An assessment of sand quality and potential impacts on corals at the Chogogo Dive and Beach Resort artificial beach – Part 2: Microbial Communities

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Wageningen University &
Research report: C004/24

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Wageningen Marine Research

Wageningen Marine Research
Den Helder, January 2024

Wageningen Marine Research report C004/24

Keywords: Artificial beach sand, coral reef, microbes, Bonaire.

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This report can be downloaded for free from https://doi.org/10.18174/647214

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KvK nr. 09098104,
WMR BTW nr. NL 8113.83.696.B16.
Code BIC/SWIFT address: RABONL2U
IBAN code: NL 73 RABO 0373599285

Contents

Samenvatting deel 2: Microbiële gemeenschappen[1] **4**

Summary **6**

1 Introduction **7**

2 Results and observations **8**

2.1 Microbial Communities 8
2.2 Observations: possible human pathogens 12

3 Conclusions and recommendations **12**

Acknowledgements **14**

Quality Assurance **14**

References **14**

Justification **16**

Appendices **17**

Appendix 1: Statistical analyses 17
Community Differences 17
Community Heterogeneity 17
Community Correlation to Organic Matter 17
Sphingomonas Relative Abundances 18
Kocuria Relative Abundances 19
Appendix 2: Materials & Methods 19

Samenvatting deel 2:

Microbiële gemeenschappen[1]

De Overheid van Bonaire (OLB) heeft Wageningen Marine Research (WMR) verzocht om onderzoek te doen naar de samenstelling van het zand dat gebruikt wordt voor de aanleg van het kunstmatige strand van het Chogogo Beach and Dive Resort. We verwijzen naar Meesters et al. 2022[2] (deel 1), waar de sedimentanalyse wordt gepresenteerd dat concludeert dat het kunstmatige zand bestaat uit fijnere korrelgroottes en een lager organisch materiaalgehalte heeft dan het natuurlijke zand. In deel twee van het rapport, 'Microbiële gemeenschappen'[1], breiden we de resultaten uit om te evalueren of deze verschillen in zandsamenstelling ook verschillende microbiële gemeenschappen laten zien en de mogelijke effecten die dit kan hebben op het zeeleven. Het kunstmatige zand van het strand van Chogogo werd op 10 mei 2022 bemonsterd. Daarnaast, om het natuurlijke zand dat rond Bonaire voorkomt te vergelijken met het kunstmatige zand, werd natuurlijk zand verzameld op vier locaties: voor Chogogo, aan weerszijden van Chogogo, en op Te Amo Beach (Figuren 1-2 van het rapport). De microbiële gemeenschappen in de zandmonsters werden gesequenced (vaststellen van volgorde van de nucleotiden in het DNA) aan de Universiteit van Hawaï.

Conclusie De resultaten laten zien dat de microbiële gemeenschap in het kunstmatige zand significant verschilt van de microbiële gemeenschap in natuurlijk lokaal zand. Het natuurlijk voorkomende zand bevat meer zouttolerante microben, terwijl het kunstmatige zand meer microben bevat die bestand zijn tegen hoge temperaturen, uitdroging, straling en oxidatieve stress. Potentiële pathogenen voor koraal en mens verschilden in hoeveelheid tussen het kunstmatige en natuurlijke zand. De resultaten van de analyse en deskundigenevaluatie hebben geleid tot de volgende antwoorden op specifieke vragen van het OLB:

1) Wat zijn de mogelijke effecten op het zeeleven, specifiek koralen, met betrekking tot het aangelegde strand?
Met betrekking tot de microbiële samenstelling zou het kunstmatige zand van het aangelegde strand op twee manieren invloed kunnen hebben op het zeeleven: 1) door veranderingen in ecologische functies en 2) door te dienen als een reservoir voor koraalpathogenen. Beide mogelijke effecten hebben echter een laag risico, maar we raden aan voorzorgsmaatregelen te nemen met betrekking tot de verspreiding van kunstmatig zand vanwege het belang van het rif voor Bonaire, zoals beschreven in Meesters et al. 2022[2].

Wat betreft het eerste punt zullen de significante verschillen in de samenstelling van de microbiële gemeenschappen, het aantal specifieke soorten en het gehalte aan organisch materiaal waarschijnlijk resulteren in verschillen in ecologische functies. Met name afzetting van zand op het rifoppervlak kan leiden tot veranderingen in de nutriëntencyclus. Veranderingen in nutriënt-concentraties in het rif kunnen negatieve gevolgen hebben voor de gezondheid van koralen, zowel omdat koralen gevoelig zijn voor deze omgevingscondities als ook omdat ze concurrenten van koralen kunnen bevorderen, zoals macroalgen en benthische cyanobacteriematten. Wat betreft punt 2 kan het geïmporteerde kunstmatige zand fungeren als een reservoir voor mogelijke koraalpathogenen. Het aangetroffen genus *Sphingomonas* wordt geassocieerd met de koraalziekte 'White Plague'. Het voorkomen van 'White Syndrome' ziekten, waaronder White Plague en andere koraalziekten met vergelijkbare symptomen, neemt toe bij cumulatieve blootstelling aan hogere temperaturen. Een hoge mate van blootstelling aan pathogenen tijdens periodes van opwarming, zoals bijvoorbeeld in september-november 2023, verhoogt het risico op ziekte-uitbraken.

