available for uptake. To better understand the effects of a strong nutrient pulse, we determined uptake rates of ammonium, nitrate and phosphate by one coral species, six macroalgal species, two benthic cyanobacteria, turf algae and phytoplankton. The macroalga *Cladophora* spp., turf algae and the benthic cyanobacterium *Lyngbya majuscula* generally had the highest nutrient uptake rates, whereas the coral (*Madracis mirabilis*) had the lowest. In most benthic taxa, ammonium uptake followed a biphasic uptake pattern with an initial surge uptake followed by a slower concentration-dependent uptake. Phosphate uptake generally showed a monophasic pattern, with concentration-dependent uptake only, except for the turf algae and benthic cyanobacteria. We additionally developed a simple model to determine which benthic organism takes up most NH_4^+ and PO_4^{3-} from a nutrient plume. This revealed that turf algae, *Dictyota* spp. and *L. variegata* had the largest share. Our results demonstrate large differences in nutrient uptake among the functional groups, which likely has major consequences for their competitive interactions.

INTRODUCTION

Coastal eutrophication increases the availability of key nutrients such as nitrogen and phosphorus above historic baselines, which can have major impacts on the species composition of coral reef communities (Smith et al. 1981, Bell 1992, Fabricius 2005). Coastal eutrophication can take a variety of forms, such as the discharge of domestic and industrial sewage, terrestrial run off at sites with coastal construction or agriculture, and effluent from aquaculture (Smith et al. 1981, Bell 1992, Devlin & Brodie 2005, Fabricius 2005, Reopanichkul et al. 2009, Herbeck et al. 2012).

Eutrophication events on coral reefs are poorly described, however. Concentrations of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) in waters overlying the reefs are often assumed to be chronically low. However, most nutrient sampling efforts on coral reefs are conducted on relatively coarse temporal and spatial sampling scales (i.e., weeks to years at widely separated measuring sites and depths; Leichter et al. 2003), and only few reports have quantified the duration and magnitude of nutrient increases during actual eutrophication events. As a

consequence, short-term eutrophication events lasting only a few hours often remain unrecorded. Figure 1 depicts examples of such short-term eutrophication events on the coral reefs of Curaçao, Southern Caribbean, after heavy rainfall. Here, brown-colored sediment plumes generated by terrestrial runoff (often augmented by coastal construction) after heavy rainfall are clearly visible, both above water (Fig. 1A,B) and under water (Fig. 1C,D). These sediment plumes have a lower salinity and likely have different DIN and DIP concentrations than the clear oceanic water masses. Nutrient inputs from terrestrial runoff can be substantial. For instance, Devlin & Brodie (2005) observed an up to 400 times higher nutrient influx from terrestrial sources into the Great Barrier Reef after periods of heavy rainfall.

It is often argued that coastal eutrophication enables macroalgae, turf algae and benthic cyanobacteria to replace corals as the dominant phototrophs of reef communities (Done 1992, Paul et al. 2005, Cheal et al. 2010, Vermeij et al. 2010), especially when levels of herbivory are low (Burkepile & Hay 2006). Surprisingly, however, little is known about the differences in nutrient uptake kinetics between these species, and how their nutrient uptake activity varies with the periodicity, duration, nutrient concentration, and nutrient composition of eutrophication events. Furthermore, nutrients entering the water column do not necessarily end up in these benthic species, but can also be taken up by phytoplankton before benthic primary producers can profit from them (Furnas et al. 2005).

To improve our understanding of the impact of episodic eutrophication events on coral reef communities, we determined nitrogen and phosphorus uptake rates of corals, macroalgae, benthic cyanobacteria, turf algae and phytoplankton after a strong nutrient pulse. We additionally developed a simple model to determine which benthic organism consumed the largest share of a nutrient plume entering the reef. This enabled us to assess how the short-term nutrient inputs of sediment plumes generated by heavy rainfall (Fig. 1) are distributed over the different species, which may have implications for their competitive relationships.

MATERIALS AND METHODS

Research site

This study was conducted during the spring (March-May) of 2012 and 2013 at research site 'Buoy 0' on the leeward side of the island of Curaçao, Southern Caribbean (12°7'29.07"N, 68°58'22.92"W; Fig. 2). The benthic community is characterized by 10% coral cover, a high abundance of turf algae and benthic cyanobacteria in shallow areas (0-10 m depth) and a high abundance of macroalgae in deeper parts of the reef (Den Haan et al. 2013).

Ambient nutrient concentrations were determined by analyzing 29 water samples taken at 10 cm above the reef bottom during the period 31 October – 10 December 2011 using a 50 ml

Terumo syringe (Terumo Europe, Leuven, Belgium). Nutrient concentrations in a sediment plume observed at the outlet of the nearby Piscadera Bay (Fig. 2) after heavy rainfall on 23 November 2011 were sampled in a similar fashion (n=6). The water samples were immediately filtered using 0.22 μm Acrodisc filters and stored in 6 ml polyethylene vials (PerkinElmer, MA, USA) that were kept at -20°C until further analysis. Concentrations of NH_4^+ (Helder & De Vries 1979), NO_3^- (Grasshoff et al. 1983) and PO_4^{3-} (Murphy & Riley 1962) were analyzed using continuous flow analysis in a Quatro auto-analyzer (Seal Analytical, UK).

Collection of benthic organisms

Benthic organisms were collected at 5 and 20 m depth to determine their nutrient uptake kinetics. We selected one abundant coral species (*Madracis mirabilis*), six macroalgal species (*Cladophora* spp., *Dichotomaria marginata*, *Dictyota menstrualis*, *Dictyota pulchella*, *Halimeda opuntia*, *Lobophora variegata*), two benthic cyanobacteria (*Dichothrix* spp. and *Lyngbya majuscula*) and turf algae. Turf algae were not collected directly from the reef, because scraping them off the rocky surface of the reef damaged the algal tissues. Instead, turf algae were grown on the exterior of 1.5 L square plastic bottles (FIJI Water Company, CA, USA), which were placed inside 1 m^3 chicken-wired cages (mesh Ø2.5 cm) to prevent grazing by large herbivores. The encaged bottles were placed at 5 and 20 m depth, about 0.5 m above the reef to avoid overgrowth by benthic cyanobacterial mats and macroalgae. After 6 weeks the bottles were covered by turf algal communities comprising all major taxa of natural turf communities on the reef, including Chlorophyta, Rhodophyta, Phaeophyceae and Cyanobacteria (Fricke et al. 2011). The turf algae were collected by cutting out plastic strips (± 25 cm^2) from these algal-covered bottles.

During collection, benthic organisms were carefully cleaned from epiphytes and detritus using tweezers, and put into darkened plastic Ziploc bags that were placed in a cool box filled with ambient seawater (27-29°C) for transport to the lab within 15 min. At the lab, samples were immediately used for nutrient uptake experiments, with the exception of *M. mirabilis*, which was collected one day earlier to ensure the polyps had at least 24 hrs to recover from sampling.

Community composition and total biomass

Percent cover of dominant benthic taxa on the reef was determined from photographs of 60 quadrats (1.5 m^2), randomly placed along both sides of a 100 m transect line laid along the 5 and 20 m isobath. Photographs were analyzed using the program Coral Point Count with Excel Extensions (CPCe) (Kohler and Gill 2006). To obtain the total biomass of hard corals, the macroalgae *H. opuntia*, *Dictyota* spp. and *L. variegata*, turf algae, and the benthic cyanobacteria *Dichothrix* spp. and *L. majuscula* on the reef (g^{-1} DW m^{-2} reef area), we multiplied the percent cover

of each benthic organisms with its areal density (i.e., dry weight per unit of area). The areal density of each benthic organism was obtained from Den Haan et al. (unpublished manuscript [in review for *Ecosystems*]).

Nutrient uptake by benthic species

In a series of laboratory experiments, we measured the nutrient uptake rates of the collected benthic organisms in response to nutrient enrichment. Five different nutrient treatments were investigated: an ammonium pulse with 5 μM ('low') and with 50 μM ('high') of NH_4Cl, a nitrate pulse with 25 μM $NaNO_3$, and a phosphate pulse with 0.88 μM ('low') and with 1.75 μM ('high') of KH_2PO_4. For each species and each nutrient treatment, we used ten 300 ml glass jars that were acid-washed (10% HCl) prior to usage. The glass jars were filled with 0.22 μm filtered (Whatman Cellulose acetate membrane filters) natural seawater that was enriched with NH_4^+, NO_3^- or PO_4^{3-} depending on the nutrient treatment. Benthic organisms were placed in 9 of the 10 glass jars, while one glass jar served as control to assess whether nutrient concentrations remained constant when organisms were absent. For experiments involving turf algae, a clean plastic strip was added to the control glass to check whether the plastic on which turf algae were propagated did not interfere with the nutrient concentrations in the glass jars. None of the controls showed significant changes in nutrient concentration (data not shown).

The ten glass jars were placed in a large aquarium (80 x 40 x 20 cm) that was constantly replenished with fresh seawater to ensure that samples in the glass jars experienced similar temperatures as they would have on the reef (27-29°C). The glass jars were aerated using a Vibra-Flo 2 or 3 Aquarium Air Pump (Blue Ribbon Pet Products, NY, USA) equipped with 0.22 μm Acrodisc filters to filter the air and minimize contamination during experimental runs. Ten 50 W spotlights provided a constant light intensity of 200 μmol photons m^{-2} s^{-1}, which is representative of the light conditions measured at 20 m depth on the reefs of Buoy 0 using a Hydrolab DS5 Sonde (OTT Messtechnik GmbH & Co., Kempten, Germany).

Uptake of NH_4^+ and PO_4^{3-} by benthic organisms was determined by monitoring the decrease of the NH_4^+ and PO_4^{3-} concentrations in the glass jars. The first water sample was always taken before the benthic organism was placed in the glass jar. At time intervals of 10 min, 5 ml water samples were taken from the glass jars, filtered through 0.22 μm Sterile Acrodisc filters (Pall Corporation, NY, USA) into 6 ml Polyethylene vials (PerkinElmer, MA, USA), and immediately analyzed for NH_4^+ (Holmes et al. 1999) and PO_4^{3-} (Murphy & Riley 1962) in a T60 Visual Spectrophotometer (PG Instruments Ltd, Wibtoft, UK). For each species, the maximum uptake rate (V_{max}) of NH_4^+ and PO_4^{3-} was estimated as the uptake rate observed during the first 10-minute time interval in the treatments with a high nutrient pulse.

Uptake of NO_3^- was determined from incorporation of the stable isotope ^{15}N. At the onset, 25 μM $Na^{15}NO_3$ (98 at%) was added to nine glass jars with benthic organisms, whereas the tenth glass jar only contained the organism but did not receive ^{15}N to serve as a control for background ^{15}N levels in the organism. After 2 hrs, samples were quickly rinsed with distilled water and stored in pre-weighed aluminum foil at -20°C. The samples were freeze-dried overnight in a Scanvac CoolSafe Freeze-dryer (Scala Scientific B.V., Ede, The Netherlands) and their dry weight was determined.

The coral *M. mirabilis* was processed differently. Live tissue of *M. mirabilis* was removed from its skeleton using a toothbrush and suspended in a 15 ml test tube with Whatman GF/F filtered seawater. This suspension was centrifuged twice at 4000 rpm for 20 min in an EBA 21 Centrifuge (Hettich Laborapparate, Bäch, Germany), so that zooxanthellae concentrated at the bottom of the tube. The zooxanthellae containing the ^{15}N were pipetted out of the tube, filtered onto a Whatman GF/F filter that was pre-combusted at 450°C for 4 hrs using an Air Recirculating Chamber Furnace (Carbolite, Hope Valley, UK), and stored at -20°C for at least three days. Subsequently the filters were freeze-dried using a Scanvac CoolSafe Freeze-dryer. Dry weight of *M. mirabilis* was approximated from its surface area according to Hardt (2007).

Samples were analyzed for their tissue N content and $\delta^{15}N$ content using a Thermofinnigan Delta Plus isotope ratio mass spectrometer (Bremen, Germany) connected to a Carlo Erba Instruments Flash 1112 Element Analyzer (Milan, Italy). Each sample was first grinded into powder and packed inside a tin capsule that was folded into a small pellet. For *M. mirabilis*, Whatman GF/F filter loaded with zooxanthellae were directly packed into the tin capsule and folded into a small pellet. The pellets were weighed and their $\delta^{15}N$ content (in ‰) was quantified as:

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \tag{1}$$

where R_{sample} is the isotope ratio $^{15}N/^{14}N$ of the sample and $R_{standard}$ is the isotope ratio of atmospheric N_2 (i.e., $R_{standard} = 0.0036765$). The $\delta^{15}N$ measurements were calibrated against the laboratory standards urea ($\delta^{15}N = -40.81‰$) and acetanilide ($\delta^{15}N = 1.3‰$). The NO_3^- uptake rate (in μmol N g^{-1} DW h^{-1}) of each species was calculated as:

$$V = \frac{Q_N}{t} \times R_{standard} \times (\delta^{15}N_{treatment} - \delta^{15}N_{control}) \times \frac{100}{at_w} \tag{2}$$

where Q_N is nitrogen content of the tissue (in mmol N g^{-1} DW), t is incubation time, $\delta^{15}N_{treatment}$ is the $\delta^{15}N$ of ^{15}N-enriched samples, $\delta^{15}N_{control}$ is the $\delta^{15}N$ of the control sample, and at_w is the at% of ^{15}N in the nitrate pulse that we supplied.

To determine the tissue P content of the organisms, grinded samples were first treated with 6 M HNO_3, 30% H_2O_2, 2 M H_2SO_4, 48% HF, and 2 M HCl inside platinum crucibles that were gently heated to ~300ºC using a Cimarec 3 ceramic-top plate to dissolve total P of the samples (Barnstead-Thermolyne Corp., Iowa, USA). P contents were then measured in an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 3000XL, Perkin Elmer, Waltham, MA, USA).

Nutrient uptake by phytoplankton

Phytoplankton was collected at Buoy 0 by filling 20 Plexiglas incubation tubes of 5.3 l each (Röhm GmbH & Co KG, Darmstadt, Germany) with seawater from 5 and 20 m depth. The incubation tubes were darkened and transported to our nearby laboratory, where they were filtered within one hour after collection through a double-mesh filter (50 and 150 μm) to remove most zooplankton and debris. The filtered seawater was put back in the incubation tubes, which were returned to the reef to study NH_4^+ and NO_3^- uptake by phytoplankton.

The phytoplankton concentrations were too low to create substantial changes in added nutrient concentrations, and therefore their nitrogen uptake rates were estimated from incorporation of the stable isotope ^{15}N. At the start of the incubation, eight tubes were enriched with 50 μM $^{15}NH_4Cl$ (98 at%), eight tubes were enriched with 25 μM $Na^{15}NO_3$ (98 at%), and four tubes served as controls. Immediately after nutrient addition, the incubation tubes were closed, shaken and attached to a vertical rope running from 30 m depth to the water surface. Ten incubation tubes (4×NH_4^+, 4×NO_3^-, 2 controls) were attached at 5 m depth and the other ten tubes (4×NH_4^+, 4×NO_3^-, 2 controls) at 20 m depth. After two hours, the incubation tubes were taken off the rope, darkened, and returned to the laboratory. At the lab, their contents were immediately filtered over a pre-combusted, pre-weighed Whatman GF/F filter placed inside a Ø25 mm Polycarbonate filter holder (Cole Palmer, Chicago, IL, USA). The loaded filters were stored in aluminum foil at -20°C for three days, freeze-dried overnight, and weighed. Subsequently, the samples were analyzed for $\delta^{15}N$ content (as described above) and nitrogen uptake rates were calculated with equation 2. The phytoplankton incubations were repeated on 5 different days within a 2-weeks period.

Statistical analyses

To test whether nutrient concentrations in clear oceanic water at Buoy 0 differed from those in sediment plumes after heavy rainfall we applied the two-sample Student's t-test (for equal variances) or the Welch's t-test (for unequal variances). Two-way analysis of variance was used to test whether nutrient uptake rates differed between species and between depths. The data

were log-transformed if this improved 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. It is known that nutrient uptake rates can sometimes be substantially higher immediately after a nutrient pulse, and then settle at a lower uptake rate (D'Elia & DeBoer 1978, Dy & Yap 2001). The presence of such biphasic patterns in nutrient uptake was tested by comparison of the nutrient uptake rates during the first 10 minutes of the experiments (time interval t_{0-10}) and the subsequent 10 minutes (time interval t_{10-20}) using the paired samples t-test.

RESULTS

Nutrient enrichment after rainfall

Seawater collected at 0.5 m above the reef surface at research site Buoy 0 contained low ambient nutrient concentrations (±SE) of 1.45 ± 0.14 µM NH_4^+, 0.15 ± 0.02 µM NO_3^- and 0.032 ± 0.003 µM PO_4^{3-}. In contrast, seawater collected from the sediment plume observed near Piscadera Bay after heavy rainfall contained a 75-fold higher NO_3^- concentration (Welch's t-test: $t=-36.1$, df=29.1, p<0.001), 31-fold higher PO_4^{3-} concentration (Welch's t-test: $t=-44.6$, df=28.3, p<0.001), but only a 3-fold higher NH_4^+ concentration (Student's t-test: $t=-5.2$, df=33, p<0.001) than these background nutrient levels (Fig. 3). During our study, similar sediment plumes were observed at least once per month near Buoy 0, and often lasted only tens of minutes to a few hours.

Nutrient uptake kinetics

During the nutrient uptake experiments, NH_4^+ and PO_4^{3-} concentrations in the glass jars decreased, and concomitantly the tissue N and P contents of the organisms increased (see Fig. 4 and Fig. 5 for a few selected examples; Figs. S1-S4 in the Supplement for all species). Uptake rates of NH_4^+ and PO_4^{3-} gradually diminished as the external nutrient concentrations were reduced. Generally speaking, the NH_4^+ and PO_4^{3-} concentrations were not completely exhausted, but the organisms left over a residual NH_4^+ concentration of 1.5-8 µM and a residual PO_4^{3-} concentration of 0.03-0.5 µM depending on the species (Fig. 4 and 5; Figs. S1-S4 in the Supplement).

The maximum uptake rates (V_{max}) of NH_4^+, NO_3^- and PO_4^3 differed significantly between the species (Fig. 6; Table 1). Turf algae had the highest V_{max} for NH_4^+, and also the macroalgal species had a relatively high V_{max}, whereas the coral $M.$ $mirabilis$ had the lowest V_{max} for NH_4^+. The benthic cyanobacterium $L.$ $majuscula$ and green macroalga of the $Cladophora$ genus had the highest nutrient uptake rates for NO_3^-, whereas the calcified green alga $H.$ $opuntia$ had the lowest

NO_3^- uptake rate. For PO_4^{3-}, again *L. majuscula* and *Cladophora* spp. had the highest V_{max}, whereas *H. opuntia* and *M. mirabilis* had the lowest V_{max} (Fig. 6).

Furthermore, we found a significant main effect of depth on the NO_3^- uptake rate and a significant interaction effect of species x depth on the NH_4^+ and NO_3^- uptake (Table 1). However, post hoc comparison of the means showed that *M. mirabilis* was the only species for which the nutrient uptake rate (of NO_3^-) differed between 5 and 20 m depth; for all other species that occurred at both depths the nutrient uptake rates were not significantly different between 5 and 20 m depth (Fig. 6).

The uptake of NH_4^+ was biphasic for all benthic taxa, except *M. mirabilis* and *D. marginata*. More specifically, the NH_4^+ uptake rates of turf algae, benthic cyanobacteria and the macroalgae *L. variegata*, *Cladophora* spp., *H. opuntia* and *Dictyota* spp. were significantly higher during the first 10 min than in the subsequent time intervals (Table 2). When this initial surge uptake was removed from the data, the NH_4^+ uptake rates of almost all species followed a linear function of the ambient NH_4^+ concentration (Fig. 7).

The uptake of PO_4^{3-} showed a biphasic pattern only for turf algae, *Dichothrix* spp. and *L. majuscula*, but not for the other species (Table 2). For most species, the PO_4^{3-} uptake rates could be adequately described as a linear function of the ambient PO_4^{3-} concentration (Fig. 8).

Distribution of nutrient influx over the different species

We can use our nutrient uptake measurements to estimate, roughly, how episodic nutrient pulses are distributed over the different species in the community. The consumption of a pulse of nutrients by the coral reef community can be described by the following differential equation:

$$\frac{dN}{dt} = -\sum_{i=1}^{n} u_i(N)B_i - DN \tag{3a}$$

$$\frac{dQ_i}{dt} = u_i(N)B_i \tag{3b}$$

Here, N is the nutrient concentration in the nutrient plume, Q_i is the amount of nutrient in species i, $u_i(N)$ is the biomass-specific nutrient uptake rate of species i as function of the nutrient concentration N, B_i is the biomass of species i, and D is the rate at which the nutrient concentration in the plume erodes by other processes (e.g., mixing with open ocean water, sedimentation, denitrification). In our case, we found that the nutrient uptake rates could be adequately described as linear functions of the ambient nutrient concentration, i.e., $u_i(N)=a_iN$. Hence, differential equation (3a) can be easily solved, and the decrease of nutrient concentration in time can be described as:

$$N(t) = N_0 e^{-(\sum a_j B_j + D)t} \tag{4}$$

where N_0 is the initial nutrient concentration in the nutrient plume. Nutrient acquisition by the different species is obtained by insertion of this equation into equation (3b) and subsequent integration, which gives:

$$Q_i(t) = Q_{i,0} + \frac{a_i B_i}{\sum a_j B_j + D}\left(1 - e^{-(\sum a_j B_j + D)t}\right)N_0 \qquad (5)$$

where $Q_{i,0}$ is the initial amount of nutrients in the tissue of species i. This equation shows that each species acquires a fraction $a_i B_i/(\sum a_j B_j + D)$ of the nutrient pulse. Accordingly, in line with common intuition, species with more biomass and higher biomass-specific nutrient uptake rates will acquire a larger fraction of the total nutrient pulse, and also the rate at which a nutrient plume is eroded by other processes is a major determinant of the amount of nutrients that species can absorb from a nutrient plume.

Since we have measured the nutrient uptake rates of the different species (Figs. 7 and 8) and their biomass distribution at our study site (Fig. 9A), we can now calculate how the nutrients taken up from a nutrient plume are distributed over the different species. However, we made some assumptions for this model:

- a_i is similar for benthic organisms that occur at both 5 and 20 m depths. Table 1 revealed that the uptake of NH_4^+ and PO_4^{3-} remained unaffected by depth-origin of the organism.

- a_i is solely based on the linear uptake rate of the benthic organisms as depicted in Figs. 7 and 8, hence for benthic organisms that had a biphasic uptake pattern we only included the linear uptake after 10 min to determine a_i.

- a_i of *D. menstrualis* is the same the a_i of *D. pulchella*.

- *M. mirabilis* represents the NH_4^+ and PO_4^{3-} uptake of all hard corals.

