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Substrate as a driver of sponge distributions in mangrove ecosystems

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ABSTRACT: Caribbean mangrove-associated sponge communities are very distinct from sponge communities living on nearby reefs, but the mechanisms that underlie this distinction remain uncertain. This study aimed to elucidate the relative importance of substrate and habitat in determining the ability of sponges to persist in mangrove ecosystems, and to evaluate the role of bacterial symbiont composition and carbon uptake in sponge distribution. Two reef species (*Aplysina archeri* and *Desmapsamma anchorata*) were transplanted to mangrove roots and PVC tubes at a mangrove stand and a reef site. The mangrove species *Mycale microsigmatosa* was transplanted to both substrates in mangroves as control and showed complete survival. In contrast, lowest survival for *D. anchorata* was observed on roots in mangroves and intermediate survival on both PVC in mangroves and roots on the reef. Complete survival was observed on PVC on the reef. *A. archeri* had reduced survival in all treatments, but was most affected by the root substrate in mangroves. These results reveal that the inability of typical reef species to survive in mangrove ecosystems is related to habitat and substrate. The symbiotic bacterial communities were host specific and very similar before and after transplantation. The cluster analysis of metabolic diversity of bacterial communities in *A. archeri, M. microstigmatosa* and *D. anchorata* showed strong separation between host species and the surrounding water. It is speculated that compositional differences in dissolved organic matter (DOM) composition and symbiotic bacteria are potentially important in structuring sponge communities, explaining the exclusion of typical reef species and persistence of mangrove species in mangrove ecosystems.

KEY WORDS: Dissolved organic matter · Sponges · Bacterial symbionts · Reef · Mangrove roots

INTRODUCTION

Submerged roots of mangroves along sub-tropical and tropical Caribbean coasts serve as a substrate for a diverse and dense sponge community. The species composition of these mangrove-associated sponge communities is very distinct from sponge communities living on nearby reefs (e.g. van Soest 1978, 1980, 1984, Wulff 2004, Diaz 2012), but the mechanisms that underlie this distinction remain uncertain (for review see Wulff 2012). Transplantation experiments of typical reef sponges to roots on off-shore mangrove stands embedded in coral reefs revealed that reef species were able to grow well on roots and compete with typical mangrove sponges in the presence of spongivorous fishes (Wulff 2005). This suggests that biological interactions play a major role in steering sponge species composition. In contrast, typical reef sponges deteriorated quickly after transplantation to coastal mangroves, even in the absence of predation (Farnsworth & Ellison 1996, Wulff 2004, Pawlik et al. 2007), suggesting that other factors

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are also important for sponge survival and perseverance in mangrove ecosystems, although it remained uncertain which factor(s) was the key controlling variable(s).

Previous studies indicated that mangrove sponge community assembly mainly relies on small scale (i.e. among-root scale) processes (Guerra Castro et al. 2011), which might be directly related to the root substrate (Hunting et al. 2010a,b). Mangrove-derived organic matter leaching from the roots and decomposing litter are the primary carbon sources for sponges living in mangrove habitats (Granek et al. 2009), and bacterial symbionts play an important role in assimilating these organic carbon sources (de Goeij et al. 2008a,b, Ribes et al. 2012). However, mangrove-derived dissolved organic matter (DOM) consists mainly of tannins and polyphenolic compounds (Maie & Jaffe 2006), which are structurally complex and recalcitrant to biodegradation (Field & Lettinga 1992, Koch et al. 2005, Kristensen et al. 2008). Increasing evidence suggests that only a limited number of bacterial and fungal species are able to degrade complex polyphenols and tannins (Bhat et al. 1998) and it has indeed been demonstrated that sponges commonly associated with mangrove roots contain bacteria that are capable of degrading mangrove-derived DOM, while bacterial communities associated with sponges that are typical to reef environments appear less proficient in degrading mangrove-derived DOM (Hunting et al. 2010a). It is therefore hypothesized that the presence of bacterial endosymbionts that are capable of degrading mangrove-derived DOM may explain the observed differences in species composition between mangrove and reef sponge communities.