2) Wat zijn de mogelijke effecten op de gezondheid van de koralen onder normale weersomstandigheden?
Aangezien het kunstmatige zand al mengt met natuurlijk zand en naar zee wordt getransporteerd, is het waarschijnlijk dat microben die normaal niet in het gebied worden aangetroffen al zijn geïntroduceerd. Dit is met name het geval voor stressbestendige microben zoals *Deinococcus*. Het effect van deze introducties is moeilijk te voorspellen. Er zijn geen negatieve effecten bekend van deze microben voor het zeeleven, maar het introduceren van onbekende microben in het milieu kan risico's met zich meebrengen.

3) Wat zijn de mogelijke effecten op de gezondheid van de koralen tijdens een storm of windomkering?
Grote hoeveelheden kunstmatig zand die op het rif worden afgezet kunnen leiden tot de vestiging van exotische microben in de bovenste sediment laag, wat op lokale schaal kan leiden tot veranderingen in de nutriëntencyclus. De omvang van dit effect en de negatieve gevolgen ervan voor koraal zijn moeilijk te voorspellen aangezien dit afhankelijk is van de concurrentie tussen de geïntroduceerde en inheemse microben. Veranderde nutriëntencycli kunnen de vestiging van concurrenten van koraal, zoals macroalgen en benthische cyanobacteriematten bevorderen. Bovendien kunnen koralen die bedekt zijn met zand vatbaarder zijn voor infecties. Dit, in combinatie met de hogere aanwezigheid van *Sphingomonas* in het kunstmatige zand, kan leiden tot een toename van het voorkomen van 'White Plague' ziekte in het gebied.

4) Hoe belangrijk is de oorsprong van het zand, het type zand (rivier- of carbonaatzand), de hoeveelheid zand, de indeling van het strand en de aangelegde muur voor de effecten?

Wat betreft microbiële gemeenschappen zijn al deze factoren belangrijk omdat ze bepalen welke exotische microben (inclusief pathogenen) aanwezig zijn en de waarschijnlijkheid dat ze permanent in het milieu worden geïntroduceerd. De oorsprong en behandeling van het zand bepalen de microben die in het eindproduct kunnen worden gevonden. Het is waarschijnlijk dat stressbestendige microben zijn aangetroffen in het kunstmatige zand vanwege de behandeling die het heeft ondergaan voordat het werd afgeleverd. Zoals beschreven in het hoofdrapport van Meesters et al. 2022, beïnvloeden de hoeveelheid zand, de indeling van het strand en de aanwezigheid van de aangelegde muur de erosie van het strand. Een frequente erosie van het kunstmatige strand of het transport van grotere hoeveelheden zand naar het rif vergroot de kans dat uitheemse microben zich vestigen en een negatieve invloed uitoefenen op de koraalgemeenschap.

[1] Kemenes, T., Meesters, E., Martinez, S., Becking, L.E. (2024). An assessment of sand quality and potential impacts on corals at the Chogogo Dive and Beach Resort artificial beach – Part 2: Microbial Communities (Report No. C004/24). pp. 19

[2] Meesters, E., de Hart, M., & Dogruer, G. (2022). An assessment of sand quality and potential impacts on corals at the Chogogo Dive and Beach Resort artificial beach (Report No. C062/22). Wageningen University & Research. https://edepot.wur.nl/579171.

Summary

The Government of Bonaire (OLB) has requested Wageningen Marine Research (WMR) to research the composition of the sand used to construct the artificial beach of the *Chogogo Beach and Dive Resort*. The main aim was to evaluate whether the artificial sand that is being used could harm the corals in the marine park. The artificial sand of the beach of Chogogo was sampled on the 10th of May 2022. Additionally, to compare the sand that naturally occurs around Bonaire to the artificial sand, natural sand was collected at four locations: in front of Chogogo, on either side of Chogogo, and at Te Amo beach (Figures 1-2). The microbial communities in the sand samples were sequenced at the University of Hawaii.