- The biomass distribution of *Dichothrix* spp. and *L. majuscula* are similar to those of the benthic cyanobacterial mats from Den Haan et al. (unpublished manuscript [in review for *Ecosystems*])

Our results show that turf algae make up ~40% of the total biomass in the shallow reef (Fig. 9A). However, our model indicates that turf algae obtain an even larger share of the NH_4^+ (~78%) and PO_4^{3-} (~83%) nutrient pulses (Fig. 9B,C). In contrast, corals make up ~44% of the total biomass in the shallow reef (Fig. 9A), but they acquire only ~8% of the NH_4^+ and ~2% of the PO_4^{3-} nutrient plumes (Fig. 9B,C). Likewise, at the deeper reef, corals have a high total biomass (~73%; Fig 9A), yet only obtain a small share of the NH_4^+ and PO_4^{3-} nutrient plumes (~12% and ~7% respectively; Fig. 9B,C). Here notably the macroalgae *Dictyota* spp. and *Lobophora variegata* took up a large share of the NH_4^+ (~17% and ~62% respectively) and PO_4^{3-} plumes (~29% and ~41% respectively) (Fig. 9B,C). Turf algae only took up a fraction of the

NH_4^+ nutrient plume at 20 m depth (~7%; Fig. 9B) due to a very low total biomass (~4%; Fig. 9A), yet surprisingly still acquired up to ~17% of the PO_4^{3-} plume (Fig. 9C).

DISCUSSION

In this study, we determined the NH_4^+, NO_3^- and PO_4^{3-} uptake kinetics of abundant phototrophic organisms on Curaçaoan reefs in a series of laboratory experiments. Nutrient uptake rates varied considerably among these phototrophic organisms. Generally, filamentous organisms like turf algae, *Cladophora* spp., and *L. majuscula* had the highest NH_4^+, NO_3^- and PO_4^{3-} uptake rates, whereas the coral *M. mirabilis* and the green macroalga *H. opuntia* consistently showed the lowest. Phytoplankton did not have higher nitrogen uptake rates than most benthic organisms studied. Most benthic organisms had a biphasic NH_4^+ uptake: during the first ten minutes we observed maximum nutrient uptake rates (V_{max}), a so-called surge uptake, followed by linear non-saturating uptake. The uptake of PO_4^{3-} for most benthic organisms was linear and non-saturating, except for turf algae, *Dichothrix* spp. and *L. majuscula*. Further, our model indicated that turf algae contributed most to the uptake of the NH_4^+ and PO_4^{3-} plumes at 5 m depth, whereas at 20 m depth the macroalgae *Dictyota* spp. and *L. variegata* had the largest share. Corals only played a minor role at both depths, even though they dominated the benthic substrate in terms of total biomass.

NH_4^+, NO_3^- and PO_4^{3-} uptake rates by reef phototrophic organisms

In our study, *M. mirabilis* had consistently amongst the lowest NH_4^+, NO_3^- and PO_4^{3-} uptake rates. This is not surprising, as corals not only take up nutrients directly from the water column, but also acquire them via heterotrophic feeding (Goreau et al. 1971, Szmant-Froelich & Pilson 1984, Houlbrèque & Ferrier-Pagès 2009). The latter is essential as direct uptake of nutrients by zooxanthellae is insufficient to meet corals' energetic demands (Bythell 1988, Muscatine et al. 1989, Houlbrèque & Ferrier-Pagès 2009). Bythell (1988) reported that heterotrophic feeding supplied 70% of the nitrogen demand in the Elkhorn coral *Acropora palmata*, and Anthony (1999) showed that one third of the nitrogen required for coral tissue growth of *Pocillopora damicornis* was obtained heterotrophically. Since *M. mirabilis* is known for its high zooplankton capture rate (Sebens et al. 1996), it did not come as a surprise that we observed low uptake rates of dissolved inorganic nitrogen and phosphorus by *M. mirabilis* in this study.

Macroalgae, turf algae and benthic cyanobacteria acquire nitrogen and phosphorus by means of diffusion and/or active transport (Lapointe 1997, Larned 1998, Kuffner & Paul 2001, Vermeij et al. 2010). A more filamentous morphology increases the surface:volume ratio, which in turn corresponds to an improved capacity to take up nutrients (Littler & Littler 1980,

Rosenberg & Ramus 1984, Pedersen & Borum 1997). In this study, we indeed found support for the notion that taxa with high surface:volume ratios were characterized by higher nutrient uptake rates. The filamentous turf algae, *Cladophora* spp. and *L. majuscula* had the highest uptake rates of NH_4^+ and NO_3^-. However, *Dichothrix* spp., which is also filamentous, had comparatively low uptake rates. Littler & Littler (1980) found that a tightly clumped morphology (as is the case with *Dichothrix* spp.) can result in decreased water movement along the thalli causing diffusion gradients to overlap so that neighboring thalli have to compete for the same nutrients resulting in lower overall nutrient uptake rates (Littler & Littler 1980). Besides a high surface:volume ratio, other factors such as storage capacity (Fujita 1985, Lapointe 1989, Fong et al. 1994, Fong et al. 2004) and efficiency of internal nutrient recycling (Duarte 1995) will also affect nutrient uptake rates. Though not investigated in the present study, these factors will to some degree determine which phototrophic organism is most successful in taking up available nutrients.

Whether dissolved inorganic nutrients entering coral reefs from land actually reach the benthos is highly dependent on the distance of the nutrient source to the reef (Smith et al. 1981, Devlin & Brodie 2005, Fabricius 2005). Often phytoplankton, protozoa, flagellates and bacteria take up these nutrients in the water column before they reach the benthos (Furnas et al. 2005). Consequently, these nutrients are not directly available for the benthos, as they are temporarily 'locked' in the tissues of phytoplankton (i.e., as organic matter) (Duarte & Cebrian 1996, Furnas et al. 2005). To better understand the competition of phytoplankton for nutrients with the benthos, the uptake of nitrogen by phytoplankton was also included in this study. Though we hypothesized that phytoplankton would be superior in nitrogen uptake, as phytoplankton have a larger surface:volume ratio than macroalgae (Hein et al. 1995), we did not observe this in our study. Phytoplankton in fact had comparable nitrogen uptake rates (NH_4^+ and NO_3^-) as the benthic taxa. This does however not mean that phytoplankton only plays a minor role in the competition with benthic phototrophs for nutrients since they are often not in direct competition but spatially separated. As can be seen from the pictures of the sediment plumes (Fig. 1), these plumes are mainly concentrated in the upper water layers where the nutrients are available to phytoplankton however not (yet) to the benthos. If the water is mixed deeper or if particles form the sediment plume sinks down, the benthos are more likely to profit from the increased nutrient concentrations found in such sediment plumes.

Biphasic (NH_4^+) and phasic (PO_4^{3-}) uptake patterns of benthic organisms

The NH_4^+ uptake kinetics of most benthic organisms followed a biphasic pattern. Here, the initial surge uptake (i.e., uptake during the first 10 min) was followed by concentration dependent linear, non-saturating uptake. Lobban & Harrison (1997) indicated that during the first

minutes of nutrient availability, algae can rapidly take up these nutrients via passive diffusion into the free space between the cell wall and plasma membrane. Especially when the external seawater is very eutrophic, surge uptake of NH_4^+ is expected. Indeed, we observed surge uptake in this study after adding a pulse of a high NH_4^+ concentration. As the free space between the cell wall and plasma membrane is quickly filled with NH_4^+, uptake hereafter will be controlled by concentration-dependent active transport. Here, a higher external NH_4^+ concentration results into a higher NH_4^+ uptake as long as sufficient membrane transport carriers can be utilized to actively transport NH_4^+ through the plasma membrane into the cytoplasm. This will reach a plateau (V_{max}) at high NH_4^+ concentrations, but even the highest external concentrations (i.e., ~50-fold NH_4^+-enrichment) we used were not saturating. This may indicate that not all membrane transport carriers were utilized, hence increased uptake at even higher NH_4^+ concentrations can be expected than those used in this study. This highlights that benthic organisms will continue to take up more NH_4^+, even during large eutrophication events.

Various NH_4^+ uptake patterns by macroalgae and corals have previously been reported, these include linear, non-linear, and biphasic uptake patterns (D'Elia & DeBoer 1978, Muscatine & D'Elia 1978, Friedlander & Dawes 1985, Fujita 1985, Fujita et al. 1988, Dy & Yap 2001, Smit 2002, Abreu et al. 2011), as well as NH_4^+ uptake patterns that follow the Michaelis-Menten model (e.g. Campbell 1999, Runcie et al. 2003, Luo et al. 2012). The Michaelis-Menten model illustrates how the NH_4^+ uptake rate increases asymptotically with increasing external NH_4^+ concentration until a V_{max} is reached. Though the Michaelis-Menten model has often been used to describe nutrient uptake rates of macroalgae (e.g. Wallentinus 1984, Pedersen et al. 1997, Cohen & Fong 2005), initial surge uptake can dispute the Michaelis-Menten model as uptake can then appear unsaturable with increasing external nutrient concentrations (e.g. Harrison et al. 1989, Pedersen 1994), hence it seems no V_{max} can be reached. In our study, we did observe initial surge uptake, however since the uptake after 10 min proved to be linearly unsaturable, we were not able to fit the Michaelis-Menten model to our data. Instead we observed a biphasic NH_4^+ uptake pattern.

The uptake of PO_4^{3-} followed a strict linear, non-saturating pattern with increasing PO_4^{3-} concentration for most benthic organisms. Highest PO_4^{3-} uptake rates were achieved at the highest external PO_4^{3-} concentration (i.e., ~50-fold PO_4^{3-} enrichment), which was similar to the NH_4^+ uptake experiments. However, no initial surge uptake was observed with the PO_4^{3-} uptake experiments, indicating PO_4^{3-} uptake was probably only regulated via active transport, except for turf algae, *Dichothrix* spp. and *L. majuscula*, which did have an initial surge uptake. Previous studies often argued the applicability of the Michaelis-Menten model for PO_4^{3-} in macroalgae (Wallentinus 1984, Lavery & McComb 1991, Luo et al. 2012), yet other studies have also found

linear, non-linear and biphasic PO_4^{3-} uptake patterns (Chisholm & Stross 1976, Friedlander & Dawes 1985, Martínez & Rico 2004).

Possible consequences of short-term eutrophication events on the reef

Our results indicate that notably *Cladophora* spp., turf algae and benthic cyanobacteria (i.e., *L. majuscula*) that have previously been documented to cause large nuisance blooms on reefs around the world (Lapointe 1997, Albert et al. 2005, Paul et al. 2005, Smith et al. 2005, Dailer et al. 2012) quickly capitalize on newly available nutrients, and never became nutrient-saturated at very high nutrient concentrations (i.e., 50x the ambient NH_4^+ and PO_4^{3-} levels). Consequently, during subsequent eutrophication events, ephemeral, fast-growing algae and cyanobacteria will constantly take in more nutrients that can be used to increase their growth rate (Kuffner & Paul 2001, Fong et al. 2004, Smith et al. 2005). Slow-growing corals and macroalgae (i.e., *H. opuntia*, *D. menstrualis*, *D. pulchella*, *L. variegata* and *D. marginata*) are better capable at storing nutrients for future growth (Reef et al. 2012, Kopp et al. 2013), and thus seem better adapted to circumstances where nutrient availability is episodic due to e.g., upwelling events, internal tides or irregular rain-induced land run-off (Andrews & Gentien 1982, Furnas & Mitchell 1996, Szmant & Forrester 1996, Leichter et al. 2003, Fabricius 2005). Interestingly, our model - though highly simplified - confirmed that turf algae consumed the largest shares of the NH_4^+ and PO_4^{3-} plumes distributed onto the shallow reef, yet also indicated that *Dictyota* spp. and *L. variegata* consumed most of these plumes at the deeper parts of the reef. Though the level of nutrient enrichment onto a reef cannot solely explain the benthic community structure, as other factors such as the level of herbivory play a major role (McClanahan et al. 2003, Burkepile & Hay 2006, 2009), the results of our model are appealing, especially since they agree well with the general observation that many degraded reefs in the Caribbean are now dominated by notably turf algae, *L. variegata* and *Dictyota* spp. (e.g., Hughes 1994, Williams & Polunin 2001, Mumby et al. 2005, Nugues & Bak 2008, Vermeij et al. 2010).

None of the benthic organisms that we studied were capable of exhausting their nutrient supply below the ambient nutrient concentrations measured at Buoy 0. This suggests benthic organisms are highly dependent on external nutrient supply from episodic eutrophication events, such as those observed and mimicked in this study. Hence, minimizing the amount of nutrients entering coral reefs from land is essential, as notably undesirable algae and benthic cyanobacteria benefit disproportionally from high nutrient availability, which in turn can increase their abundance at the expense of coral.

ACKNOWLEDGEMENTS

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TABLES

Table 1. Two-way analysis of variance of the nutrient uptake rates, with species and depth as independent variables

Effect	df_1, df_2	F	p
NH_4^+:			
Species	**10, 126**	**92.981**	**<0.001**
Depth	1, 126	1.024	0.313
Species × Depth	**3, 126**	**5.800**	**<0.001**
NO_3^-:			
Species	**7, 108**	**93.556**	**<0.001**
Depth	**1, 108**	**34.090**	**<0.001**
Species × Depth	**3, 108**	**8.678**	**<0.001**
PO_4^{3-}:			
Species	**9, 103**	**284.222**	**<0.001**
Depth	1, 103	1.655	0.201
Species × Depth	3, 103	2.380	0.074

Columns indicate the investigated effects, degrees of freedom ($df1$ and $df2$), the F-statistic ($Fdf1,df2$) and the

corresponding probability (p). Significant results ($p<0.05$) are indicated in bold.

Table 2. Comparison of nutrient uptake rates (for NH_4^+ and PO_4^{3-}) between the initial time interval t_0-t_{10} and the

subsequent time interval t_{10}-t_{20} using the paired samples t-test

Species	NH_4^+			PO_4^{3-}		
	t	df	p	t	df	p
Turf algae	**3.743**	**33**	**<0.001**	**3.176**	**31**	**0.003**
Lobophora variegata	**3.320**	**16**	**0.004**	0.102	15	0.920
Cladophora spp.	**4.075**	**16**	**<0.001**	1.324	17	0.203
Dichotomaria marginata	1.619	26	0.118	0.921	35	0.363
Halimeda opuntia	**4.869**	**33**	**<0.001**	2.015	31	0.053
Dictyota menstrualis	**2.954**	**16**	**0.009**	1.007	15	0.330
Dictyota pulchella	**2.408**	**16**	**0.028**	1.889	17	0.076
Lyngbya majuscula	**5.418**	**25**	**<0.001**	**4.972**	**26**	**<0.001**
Dichothrix spp.	**2.502**	**16**	**0.024**	**2.322**	**17**	**0.033**
Madracis mirabilis	-0.892	33	0.379	0.605	35	0.549

For each species, nutrient uptake rates were obtained from 9 replicate time series at 5 and/or 20 m depth and for 2

initial nutrient concentrations. Significant results ($p<0.05$) are indicated in bold.

FIGURES
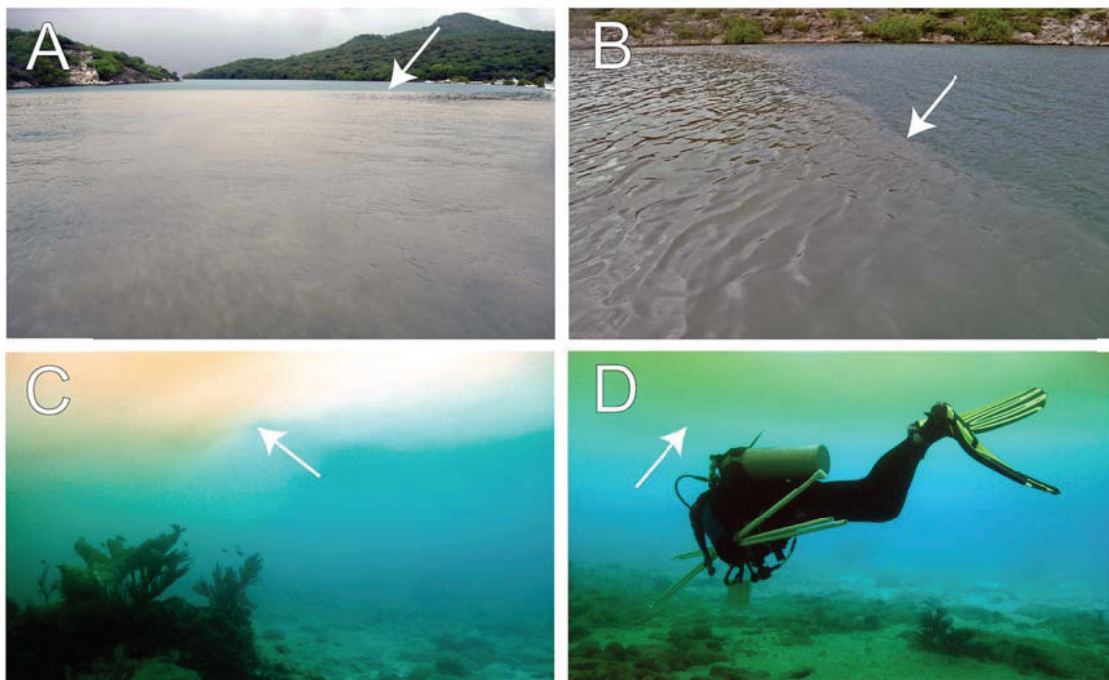
Fig. 1 Short-term eutrophication events (A,B) Sediment plume at the outlet of Piscadera Bay on the island of Curaçao (12°7'21.42"N, 68°58'12.52"W; marked with letter 'A' in insert Fig. 2) after a period of heavy rainfall on 23 November 2011; the arrows indicate the front between the sediment plume and clear oceanic water. (C,D) An underwater view of the sediment plume, which extends from the water surface to ~3 m depth. at dive site 'Pest Bay' on 21 October 2010 (12°9'59.77"N, 69°0'43.91"W; marked with letter 'B' in insert Fig. 2). Image credit: Hannah J. Brocke and Joost den Haan
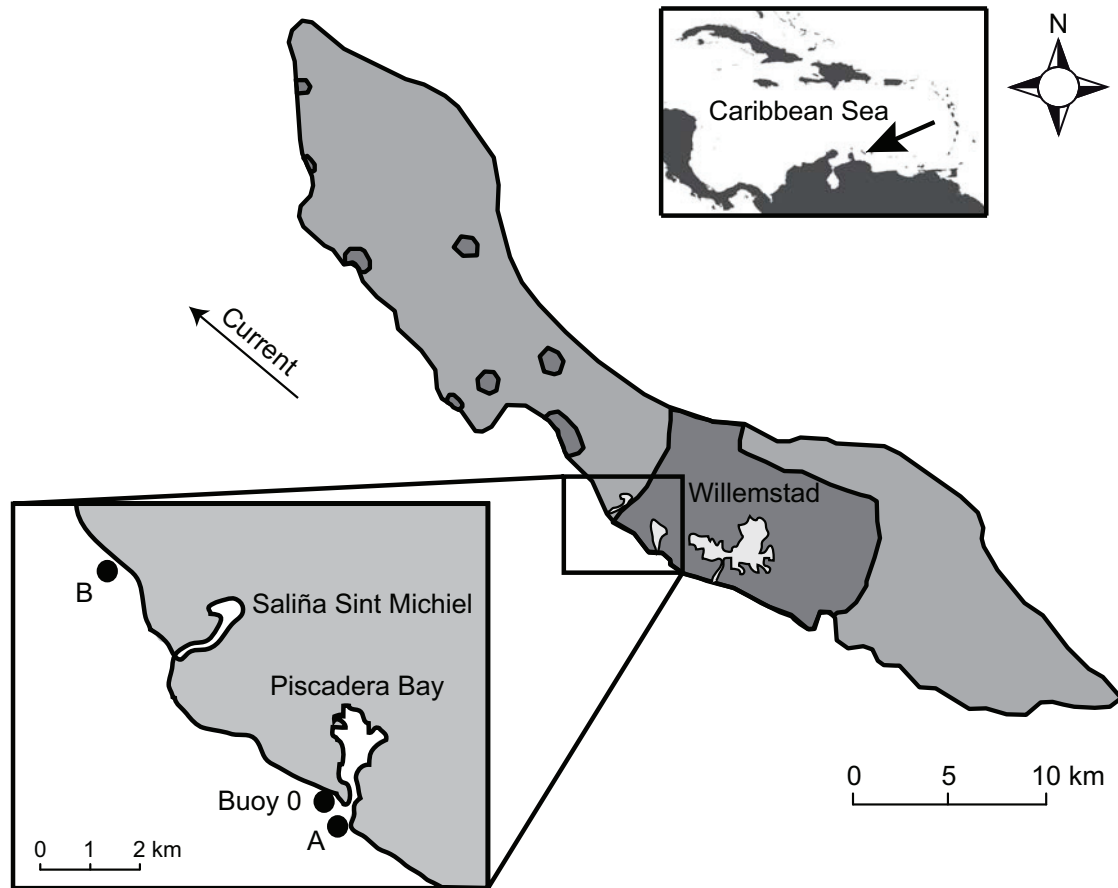
Fig. 2 Map of Curaçao

Map with collection site Buoy 0 on the island of Curaçao, Southern Caribbean (12°10'N, 68°58'W). Shading indicates urban areas (dark grey zones) and the commercial harbor (striped area). Included are also the sediment plumes observed at Piscadera Bay (A) and Pest Bay (B)
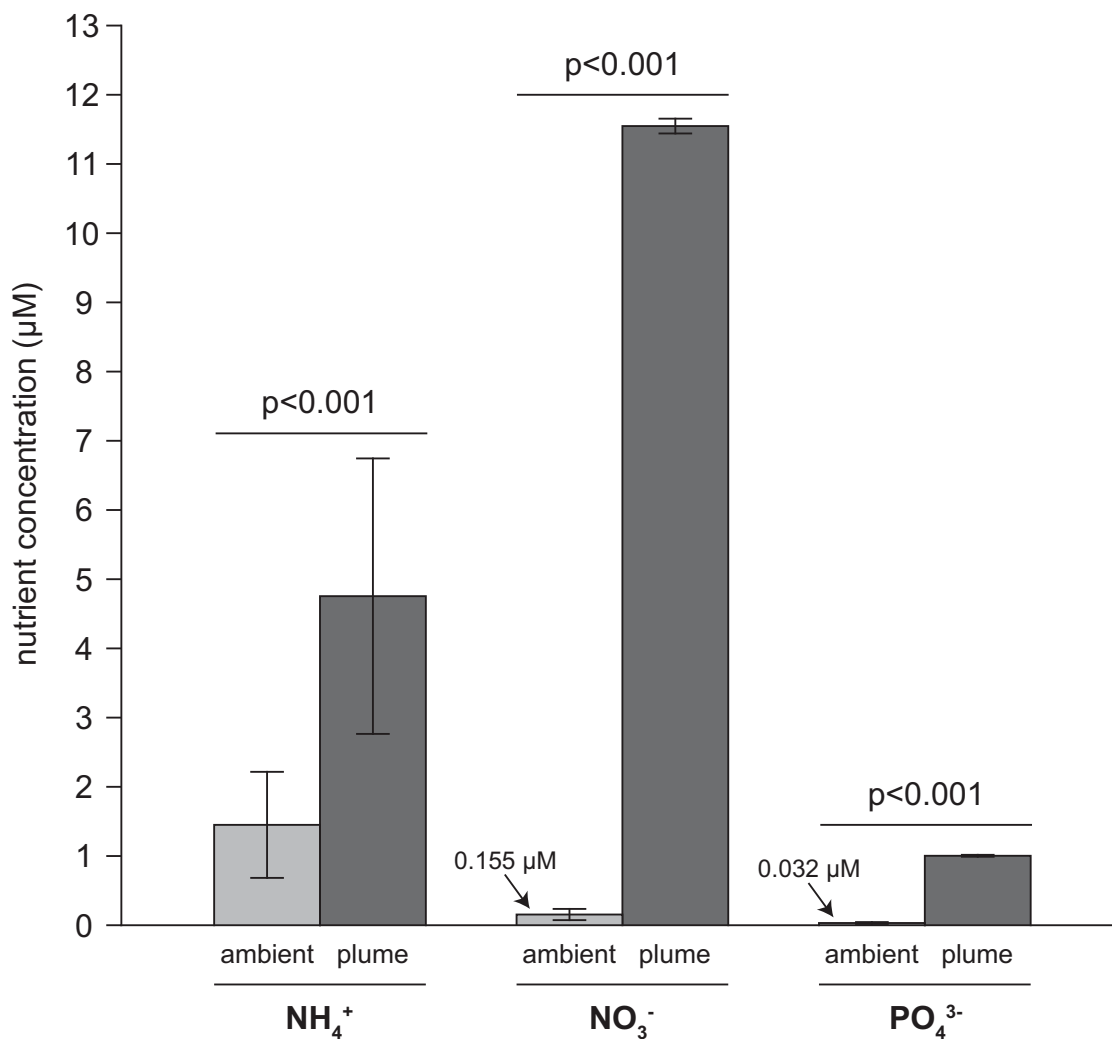
Fig. 3 Nutrient enrichment due to a short-term eutrophication event