Evaluating the role of habitat, substrate and symbiotic bacteria in driving sponge distributions thus requires experiments that discriminate effects of substrate and habitat (i.e. surrounding water) on the survival and perseverance of sponges in mangrove ecosystems and assess symbiotic bacterial community composition and metabolic diversity upon transplantation. We therefore (1) monitored survival and condition of typical mangrove and reef sponge species after *in situ* reciprocal transplantation to DOM-releasing mangrove roots and DOM-free surrogate roots (PVC tubes) in both mangrove and reef environments, (2) determined the structure and stability of the symbiotic bacterial community in the sponge host before and after transplantation and (3) evaluated carbon utilization patterns of the symbiotic bacterial communities of the individual sponge species.

MATERIALS AND METHODS

Study site

For this study a location was chosen where reef and mangrove ecosystems were closely connected. Therefore, experiments were performed at the 'Spaanse Water' (12° 4′ 14.5″ N, 68° 51′ 36.8″ W), and 'Caracasbaai' (12° 4′ 11.4″N, 68° 51′ 43.8″W), on the island of Curaçao, Netherlands Antilles, Caribbean Sea (Fig. 1). The inner bay, Spaanse Water, is connected to the open sea by a small channel, and monopolized by the red mangrove *Rhizophora mangle*. Sponges are the dominant epibionts on fringing roots (on average >10% coverage per root; Hunting et al. 2008). Tidal ranges are approximately 10 cm, which does not cause emergence of the resident sponge community. Further details on physicochemical characterization are provided elsewhere (Hunting et al. 2008). Caracasbaai is an adjacent reef dominated by corals and sponges. Both sites were used for sponge collection and as transplantation sites.

Reciprocal transplantation experiment

Three sponge species were selected in April and July 2010 to investigate the effects of substrate and habitat on survival after transplantation: (1) *Mycale microsigmatosa*, which inhabits mainly mangrove habitats and is a dominant species in mangroves in the western Atlantic (Hunting et al. 2008, Wulff 2009), (2) *Desmapsamma anchorata*, an opportunistic

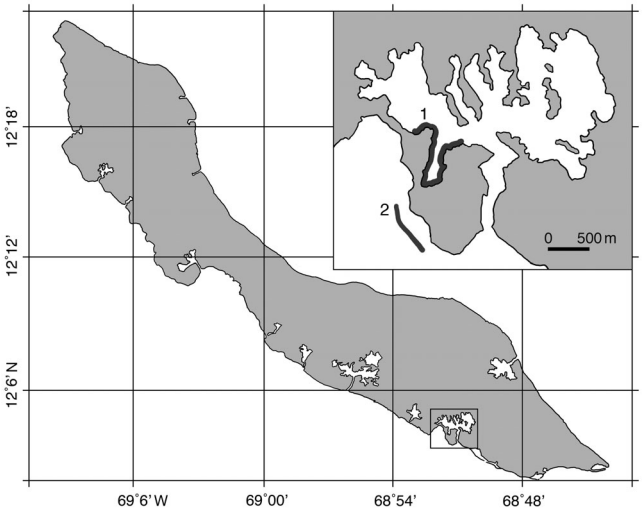

Fig. 1. Study areas on Curaçao. (1) Spaanse Water (mangrove site), (2) Caracasbaai (reef site)

species that abounds on reefs, but sometimes also occurs in mangrove habitats (McLean & Yoshioka 2008) and (3) *Aplysina archeri*, a vasiform species, commonly found on Caribbean reefs, but absent in mangroves, and therefore chosen to represent a typical reef species. Fragments of *M. microsigmatosa* (2 to 3 cm^2) were collected from mangrove roots and adjacent substrata in Spaanse Water at depths ranging from 0.9 to 2.3 m. Fragments of *A. archeri* (6 to 10 cm^3) and *D. anchorata* (2 to 6 cm^3) were collected from the Caracasbaai reef at depths ranging from 9.8 to 22.3 m and 4.8 to 8 m, respectively. Sponge fragments were transported in 25 l containers filled with natural seawater.