Overall, the microbial community in the artificial sand is significantly different than the microbial community in natural local sand. The naturally occurring sand contains more salt-tolerant microbes while the artificial sand contains more microbes that are resistant to high temperatures, desiccation, radiation, and oxidative stress. Potential coral and human pathogens differed in abundance between the artificial and natural sands. The results of the analysis and expert evaluation have resulted in the following responses to specific questions that were posed by OLB:

1) What are the potential effects on marine life, specifically corals, in relation to the constructed beach?
We refer to Meesters et al. 2022[1], where the sediment analysis is presented and shows that the artificial sand is composed of finer grain sizes and lower organic matter content than the natural sand. Here we extend the results to evaluate whether this different sand composition also shows different microbial communities and the effects that may have on marine life. With respect to the microbial composition, the artificial sand of the constructed beach could potentially impact marine life in two ways: changes in ecological functioning and possible reservoir for coral-pathogens. Both possible impacts have a low risk, however, we recommend that precautions regarding the spread of artificial sand are implemented due to the importance of the reef to Bonaire, as outlined in Meesters et al. 2022[1].

First, the significant differences in 1) composition of microbial communities, 2) abundances of specific species and 3) organic matter content, likely will result in differences in ecological functioning. Particularly, deposition of sand on the reef surface may result in altered nutrient cycling. Changes to nutrient concentrations in the reef can have negative consequences for coral health, both because corals are sensitive to these environmental conditions and because they can promote coral competitors such as macroalgae and benthic cyanobacterial mats.

Second, the imported artificial sand may act as a reservoir for potential-coral-pathogens (*Sphingomonas*)[2]. *Sphingomonas* has been associated with the coral disease White Plague. The prevalence of White Syndrome diseases, which includes White Plague and other coral diseases with similar symptoms, increases with cumulative heat exposure[3,4,5]. During warming events such as the one seen in September-November 2023[6], increased exposure to pathogens linked to these syndromes increases the risk of disease outbreaks[3,7,8].

2) What are the potential effects on the health of the corals under normal weather conditions?
As the artificial sand is already mixing with natural sand while being transported towards the sea, it is possible that microbes not normally found in the area have already been introduced to the environment. This is particularly the case for stress-resistant microbes such as *Deinococcus*. The effect of these introductions is difficult to predict. No known negative effects for marine life are recorded for these microbes. Nevertheless, introducing microbes may carry unknown risks[9,10].

3) What are the potential effects on the health of the corals during a storm or wind reversal?
Large amounts of artificial sand deposited on the reef may result in the establishment of foreign microbes in the top sediments, leading to altered nutrient cycling at local scales. The extent of this effect and its negative consequences on coral are difficult to predict, as this depends on the competition between the introduced and native microbes. Altered nutrient cycling can favor the establishment of coral competitors such as macroalgae and benthic cyanobacterial mats. Furthermore, corals smothered by sand can be more susceptible to infections. This, in combination with the higher abundance of *Sphingomonas* in the artificial sand, may lead to an increase in the occurrence of White Plague disease in the area.

4) How important is the origin of the sand, the kind of sand (river or carbonate sand), the quantity of sand, the layout of the beach, and the constructed wall for the effects?
In terms of microbial communities, all of these factors are important because they determine the presence of foreign microbes (including pathogens), and the likelihood of introducing them permanently to the environment. The origin and treatment of the sand will determine the microbes that can be found in the final product. It is likely that stress resistant microbes are found in the artificial sand due to the treatment that it underwent prior to delivery. As outlined in the main report of Meesters et al. 2022[1], the quantity of sand, layout of the beach, and the presence of the constructed wall influence the erosion of the beach. More frequent erosion of the artificial beach or transport of larger

amounts of sand onto the reef increases the likelihood that foreign microbes become established and exert a negative impact on the coral community.

1 Introduction

The Government of Bonaire has requested Wageningen Marine Research (WMR) to investigate the composition of the sand used to construct the artificial beach of the *Chogogo Beach and Dive Resort* (Chogogo) (Terms of Reference in the email of the 18th of January 2022). The sand of the beach of Chogogo was sampled on the 10th of May 2022 and additionally, the natural sand in front of Chogogo and several other places was sampled to better compare the sand that naturally occurs around Bonaire to the artificially placed sand. All sand samples were sequenced at the University of Hawaii at Manoa to characterize microbial communities. The Center for Microbial Oceanography is a respected center of excellence for the analysis of microbial communities, carrying out research on nutrient feedbacks in coral reefs as well as long-term monitoring of Pacific reefs for NOAA and the microbiology of Mo'orea's coral reef.

WMR was asked to evaluate whether the sand used to construct the beach of Chogogo Beach and Dive Resort can harm the corals in the marine park. More specifically, the following questions were posed:

1. What are the potential effects on marine life, specifically corals, in relation to the constructed beach?
2. What are the potential effects on the health of the corals under normal weather conditions?
3. What are the potential effects on the health of the corals during a storm or wind reversal?
4. How important are the origin of the sand, the kind of sand (river or carbonate sand), the quantity of sand, the layout of the beach, and the constructed wall for the effects?

Figure 1. Samples taken at Chogogo. Black filled circles are samples for DNA analysis, blue circles denote sand samples for grain size analysis. Numbers refer to waypoints used in sample and data processing.