Difference between ambient nutrient concentrations found at Buoy 0 (light grey bars) and those of a short-term eutrophication event observed as a sediment plume in the reef water on 23 November 2011 (dark grey bars). Included bars represent NH_4^+, NO_3^- and PO_4^{3-}. Error bars represent s.d. of means. The difference between the two nutrient concentrations was tested by a one-way analysis of variance
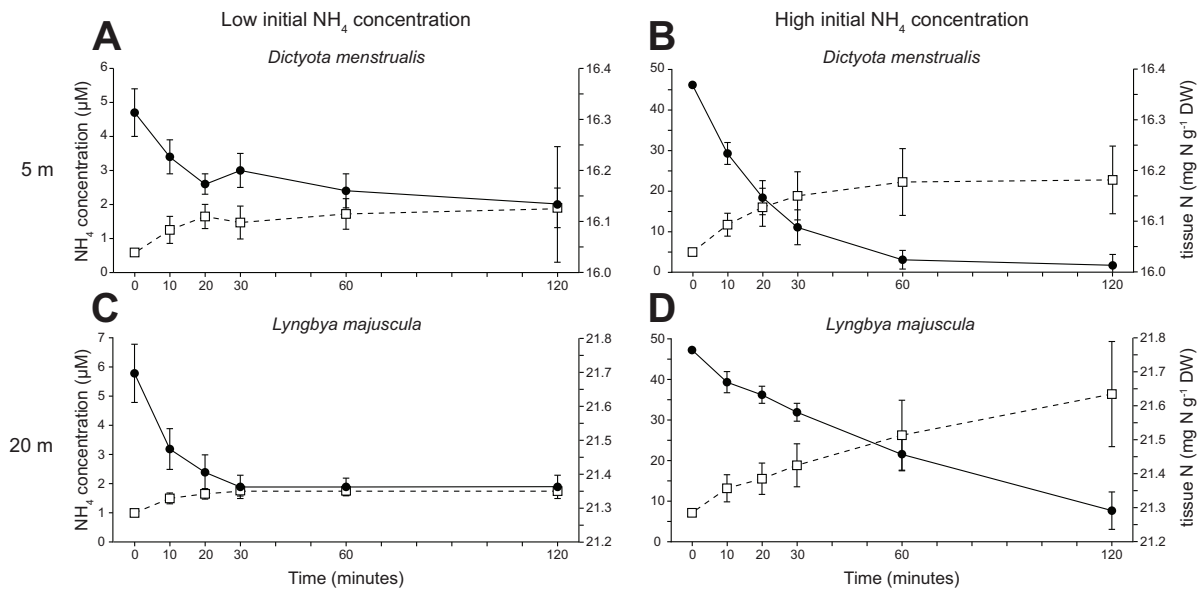
Fig. 4 Examples of NH_4^+ uptake by benthic organisms

Decrease of the NH_4^+ concentration (μM) inside glass jars due to the uptake by the macroalga *Dictyota menstrualis* (from 5 m depth; A,B) and the benthic cyanobacterium *Lyngbya majuscula* (from 20 m depth; C,D) over a 2-hour period (solid black circles). As a consequence, its tissue N content increased (mg N g^{-1} DW) as indicated by the open squares. Included are results with low and high initial NH_4^+ concentrations. Error bars represent s.d. of means. Results from all other benthic organisms studied are shown in Supplement 1 (those originating from 5 m depth) and Supplement 2 (those originating from 20 m depth)
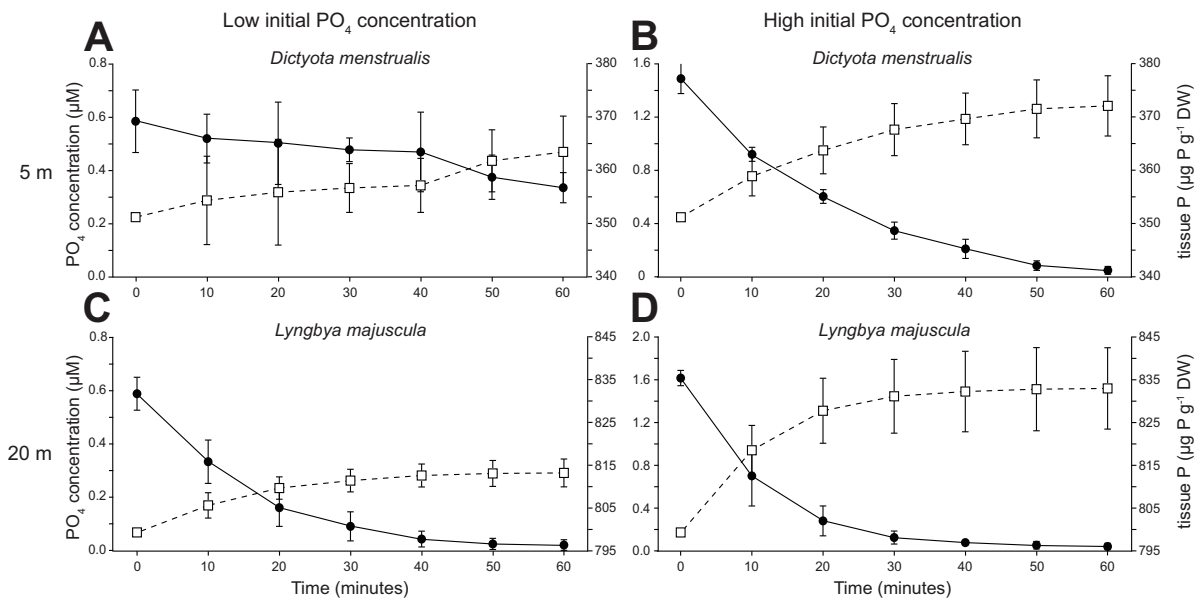
Fig. 5 Examples of PO_4^{3-} uptake by benthic organisms

Decrease of the PO_4^{3-} concentration (μM) inside glass jars due to the uptake by the macroalga *Dictyota menstrualis* (from 5 m depth; A,B) and the benthic cyanobacterium *Lyngbya majuscula* (from 20 m depth; C,D) over a 2-hour period (solid black circles). As a consequence, its tissue P content increased (μg P g^{-1} DW) as indicated by the open squares. Included are results with low and high initial PO_4^{3-} concentrations. Error bars represent s.d. of means. Results from all other benthic organisms studied are shown in Supplement 3 (those originating from 5 m depth) and Supplement 4 (those originating from 20 m depth)
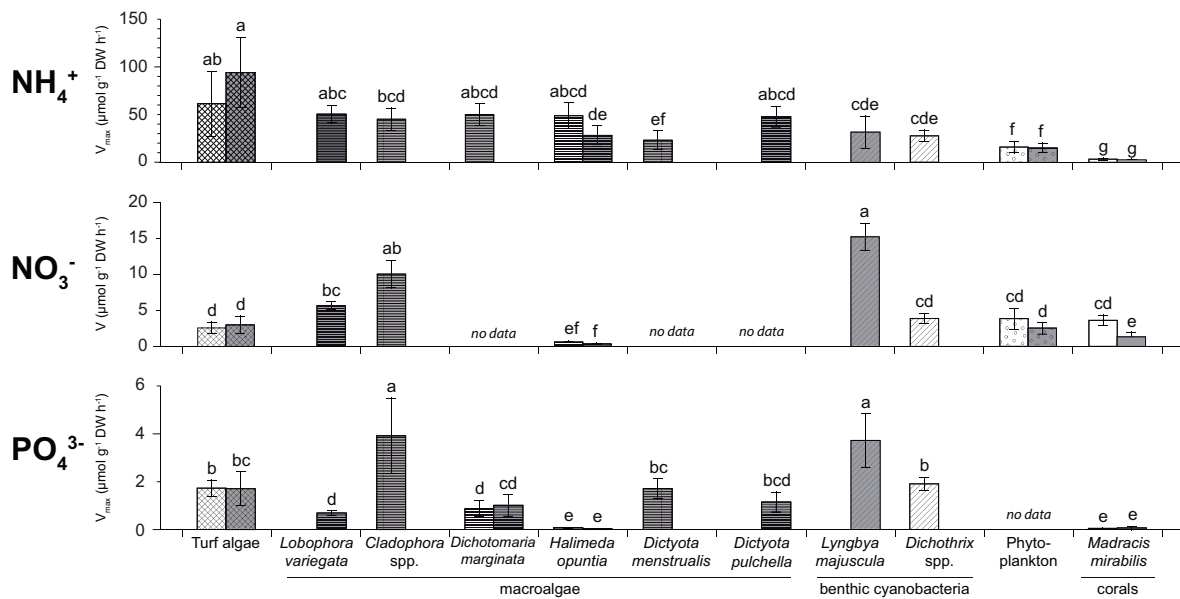
Fig. 6 V of benthic organisms

Comparison of the nutrient uptake rates (NH_4^+, NO_3^- and PO_4^{3-}) by benthic organisms (V). Included are only the results from the high initial nutrient addition (i.e. ~50 μM NH_4^+, ~25 μM NO_3^- and ~1.75 μM PO_4^{3-}). Patterns of the bars represent the five functional groups studied, including turf algae (checkered), macroalgae (horizontally dashed), benthic cyanobacteria (45° angled lines), phytoplankton (small circles) and corals (blank). Error bars represent s.d. of 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
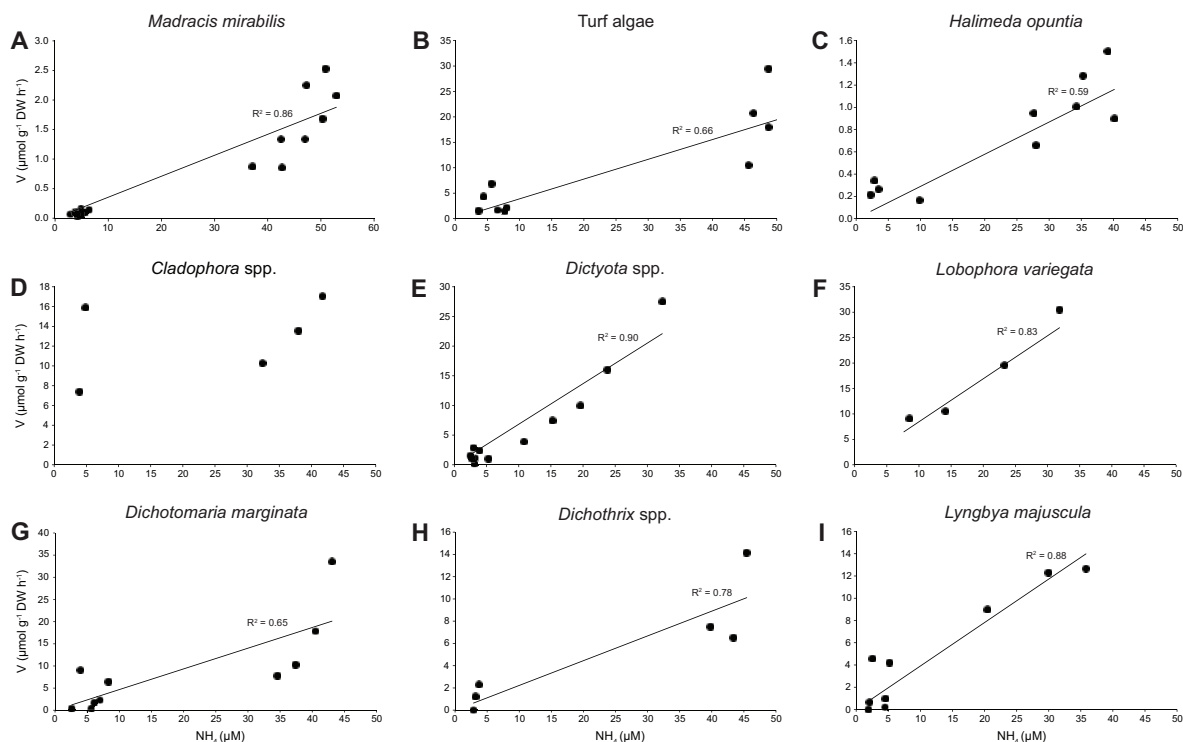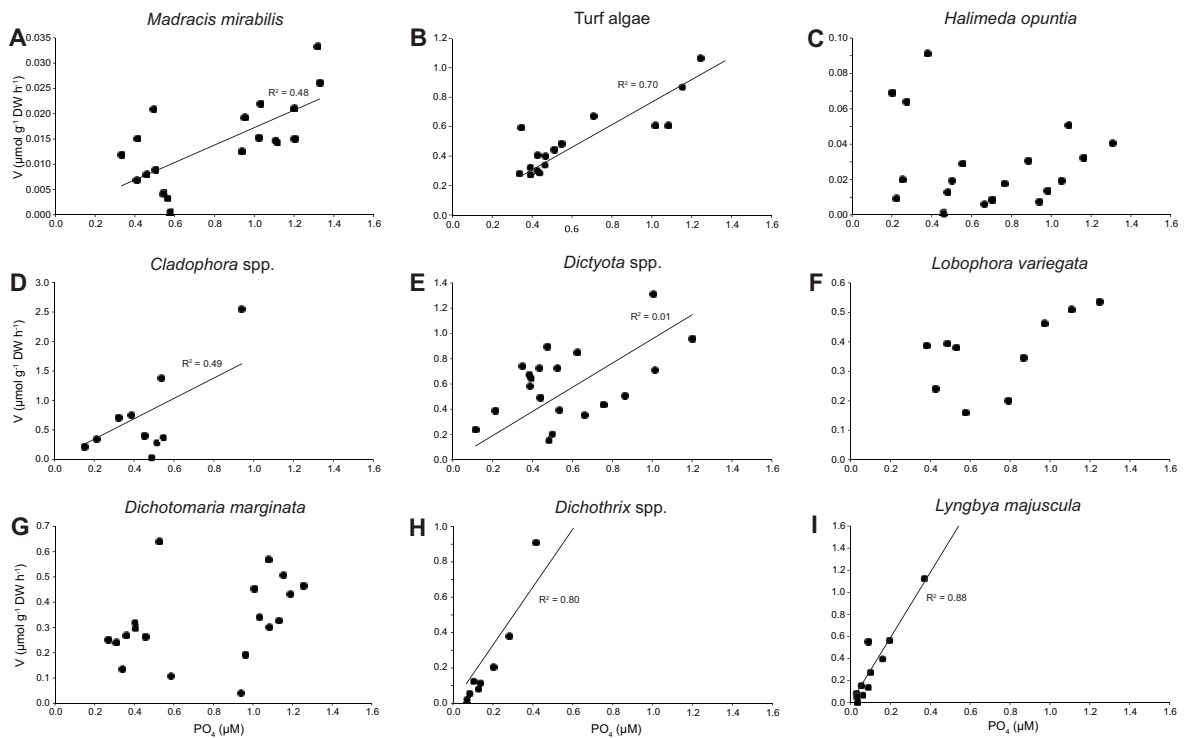
Fig. 7 External NH_4^+ concentration vs. NH_4^+ uptake rate

Relationship between the external NH_4^+ concentration (inside glass jars) and the uptake rate of all benthic organisms studied. Per benthic organism, data is included that was obtained from uptake experiments with both low and high initial NH_4^+ concentrations (~5 and ~50 μM) and if applicable, from both 5 and 20 m depth. Each data point in each graph is an average of three consecutive time points at which nutrient concentrations inside the glass jars were measured. Since the uptake of NH_4^+ proved to be biphasic for most benthic organisms and a linear NH_4^+ uptake pattern was only present 10 minutes after the initiation of the uptake experiment, the first data point (t_0) was not included. The only exceptions here were *M. mirabilis* and *D. marginata*, which did not have a biphasic NH_4^+ uptake rate, and therefore also data from t_0 was included in the graphs. If the linear regression proved to be significant ($p < 0.05$), solid regression lines are depicted