To distinguish between possible substrate and habitat effects, we used freshly cut prop roots of *Rhizophora mangle* and PVC tubes (∅ = 40 mm) as surrogate roots. PVC tubes were placed at both the mangrove site and reef site approximately 1 wk before the start of the experiment to allow development of a biofilm required for sponge attachment (Ellison et al. 1996). Cleaned prop roots were cut 2 d before the start of the experiment. All substrates were placed at 2 m depth in both habitats. Fragments of the reef sponges *Aplysina archeri* and *Desmapsamma anchorata* (n = 80 per treatment for both species) were subsequently transplanted to both cut mangrove roots and PVC tubes in both the mangrove site and reef site (total of 320 specimens per species). This resulted in 4 treatments for both reef species: Mangrove+Root, Mangrove+PVC, Reef+Root and Reef+PVC. Fragments of *Mycale microsigmatosa* (n = 80 per treatment) were transplanted as control to both cut mangrove roots and PVC tubes within the mangrove habitat to enable us to exclude potential effects of transplantation and cutting of the root substrate (total of 160 specimens), although this did not allow us to rule out potential negative effects of root cutting on reef sponges. Plastic cable ties, which did not affect the sponges during the experiment, were used for attachment. Survival was visually inspected after 0, 2, 4, 6, 8, 10, 14, 18, 24, 32 and 42 d. Newcombe (1998) was followed to approximate 95% confidence limits (CL) of the obtained proportions.

Desmapsamma anchorata is known for its unusually fast growth (Wulff 2005) and therefore an additional reciprocal transplantation experiment was performed to monitor changes in sponge tissue after transplantation of *D. anchorata* (n = 20 per treatment) to mangrove roots and PVC tubes in both the mangrove and reef habitat. Transplants were photographically monitored (Nikon digital underwater camera) on 5 occasions during 2 wk, and images were evaluated for survival, necrosis (formation of white patches) and development of new oscula. Newcombe (1998) was followed to approximate 95% CL of the obtained proportions.

Bacterial community structure

Subsamples were taken from the transplanted sponges to measure changes in bacterial community composition upon transplantation. Triplicate samples of all treatments were sampled immediately upon transplantation and 25 d after initial sampling for *Mycale microsigmatosa*. Triplicates were sampled for *Aplysina archeri* and *Desmapsamma anchorata* at the point that >60% of the reef sponge transplants died (50 and 29 d after initial sampling, respectively). In accordance with Dawson et al. (1998), subsamples were taken from the internal, healthy tissue (without noticeable necrosis) and stored in 100% dimethyl sulfoxide (DMSO). Bacterial DNA of the sponge-bacterial consortia was isolated in accordance with Hardoim et al. (2009). In brief, sponges stored in 100% DMSO were washed in 6.5 ml autoclaved water for 15 to 90 min and ground under liquid nitrogen to disrupt the sponge tissue and endoskeleton (mesohyl, mineral spicules and spongin fibers). DNA of ground samples was subsequently isolated using the UltraClean Microbial DNA Isolation Kit (MO BIO). An additional seawater sample (50 ml) at both sites was taken as control. DNA from filtered (0.2 µm NC 20 cellulose nitrate filter, Whatman) seawater samples was isolated using a PowerWater DNA Isolation Kit (MO BIO). Isolated DNA was amplified using the general bacterial forward primer F357 (GC CGC CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG CCT ACG GGA GGC AGC AG), which contained a GC clamp (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C) at the 5′ end. The reverse primer used was R518 (ATT ACC GCG GCT GCT GG) (Muyzer et al. 1993). The variable V3 region of the 16S ribosomal RNA gene was amplified on an MJ Research PTC-200 Thermo Cycler (St. Bruno), using the following conditions: initial denaturation at 94°C for 5 min; 35 cycles of denaturation (94°C, 30 s), annealing (54°C, 30 s) and extension (72°C, 1 min); final elongation at 72°C for 8 min; and cooling at 10°C for 15 min. DGGE was performed on a Bio-Rad DCode system, using 1 mm thick gels consisting of 8% (w/v) polyacrylamide (acrylamide:bisacrylamide ratio = 37.5:1). A linear denaturing gradient from 30 to 55% was used, where

100% denaturing solution contained 7 M urea and 40% (v/v) deionized formamide. The gels were run in 1× TAE buffer (Tris, acetic acid and EDTA) at 60°C and 200 V for 4 h, and stained with ethidium bromide. Images of banding patterns were subsequently analyzed with Gelcompar II (Applied Maths). Banding patterns of individual species were correlated by means of the unweighted pair group method with arithmetic mean (UPGMA) and Pearson's correlation coefficients, and differences between sponge species were tested with Mann-Whitney *U*-tests, where the within-group similarities were tested against the between-group similarities for each combination of species.