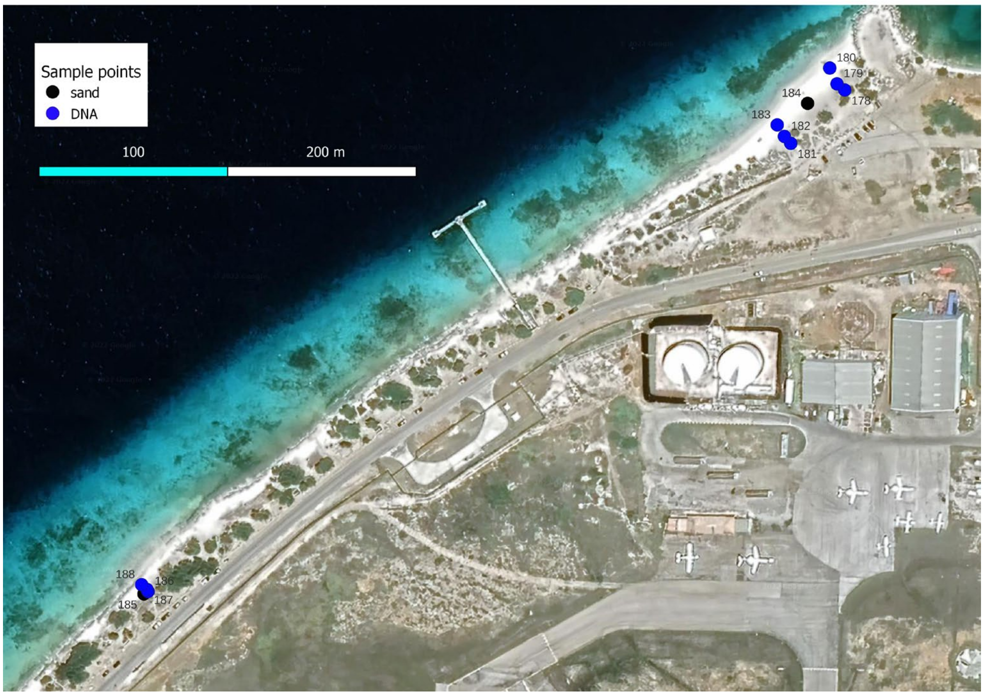

Figure 1. Samples taken at other locations. Symbols as in Figure 1.

2 Results and observations

2.1 Microbial Communities

The microbial communities found in the artificial sand are significantly different from those found in the natural beach sand in the area (Figures 3-4; PERMANOVA, $p < 0.001$). The artificial sand samples are more homogeneous across samples compared to the natural sand (ANOVA on distances to group centroids with betadisper, $F_{1,18} = 9.96$, $p = 0.006$). The introduction of artificial sand to the reef may thus modify the microbial composition locally. This is already apparent in samples taken near the path separating the artificial sand and the natural sand (Figure 1), where mixing was already observed at the time of sampling (Figures 5-7). Specifically, locations 108 and 114 in Figure 3, both close to the path, show intermediate composition between those seen in the artificial sand and the natural sand. Different microbial communities are likely to perform different functional roles because they have different metabolisms (see also Table 1), which affects the concentrations of different inorganic nutrients in the environment[11,12]. The microbial communities were significantly correlated to the organic matter (OM) content of the nearest sediment sample collected for OM analysis ($R^2 = 0.65$, $p < 0.001$), supporting likely functional differences between the microbes found in each sand type.