**Fig. 8** External PO_4^{3-} concentration vs. PO_4^{3-} uptake rate

Relationship between the external PO_4^{3-} concentration (inside glass jars) and the uptake rate of all benthic organisms studied. Per benthic organism, data is included that was obtained from uptake experiments with both low and high initial PO_4^{3-} concentrations (~0.88 and ~1.75 μM) and if applicable, from both 5 and 20 m depth. Each data point in each graph is an average of three consecutive time points at which nutrient concentrations inside the glass jars were measured. Since the uptake of PO_4^{3-} proved to be monophasic for most benthic organisms, the first data point (t_0) was also included. The only exceptions here were turf algae, *Dichothrix* spp. and *L. majuscula*, which did have a biphasic PO_4^{3-} uptake pattern, hence here t_0 was not included in the graphs. If the linear regression proved to be significant (p<0.05), solid regression lines are depicted.
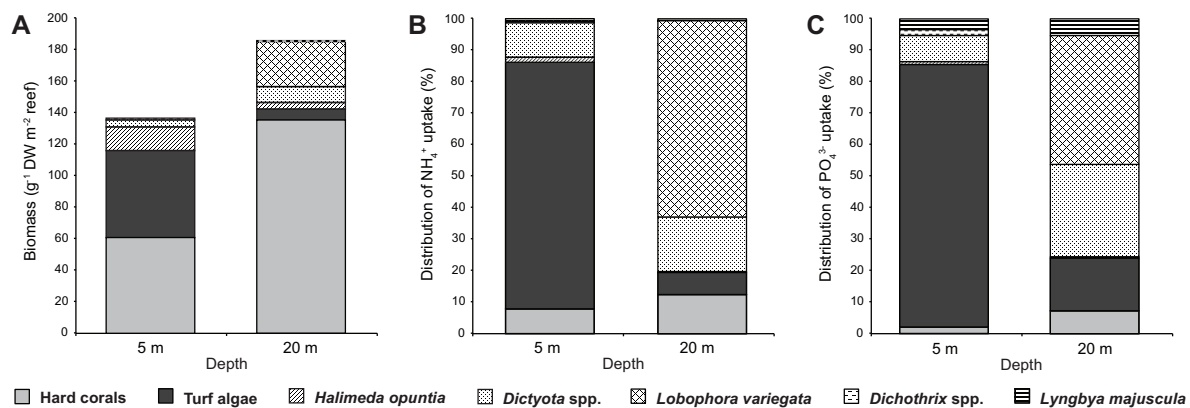
Fig. 9 Composition of the benthic community at Buoy 0 and distribution of nutrient plumes (A) Biomass of hard corals, turf algae, macroalgae and benthic cyanobacteria at Buoy 0, expressed as g^{-1} DW m^{-2} reef, (B,C) Distribution of the NH_4^+ and PO_4^{3-} plumes, respectively, among the benthic community.
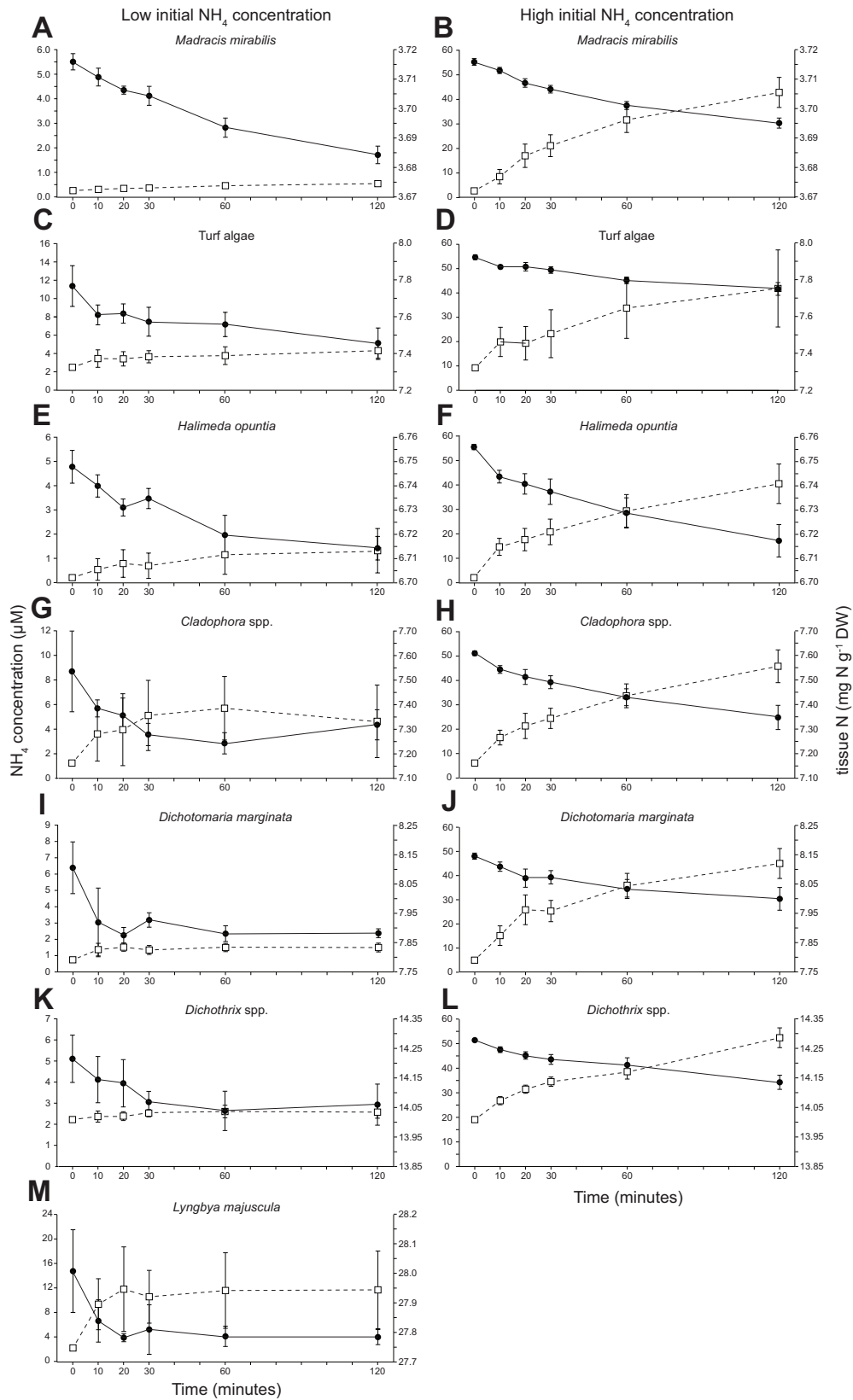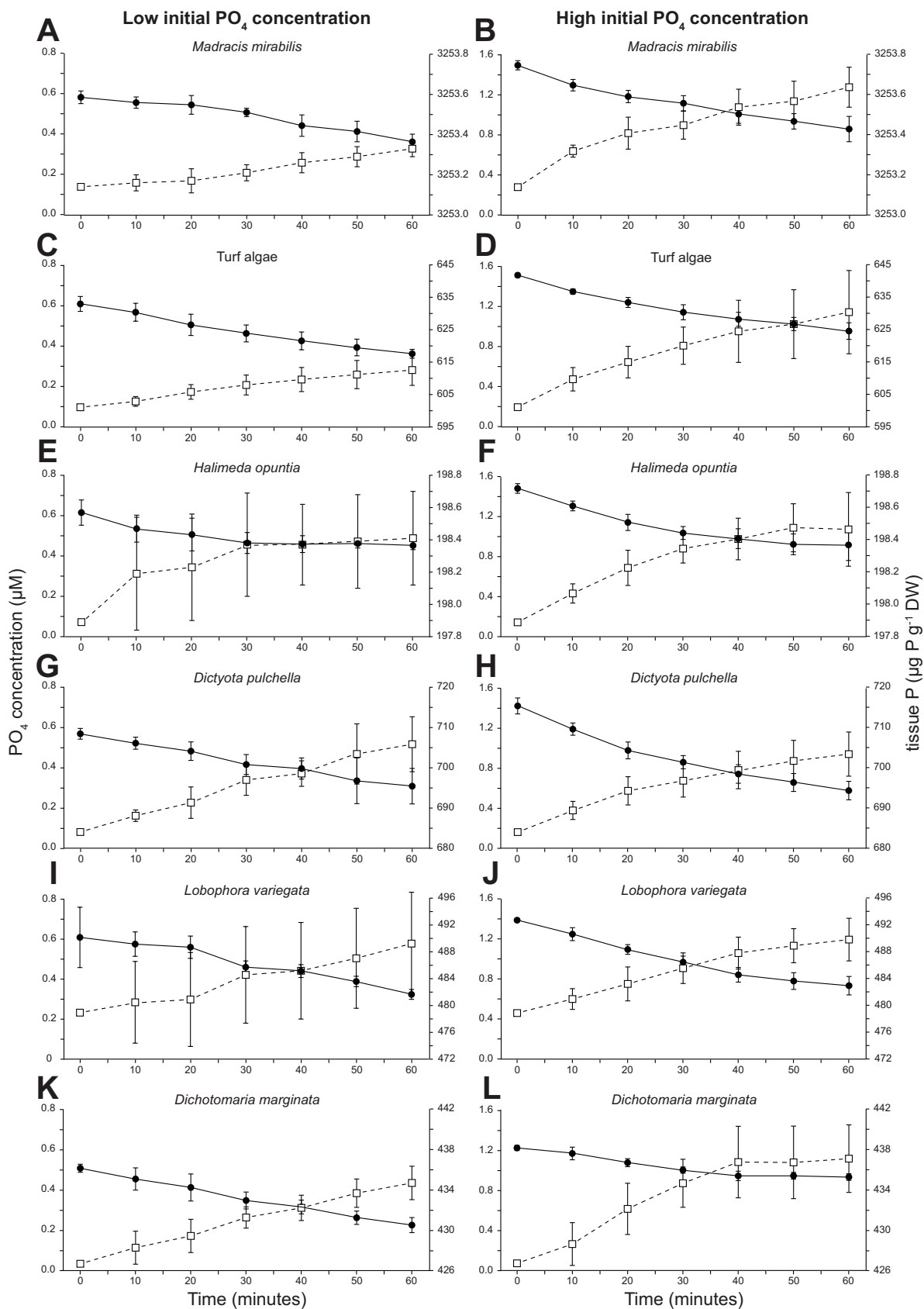
Supplement 1 Examples of NH_4^+ uptake by benthic organisms originating from 5 m depth.
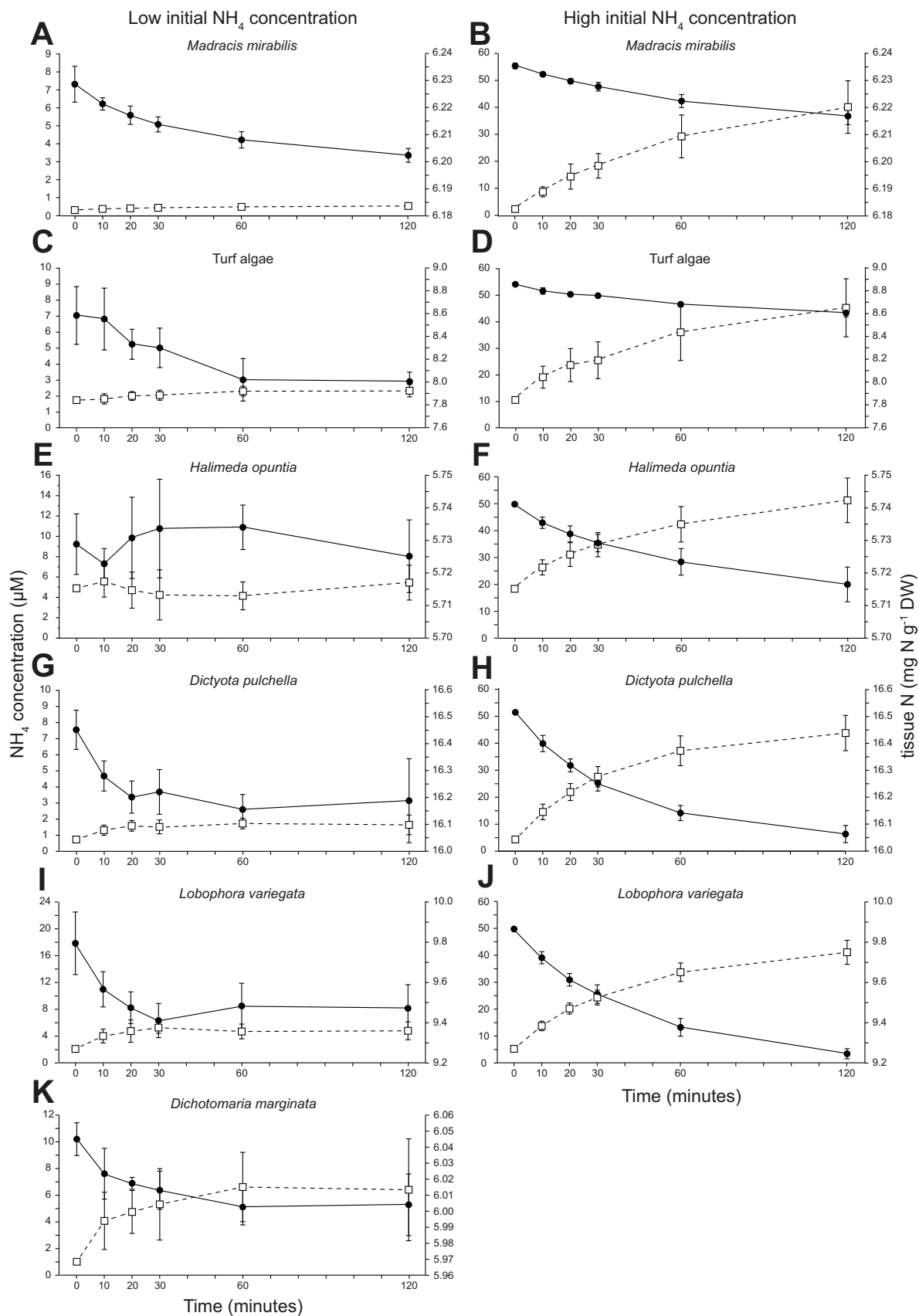
Supplement 2 Examples of NH$_4^+$ uptake by benthic organisms originating from 20 m depth.
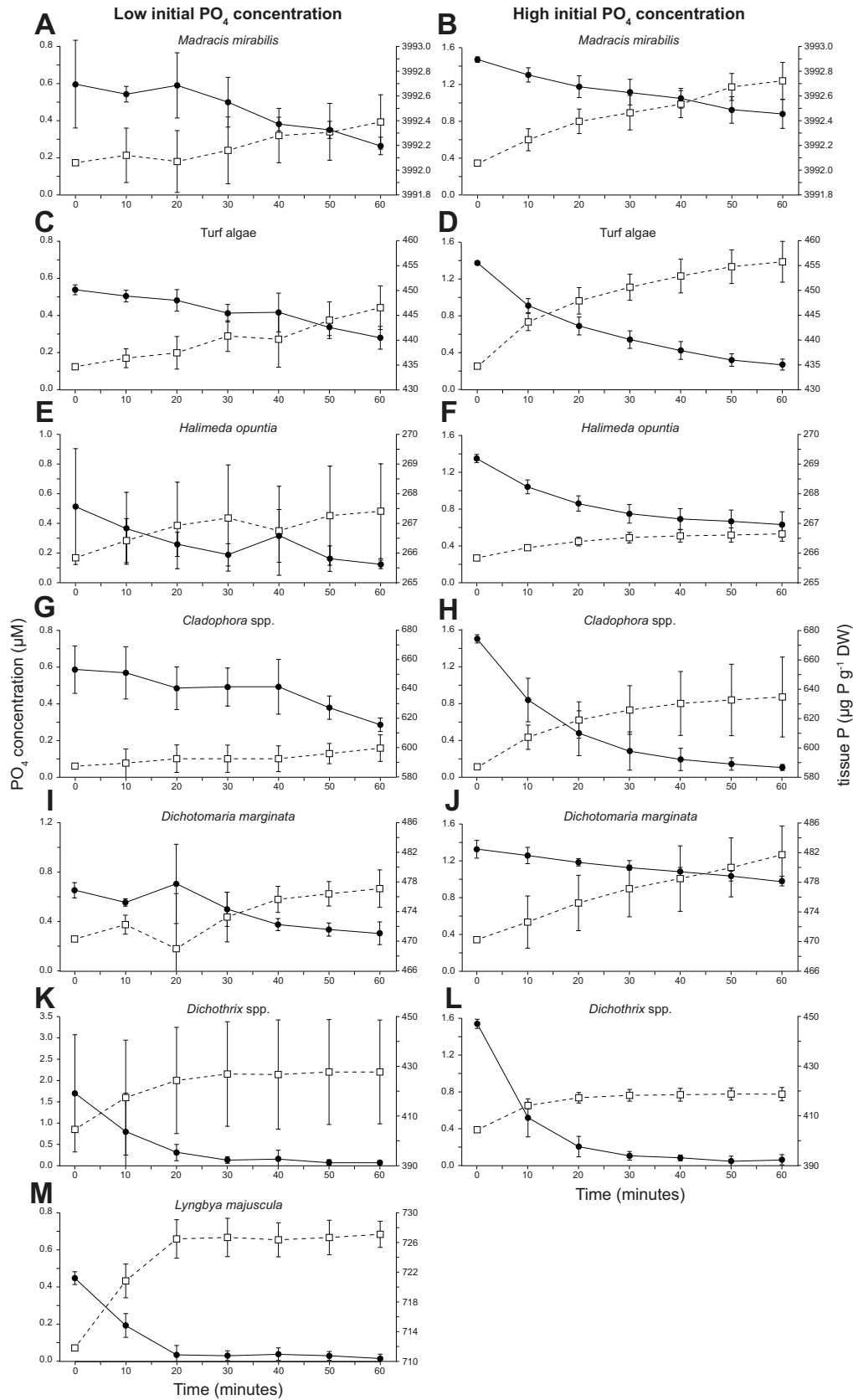
Supplement 3 Examples of PO$_4$$^{3-}$ uptake by benthic organisms originating from 5 m depth.

Low initial PO$_4$ concentration

High initial PO$_4$ concentration

Supplement 4 Examples of PO$_4^{3-}$ uptake by benthic organisms originating from 20 m depth.

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6. Manuscript V

MS V: Shifts in primary productivity during the transition from coral to algal dominance in a reef community

(In review)

Shifts in primary productivity during the transition from coral to algal dominance in a reef community

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KEY WORDS: coral reef, eutrophication, primary production, ^{13}C, turf algae, phase shift, crustose coralline algae

ABSTRACT

During recent decades, many coral reef ecosystems have shifted from coral to algal dominance. To what extent this shift in dominance pattern alters the primary productivity of reefs has received relatively little attention. In this study we assessed the contribution of different benthic primary producers and phytoplankton in the above water column to the total primary productivity of a degraded coral reef in the Caribbean using ^{13}C-labelling. This showed that primary productivity per unit biomass was highest for benthic cyanobacteria, followed by macroalgae and turf algae. Corals had the lowest primary productivity per unit biomass. To calculate primary productivity at the reef scale, we assessed the cover of the different benthic species at 5 and 20 m depth, converted this to biomass and subsequently calculated their productivity per m^2 of reef surface. Our results show that benthic productivity decreased with depth, but still exceeded pelagic productivity even at 20 m depth. Due to their high abundance, turf algae contributed most (more than 60%) to the total primary productivity of shallow reef communities. In deeper parts of the reef, hard corals, macroalgae and phytoplankton each contributed ~30% to the total primary productivity whereas the contribution of turf algae and benthic cyanobacteria was minor (<5%). In comparison to a similar study conducted at the same research site 40 years ago, the total primary productivity of the entire reef community has not increased but turf algae and macroalgae have replaced corals and crustose coralline algae as the main primary producers.

INTRODUCTION

Coral reefs occur in oligotrophic waters, but represent one of the most productive ecosystems in the world (Odum and Odum 1955; Hatcher 1990; De Goeij et al. 2013). A wide variety of different species including corals, sponges, macroalgae, turf algae, crustose coralline algae, benthic cyanobacteria and pelagic phytoplankton all contribute to the primary productivity of coral reef ecosystems. Because photosynthetic rates differ among these functional groups (e.g., Odum and Odum 1955; Jantzen et al. 2013; Naumann et al. 2013), changes in their relative abundances can affect the total productivity of the entire reef community. However, studies quantifying the contributions of different phototrophic species to the total productivity of coral reefs are limited. Furthermore, primary productivity estimates rarely focus on both the benthic

and pelagic community (e.g., Charpy 1996; Furnas et al. 2005; Lefebvre et al. 2012; Naumann et al. 2013). Interestingly, however, a few classic studies have quantified the primary productivity of coral reefs in great detail, at a time that corals still dominated reef communities (e.g., Odum and Odum 1955; Gordon 1971; Wanders 1976).

Many coral reefs have undergone major shifts in community composition in recent decades (McManus and Polsenberg 2004; Hughes et al. 2007; Cheal et al. 2010). Coral abundance has declined (Gardner et al. 2003; Bruno and Selig 2007; Rogers and Miller 2013), phytoplankton concentrations in the water column have increased due to enhanced nutrient availability (Furnas et al. 2005) and macroalgae have come to dominate many reef communities (Done 1992; Cheal et al. 2010; Hughes et al. 2010). In addition, turf algae and benthic cyanobacteria have also increased in abundance in many coral reef communities (Paul et al. 2005; Barott et al. 2009; Haas et al. 2010; Vermeij et al. 2010; Wangpraseurt et al. 2012). Therefore, primary productivity assessments made in the past provide useful historic reference points, but are likely no longer representative of present-day reef communities due to the changed abundances of the dominant primary producers.

In this study, we aimed to determine the contribution of different benthic and pelagic primary producers to the total primary productivity of a once coral dominated, but now degraded Caribbean coral reef. To quantify the relative contribution of each functional group to the primary productivity of the entire community, we (1) determined the cover of the most abundant benthic primary producers at 5 and 20 m depth, (2) determined phytoplankton concentrations in the overlying water column, (3) measured the photosynthetic light-response of different phototrophic species, and (4) estimated their primary productivity based on the incorporation of ^{13}C-labelled bicarbonate. The primary productivity of the different functional groups was also investigated at our study site about 40 years ago (Wanders 1976), before this reef shifted from a coral-dominated to an algal-dominated ecosystem, which offered a unique opportunity to compare our findings to a historic baseline.

MATERIALS AND METHODS

Research site

This study was conducted during the spring (March–May) of 2012 and 2013, on a degraded coral reef at research site 'Buoy 0' on the leeward side of the island of Curaçao, Southern Caribbean (12°7'29.07"N, 68°58'22.92"W; Figure 1A). Buoy 0 is near the island's capital (Willemstad) with its industrial harbor and situated just at the outlet of the eutrophied Piscadera Bay.

Community composition

Percent cover of dominant benthic taxa was determined from photographs of 60 quadrats (1.5 m^2), randomly placed along both sides of a 100 m transect line laid along the 5 and 20 m isobath. Photographs were analyzed using the program Coral Point Count with Excel Extensions (CPCe) (Kohler and Gill 2006).

We determined the areal density of each benthic taxon as the dry weight per m^2 cover of this species. The areal density of the coral *Madracis mirabilis* was obtained from Hardt (2007). For macroalgae, we photographed 25 small quadrats of 0.25 m^2 placed on patches of *Halimeda opuntia*, *Dictyota* spp. and *Lobophora variegata*. The photographs were analyzed for total algal surface area using the program ImageJ (Abràmoff et al. 2004). Subsequently, the macroalgae were collected from the quadrats, manually cleaned from epiphytes and detritus, and dried at 60ºC for at least 3 days to determine their dry weight. The areal density of turf algae was obtained from 35 strips cut from plastic bottles incubated at 5 and 20 m depth for six weeks (see below). Each strip was photographed and the surface area covered by turf algae was measured. Turf algae were scraped off the plastic strips after freeze-drying in a Scanvac CoolSafe Freeze-dryer (Scala Scientific B.V., Ede, The Netherlands) to determine their dry weight. We multiplied the dry weight of turf algae with a factor 1.5 to correct for the fact that the actual reef surface is topographically more complex than the plastic strips from which we sampled turf algae (Jantzen et al. 2013).

Cyanobacterial mats and attached sediment were collected at both 5 and 20 m depth, from 10 small quadrats of 0.01 or 0.04 m^2 with 100% cyanobacterial cover using a 50 ml Terumo syringe (Terumo Europe, Leuven, Belgium). The cyanobacteria were freeze-dried using a Scanvac CoolSafe Freeze-dryer, and combusted at 450°C for 4 h using an Air Recirculating Chamber

Furnace (Carbolite, Hope Valley, UK). Their areal density was determined from the weight loss of the combusted samples.

To quantify phytoplankton abundance, water samples from 5 and 20 m depth were filtered through Whatman GF/F filters for chlorophyll extraction with 80% acetone. Chlorophyll *a* was measured spectrophotometrically (T60 Visual Spectrophotometer, PG Instruments Ltd, Wibtoft, UK) at 647 and 664 nm according to Porra et al. (1989).

Light conditions

Light profiles of the water column (0-20 m depth) were measured on 6 different days in January-April with a Hydrolab DS5 Sonde (OTT Messtechnik GmbH & Co., Kempten, Germany). Light attenuation coefficients were calculated as the slope of the linear regression of ln-transformed light intensities versus depth.

Photosynthesis-irradiance curves

We measured photosynthesis-irradiance relationships of the dominant taxa *in situ* using a Diving-PAM/B Underwater Fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany). Rapid Light Curves (RLCs) (Ralph and Gademann 2005) were measured at both 5 and 20 m depth (n=5 per depth) between 08:45 and 09:45 AM to minimize interference from day-dependent light acclimation (Serôdio et al. 2005). We used pre-determined positions on the tuft, thallus or stalk of the benthic organisms that were larger than the PAM sensor itself to ensure RLCs were conducted consistently.

Phytoplankton concentrations in the water column were too low to measure RLCs *in situ*. Therefore, the RLCs of phytoplankton were determined from GF/F filters loaded with phytoplankton collected at both 5 and 20 m depth (n=10 per depth).

Prior to each RLC measurement, samples were dark adapted for at least 1 min to relax photochemical quenching (Iglesias-Prieto et al. 2004). After dark adaptation, the actinic light intensity of the PAM fluorometer was increased by eight incremental steps of 10 seconds each, from 4 to 974 μmol photons m^{-2} s^{-1}. Each 10-second intensity was followed by a saturating light pulse to estimate the quantum yield of photosystem II (Φ_{PSII}) according to Genty et al. (1989). The quantum yield is a measure of photosynthetic efficiency, and expresses the fraction of absorbed photons utilized for photosynthetic electron transport (e.g., Maxwell and Johnson

2000). The relative electron transport rate (rETR) was obtained by multiplying the quantum yield with irradiance (Ralph and Gademann 2005). The rETR is closely related to photosynthetic activity, as it reveals the overall shape of the photosynthesis-irradiance relationship but not its absolute value.

A saturating photosynthesis-irradiance model was fitted to our rETR data according to Platt et al. (1980):

$$rETR = rETR_{max} \times \left(1 - e^{-\frac{\alpha I}{rETR_{max}}}\right) \tag{1}$$

where $rETR_{max}$ is the maximum electron transport rate at saturating light and α is the initial slope of the RLC at low light conditions. The saturation irradiance (E_k) represents the intersection between a and $rETR_{max}$, and was calculated as $E_k = ETR_{max} / a$.

Field incubations of benthic organisms

Collection of benthic organisms To determine the primary productivity based on ^{13}C incubations, benthic organisms were collected at 5 and 20 m depth. We selected branches (~5 cm length) of the most abundant coral species (*M. mirabilis*), leaves (~10 cm^2 per sample) of six macroalgal species (*Cladophora* spp., *Dichotomaria marginata*, *Dictyota menstrualis*, *Dictyota pulchella*, *H. opuntia*, *L. variegata*), and tufts (~10 cm^2 per species) of two benthic cyanobacteria (*Dichothrix* spp., *Lyngbya majuscula*). We also collected turf algae but scraping them off the rocks resulted in damage to the algal tissues. To prevent this problem, turf algae were grown on the exterior of 1.5 L square plastic bottles (FIJI Water Company, CA, USA). The bottles were placed 0.5 m above the reef to avoid overgrowth by benthic cyanobacterial mats and macroalgae, and were placed inside cages (1x1x1m) made of chicken-wire (mesh Ø2.5 cm) to prevent grazing by large herbivores. Leaving the bottles for 6 weeks under these conditions ensured the establishment of turf algal communities comprising all major taxa including Chlorophyta, Rhodophyta, Phaeophyceae and Cyanobacteria (Fricke et al. 2011). Subsequently, turf algae were collected by cutting out plastic strips (~6 cm^2).

Immediately after collection, all sampled organisms were cleaned from loosely attached epiphytes and detritus, and put into darkened plastic Ziploc bags that were placed in a cool box filled with ambient seawater (27-29 °C) for transport to the lab within 15 min. In the laboratory, remaining epiphytes and detritus were removed using forceps, after which each species was incubated in a

separate acid-washed (10% HCl) glass jar (0.175 L) that was completely filled with filtered seawater (0.22 μm pore size Whatman cellulose acetate membrane filters). The jars were then placed inside a flow-through aquarium for one night to allow sampled organisms to recover from collection and cleaning (water from the aquarium could not mix with water inside the jars). Samples were kept at 27-29°C and under shaded conditions (~100 μmol photons m^{-2} s^{-1} as measured with a Hydrolab DS5 Sonde).

Field incubations The following day, the samples were transferred to new acid-washed glass jars containing freshly filtered seawater (similar as above). To determine the primary productivity of each sample, the stable isotope ^{13}C was added by dissolving $NaH^{13}CO_3$ (98% Sigma Aldrich, Zwijndrecht, The Netherlands) at a final concentration of 180 μM ^{13}C. The amount of stable isotope corresponded to ~10% of the total dissolved inorganic carbon (DIC) in the reef's waters, which is a recommended value for primary productivity measurements (Mateo et al. 2001; Mulholland and Capone 2001; Hashimoto et al. 2005). Controls consisted of samples without added ^{13}C, so that the natural abundance of ^{13}C in all sampled groups could be determined.