Metabolic diversity of sponge bacterial symbionts

Additional triplicate samples of *Mycale microsigmatosa, Desmapsamma anchorata* and *Aplysina archeri* were collected in January 2013. These were used to study differences in carbon resource utilization of the targeted sponge species' bacterial symbionts. These samples were stored at 4°C for 1 wk until analysis. Triplicate samples of the water of both the mangrove site (Spaanse Water) and the reef site (Caracasbaai) were included as control. Sponge samples were rinsed with ethanol (70%) for 30 s to reduce the potential contribution of superficial bacteria in the analysis. Bacterial endosymbionts of the sponge specimens were extracted from subsamples of sponge tissue (0.1 cm^3) in a Precellys 24 lysis/homogenizer (Bertin Technologies) using ∅ = 0.1- and 0.5-mm beads. Subsequent centrifugation was performed for 30 s at 11000 × *g* (Hunting et al. 2010a). We assessed bacterial metabolic diversity by community level physiological profiling using Biolog GN microplates containing 95 unique single substrates (Garland & Mills 1991, Garland 1997). The 95 substrates in the GN plate are comprised of simple, common substrates (e.g. sucrose, mallose and citric acid), selected on their ability to discriminate among bacterial isolates (Bochner 1989). Biolog GN plates do not include recalcitrant substrates or substrates typical of mangrove DOM and therefore this approach can only be used to illustrate that microbial communities are functionally similar or distinct (Garland 1999). Samples were incubated for 48 h and utilization patterns of 95 different single carbon sources were analyzed using a Jaccard-based cluster analysis and 1-way ANOSIM and a Bonferroni corrected pair-wise comparison (Hammer et al. 2001).

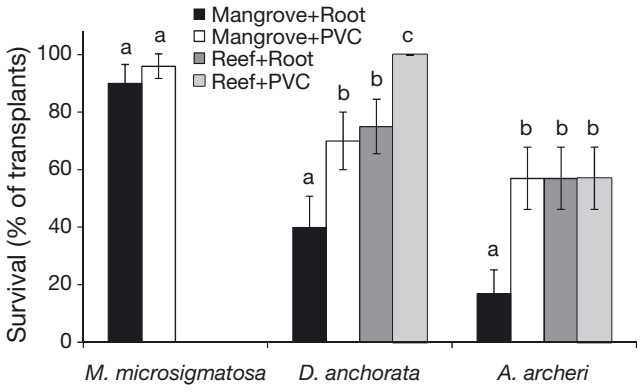

Fig. 2. Survival of *Aplysina archeri* (n = 320, *t* = 42 d) and *Desmapsamma anchorata* (n = 320, *t* = 15 d) after transplantation from the reef site to mangrove roots and PVC tubes at the reef and mangrove site, and *Mycale microsigmatosa* (n = 160, t = 57 d) after transplantation within the mangrove site to mangrove roots and PVC tubes. Error bars = approximated 95% CL. Bars sharing the same letters are not significantly different ($p < 0.05$)

RESULTS

Survival and condition of sponge species

In the control treatments, almost all specimens (>90%) of the sponge *Mycale microsigmatosa* survived transplantation to mangrove roots and PVC tubes within the mangrove system (Fig. 2), with no significant ($p > 0.05$) difference observed in survival between treatments. In contrast, only a small proportion (17%) of transplants of the sponge *Aplysina archeri* survived transplantation in the Mangrove+Root treatment. This was three times lower than transplants to mangrove roots and PVC tubes on the reef or onto PVC tubes in mangroves (all 57% survival) (Fig. 2). The poor survival rates of *A. archeri* in all treatments indicate that this species was negatively affected by the transplantation. The fact that the lowest survival was observed in the Mangrove+Root treatment, suggests that only substrate affected the survival of *A. archeri*. Transplants of the opportunistic sponge *Desmapsamma anchorata* performed better compared to transplants of *A. archeri*. *D. anchorata* showed a more gradual transition in mortality between the 4 categories than *A. archeri*, with lowest survival in the Mangrove+Root treatment, intermediate survival in the Mangrove+PVC and Reef+Root treatments, and complete survival in the Reef+PVC treatment (Fig. 2). These results indicate a combined effect of substrate and habitat on the survival of *D. anchorata.*