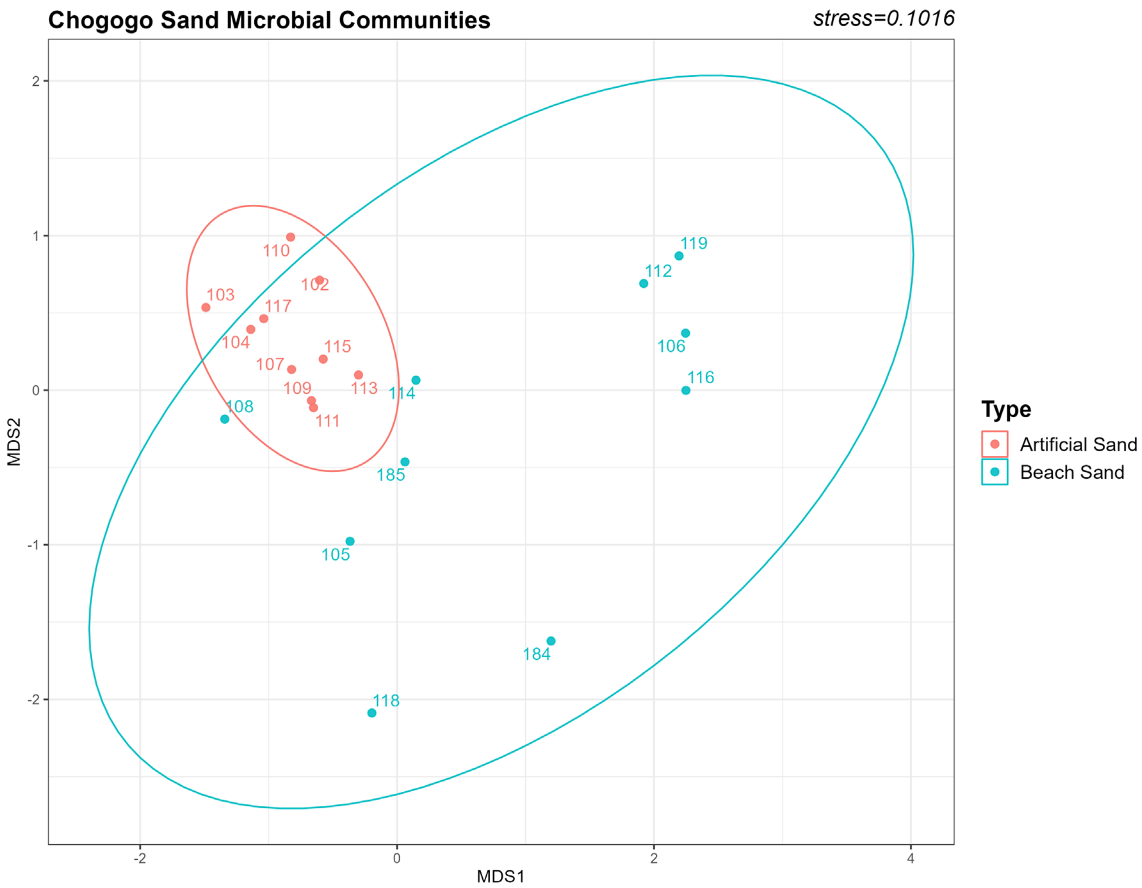

Figure 3. Ordination plot of the microbial communities from the artificial (red) and beach (blue) sands. Numbers refer to the samples and locations as shown in Fig. 1. Each dot refers to the composition of the microbial community in a sample. Dots close to each other indicate microbial communities that are more similar than dots (samples) further away. Samples from the artificial sand as a group are closer together than samples from the normal sand. This means that, in terms of microbes, the samples from the artificial beach are all relatively similar in composition and relative abundance. Some normal beach samples are ordinated close to the artificial beach samples (108, 114) which may indicate mixing of the two sand sources.

The microbial communities are significantly different because of 1) differences in the composition, i.e. presence or absence of species among samples, 2) differences in abundances of the same microbes in each sample. In the results presented there are also microbes that are not shared between the artificial and natural sand (Figure 4, Table 1). Microbes present in the natural sand that are absent in the artificial sand are generally salt-tolerant (e.g. Halobacterota, *Salinimicrobium*) (Table 1). Microbes present in the artificial sand that are absent in the natural sand are generally tolerant to various forms of stress including high temperatures, desiccation, radiation, and oxidizing agents (Table 1). This does not necessarily mean that the presence of these microbes will lead to impacts on the coral community, as none of these microbes are known to be pathogenic. However, stress tolerance suggests that these microbes are likely to remain in the environment once introduced.

One potential coral pathogen was found in both the artificial and natural sands, namely *Sphingomonas*. While the causative pathogen behind White Syndrome diseases has not been determined[13], this genus has been associated to White Plague, occurring in high abundances in infected corals of multiple species[2,14]. This genus was found to be significantly more abundant in artificial sand than in natural sand (Figure 4, Welch Two Sample t-test on 4^{th}-root transformed relative abundance data, $t = 2.86$, $df = 10.403$, $p = 0.016$). The median abundance of *Sphingomonas* in natural sand was 0.11%. One exception was the control sampling location 185 (Te Amo beach, Figure 2), which showed an abundance of 2.13%. This is similar to the median abundance of 2.11% in the artificial sand. Thus, *Sphingomonas* can be found in abundances of around 2% in natural sand on Bonaire. However, the only samples with similar abundances in the natural sand at Chogogo were located next to the path were mixing is occurring. These communities may not be representative of the rest of the natural sand in Chogogo, in which *Sphingomonas* is less abundant or absent (Figure 4). Therefore the high abundance of *Sphingomonas* in the artificial sand is highlighted as a change in the local microbial communities of Chogogo.