At Buoy 0, all glass jars containing benthic organisms originating from 5 m depth (*M. mirabilis*, *Cladophora* spp., *D. marginata*, *D. menstrualis*, *H. opuntia*, *Dichothrix* spp., *L. majuscula*, turf algae) were tied down horizontally onto a PVC-frame platform (1.5 x 0.8 m) deployed at 5 m depth. A second platform was deployed at 20 m depth for benthic organisms collected from 20 m depth (*M. mirabilis*, *D. marginata*, *D. pulchella*, *H. opuntia*, *L. variegata*, *L. majuscula*, turf algae). Each platform hovered horizontally at ~1 m above the reef, connected to cement blocks on the sediment and a buoy at the surface. This construction ensured gentle movement of three acid-washed glass marbles (Ø10 mm) that had been added to the jars to enhance mixing and minimize nutrient-depleted boundary layers around the incubated organisms. The small size of the glass marbles ensured that the organisms were not damaged during the field incubations. The light intensity in the glass jars was ~98% of the ambient light intensity (measured with a LI-250 light meter; LI-COR, Lincoln, Nebraska, USA), and the light spectrum remained unchanged (measured with a miniature fiber optic spectrometer USB4000; Ocean Optics, Dunedin, Florida, USA).

The glass jars were left on the platform from 11:00 AM to 2:00 PM, and a Hydrolab DS5 Sonde was placed directly next to the platform to measure light intensity (PAR, 400-700 nm) at 30-second intervals during this 3-hour incubation. At 2:00 PM, all glass jars were collected, placed

inside a black plastic bag, and returned to the laboratory. The field incubations were repeated on six different days within a two-months period, resulting in six replicates (n=6) per phototrophic group at both depths.

Post-incubation In the laboratory, each sample was rinsed with fresh water to remove salts and stored in pre-weighed aluminum foil at -20°C for at least two days. Samples were subsequently freeze-dried overnight using a Scanvac CoolSafe Freeze-dryer to determine their dry weight and ^{13}C content. Turf algae were collected by scraping them off the plastic strips after freeze-drying. The coral *M. mirabilis* was processed differently. Live tissue of *M. mirabilis* was removed from its skeleton using a toothbrush and suspended in a 15 ml tube containing filtered seawater (Whatman GF/F). This suspension was centrifuged twice at 4000 rpm for 20 min in an EBA 21 Centrifuge (Hettich Laborapparate, Bäch, Germany), so that the zooxanthellae, but not the coral tissue, concentrated at the bottom of each tube. The zooxanthellae were pipetted out of the tube and filtered onto a pre-weighed Whatman GF/F filter that was pre-combusted at 450°C for 4 hrs using an Air Recirculating Chamber Furnace (Carbolite, Hope Valley, UK). The loaded filters were dried at 60°C to determine dry the weight and ^{13}C content of the zooxanthellae.

Field incubations of phytoplankton

Collection of phytoplankton Phytoplankton was collected directly above the reef slope at Buoy 0. At both 5 and 20 m depth, two custom-made 5.3 L Plexiglas incubation tubes (Ø 10 cm; Röhm GmbH & Co KG, Darmstadt, Germany) were filled with seawater. The incubation tubes were darkened with a towel and transported to the laboratory where they were filtered within one hour after collection through a double-mesh filter (50 and 150 μm) to remove zooplankton and other large debris. The filtered seawater containing phytoplankton was again put in 5.3 L Plexiglas incubation tubes and returned to Buoy 0 to start the incubation.

Field incubations Prior to each incubation, one of the tubes collected at each depth was enriched (10%) with ^{13}C-labelled bicarbonate following the procedures mentioned above, whereas the other tube served as control. A vertical rope was spanned from the sediment to the water surface, and the two tubes with water collected at 5 m depth were attached to the rope at 5 m depth while the other two tubes were attached at 20 m depth. Light transmission through the Plexiglas tubes was ~92% and the light spectrum was again unaffected. A Hydrolab DS5 Sonde was attached to the rope at 5 m depth to monitor light intensity at 30-second intervals. The field incubations were

conducted from 11:00 to 14:00 hrs, and repeated on five different days over a two-week period to obtain five independent replicates.

Post-incubation After three hours, the incubation tubes were taken off the rope, darkened using a towel and returned to the laboratory within 15 min. In the laboratory, the contents of each incubation tube were filtered over a pre-combusted, pre-weighed Whatman GF/F filter placed in a Ø25 mm Polycarbonate filter holder (Cole Palmer, Chicago, IL, USA). The loaded filters were then stored in pre-weighed aluminum foil at -20°C for two days and freeze-dried in a Scanvac CoolSafe Freeze-dryer, so that their dry weight could be determined.

Analysis of ^{13}C content

To determine their ^{13}C content, the freeze-dried samples were grinded to powder using mortar and pestle. The powder was packed in tin capsules that were folded into small pellets. For filtered samples (*M. mirabilis* zooxanthellae and phytoplankton), small pieces (Ø 7 mm) of the loaded filters were used. The ^{13}C content of the benthic samples was analyzed using an Isoprime 100 Isotope Ratio Mass Spectrometer (IRMS) (Manchester, United Kingdom) connected to a VarioIsotope Cube Element Analyzer (Hanau, Germany). The ^{13}C content of phytoplankton samples was analyzed using a Thermofinnigan Delta Plus IRMS (Bremen, Germany) connected to a Carlo Erba Instruments Flash 1112 Element Analyzer (Milan, Italy). All samples were measured in duplicate and ^{13}C abundances were reported as atom%. Isotopic data were calibrated using a two-point calibration curve and linear regression (Paul et al. 2007; Skrzypek 2013) with IAEA-CH6 sucrose (δ^{13}C = -10.449±0.033) and IAEA-309B, UL-glucose (δ^{13}C = 535.3±4.75) as reference standards.

The primary productivity (P, in mg C g^{-1} DW h^{-1}) was calculated according to Hama et al. (1983):

$$P = \frac{C}{t} \times f \times \left(\frac{at_e - at_c}{at_w - at_c} \right) \qquad (2)$$

where *C* is the total organic carbon content of the sample (in mg C g^{-1} DW), *t* is the duration of the ^{13}C incubation (three hours), *f* is the fractionation factor correcting for the preference of the Rubisco enzyme for ^{12}C instead of ^{13}C (*f*=1.025; Hama et al. 1983), at_e is the atom% of ^{13}C in the ^{13}C-enriched sample, at_c is the atom% of ^{13}C in the control sample, and at_w is the sum of the atom% of ^{13}C present naturally in seawater of Curaçao (1.11%) and ^{13}C added as a tracer.

Primary productivity per unit surface area

To estimate primary productivity per unit surface area, we projected an area of 1 m^2 extending from the water surface to the benthic reef community at 5 and 20 m depth (Figure 1B), where the footprint of this column was 1.25 m^2 due to the sloping reef bottom (53°). For each benthic species, primary productivity per m^2 was calculated from the product of primary productivity per unit dry weight based on ^{13}C incorporation, areal density (dry weight per m^2 of cover) and percent cover of that species. For phytoplankton, primary productivity per unit surface area was calculated from the product of their primary productivity per m^3 based on ^{13}C incorporation and the depth of the water column. For the 5-m water column we used the phytoplankton productivity measured at 5 m depth; for the 20-m water column we averaged their productivity at 5 m and 20 m depth.

Statistics

For each species, we used the two-sample Student's *t*-test to determine whether their photosynthetic parameters and primary productivity estimates differed between 5 and 20 m depth. The data were log-transformed if this improved homogeneity of variance, as tested by Levene's test. In the few cases without homogeneity of variance, we applied the two-sample Student's *t*-test for unequal variances (also known as Welch's *t*-test).

RESULTS

Reef community composition

The benthic composition of research site Buoy 0 differed between 5 and 20 m depth (Figure 2A). The cover of turf algae (43.5±3.3% (SE)) and sand (28.3±2.7%) dominated the reef at 5 m depth, whereas the macroalgae *L. variegata* (28.5±1.9%) and *Dictyota* spp. (14.0±1.0%) dominated the reef at 20 m depth. Hard coral cover was low at 5 m depth (7.4±1.7%), but higher at 20 m depth (16.5±1.5%). Corals consisted mainly of *M. mirabilis* and *Diploria* spp. at 5 m depth, *Agaricia* spp. were more abundant at 20 m depth, while *Montastraea* spp., *Porites* spp. and *Colpophyllia natans* were found at both depths. Crustose coralline algae were rare at both 5 m depth (0.5±0.2%) and 20 m depth (0.9±0.3%).

Total biomass per m^2 reef area of each benthic species was calculated using the areal densities reported in Table 1. Mean total biomass of hard corals, turf algae, macroalgae and benthic

cyanobacteria per m^2 reef area was lower at 5 m depth (137 g DW m^{-2}) than at 20 m depth (185 g DW m^{-2}), mainly due to the lower abundance of corals at 5 m depth (Figure 2B). At 20 m corals dominated total reef biomass, while at 5 m corals and turf algae contributed similarly. The chlorophyll *a* concentration (±SD) of ambient seawater was 0.11±0.01 µg l^{-1} (n=4).

Light conditions

Vertical light profiles revealed that the light intensity at each depth was about two times higher during sunny than during cloudy days (Figure 3). Water clarity showed little variation throughout the study, with light attenuation coefficients (±SD) of K_d=0.098±0.017 m^{-1} (n=6) during both sunny and cloudy days. The average (± SD) light intensity that the phototrophic species experienced during our field incubation was three times higher at 5 m depth (641 ± 341 µmol photons m^{-2} s^{-1}) than at 20 m depth (203 ± 158 µmol photons m^{-2} s^{-1}).

Photosynthesis-irradiance curves

The RLCs revealed substantial variation in photosynthetic traits among species (Table 2; Figure 4 and Electronic Supplementary Material (ESM)). Turf algae were best adapted to low light intensities as their saturation irradiance (E_k) was lowest for all species (53 µmol photons m^{-2} s^{-1} at 20 m depth). Macroalgae and the cyanobacterium *L. majuscula* had intermediate saturation irradiances of 100-300 µmol photons m^{-2} s^{-1}. High saturation irradiances of 414, 657 and 834 µmol photons m^{-2} s^{-1} were found at 5 m depth in the cyanobacterium *Dichothrix* spp., the coral *M. mirabilis* and the phytoplankton community, respectively.

For all 7 species that we had sampled at both depths, the saturation irradiance (E_k) was lower at 20 m than at 5 m depth, although this difference was significant at *P*<0.05 only for *M. mirabilis*, *H. opuntia*, *Dictyota* spp. and *D. marginata* (Student's *t*-test). Similarly, all species, except *L. majuscula*, had a lower maximum electron transport rate ($rETR_{max}$) at 20 m than at 5 m (Table 2), and this difference was again significant at *P*<0.05 for *M. mirabilis*, *H. opuntia*, *Dictyota* spp. and *D. marginata* (Student's *t*-test). This depth-related variation in the photosynthetic parameters indicates that the species were acclimated to the local light conditions at 5 and 20 m depth (Table 2; Figure 4). In contrast, the initial slope of the RLCs (α) was similar at 5 and 20 m depth for most species. However, the coral *M. mirabilis* had a significantly higher α at 20 m depth (Student's t-test, *P*<0.05) indicating that it was more efficient in capturing low light intensities at 20 m than at 5 m depth.

Primary productivity

Primary productivity rates measured by ^{13}C incubation varied among the different species (Figure 5). Primary productivity per unit biomass was lowest for the coral *M. mirabilis* and 20 times higher for the cyanobacterium *L. majuscula*, which had the highest primary productivity per unit biomass of all species. For most species, the primary productivity per unit biomass tended to be higher at 5 m than at 20 m depth (Figure 5). Yet, for many species this pattern was not statistically significant, presumably because the light conditions at 20 m depth were still sufficient to sustain relatively high photosynthetic rates (cf. Figures 3 and 4). The decrease of primary productivity with depth was significant only for turf algae, whose primary productivity was two times lower at 20 m than at 5 m depth (Student's *t*-test with unequal variances: $t=6.626$, df=5.44, $P<0.001$). On a volumetric basis, primary productivity ($\pm$SD) of phytoplankton was 2.2 ± 0.9 µg C l^{-1} h^{-1} at 5 m depth and 2.0 ± 0.4 µg C l^{-1} h^{-1} at 20 m depth.

We combined the primary productivity rates of the different species with their biomass on the reefs (Figure 2B) to calculate the depth-integrated primary productivity per unit surface area (Figure 6). Pelagic primary productivity of the phytoplankton above the deep reef was much higher than above the shallow reef (41 vs. 12 mg C m^{-2} h^{-1}, respectively), essentially because pelagic productivity was integrated over a larger depth range. Conversely, benthic primary productivity of the deep reef was substantially lower than of the shallow reef (103 vs. 172 mg C m^{-2} h^{-1}, respectively; Figure 6). The total depth-integrated primary productivity of the benthic and pelagic combined was higher at the shallow reef than at the deep reef (184 vs. 144 mg C m^{-2} h^{-1}, respectively).

At the shallow reef, turf algae were the major primary producers accounting for 63.5% of the total productivity, whereas hard corals, macroalgae and benthic cyanobacteria contributed only 14.9%, 10.5% and 4.8%, respectively. At the deep reef, hard corals, macroalgae and phytoplankton each contributed an equal share of about 30% to the total productivity, whereas the contribution of turf algae (4.9%) and benthic cyanobacteria (2.2%) was only minor (Figure 6).

DISCUSSION

Changes in community structure

The reef community at our research site was investigated in the 1970s, when Wanders (1976, p. 238-239) wrote enthusiastically:

"along the south-west coast a luxuriant coral reef system composed of mainly scleractinian corals, hydrocorals and gorgonians covers the submarine plateau. Crustose coralline algae are conspicuous, but other algae constitute only an unobtrusive vegetation … corals, including fragile species such as Madracis asperula *Milne-Edwards & Haime, form extensive aggregates ... Gorgonians are characteristic for the depth zone of 3-7 m … The shallowest part … is covered by a solid reef dominated by* Acropora palmata *Lamarck (elkhorn coral),* Millepora *spec. (fire coral) and the encrusting coralline alga* Porolithon pachydermum *(Fosl.) Fosl."*

During the past 40 years, the reef community composition at Buoy 0 has changed completely. According to Wanders (1976, Table V), hard corals comprised 38% of the total benthic cover at 5-10 m depth. Hard corals now cover less than 10% at 5 m depth, gorgonians and *Millepora* spp. less than 1%, and elkhorn coral has almost completely disappeared from Buoy 0 and its surroundings. The decline of crustose coralline algae (CCA) is particularly dramatic. CCA comprised almost 40% of the benthic cover in the 1970s, but nowadays they cover less than 1% of the surface. Corals and CCA have largely been replaced by turf algae and macroalgae, which have increased from an "unobtrusive vegetation" comprising only ~15% of the total benthic cover 40 years ago to their present-day dominance with more than 50% cover for turf algae and macroalgae combined.

In deeper parts of the reef, at 20 m depth, hard corals still persist with a cover of 17%, although also at this depth the coral cover at our research site was much higher in the 1970s (40%; Bak et al. 2005). The reef community at 20 m depth is now dominated by *L. variegata*, a macroalga that has increased in percent cover from only 1% in 1998 to 18% in 2006 (Nugues and Bak 2008) and to 28.5% in the present study.

Implications for primary productivity

Our results show that the shift from a coral-dominated to an algal-dominated community is reflected in a redistribution of the primary productivity among the different species. Wanders (1976, Table X) estimated primary productivity of the dominant functional groups based on net

oxygen production of field samples incubated at 0.5 - 3 m depth. At the time, corals contributed ~65% of the total primary productivity, while CCA and other algae contributed 16% and 19%, respectively. In our study, 40 years later, the roles are completely reversed: 68% of the total primary productivity at 5 m depth is now due to turf algae, whereas corals contribute only 16%.

Interestingly, the total benthic primary productivity measured in our study was lower than the total benthic primary productivity measured by Wanders (1976). We found a benthic primary productivity of 172 mg C m^{-2} h^{-1} at 5 m depth based on ^{13}C incorporation. Wanders (1976) estimated a benthic primary productivity of 265 mg C m^{-2} h^{-1} at 0.5–3 m depth based on O$_2$ production, where we assume that one molecule of CO$_2$ is fixed for each molecule of O$_2$ released. Wanders' estimate compares well with the productivity range of other reef ecosystems described in the older literature (e.g., Sargent and Austin, 1949, 1954; Odum and Odum 1955, Kohn and Helfrich 1957). Mateo et al. (2001) have shown that results from ^{13}C incorporation and O$_2$ production can be directly compared when the incubation period is shorter than 4 hours. A possible explanation for the higher primary productivity in Wanders (1976) might be that he investigated shallower parts of the reef with a higher light availability. Furthermore, nowadays, almost 30% of the surface area of the reef at 5 m depth is not occupied by living organisms but covered by sand. If we would consider only the vegetated area, the total benthic primary productivity estimated by our study is 240 mg C m^{-2} h^{-1}, which is remarkably close to Wanders' estimate of the 1970s.

Although Wanders (1976) did not estimate the primary productivity of phytoplankton above the reef, our estimates of phytoplankton productivity (ca. 2 µg C l^{-1} h^{-1}) are comparable to values reported by other studies, e.g. in the Great Barrier Reef (Furnas et al. 1990) and at the fringing reef of Miyako Island, Japan (Ferrier-Pagès and Gattuso 1998). When expressed per unit of projected surface area, benthic productivity greatly exceeded pelagic productivity at the shallow reef, where phytoplankton contributed only 6.4% to the total primary productivity. However, benthic productivity decreased with depth, whereas phytoplankton productivity per unit surface area increased when it was integrated over a deeper water column. As a consequence, phytoplankton provided a much larger contribution (almost 30%) to the total primary productivity of the reef at 20 m depth.

The proliferation of turf algae

In the last decades, many Caribbean coral reefs experienced a major loss of live coral cover (Gardner et al. 2003; Burke et al. 2011). The degradation of coral reef communities is often associated with increasing abundances of macroalgae (Done 1992; McManus and Polsenberg 2004; Hughes et al. 2007). At our study site, however, turf algae have become the single-most dominant benthic component of shallow reefs. This strong proliferation of turf algae has also been observed on many other shallow reefs in the Caribbean (Kramer 2003; Vermeij et al. 2010), and also on degraded reefs in, e.g., the Red Sea (Haas et al. 2010), Central Pacific (Barott et al. 2009; Barott et al. 2012) and Indonesia (Wangpraseurt et al. 2012).

The prevalence of turf algae in shallow waters is in agreement with previous studies (Van den Hoek et al. 1975; Adey and Goertemiller 1987; Steneck and Dethier 1994), and confirmed by a much larger biomass development of turf algae on plastic bottles placed within the upper 10 m of the water column than on deeper bottles (Den Haan et al. 2014). These bottles were protected from grazing by herbivores larger than 2.5 cm in diameter, and therefore the prevalence of turf algae in shallow waters cannot be attributed to depth-dependent variation in grazing by, e.g., fish. Several studies have pointed out that turf algae have relatively high light requirements (e.g., Carpenter 1985; Klumpp et al. 1987). This is contradicted by the RLCs, which indicated that the turf algae in our study had the lowest saturation irradiance (E_k) of all functional groups (Figure 4C,D). However, the RLCs do show a strong reduction in maximum photosynthetic activity ($rETR_{max}$) of turf algae grown at greater depths. This matches our ^{13}C measurements, which showed a relatively large reduction in the primary productivity of turf algae at greater depth in comparison to other species. In fact, turf algae were the only functional group that showed a significantly higher primary productivity at 5 m than at 20 m depth (Figure 5), which may offer a plausible explanation for their prevalence at shallow reefs.

Possible causes for the shift to algal dominance

A phase shift from coral to algal dominance is often associated with coastal eutrophication (Smith et al. 1981; Fabricius et al. 2005, Bell et al. 2014). In view of the proximity of our study site to an expanding city with extensive coastal development and one of the largest industrial harbors in the Caribbean, it is indeed likely that eutrophication has contributed to degradation of the reef community. Field experiments at our research site have shown that nutrient enrichment enables turf algae to overgrow corals (Vermeij et al. 2010). A recent spatial comparison showed that the coastal waters at our study site had significantly higher concentrations of nitrate and

phosphate in the upper layers of the water column, and a slightly but significantly higher turbidity than the more remote site of Playa Kalki at the northwest point of the island, indicating that Buoy 0 is more eutrophied than Playa Kalki (Den Haan et al. 2013, 2014).

Unfortunately, data on changes in nutrient levels, water clarity and phytoplankton concentrations during the shift from coral to algal dominance are scarce. In 1994, an average chlorophyll *a* concentration of 0.27 μg l^{-1} was measured at our study site (Van Duyl et al. 2002), which exceeds the chlorophyll concentration in our present data (0.11±0.01 μg l^{-1} at 0-10 m depth). Vertical light profiles were measured in 1998, and revealed a light attenuation coefficient (±SD) of K_d=0.093±0.010 m^{-1} (PAR range; Visser et al. 2002), which is very similar to the light attenuation coefficient of K_d=0.098±0.017 m^{-1} that we found in our study. Hence, comparison with the available data do not point at a major reduction in water clarity or increase in chlorophyll concentration during the past 20 years. This is remarkable since the common expectation is that eutrophication of coral reefs will result in substantially higher phytoplankton concentrations and lower water clarity (Bell 1992; Cheal et al. 2013). Our data do not support this paradigm.

High water clarity does not refute the possibility that eutrophication contributes to the phase shift from corals to turf algae at our research site. Extensive exchange with the open ocean may continuously refresh the water column above the reef with clear oceanic water, while high abundances of turf algae, cyanobacterial mats and macroalgae may effectively absorb the surplus of externally supplied nutrients. However, in addition to eutrophication, other factors might also play a role in the shift to algal dominance, such as the mass mortality of the black sea urchin *Diadema antillarum* across the Caribbean in 1983/1984 (Bak et al. 1984; Lessios et al. 1984; Edmunds and Carpenter 2001) or the collapse of herbivorous fish populations that kept algal growth under control (Hughes 1994; Jackson et al. 2001). In particular, field experiments by Wanders (1977) performed at our research site point at the major significance of grazing fish, as their experimental exclusion led to rapid overgrowth of CCA by turf algae.

In conclusion, although we did not find a major change in total primary productivity of the entire reef community, our results show a large shift from corals and CCA to turf algae and macroalgae as the dominant primary producers. This shift is likely to have a major impact on numerous other species, in particular on the herbivores that ultimately depend on the productivity, species composition and nutritional quality of the primary producers on the reef.

ACKNOWLEDGEMENTS

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Tables

Table 1. Areal density (i.e., dry weight per unit of area) of the different benthic species.

Benthic species	Depth (m)	Areal density (g DW m^{-2})
Hard corals*	5	820
	20	820
Turf algae	5	127
	20	68
Halimeda opuntia	5	2590
	20	2590
Dictyota menstrualis	5	70
Dictyota pulchella	20	70
Lobophora variegata	20	100
Cyanobacterial mats	5	11.1
	20	6.2

*Based on estimates for *Madracis mirabilis* by Hardt (2007)

Table 2. Photosynthetic parameters of the phototrophic species on the reef.