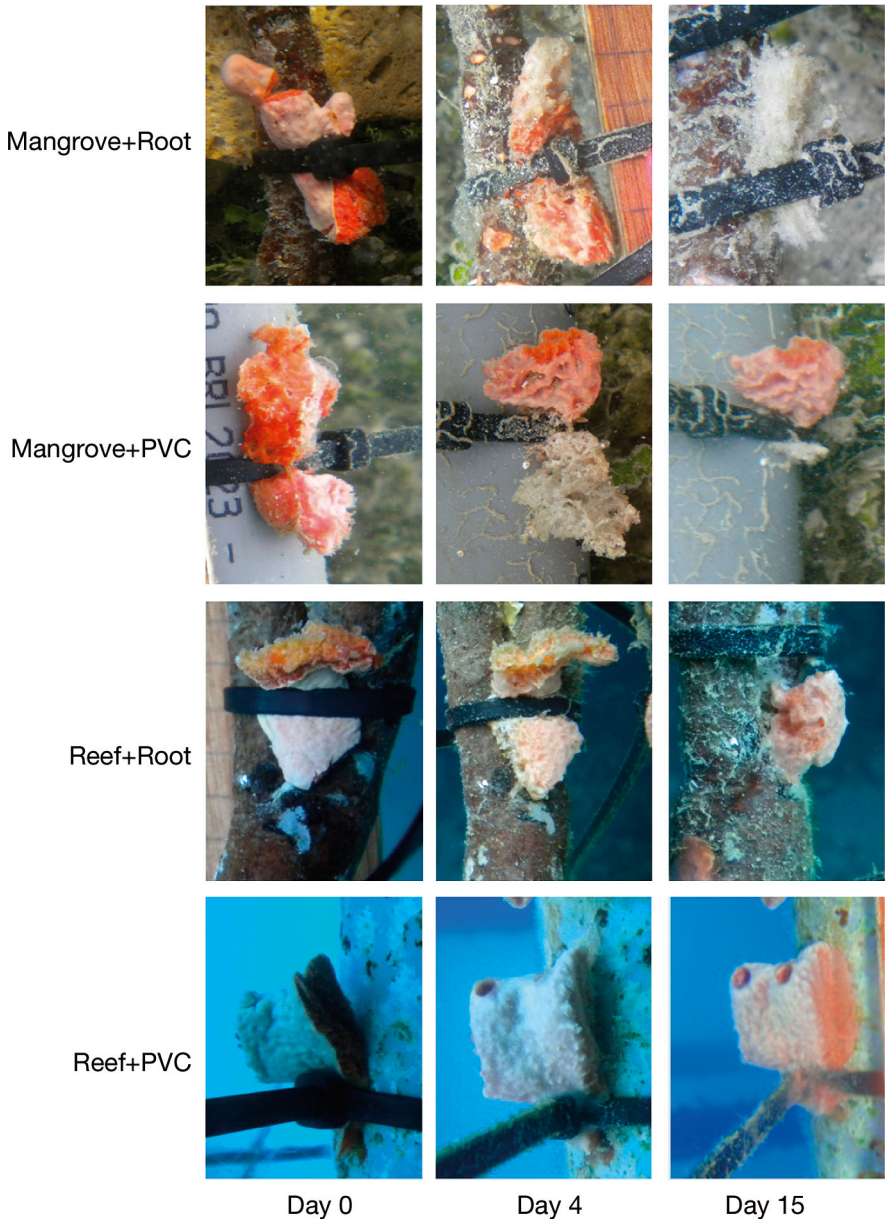

Fig. 3. Development of the sponge *Desmapsamma anchorata* after transplantation. Photographs were taken on Days 0, 4 and 15. The photographs are representative of all replicates for each treatment