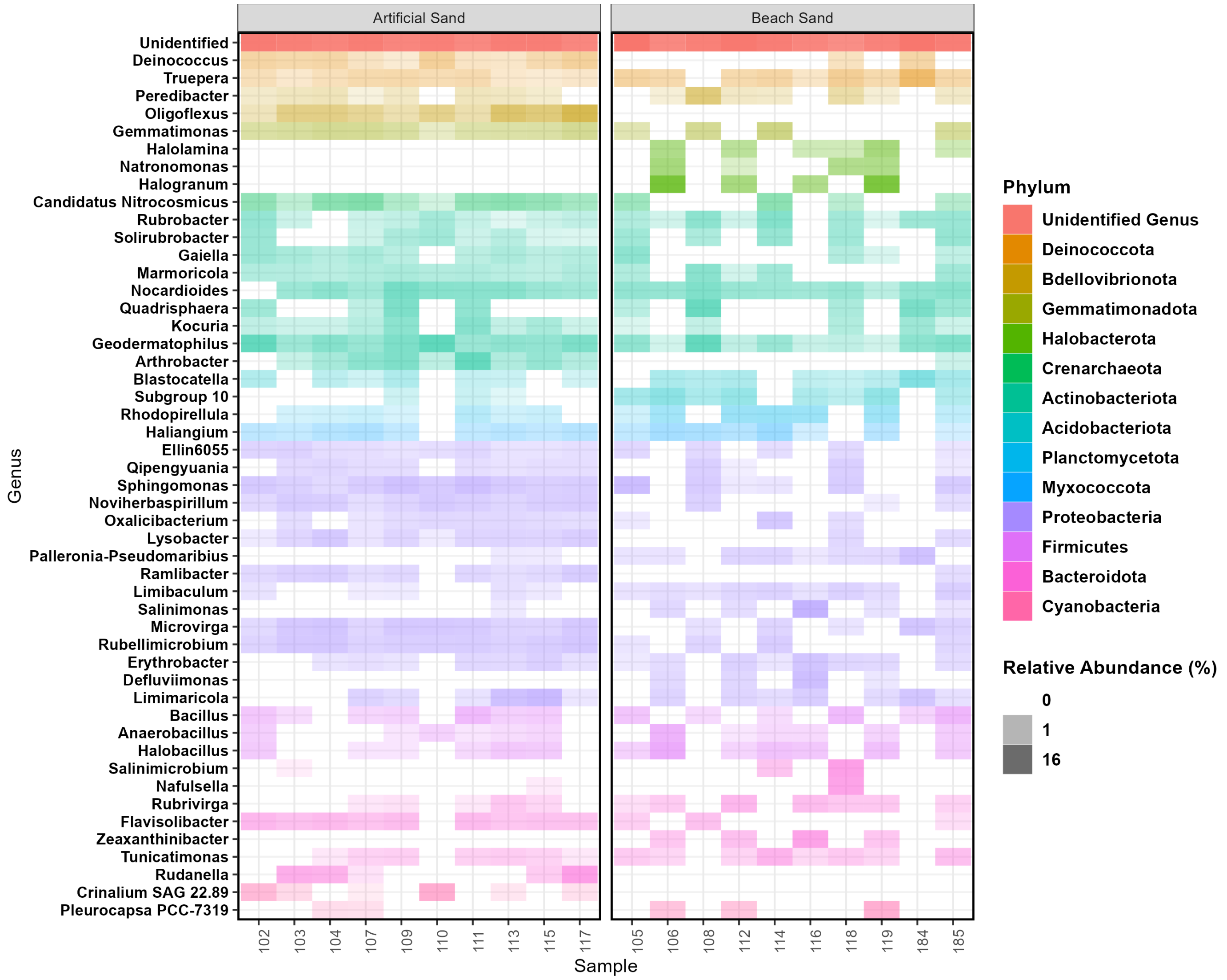

Figure 4. For each sample the composition is shown. The intensity of the color indicates the relative abundance in the sample (darker means higher abundance). Differences between the two sand sources are clear. Some genera are almost completely lacking in the (natural) beach sand and vice versa, indicating that the microbial communities as a whole differ strongly and likely function differently ecologically.

Table 1. Discriminating microbial genera in the two sand sources.

	Mainly Present in Artificial Sand	**Mainly Present in Natural Beach Sand**
Genera	*Deinococcus* *Oligoflexus* *Arthrobacter* *Lysobacter* *Ramlibacter* *Rudanella* *Crinalium SAG 22.89*	3 Halobacterota genera *Palleronia-Pseudomaribius* *Salinimonas* *Defluviimonas* *Salinimicrobium* *Nafulsella* *Zeaxanthinibacter*
Associated Features	- *Deinococcus* is known as an extremophile highly resistant to radiation, desiccation, and oxidizing agents[15]. - Many of these genera are composed of species that are aerobes (organisms that use oxygen)[16,17] - Most of these genera are known to have species that are resistant to various forms of stress (temperature, desiccation, radiation, Reactive Oxygen Species)[15,16,18,19]	- Many of these are specifically associated to high salinity (halophiles)[20,21,22]

Figure 5. Sand can be seen accumulating along the retention wall.

Figure 6. On the southern side of the Chogogo area, the artificial sand has mixed with the natural sand.

Figure 7. The sand in several places is already being transported over the edge of the wall.