Species	Depth (m)	α	$rETR_{max}$	E_k	R^2
Madracis mirabilis	5	0.583±0.009	382±29	657±52	1.000
	20	0.639±0.019	242±17	382±37	0.999
Turf algae	5	0.261±0.029	23.2±5.9	85.9±17.7	0.980
	20	0.251±0.039	13.4±3.5	53.6±13.9	0.930
Halimeda opuntia	5	0.632±0.025	181±26	292±51	0.998
	20	0.644±0.049	76.9±11.1	120±17	0.998
Dictyota spp.	5	0.751±0.032	195±23	258±23	0.999
	20	0.792±0.039	125±16	155±14	0.999
Cladophora spp.	5	0.406±0.030	50.9±7.4	131±24	0.988
Dichotomaria marginata	5	0.486±0.026	115±10	236±13	0.999
	20	0.515±0.030	65.1±10.3	124±14	0.998
Lobophora variegata	20	0.867±0.062	94.0±4.7	112±13	0.999
Lyngbya majuscula	5	0.278±0.054	40.4±8.8	191±64	0.935
	20	0.312±0.018	43.0±9.1	136±27	0.990
Dichothrix spp.	5	0.662±0.088	255±29	414±71	1.000
Phytoplankton	5	0.054±0.011	27.1±7.8	834±309	0.790
	20	0.058±0.004	11.3±2.3	206±53	0.729

The photosynthetic parameters (estimate ± SE) are based on n=5 (for benthic organisms) and n=6-8 (for phytoplankton) rapid light curves per depth, fitted to Eqn (1) using nonlinear regression.

Figures
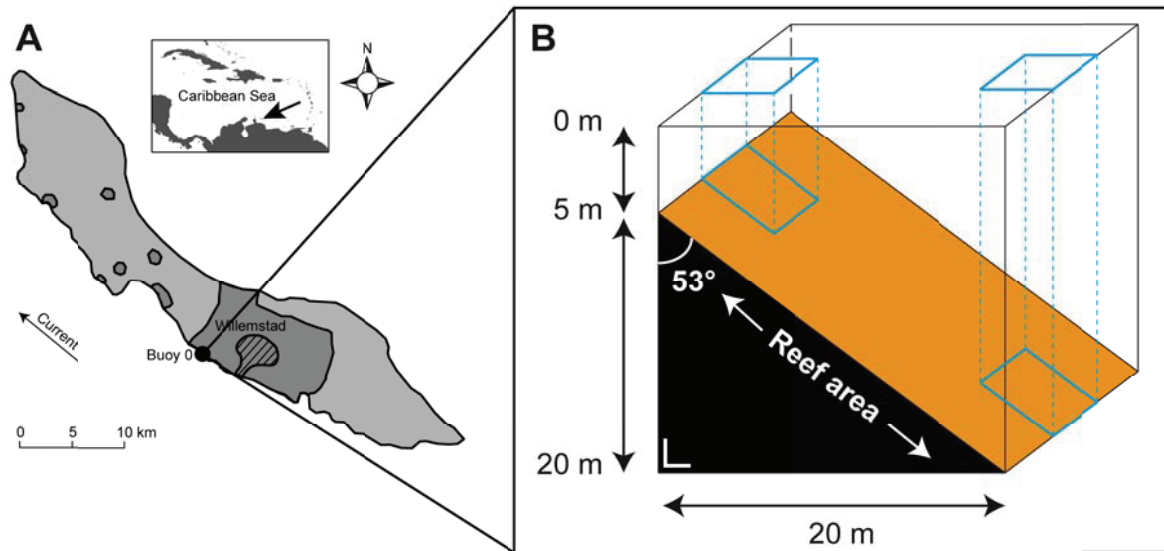
Figure 1. A Map indicating the location of research site Buoy 0 on the island of Curaçao, Southern Caribbean. Dark-grey zones are urbanized areas, including the capital Willemstad and its industrial harbor (hatched area). **B** Schematic of the reef investigated at Buoy 0. The two vertical columns indicate that the primary productivity per unit surface area was calculated for phytoplankton in the water column and for benthic organisms on the reef at 5 m and at 20 m depth.

Benthic composition Buoy 0
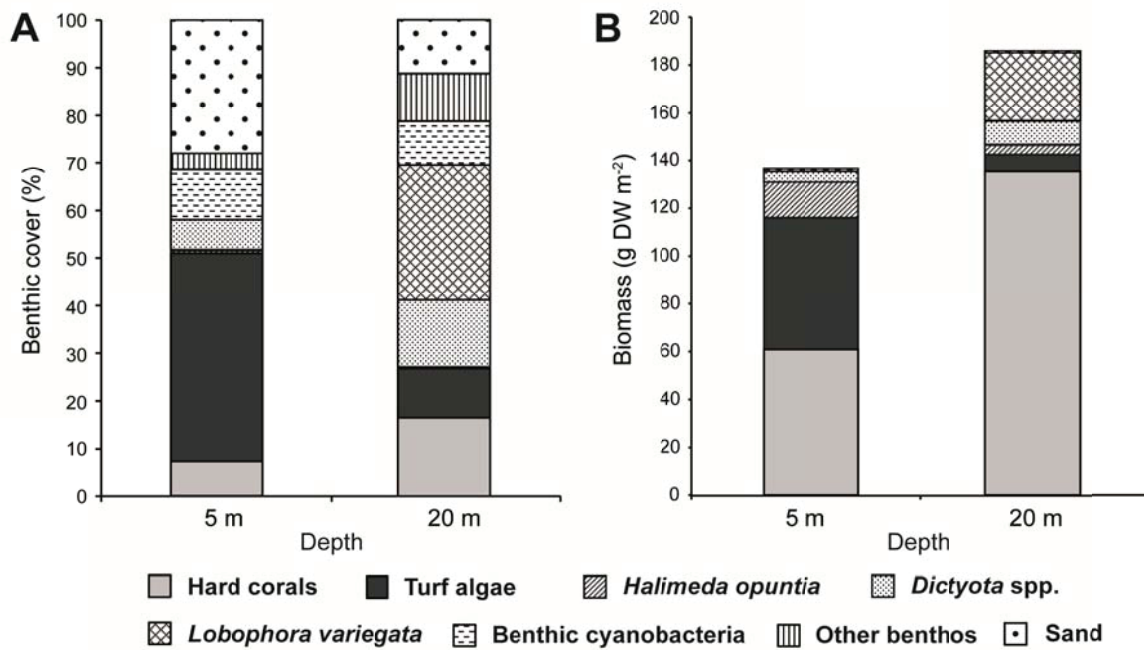
Figure 2. Composition of the benthic community. **A** Percent cover of hard corals, turf algae, macroalgae (*Dictyota* spp., *Halimeda opuntia*, *Lobophora variegata*), benthic cyanobacteria, other benthic organisms (e.g., sponges) and sand at 5 m and at 20 m depth. **B** Biomass distribution per unit surface area of the same functional groups.
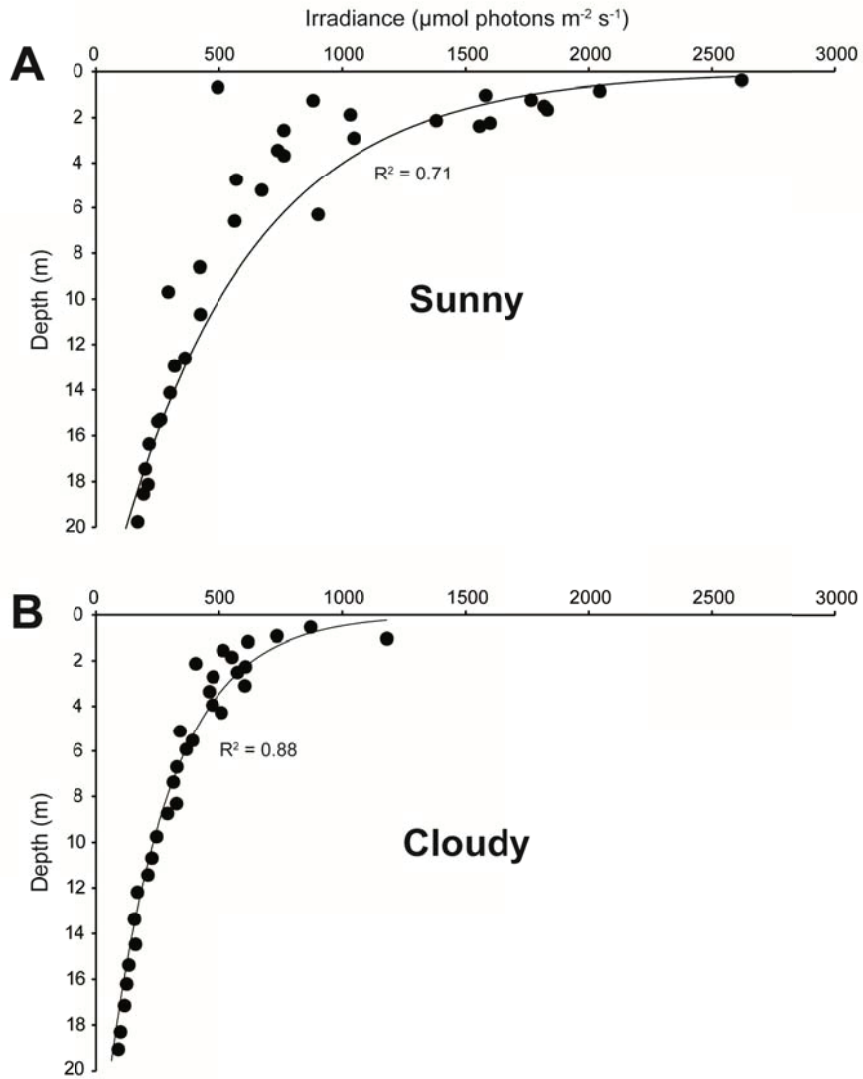
Figure 3. Light profiles measured at midday during (**A**) a sunny day and (**B**) a cloudy day. Solid lines are based on linear regression of ln-transformed irradiance versus depth.
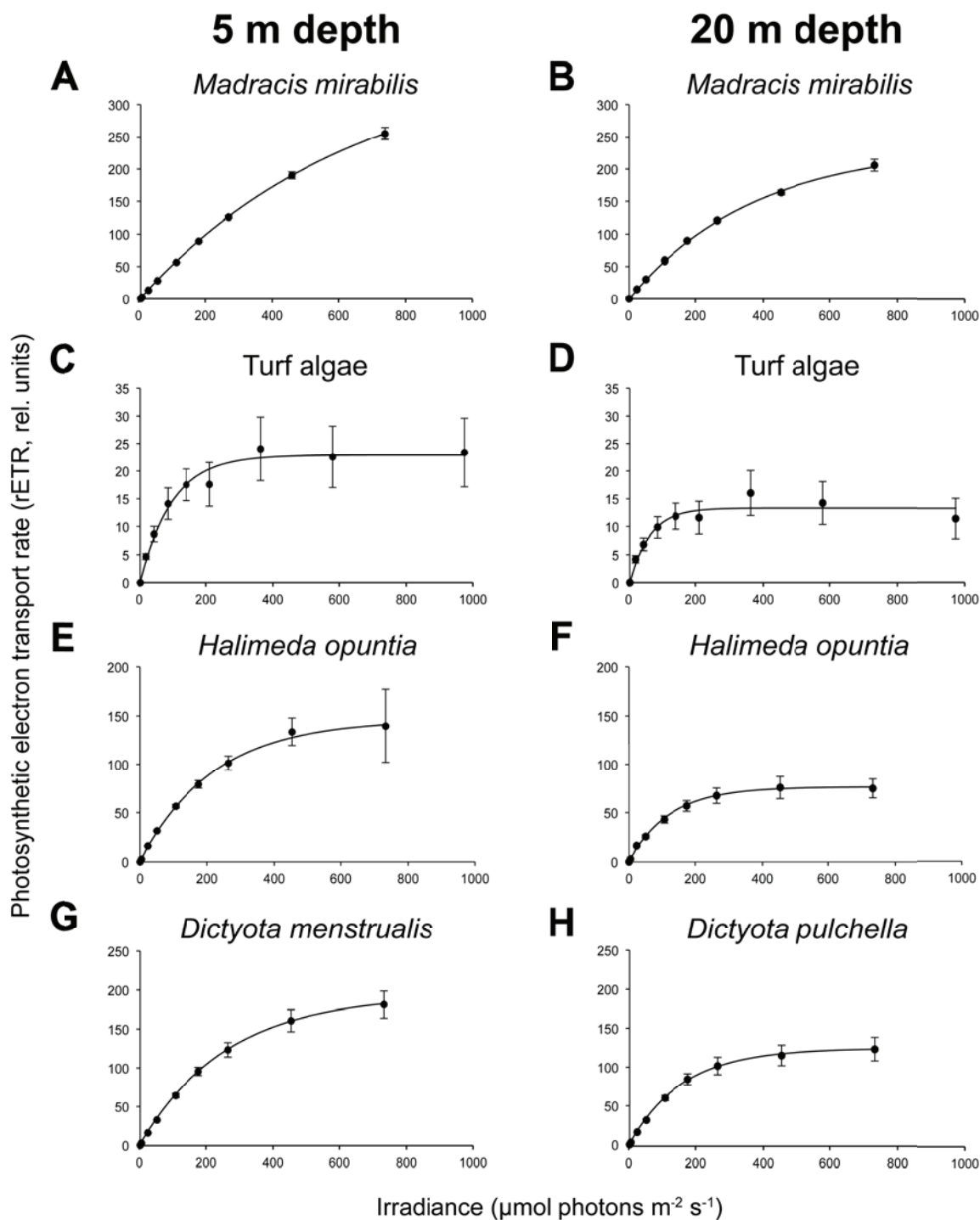
Figure 4. Rapid light curves of (**A,B**) the coral *M. mirabilis*, (**C,D**) turf algae, (**E,F**) the macroalga *H. opuntia*, and (**G,H**) two species of the macroalgal genus *Dictyota*. Data points show photosynthetic rates (expressed as relative electron transport rate, rETR) as function of irradiance, measured *in situ* on the reefs with a PAM fluorometer. Error bars represent s.e. of the mean (n=5); solid lines represent photosynthesis-irradiance curves fitted to the rETR data using Eqn (1).
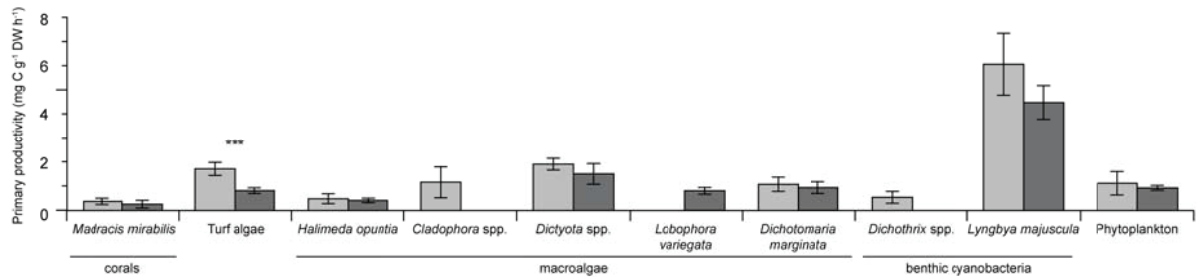
Figure 5. Primary productivity per unit biomass of the different phototrophic species on the reef, measured by ^{13}C incorporation at 5 m depth (light grey bars) and 20 m depth (dark grey bars). Error bars represent s.d., *** represents a significant difference between 5 m and 20 m depth (Student's *t*-test; $P<0.001$); for the other species we did not find a significant depth effect.
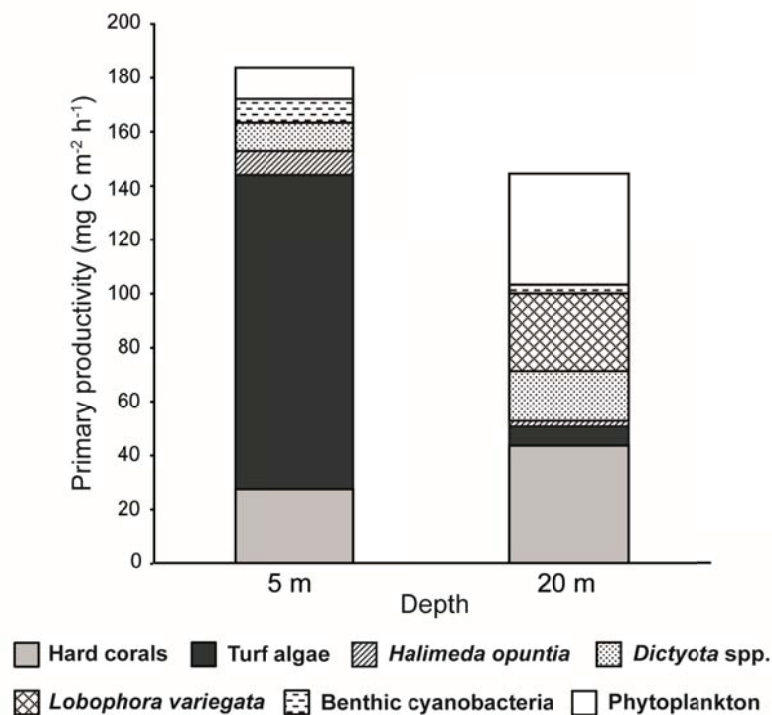
Figure 6. Contributions of different functional groups to the primary productivity per unit surface area, for a shallow part (5 m) and deep part (20 m) of the reef.

ELECTRONIC SUPPLEMENTARY MATERIAL

5 m depth   20 m depth
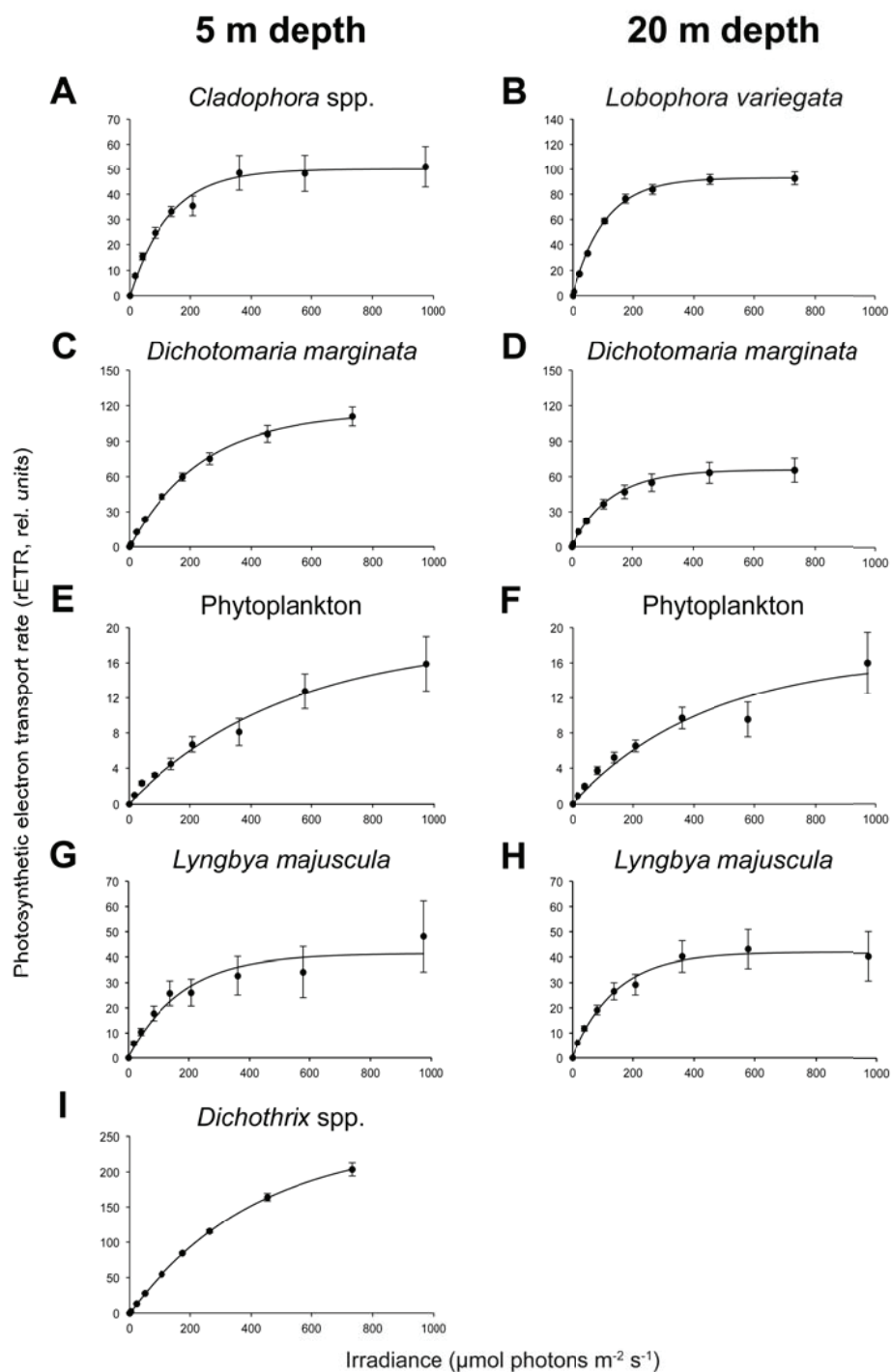
ESM Figure. Rapid light curves of several additional phototrophic species. Data points show photosynthetic rates (expressed as relative electron transport rate, rETR) as function of irradiance, measured *in situ* on the reefs with a PAM fluorometer. Error bars represent s.e. of the mean (n=5); solid lines represent photosynthesis-irradiance curves fitted to the rETR data using Eqn (1).

7. Manuscript VI

MS VI: Diel vertical migration of Beggiatoaceae in benthic cyanobacterial mats

(In preparation)

Diel vertical migration of *Beggiatoaceae* in benthic cyanobacterial mats

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During night dives on coral reefs in Curaçao (12°N, 69°W), we observed *Beggiatoaceae* moving up to the surface of brown-colored benthic cyanobacterial mats (Fig. 1a,b). The brown mats, mostly dominated by *Oscillatoria bonnemaisonii* P.L.Crouan & H.M.Crouan ex Gomont, occurred over sand between 5 and 14 m depth. *Beggiatoaceae* emerged as a white layer on top of the mats between 0200 and 0600 hrs. The diameters of their filaments varied between 2 and 80 μm, indicating the presence of several taxa.

Beggiatoaceae are single filamentous, gliding sulfur-oxidizing bacteria, which can form thick and long filaments visible to the human eye. They appear white due to intracellular inclusions of stored elemental sulfur which they produce by sulfide oxidation (Fig. 1c). They are indicator species for high organic and sulfide levels in sediments (Elliott et al. 2006). Increase in organic carbon leads to the production of hydrogen sulfide during bacterial reduction of sulfate, which provides their principal energy source. Thus they are typically found in habitats that have high levels of hydrogen sulfide, including sulfur springs, organic-rich marine and freshwater sediments and sewage outlets (Larkin and Strohl 1983). On coral reefs, they are a component of the microbial consortium causing black band disease in scleractinian corals (Carlton and Richardson 1995).

Since they require oxygen to oxidize sulfide but avoid high oxygen levels, we hypothesize that, during nighttime, hypoxia occurs in the sediment and overlying cyanobacterial mat, causing

them to migrate upwards to sustain their metabolism. During daytime, oxygen becomes oversatured due to cyanobacterial photosynthesis, causing them to migrate downwards. Similar vertical migration and explanatory mechanisms have been demonstrated in black band disease (Carlton and Richardson 1995).

We suggest that the abundance of *Beggiatoaceae* on coral reefs indicates high concentrations of organic matter and hydrogen sulfide. Since hypoxia and hydrogen sulfide are toxic to many organisms (Elliott et al. 2006), it remains to be investigated how such conditions affect infaunal coral reef communities.