In the photographic monitoring experiment, clear differences in survival, necrosis and oscula formation after transplantation of *Desmapsamma anchorata* were visible between the 4 categories studied. Representative photographs of the most dominant changes per treatment are shown in Fig. 3, and proportions are presented in Fig. 4. In the Mangrove+Root, Mangrove+PVC and Reef+Root treatments, necrosis was already clearly visible on Day 4, eventually resulting in total mortality in Mangrove+Root transplants and partial survival in specimens transplanted to Mangrove+PVC and Reef+Root. The stress caused by the mangrove habitat, particularly the Mangrove+Root combination, was visible as tissue degradation and alteration. In contrast, all specimens in the Reef+PVC treatment appeared healthy and formed a number of new oscula at the end of the experiment (Day 15), which was also the case for the majority of specimens (~60%) that survived transplantation in the Reef+Root treatment. Sponge condition generally reflected the same ranking as shown in Fig. 2, i.e. the worst condition was observed in the Mangrove+Root treat-

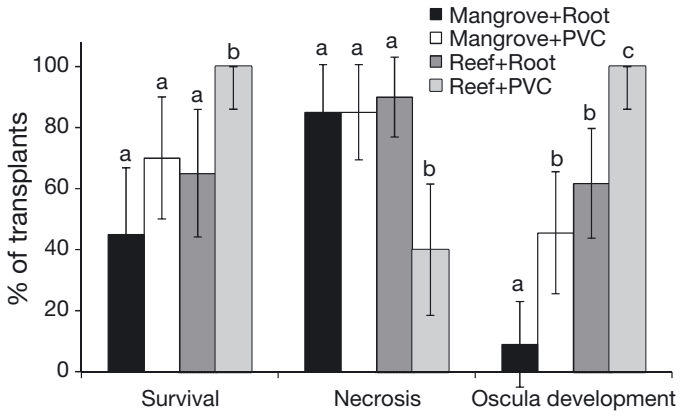

Fig. 4. Condition of the sponge *Desmapsamma anchorata* after transplantation. Condition is categorized by survival (after 15 d), necrosis (during experiment), and oscula development (after 15 d) as percentages of total number of transplants. Error bars = approximated 95% CL. Bars sharing the same letters are not significantly different ($p < 0.05$)

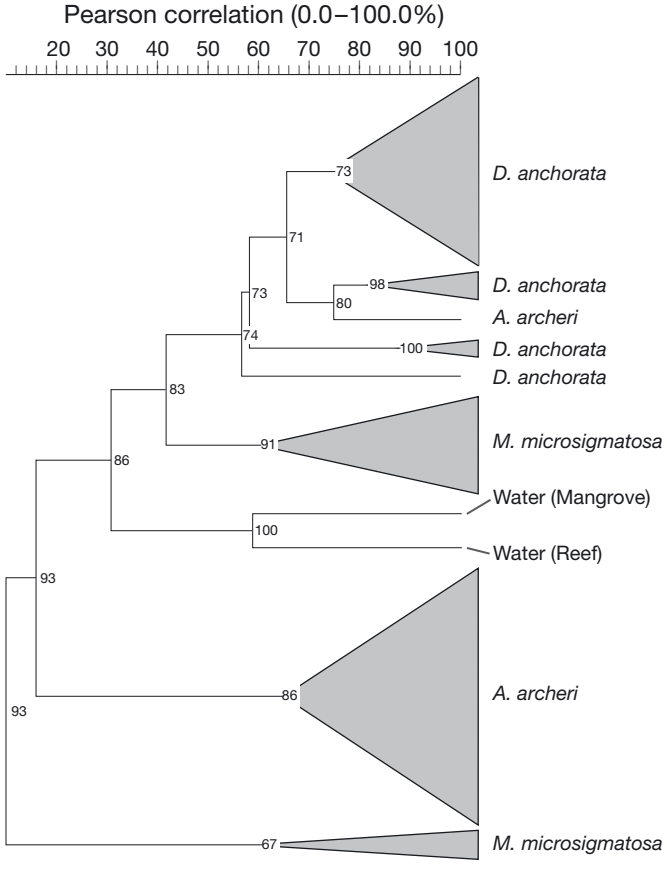

Fig. 5. Pearson's-based cluster analysis of sponge-associated bacterial community composition of samples of *Mycale microsigmatosa*, *Desmapsamma anchorata* and *Aplysina archeri* before and after transplantation. Microbial community composition was significantly different between species for all species combinations (Mann-Whitney *U*, $p < 0.001$)

ment, intermediate conditions in the Mangrove+PVC and Reef+Root treatments, while all specimens in the Reef+PVC treatment performed very well.