2.2 Observations: possible human pathogens

As an observation we also note the occurrence of potential human pathogens in the microbial communities. All of the identified human pathogens are common in the environment, but are known to lead to infections in hospital settings where patients have weakened immune systems. We recommend further assessment of the risk posed by these microbes. First, the genus *Kocuria* was more abundant in the artificial sand than the natural sand (Welch Two Sample t-test on 4^{th}-root transformed relative abundance, $df = 17.9$, $t = 2.12$, $p = 0.048$). *Kocuria* is a genus that is generally not pathogenic, but it has been described as an emerging bacterial infection[23]. Second, when identifying microbes with the GenBank database, two sequences that were present <u>only in the artificial sand</u> had a 97% match with *Clostridium perfringens* and *Acinetobacter baumannii*. The relative abundance of each of these sequences was in the top 25% of identified microbes. As with *Kocuria*, these microbes commonly occur in the environment, but have been associated to opportunistic infections in hospitals[24,25]. Note that these sequences also matched other related species in the same genera. Therefore, it is not clear if these bacteria pose additional risk and they are only highlighted for further investigation.

3 Conclusions and recommendations

Below are the questions that were asked to Wageningen Marine Research and the provided answers:

Question 1: What are the potential effects on marine life, specifically corals, in relation to the constructed beach?
Answer: Meesters et al. 2022[1] described the effect of sediment load on corals, namely smothering, shading, abrasion, and inhibition of coral recruitment. With respect to the microbial composition, there are two potential effects of the constructed beach. The risk of both effects is low, but we suggest precautions against the erosion of the artificial sand are maintained given the importance of reefs to Bonaire:

First, the ecological functioning of the microbial fauna in the deposited substrate will likely differ due to the different microbial composition and differences in organic matter. Microbial communities in reef sediments play an important role in nutrient cycling[11,12], which may be disturbed by introducing foreign sand with different microbial communities. Nutrient concentrations in the reef are important for coral health, and changes to these can promote the growth of coral competitors such as macro-algae. Whether a negative impact will occur is difficult to predict, as it depends on the competition between microbes in the sand and the amount of artificial sand deposited on the reef.

Second, the artificial sand contains a high abundance of *Sphingomonas*, which has been associated with White Plague disease in corals[2,14]. The artificial sand thus acts as a reservoir for this potential coral pathogen[7,13]. The abundance of *Sphingomonas* was however found to be similarly high at other beach locations in Bonaire (Te Amo beach), but not in the natural beach in front of Chogogo. Whether the presence of this microbe at higher abundance translates into higher occurrence of coral disease is unknown. In the context of other stressors, such as the high temperatures and associated coral bleaching seen between September-November 2023, it is advisable to minimize further risks to coral health. The prevalence of White Syndrome diseases, which includes White Plague and other coral diseases with similar symptoms, has been observed to increase with cumulative heat exposure[3,4,5]. White Plague affects important reef-building corals. Therefore, as mentioned in Meesters et al.[1], raising the retention wall and regular re-distribution of the artificial sand would be recommended as a precaution against possible impacts from microbes.

Question 2: What are the potential effects on the health of the corals under normal weather conditions?
Answer: At the time of sampling the artificial sand was already observed being transported towards the sea. The overlap of microbial communities coinciding with samples at the border between the artificial sand and the beach suggests that microbes from the artificial sand have already been introduced into the surrounding area. Their persistence will depend on competition with native microbial communities, but continuous mixing would ensure presence of these microbes regardless. The persistence of microbes from the artificial sand is more likely because of the presence of stress-resistant microbes such as *Deinococcus*. However, the effect of these introductions is difficult to predict. No known negative effects for marine life are recorded for these microbes. Nevertheless, introducing microbes may carry unknown risks[9,10].

Question 3: What are the potential effects on the health of the corals during a storm or wind reversal?
Answer: As outlined in the main of Meesters et al. 2022[1], storms and wind reversals may result in large amounts of artificial sand being deposited onto the reef in front and up-current from the artificial beach. This may result in the establishment of foreign microbes, leading to altered nutrient cycling. The extent of this effect and its negative consequences on corals are difficult to predict, as this depends on the competition between the introduced and native microbes. Altered nutrient cycling may favor the establishment of coral competitors such as benthic cyanobacterial mats. Furthermore, corals that are covered or smothered by sand can be more susceptible to infections, which in combination with the higher abundance of *Sphingomonas* in the artificial sand may lead to an increase in the occurrence of White Plague disease in the area.