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Elliott JK, Spear E, Wyllie-Echeverria S (2006) Mats of *Beggiatoa* bacteria reveal that organic pollution from lumber mills inhibits growth of *Zostera marina*. Mar Ecol-Evol Persp 27:372-380

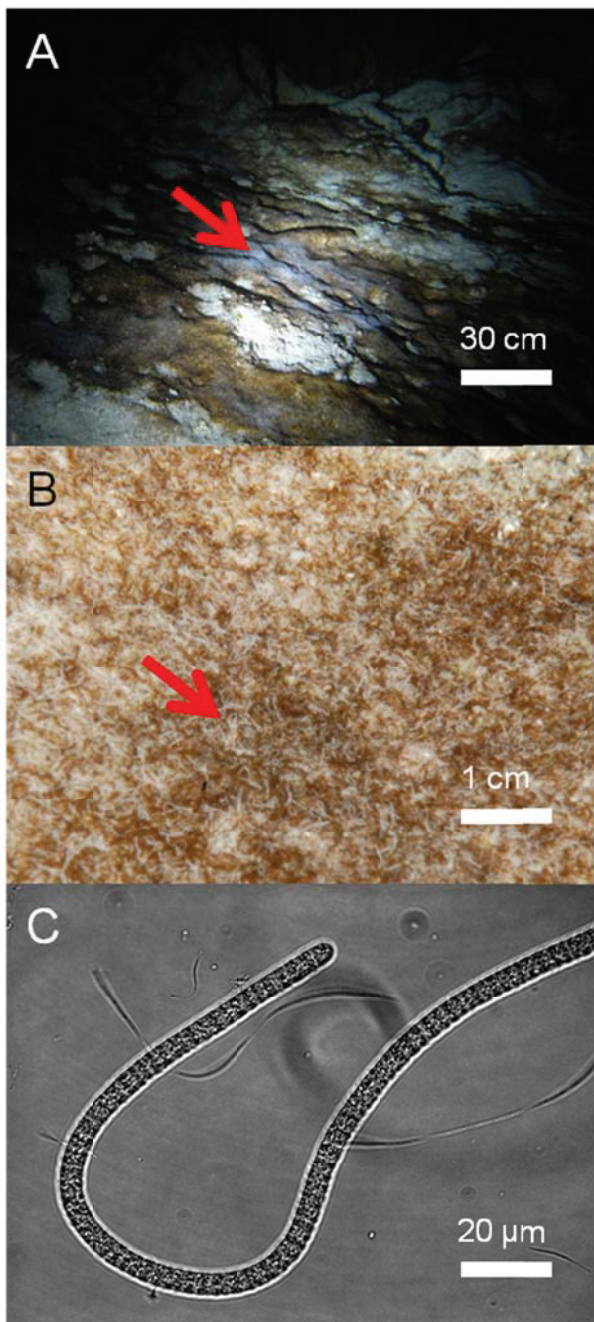Larkin JM, Strohl WR (1983) *Beggiatoa*, *Thiothrix*, and *Thioploca*. Annu Rev Microbiol 37:341-367

Fig. 1 **a** Benthic cyanobacterial mat (brown filaments) with surfacing *Beggiatoaceae* (indicated by white arrows) at night at 7 m depth in Curaçao; **b** Close-up of mat with cyanobacteria (brown filaments) and *Beggiatoaceae* (white filaments shown by white arrows); **c** *Beggiatoaceae* filament with refractive structures of intracellular sulfur globules (white arrows).
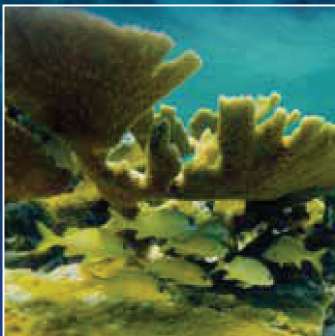
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8. Manuscript VII

MS VII: Preventing blooms of cyanobacterial mats

(Printed June 2014)

Towards Reef Resilience and Sustainable Livelihoods

Preventing blooms of cyanobacterial mats

Dense cyanobacterial mats.

Recent research suggests that mats grow faster when exposed to higher levels of organic matter. Corals are adapted to life in a nutrient poor environment. Excess organic matter leads to the release of nutrients by microbial degradation, which stimulates the growth of cyanobacterial mats at the expense of corals. It is thus expected that measures decreasing the input of nutrients and organic matter on reefs will reduce the abundance of cyanobacterial mats and help to maintain reef health.

THE EVIDENCE

Research along the coast of Curaçao compared the distribution of cyanobacterial mats with land usage and wave energy along the coastline. In the uninhabited south east part of the island, the mat abundance was very low (<1% cover), whereas it increased near industrialized and populated areas, especially close to estuaries (>30% cover). The only areas close to industrialized and populated areas where mats were less abundant were those exposed to high wave energy where nutrients are diluted faster and organic matter settles less on the seafloor. Sediments from sites with high abundance of mats were rich in organic matter compared to sites harboring few mats. Sediments are known to be a sink of organic matter. Researchers found that organic matter added to sediments can fuel the growth of cyanobacterial mats.

Diver checking cyanobacteria.

Cyanobacteria, also called blue-green algae, are a type of bacteria found in both aquatic and terrestrial environments. In the sea they occur as cells floating in the water column, as well as mats largely composed of intertwined cyanobacterial filaments covering the seafloor. On coral reefs cyanobacterial mats are normally rare. However, in recent years they have increased in abundance on many Caribbean reefs, sometimes reaching close to 50% cover in some areas.

Their increase can have a variety of negative consequences for the reef and the benefits that people derive from reefs. These go well beyond the simple loss of reef 'attractiveness' to divers. Cyanobacterial mats can grow quickly over the reef. They can prevent coral larvae from settling on reefs, can overgrow juvenile and adult corals and can act as coral pathogens. Furthermore, they can produce chemicals which deter the grazing of fishes and urchins and have been linked to mass reef fish die-offs.

Map of cyanobacteria coverage and human development on the island of Curaçao.

Are cyanobacterial mats poisonous?

Most cyanobacteria contain poisons. Blooms of cyanobacterial mats have been associated with mass mortalities of fishes. Direct contact may cause skin, eye and respiratory problems for humans

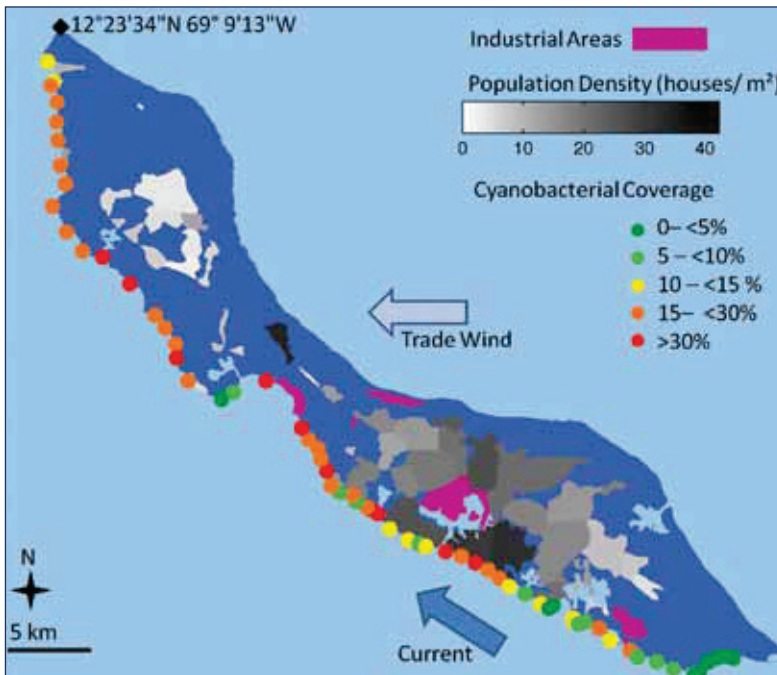**Does anything feed on cyanobacteria?**

Cyanobacteria produce chemicals that fishes and urchins do not like to eat. There seems to be minimal grazing on cyanobacteria. A handful of small invertebrates are thought to feed on cyanobacterial mats.

Is there any economic value to be gained from cyanobacterial mats?

None has been found so far.

Cyanobacterial mats come and go on coral reefs, so should we worry?

Occasional mats are natural but an increase in their cover and frequency of occurrence could indicate a problem.

MANAGEMENT IMPLICATIONS

High coverage of cyanobacterial mats on a reef indicates an increase in nutrients and organic matter which principally come from land-based run-off and pollution. Septic tanks, landfills and waste dumping areas leak nutrients into groundwater which can subsequently flow out onto reefs. Efforts could be made to reduce or stop untreated waste water and run-off from agricultural land reaching the reef.

Bays and other partially enclosed coastal areas have narrow or shallow openings towards the sea, naturally preventing nutrients from reaching the reefs. **Dredging and other activities that change water flow and increase flushing effects can lead to higher nutrient levels and, potentially, more cyanobacteria.**

Reducing nutrient inputs will not only reduce the abundance of cyanobacterial mats, it can also decrease the growth of macroalgae and bioeroders and slow down the spread of coral diseases.

Cyanobacteria.

Massive discharge of sediment loads by a river entering the Caribbean Sea off the Meso-American coast.

9. Synopsis

9. Synopsis

Benthic cyanobacterial mat proliferation is becoming more and more pronounced in freshwater and marine ecosystems all around the globe, including coral reefs. Efforts to identify the drivers and impacts of these unwanted manifestations have never been more vigorous. In this thesis we explored the mechanisms and causes of cyanobacterial blooms on coral reefs. The publications in this thesis revealed how benthic cyanobacterial mats function on the reef, especially when they are dominating the reef benthic substrate. Data to support our findings was obtained from an extensive two-year research program, which we conducted along the coast of Curaçao during both the dry and wet season. We used monitoring surveys to estimate benthic cyanobacterial abundance at 64 dive sites, recorded various environmental parameters at randomly selected dive sites (i.e. sites with high and low benthic cyanobacterial mat coverage), investigated the species composition within the benthic cyanobacterial mats and determined how this is affected by location and depth. Furthermore, we investigated the physiological functioning of the benthic cyanobacterial mats using e.g. microsensors *in situ*. We studied cyanobacterial growth responses to organic matter enrichments, the capability of the mats to fix nitrogen, estimated net fluxes (i.e., O_2, dissolved inorganic carbon, nutrients and DOC), determined how quickly cyanobacterial mats are capable of taking up NH_4^+, NO_3^- and PO_4^{3-}, and estimated its primary productivity. Nutrient uptake rates and primary productivity were compared to other major primary producers on the reef, including corals, macroalgae, turf algae and even phytoplankton.

The following paragraphs will discuss how the results of our publications are connected with one another, and will also discuss them in a broader perspective. Paragraph 9.1 discusses the main drivers and causes of cyanobacterial blooms on coral reefs. Paragraph 9.2 explains the (potential) effects of cyanobacterial proliferation on the reef, especially when they start dominating the benthic substrate. The following paragraph (9.3) will project future scenarios of coral reefs with regard to cyanobacterial proliferation. In paragraph 9.4, the perspectives for future research are discussed. Throughout the first three paragraphs, Figure 1 will act as a guide to visualize the mechanisms and causes for the proliferation of benthic cyanobacterial mats on present-day coral reefs.

9.1. Drivers and causes of benthic cyanobacterial blooms

The second chapter of this thesis (**MS I**) provides the very first demonstration that organic matter acts as a highly important mediator of essential nutrients that ultimately drive the proliferation of benthic cyanobacterial mats on the reef. We propose a model that visualizes the sources of nutrients stimulating the cyanobacterial proliferation, yet we also indicate how these nutrients are cycled throughout the coral reef ecosystem, which is substantiated by the other publications presented in this thesis (see Figure 1 for the proposed model).

Eutrophication and organic matter accumulation on coral reefs

Previous studies have shown that the growth of benthic cyanobacterial mats is stimulated when they are exposed to dissolved inorganic nutrients (NH_4^+, NO_3^-, PO_4^{3-}, but also Fe) (see e.g. Fong et al. 1993, Kuffner & Paul 2001, Ahern et al. 2006a, 2008, Arthur et al. 2009). Hence, nutrients were proposed to be the main driver of cyanobacterial proliferation on the reef. However, no study has reported that concentrations of these nutrients in the water column directly above the benthic cyanobacterial mats were in fact elevated, even when they dominated the benthic substrate (Richardson 1998, Thacker & Paul 2001). This thesis confirmed that concentrations of dissolved inorganic nutrients were not elevated above the benthic cyanobacterial mats (**MS I**). In our nutrient uptake study (**MS IV**), we could however clearly demonstrate that land-runoff after rainfall led to elevated concentrations of nitrate (75-fold), ammonium (3-fold) and phosphate (31-fold), and are taken up the quickest by benthic cyanobacteria, turf algae and filamentous macroalgae. Other reef phototrophic organisms, including corals, phytoplankton and non-filamentous macroalgae had substantially slower nutrient uptake rates (**MS IV**). As a consequence, the nutrients become (temporarily) unavailable, as they are now in organic form, either in the water column (i.e., plankton) or bound by benthic organisms on the seafloor. Organic matter will settle back on the reef or will be carried away from the reef via current flows (Kemp & Boynton 1984, Calvert 1987, Blair & Aller 2012). In accordance, in **MS I** we could clearly show that benthic cyanobacterial mat proliferation was most pronounced in those areas of the reef that were most sheltered or were in close vicinity of the urbanized coast. Here we could find significantly higher organic matter concentrations in the sediment, which we then used as a proxy to determine the eutrophication status of these reef sites (**MS I**). Sediment is a sink of organic matter (Clavier & Garrigue 1999, Rasheed et al. 2003, Rusch et al. 2006, Werner et al. 2006, Chipman et al. 2010). Most of the organic matter here will be remineralized by bacteria and other detritivorous organisms, however, depending on the input of organic matter, the sediment
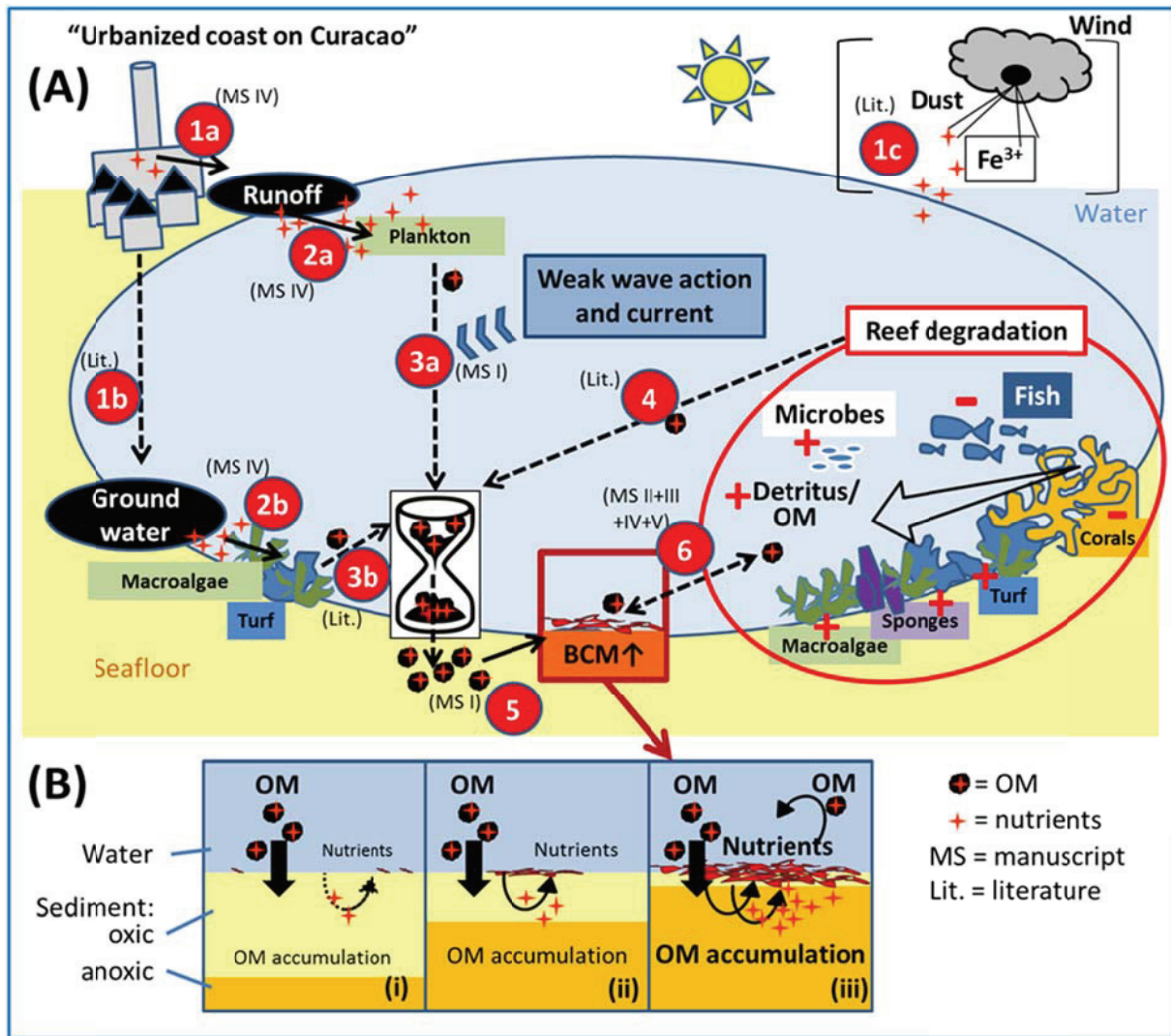
Fig 1: **(A)** Proposed model explaining the sources and cycling of nutrients that stimulate the proliferation of benthic cyanobacterial mats as well as the (potential) functional role cyanobacterial mats have within the reef ecosystem. Nutrient inputs via land runoff (**1a**) or groundwater seepage (**1b**). [In the Caribbean, Fe is largely available as a result of long-term Fe addition by the African dust (**1c**). Therefore, dissolved inorganic phosphate and nitrogen are the main nutrients limiting growth in this region.]. Nutrients are then rapidly taken up by the phytoplankton community (**2a**) and other benthic primary producers (**2b**). Nutrients are consequently trapped as organic matter and as a result measured concentrations of dissolved inorganic phosphate and nitrogen are low in the water column (i.e., oligotrophic waters). (**3a**) The phototrophic blooms (pelagic and benthic) decay and produce particulate organic matter, which can settle on the seafloor, depending on wave action, current velocity and time. (**3b**) The coral reef community takes up, produces and releases organic matter. (**4**) Reef degradation leads to a detritus-based food web and enhances organic matter accumulation on the seafloor. (**5**) Increased organic matter loading leads to cyanobacterial growth. (**6**) Cyanobacterial mats fix nitrogen, release dissolved organic carbon, and hence stimulate further reef degradation and so generate even more favorable conditions for the benthic cyanobacterial mats. **(B)** Schematic drawing of the water/sediment interface. Drawings from far away from (**i**), near to (**ii**), and middle of (**iii**) cyanobacterial mats are used as analogs for what we anticipate will happen as organic matter accumulates over time. Increased organic matter concentrations in the sediment result in a thinner oxygenated surface layer and an increased nutrient release, which triggers cyanobacterial growth. When cyanobacterial mats develop, they produce organic matter and fix nitrogen, release parts as dissolved organic carbon sustaining their bacterial community and trap organic matter/mineral particles from the water column to grow and expand. MS + roman number means number of manuscript that includes proof for the assumption made in the proposed model.

will thus accumulates organic matter over time (Clavier & Garrigue 1999, Rasheed et al. 2003, Rusch et al. 2006, Paerl 2008). When the organic matter concentration in the sediment reaches a certain threshold (in our study; >0.3% C_{org}), cyanobacterial mats can develop (**MS I**). Cyanobacterial mats contain heterotrophic bacteria and sulfate reducing bacteria which can degrade the organic matter in the sediment, but also remineralize them back to dissolved inorganic nutrients to the benefit of the cyanobacterial communities of the mat (Van Gemerden 1993, Paerl et al. 2000, Werner et al. 2006) (also paragraph 1.1.2).

Which nutrient limits cyanobacterial growth?

Phosphate, nitrogen and iron often limit the growth of cyanobacteria (Paerl 2008). Yet, the type of nutrient that in fact limits growth will strongly depend on the region where the cyanobacteria occur. Consequently, manipulative studies that investigated nutrient limited growth of benthic cyanobacterial mats across different locations and time found dissimilar results; phosphate limited growth in Guam and Mexico (Fong et al. 1993, Kuffner & Paul 2001), chelated iron in Australia and Florida (Ahern et al. 2006a, Arthur et al. 2009), mixed iron and phosphate in Australia (Ahern et al. 2007, 2008), and mixed nitrogen and phosphate in Florida (Miller et al. 1999).

In our studies we did not determine which nutrient limits the growth of benthic cyanobacterial mats on Curaçao, however our data suggest that the amount of phosphate directly released via land runoff but also bound to sediment particles is the limiting factor in driving benthic cyanobacterial growth (**MS I+II**). It is unlikely that Fe is a limiting factor on Curaçao, since the Caribbean region is known to have a large Fe input via atmospheric dust from the African continent (Roff & Mumby 2012). Luo et al. (2012) in this respect argued that the Caribbean region is a hotspot for pelagic nitrogen fixation, as here the cyanobacterial blooms are not limited by iron. The equatorial and subarctic Pacific Ocean, and the Southern Ocean on the contrary do have relatively high nutrient concentrations in the water, yet do not produce large cyanobacterial blooms (these regions of the ocean are termed 'High Nutrient Low Chlorophyll' (HNLC) regions). In these HNLC regions iron is often limiting cyanobacterial growth (e.g. Boyd et al. (2000)). In our third chapter (**MS II**) we provide the first data of nitrogen fixation rates by benthic cyanobacterial mats in the Caribbean. All cyanobacterial mats that we studied were able to fix nitrogen. We compared our findings to other N_2 fixation rates by solitary benthic cyanobacteria and turf algae (which are often dominated by N_2 fixing cyanobacteria) from reefs around the world and found that benthic cyanobacterial mats from Curaçao have amongst the highest N_2 fixation rates to date. Our results indicate that nitrogen is probably not limiting the

growth of our cyanobacterial mats due to their high N_2 fixation potential, and since previous studies found that iron unlikely limits cyanobacterial growth in the Caribbean region (Luo et al. 2012), we argue that phosphate is most likely to limit benthic cyanobacterial growth on the island of Curaçao.

Which nutrient limits cyanobacterial growth?

Phosphate, nitrogen and iron often limit the growth of cyanobacteria (Paerl 2008). Yet, the type of nutrient that in fact limits growth will strongly depend on the region where the cyanobacteria occur. Consequently, manipulative studies that investigated nutrient limited growth of benthic cyanobacterial mats across different locations and time found dissimilar results; phosphate limited growth in Guam and Mexico (Fong et al. 1993, Kuffner & Paul 2001), chelated iron in Australia and Florida (Ahern et al. 2006a, Arthur et al. 2009), mixed iron and phosphate in Australia (Ahern et al. 2007, 2008), and mixed nitrogen and phosphate in Florida (Miller et al. 1999).