Bacterial community structure and metabolic diversity

Cluster analysis of the symbiotic bacterial community composition for replicates (n = 3) of the 3 sponge species (*Mycale microsigmatosa*, *Desmapsamma anchorata* and *Aplysina archeri*) and their surrounding waters revealed that the bacterial community composition depended largely on host species (Mann-Whitney *U*-test; $p < 0.001$ for all species combinations), irrespective of sampling time and habitat (Fig. 5). Samples of the surrounding waters of the reef and mangrove ecosystem also clustered together and were distinct from sponge endosymbiont communities (Fig. 5). DGGE banding patterns of individual sponge species were highly similar within each species (see Figs. S1, S2 & S3 in the Supplement at www.int-res.com/articles/suppl/m486p133_supp.pdf).

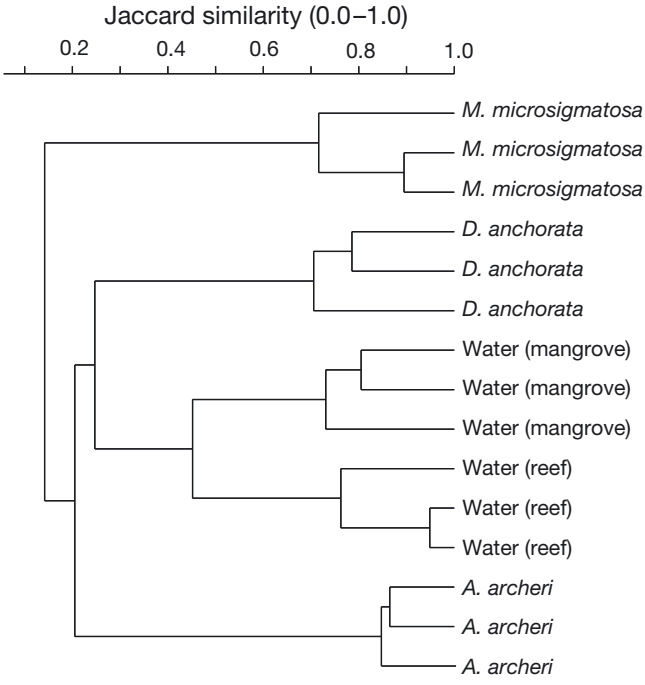

Fig. 6. Jaccard-based cluster analysis of 95 carbon sources (Biolog GN) for *Aplysina archeri*, *Desmapsamma anchorata*, *Mycale microstigmatosa*, and the control water samples at both the mangrove site and the reef site. Carbon resource utilization was significantly different between each sponge species and the control water samples (1-way ANOSIM, Bonferroni corrected pair-wise comparison, $p < 0.05$)

Bacterial communities derived from the 3 sponge species shared a number of carbon sources, but also used a number of unique carbon sources (see Fig. S4 in the Supplement), including glycerol and 2-aminoethanol (*Aplysina archeri*), D,L-camitine and succinic acid (*Desmapsamma anchorata*), and phenylethylamine and hydroxy-L-proline (*Mycale microsigmatosa*). Consequently, cluster analysis of metabolic diversity of bacterial communities in the sponge species *A. archeri, M. microstigmatosa* and *D. anchorata,* and the control water samples revealed a strong separation between sponge host species, as well as the water samples (Fig. 6) (ANOSIM, Bonferroni corrected pair-wise comparison, $p < 0.05$).

DISCUSSION

Transplantation of the typical reef sponge *Aplysina archeri* and the opportunistic sponge *Desmapsamma anchorata* onto roots in both the reef and mangrove ecosystem resulted in high mortality. This result is in line with previous transplantation experiments, where typical reef species were similarly transplanted to mangrove roots and in which potential effects of competition were excluded (Ellison & Farnsworth 1992, Pawlik et al. 2007). Photographic recordings of *D. anchorata* revealed substantial necrosis and mortality in all mangrove and root-related treatments, while specimens transplanted to PVC tubes on the reef developed very well. This outcome illustrates that, in addition to biotic interactions such as predation and competition for space (Wulff 2005) and extreme fluctuations in abiotic conditions (Pawlik et al. 2007), root substrate is important in determining survival of typical reef species in mangrove systems. A large part (~50%) of the total *A. archeri* transplants did not survive transplantation. Since *A. archeri* typically occurs much deeper than the depth used in our study, it is possible that *A. archeri* transplants were adversely affected by stressful light intensities. Despite this, survival of *A. archeri* was clearly reduced when transplanted to roots in mangroves, thereby suggesting a substrate effect. *D. anchorata* developed well on PVC tubes on the reef, but survival of *D. anchorata* was reduced when transplanted to roots on the reefs site and PVC in mangroves, demonstrating that, besides the root substrate, the surrounding water, or habitat in general, also affected *D. anchorata* in mangrove ecosystems. Both substrate and abiotic constraints thus affected survival and hampered perseverance of typical reef sponges in mangrove ecosystems.

The questions remain why typical mangrove sponge species such as *Mycale microsigmatosa* are capable of maintaining viable populations on mangrove roots in coastal mangrove ecosystems, and why typical reef species are negatively affected by the root substrate. We hypothesize that bacterial symbionts play an important role. Although there is a growing amount of literature on community composition of sponge endosymbionts, little is known about shifts in these communities induced by biotic and abiotic factors and over time (Thacker & Freeman 2012). Although we only sampled 2 time points, profiling bacterial communities by means of DGGE suggested that the symbiotic communities in the sponges remained mainly host specific, irrespective of time and treatment (substrate and habitat). This is in line with other studies that considered symbiotic bacterial communities over larger temporal scales (e.g. Hentschel et al. 2002, Yang et al. 2011, Erwin et al. 2012).

Our study further revealed that the host-specific bacterial symbionts used different carbon sources, suggesting that each sponge symbiotic bacterial community has its own specific resource niche, as frequently observed for free living environmental bacterial communities (e.g. Salles et al. 2009, Gravel et al. 2011). However, the use of Biolog plates comes with limitations, as ecological relevant substrates are not captured in this assay, and we therefore cannot directly relate substrate utilization profiles to distribution patterns of sponge-bacterial consortia. Nonetheless, we speculate that differences in resource niches could potentially be important, especially considering that DOM often differs both in composition and concentration between mangrove and reef habitats (e.g. Dittmar et al. 2006), although this was not characterized in this study. The variation in bacterial communities within sponges coincide with metabolic differences between sponge species (Weisz et al. 2007), and that rates of dissolved organic carbon (DOC) and nutrient uptake by sponges largely depend on the concentration of symbiotic bacteria (Ribes et al. 2011). Evidence for sponge resource preferences is also provided by studies that considered isotope ratios. For instance, van Duyl et al. (2011) showed that encrusting sponges in reef cavities feed mainly on DOM derived from crustose coralline algae and coral mucus, while mangrove-derived organic matter is an important carbon source for sponges that typically occur in mangrove ecosystems (Granek et al. 2009). Although the utilization profiles of simple substrates obtained in this study do not directly

relate to mangrove DOM, they complement a previous study that reported differences in the ability of bacterial symbionts to degrade mangrove DOM (Hunting et al. 2010a). Bacterial symbionts of mangrove sponges appeared proficient in degrading mangrove DOM, while symbionts of reef sponges were less capable of degrading mangrove DOM. Earlier studies also showed that common mangrove-associated sponges grew faster when attached to mangrove roots compared to PVC tubes, while typical reef species performed better on PVC than on the root substrate (Ellison et al. 1996, Wulff 2005). Although this remains speculative and requires further investigation, it suggests that typical mangrove species potentially have a competitive advantage over reef species when growing on mangrove roots, and that the composition of DOM can be of general importance to the performance of sponge–bacterial consortia. However, since mangrove DOM consists of a complex mixture of structurally diverse compounds, the identification of specific bacterial gene-clusters (e.g. through pyrosequencing) and relevant compounds in DOM (e.g. through GCMS) remains a major challenge. The ultimate challenge is to understand how these components interact and relate to the overall performance of sponge–bacterial associations under natural conditions.

In conclusion, increased mortality and poor development upon reciprocal transplantation revealed that the inability of typical reef species to survive in mangrove ecosystems is due to a combined effect of abiotic constraints and the root substrate. Our results further suggest that bacterial symbionts are largely host specific and have a specific DOM resource niche. This suggests that differences in DOM composition and corresponding differences in symbiotic bacterial communities are potentially important in structuring sponge community composition. This explains the exclusion of typical reef species, and the persistence of mangrove species, in mangrove ecosystems.

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