In our studies we did not determine which nutrient limits the growth of benthic cyanobacterial mats on Curaçao, however our data suggest that the amount of phosphate directly released via land runoff but also bound to sediment particles is the limiting factor in driving benthic cyanobacterial growth (**MS I+II**). It is unlikely that Fe is a limiting factor on Curaçao, since the Caribbean region is known to have a large Fe input via atmospheric dust from the African continent (Roff & Mumby 2012). Luo et al. (2012) in this respect argued that the Caribbean region is a hotspot for pelagic nitrogen fixation, as here the cyanobacterial blooms are not limited by iron. The equatorial and subarctic Pacific Ocean, and the Southern Ocean on the contrary do have relatively high nutrient concentrations in the water, yet do not produce large cyanobacterial blooms (these regions of the ocean are termed 'High Nutrient Low Chlorophyll' (HNLC) regions). In these HNLC regions iron is often limiting cyanobacterial growth (e.g. Boyd et al. (2000)). In our third chapter (**MS II**) we provide the first data of nitrogen fixation rates by benthic cyanobacterial mats in the Caribbean. All cyanobacterial mats that we studied were able to fix nitrogen. We compared our findings to other N_2 fixation rates by solitary benthic cyanobacteria and turf algae (which are often dominated by N_2 fixing cyanobacteria) from reefs around the world and found that benthic cyanobacterial mats from Curaçao have amongst the highest N_2 fixation rates to date. Our results indicate that nitrogen is probably not limiting the growth of our cyanobacterial mats due to their high N_2 fixation potential, and since previous studies found that iron unlikely limits cyanobacterial growth in the Caribbean region (Luo et al.

2012), we argue that phosphate is most likely to limit benthic cyanobacterial growth on the island of Curaçao.

As coral reefs degrade, more organic matter will become available

Cyanobacterial mats dominated the benthic substrate of degraded reefs that are high in algal cover and low in live coral cover (**MS I**). Further research is however needed to determine how the proliferation of cyanobacterial mats is directly connected to reef degradation, but our data suggests that the degraded reefs provide an optimal environment for benthic cyanobacterial proliferation. One of the explanations here might be the increased organic matter loading onto the reef via algal exudates (Haas et al. 2011, Barott & Rohwer 2012). It is well known and well documented that many coral reefs experienced a sharp loss in live cover of the past few decades (Jackson et al. 2014). Nowadays, many reefs are dominated by benthic algae and cyanobacteria (Jackson et al. 2001, Pandolfi et al. 2003, Paul 2008). Alterations to the benthic substrate composition will affect the quantity and chemical composition of organic materials cycling within the reef environment, including the release of dissolved organic matter by primary producers, especially macroalgae (Haas et al. 2010a,b, Barott & Rohwer 2012). Furthermore, as macroalgae and turfs become more abundant, more particulate organic matter will be trapped onto their canopies (Schaffelke 1999, Purcell & Bellwood 2001). Schaffelke (1999) nicely demonstrated that the layer of particulate matter deposited on the thalli of *Sargassum* was sufficient to supplement its nutrient supply. She postulated that a nutrient-rich diffusive boundary layer was created on the thallus surface via an epiphytic microbial community that remineralized the bound nutrients inside the particulate matter. Similarly, organic matter that accumulates on sediments could provide an important nutrient source for cyanobacterial mats, yet this still needs to be further investigated.

Benthic cyanobacterial mats impact the functioning of coral reefs worldwide. However, the sources and transport mode of nutrients that stimulate the growth of these cyanobacterial mats remain unclear. In this thesis we identified that organic matter degradation acts as a mechanism of nutrient supply to the cyanobacterial mats, which can consequently lead to their proliferation on the reef (**MS I+IV**). This thesis further demonstrates that coastal urbanization and hydrodynamics mediate the accumulation of particulate organic matter onto the seafloor, which subsequently fuels the growth of these unwanted cyanobacterial mats (**MS I**).

9.2. Impacts of cyanobacterial mats on the reef ecosystem

Large-sized cyanobacterial mats on coral reefs are a recent phenomenon (Paul 2008, Paerl & Paul 2012). As a consequence, cyanobacterial mats have for many years been overlooked within this environment. Few studies did investigate the impact of cyanobacterial mats on the reef ecosystem, yet merely focused on the toxic properties of the cyanobacterial mats (Nagle & Paul 1998, 1999, Paul et al. 2011). Therefore, as cyanobacterial mats are becoming more pronounced on coral reefs, it is vital to better understand the physiology of the cyanobacterial mats, the role they play within the carbon and nitrogen cycle of the reef ecosystem, and how they contribute to further reef degradation.

Contribution of dissolved organic carbon

Our studies are the first to investigate the release of dissolved organic matter by benthic cyanobacterial mats on coral reefs (**MS III**). As mentioned earlier, dissolved organic carbon (DOC) released by benthic algae affect the composition and increases the abundance of bacterial communities on the reef which can consequently result in coral mortality and reef degradation (Kline et al. 2006, Smith et al. 2006, Barott & Rohwer 2012). Our *in situ* incubations showed that benthic cyanobacterial mats release very high quantities of DOC during the daytime and surprisingly also during the nighttime (i.e., 1.4 and 3.5 mmol C m^{-2} h^{-1}, respectively). We combined our results with DOC release rates by other primary producers on a degraded reef and found that that cyanobacterial mats were in fact the largest contributor to the DOC pool on the reef (**MS III**). However, the quality of DOC and the responds to the bacterial community were not determined in our study and should be the main focus of future research efforts. Other studies found that certain cyanobacterial species are capable of releasing small and easily degradable organic compounds into the water column (Heyer et al. 1989, Paerl et al. 1993, Stal 1995), interestingly we found cyanobacterial species that closely relate to those species within the benthic mats from our studies (Heyer & Krumbein 1991). This signifies the great potential benthic cyanobacterial mats have in their contribution to processes that stimulate further reef degradation (**MS II+III**).

Large-sized benthic cyanobacterial mats fix notable amounts of N_2

This thesis provides novel insight to the high N_2 fixation capacity of large-size benthic cyanobacterial mats in the Caribbean (**MS II**). Biological nitrogen fixation (diazotrophy) is an important source of nitrogen to the reef ecosystem as it is vital to sustain its primary productivity in the Pacific (Wiebe et al. 1975, Larkum et al. 1988, Charpy-Roubaud et al. 2001). Unfortunately, it is not known whether this also holds true for the Caribbean region. In this thesis we investigated nitrogen fixation rates of different types of large-sized benthic cyanobacterial mats, assessed their biodiversity and up-scaled their N_2 fixation rates on a reef-wide scale. It turned out that this was rather complicated, as the benthic cyanobacterial mats were highly biodiversity in terms of species composition. Still, we found that benthic cyanobacterial mats were capable of fixing 13 mg N m^{-2} reef d^{-1}, which is enormous in comparison to both benthic and pelagic N_2 fixation rates from other tropical regions (**MS II**). Further research is necessary to determine how much bioavailable nitrogen is actually released into the reef ecosystem as for example other factors such as denitrification also take place within the cyanobacterial mat community (Van Gemerden 1993, Paerl 1996, Paerl et al. 2000, Dong et al. 2011).

Benthic cyanobacterial mats are well equipped for eutrophied reefs

Our research demonstrated that benthic cyanobacterial mats start proliferating when environmental conditions shift to their favor, yet it remained unclear where these cyanobacteria came from. Interestingly, the benthic cyanobacteria that dominate present-day benthic mats were already present 30 years ago as part of the turf communities on Curaçao (**MS II**). Therefore, as coral reefs become increasingly eutrophied, inconspicuous benthic cyanobacterial mats can become increasingly abundant, as they have – in comparison to other reef phototrophic organisms – very fast nutrient uptake rates (**MS IV**), have the potential to grow very quickly, i.e., have high primary productivity estimates (**MS V**), and quickly increased in patch-size upon organic matter enrichment (**MS I**).

In conclusion to this paragraph we can summarize that our studies provide novel insights to the physiology of cyanobacterial mats in the Caribbean. Thereby, our studies strongly indicate that cyanobacterial mats have a large impact on the coral reef ecosystem, hence could augment reef degradation processes by releasing small organic compounds into the water column. This could result in altered bacterial community compositions. Further cyanobacterial mat proliferation

could increase in the total nitrogen availability within the reef ecosystem. Detailed investigations are however necessary to determining the actual numbers.

Benthic cyanobacteria can form large mats on coral reefs, yet the impacts of these mats on the reef ecosystem remain unclear. This thesis provides novel insight into the role of dissolved organic carbon releases (DOC) by the benthic mats on the reef ecosystem. We found that benthic cyanobacterial mats contributed the majority of DOC to the total DOC pool of the reef (MS III). Further, we recorded high nitrogen fixation rates from large-sized mats (MS II) and demonstrated that the mats have a great potential to further develop on (newly) eutrophied reefs (MS II+VI+V). Our results strongly support the idea that large-scale benthic cyanobacterial mats further contribute to reef degradation processes.

9.3. High cyanobacterial mat abundances - a future scenario?

Coral reefs all around the world are predicted to further degrade and lose more of their live coral cover (Knowlton 2001, Jackson et al. 2014). Yet, what does it mean to the expansion of benthic cyanobacterial mats?

The short answer would be that the prognoses for the proliferation of cyanobacterial mats are looking pretty good on a worldwide scale. This chapter will try to interlink the findings of this thesis to other studies in order to draw a future scenario, but also to identify coral reef management tools to halt benthic cyanobacterial proliferation.

When summarizing the results of this thesis, we could generalize that when coral reefs degrade due to various reasons (i.e. overfishing, pollution, climate change), cyanobacterial mats will most likely benefit from it. We found an increased abundance of cyanobacterial mats when coral cover was reduced and algal cover was high (**MS I**). One reason could be the increase of organic matter loads (e.g. algae exudates) in these environments as discussed before (Barott & Rohwer 2012). But, there are also other factors that benefit cyanobacterial mat proliferation, such as the increase of the number of sponges on degraded reefs (González-Rivero et al. 2011). Sponges actively filter dissolve and particular organic matter from the water column, however also excrete particular organic matter onto the seafloor (Richter et al. 2001, De Goeij & Van Duyl 2007, De Goeij et al. 2013). Hence, more organic matter might be transferred to the seafloor to the benefit of the cyanobacterial mats (**MS I**). Further, the reduction of coral cover (due to e.g. increase of seawater temperature, increased coral disease pressures, hurricanes) will open up more benthic substrate

on the reef, which will quickly be occupied by turfs (i.e. cyanobacteria and algae) and cyanobacterial mats (**MS V+IV**) (Diaz-Pulido & McCook 2002, Diaz-Pulido & McCook 2004, Titlyanov et al. 2007). Furthermore, many reefs are expected to be more exposed to increasing sedimentation in the coming decades (Rogers 1990, Fabricius 2005, Risk 2014). Sediments are a dynamic substrate difficult to colonize for corals, but highly suitable to cyanobacterial mats (Palinska 2008, Charpy et al. 2012a). Also the increase of sea temperature is predicted to benefit cyanobacterial blooms, as this will increase their growth rates (Hallock 2005, Paerl & Huisman 2008, Paerl & Paul 2012). In this thesis we confirmed that benthic cyanobacterial mats notably proliferate during the warmer seasons (i.e., seawater temperature increase of 2-4 °C (**MS I**)).

On the other hand, our data indicates that when cyanobacterial mats start proliferating, they might enhance further reef degradation (see previous chapter) (**MS II+III**), which would result in an unwanted negative feedback.

Management applications

The reduction of nutrients and organic matter running into the reef ecosystem is one of the most important strategies to ensure the reduction of cyanobacterial and algal proliferation (Wiedenmann et al. 2013, D'Angelo & Wiedenmann 2014, Risk 2014). The installation of waste-water treatment plants, the stabilization of nearby land masses to reduce erosion, the maintenance of a mangrove-dominated coastline and the reduction of boat traffic especially in lagoonal areas are some of the examples that would reduce coral reef eutrophication (D'Angelo & Wiedenmann 2014, Mumby et al. 2014). The prevention of coral reef degradation must be a high priority, because as soon as large-size cyanobacterial mats have established themselves on the reef, it will become more difficult to shift back towards a coral-dominated ecosystem. In our studies we found that monitoring the levels of organic matter in the sediment was an excellent proxy to determine historical pollution and explain the (future) proliferation of cyanobacterial mats (**MS I**). Hence, we propose to use this tool to investigate the pollution state of coral reefs. To illustrate, we found that the sediment of some coral reefs studied contained such high organic matter loads that Beggiatoacea formed thick layers on the top of the cyanobacterial mats (**MS VII**). These Beggiatoacea are known as indicator species for nutrient/organic matter enrichment in other environments such as seagrass beds (Elliott et al. 2006) and thus we propose that monitoring their presence will likewise be a valuable tool to determine the current health state of the reef.

Though the aforementioned scenarios all might sound as if there is very little hope for our coral reefs, there is proof that coral reefs are capable of adapting to their changing environment. Palumbi et al. (2014) for example indicated that corals are capable of adapting to rising seawater temperatures. This type of research underlines the importance of continuing our fight to secure the survival of coral reefs so that future generations can also benefit from, and enjoy the presence of our coral reefs.

> Eutrophication and global climate change will further increase the proliferation of benthic cyanobacterial mats. Our results suggest that organic matter is a major threat to coral reef health and should be included in monitoring and management practices. The proliferation of cyanobacterial mats can only be minimized by reducing the amount of nutrients and organic matter that enter coral reefs from land.

9.4. Perspectives for future research

The scientific community only recently focused its attention to the proliferation of benthic cyanobacterial mats on coral reefs. Therefore, this field of research is relatively young. As a consequence, many scientific questions remain to better understand the impact of benthic cyanobacterial mats on the wider coral reef community.

Probably the most urgent topic that needs to be investigated is the ability of cyanobacterial mats to mobilize bound nutrients - i.e. bound to sediment or available only in organic form - in order to sustain its bacterial community and expand (for details see Fig. 2). Unraveling this topic will increase our understanding significantly to why benthic cyanobacterial mats persist once they are composed of their most vital microbial communities (e.g., cyanobacteria, heterotrophic bacteria and sulfate-reducing bacteria).

In this thesis, we focused on organic matter that drives the growth of benthic cyanobacterial mats, yet we did not investigate how nutrients underneath and inside the mats were in fact mineralized (**MS I**). Cyanobacterial mats might be capable of mobilizing nutrients that are bound to sediment particles and thus may not solely depend on settling organic matter (which contain nutrients) from the water column (Fig 2). Within sediments, phosphate and iron are often bound, hence are not in bioavailable from for any reef phototrophic organism, including corals, macroalgae, turfs, benthic cyanobacteria and phytoplankton (Sundby et al. 1992, Gunnars &
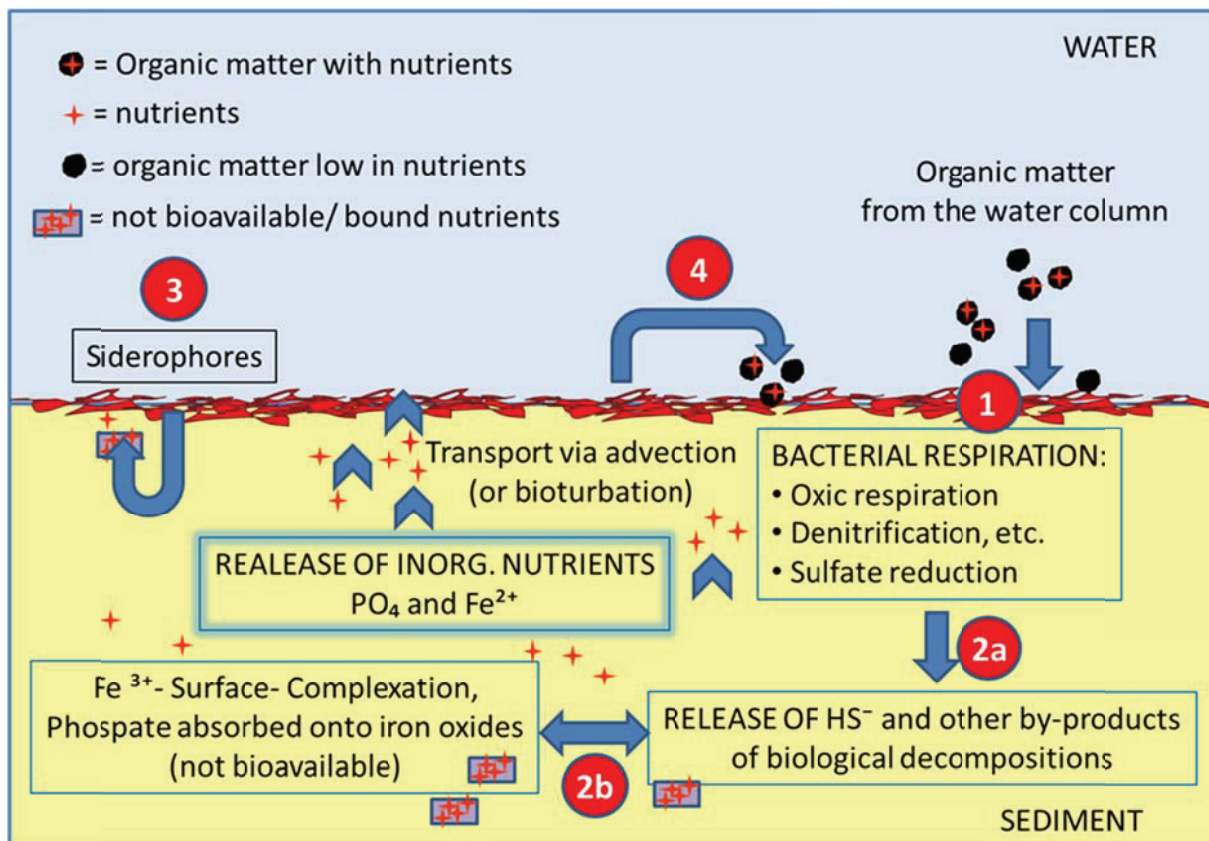
Fig 2: Schematic overview of possible pathways for nutrient mobilization inside and underneath benthic cyanobacterial mats. (**1**) Organic matter with bound nutrients is respired and nutrients are remineralized by heterotrophic and sulfate reducing bacteria. (**2a**) HS⁻ and other by-products of the biological decomposition are released by the bacterial community and (**2b**) bound and not bioavailable nutrients are mobilized and transferred into bioavailable nutrients. (**3**) Siderophores are excreted by the bacterial community (e.g. from cyanobacteria) to mobilize bound nutrients in the sediment. (**4**) Cyanobacterial mats excrete organic matter to enhance decomposition accompanied by nutrient releases (see **2a+b**). Released nutrients (i.e., PO_4 and Fe^{2+}) are taken up by the cyanobacteria. Nutrient transport modes are driven by diffusion, advection or bioturbation. Please note that the mechanisms depicted will take place in much smaller vicinities than illustrated in the figure.

Blomqvist 1997, Roden & Edmonds 1997). As a consequence, these nutrients cannot be directly utilized to the benefit of any of these aforementioned organisms. Interestingly, sediment-bound nutrients can however be released once the chemical environment within the sediment are changed, e.g. drop in pH (Jensen et al. 1995, Gomez et al. 1999). This phenomenon has been observed within many microbial mats from different aquatic environments (Revsbech et al. 1983, Paerl & Pinckney 1996), and therefore it most likely also occurs within benthic cyanobacterial mats from coral reefs. Our microsensor measurements indicated that a sharp oxygen gradient exists with depth of the mats. During the daytime, high oxygen levels were measured notably in the top-part of the mat (>700 µl O₂) whereas during the nighttime we found anoxic conditions at the top of the mats (**MS I+III**). Anoxic conditions, in combination with a high organic matter

content in the sediment (**MS I**), most likely result in sulfate reduction via sulfate-reducing bacteria present within the mats (Teske et al. 1998), which was confirmed by our nighttime observation of Beggiatoa on the mats (**MS VII**) (Schwedt et al. 2012). The hereby-produced HS$^-$ and by-products of the biological decomposition are known to induce the release of sediment-bound nutrients (Patriqui 1972, Erftemeijer & Middelburg 1993, Jensen et al. 1998, Rozan et al. 2002). Therefore, nitrogen, phosphorus and iron present within the organic matter settling on the seafloor do not necessarily need to be the sole driver for cyanobacterial growth, they could thrive on organic matter without these three nutrients. To take it even one step further, organic matter released by the cyanobacterial community might enhance the organic matter degradation process within the mat and in this way might render the mat self-sustainable. Hence, the high dissolved organic matter releases of the mats (which we measured in the water column directly above the benthic cyanobacterial mats (**MS III**)), are likely not a loss of valuable carbon, yet might provide an important food-source to the mat's bacterial communities. Another possibility how cyanobacterial mats can mobilize nutrients is the excretion of siderophores (Winkelmann & Drechsel 2008). These small compounds have a high affinity to iron and as a result can increase iron availability to the benefit of the mats. In conclusion, benthic cyanobacterial mats on coral reefs probably create optimum conditions to remobilize nutrients from sediment particles, as long as they have sufficient organic matter at their disposal to sustain growth and development. This theory could very well explain the reason why we found elevated phosphate concentrations at the surface of the cyanobacterial mats growing on both sandy sediments as well as hard substrates (**MS I**). If this is true, benthic cyanobacterial mats would thus have an additional source of nutrients, hence could sustain even in oligotrophic environments for prolonged periods of time without additional anthropogenic nutrient inputs.

A further interesting research topic is the growth rate of cyanobacterial mats. Potentially, cyanobacterial mats have very fast growth rates. In **MS IV+V** we found that they have, in comparison to corals, benthic algae and phytoplankton, the highest nutrient uptake rates and highest primary productivity. Yet, the question remains to which extent cyanobacterial mats in fact can grow. When is a mat considered 'fully developed'? One can imagine that the amount of light penetrating into the mat will ultimately diminish as filaments start overlapping, hence would result in a reduction of the photosynthetic activity. Energetically, this would be a highly undesirable scenario. Knowing the growth rates of fully developed mats, calculations can be made to the amount of nutrients necessary for them to persist on the reef. Results will most likely indicate that the growth rate of the mat will decrease with increasing mat thickness, thereby lowering the need for external nutrient supply.

The last interesting topic I would propose for future research is to better understand the supply of fixed nitrogen from the cyanobacterial community of the benthic mat to the other bacterial communities within the mat (i.e., heterotrophic bacteria, sulfate-reducing bacteria). Additionally, it would be of great interest to determine how much of the fixed nitrogen is also released to other coral reef communities (e.g. nearby corals, benthic algae etc.). In our studies, we have investigated nitrogen fixation rates using the acetylene-ethylene method (**MS II**). This is a powerful tool to investigate the total nitrogen fixation rate of the entire mat. However, it remains unclear if the fixed nitrogen stays within the cell of the nitrogen fixing organism, or whether it is released and transferred into the surrounding environment. Isotope measurements in combination with Nano Sims would be a powerful tool to investigate this. Unpublished data from our experiments indicated a release of >70% of the newly fixed nitrogen to the surrounding water column. These high rates have also been shown in other environments, such as by marine diazotrophic cyanobacteria, *Trichodesmium spp.* (Glibert & Bronk 1994, Mulholland et al. 2006, Wannicke et al. 2009). Since nitrogen fixation is an energy-demanding process, it would be of great interest to better understand why the bacterial mat community would continue fixing nitrogen, even when large quantities are expelled into the water column. Furthermore, it would be interesting to understand to what extent this additional nitrogen source aids further reef degradation.

> Research on benthic cyanobacterial mats on coral reefs is relatively new. Hence, many scientific questions remain to be unraveled to better understand its effects and persistence on coral reefs. Increased knowledge regarding nutrient mobilization within and underneath the mats in this context is vital. Furthermore, more research is needed to determine the contribution of benthic cyanobacterial mats to the nitrogen and carbon cycle of the reef ecosystem.

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10. Literature

(Introduction and Synopsis)

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