Question 4. How important is the origin of the sand, the kind of sand (river or carbonate sand), the quantity of sand, the layout of the beach, and the constructed wall for the effects?
Answer: In terms of microbial communities, all of these factors are important because they determine the presence of foreign microbes (including pathogens), and the likelihood of introducing them permanently to the environment. The origin and treatment of the sand will determine the microbes that can be found in the final product. It is likely that stress resistant microbes are found in the artificial sand due to the treatment prior to delivery. As outlined in the main report of Meesters et al. 2022[1], the quantity of sand, layout of the beach, and the presence of the constructed wall influence the erosion of the beach. More frequent erosion of the artificial beach or transport of larger amounts of sand onto the reef increases the likelihood that foreign microbes become established and exert an impact on the coral community.

General remarks
The sequencing results showed higher abundance of microbes associated to opportunistic human infections in the artificial sand compared to the natural sand. These microbes are widely present in the environment, so their detection is not directly alarming. However, because this sand is used for recreational purposes, we recommend further investigation.

Acknowledgements

We would like to thank the technicians, Elza Duim, Elsa B. Girard, and Dr. Benoit Paix at the Naturalis laboratory for their support during the DNA extraction and bioinformatic analysis of the sand samples, as well as the Center for Microbial Oceanography at the University of Hawaii for their support with the sequencing of the microbial communities.

Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

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Justification

Report C004/24
Project Number: 4315100198

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Linda Tonk
Senior researcher

Signature:

Date: 30 January 2024

Approved: Dr. A.M. Mouissie
Bussinessmanager

Signature:

Date: 30 January 2024

Appendices

Appendix 1: Statistical analyses

Community Differences

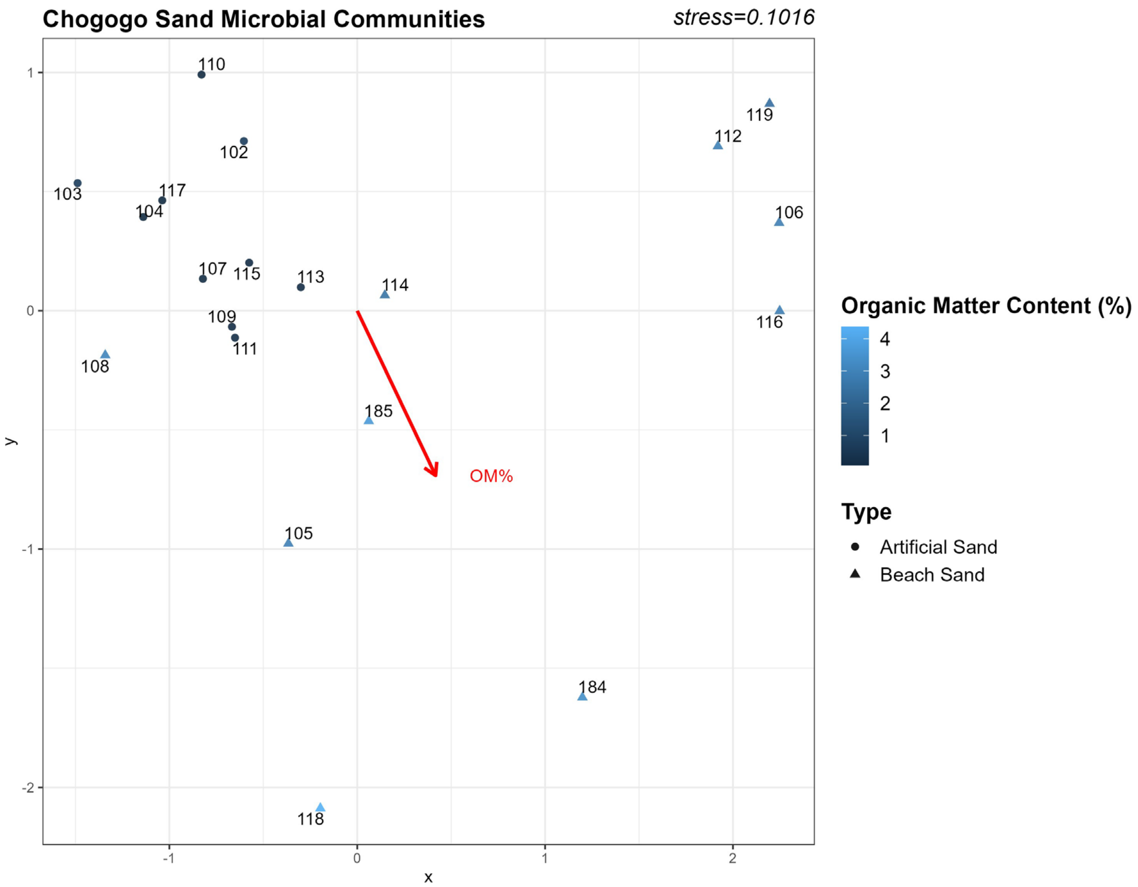
```
Permutation test for adonis under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 9999

adonis2(formula = transformed community relative abundances ~ sand source, permutations = 9999, method
= "bray", na.action = na.omit)
                     Df SumOfSqs      R2      F Pr(>F)
Sand Source           1   1.2081 0.15144 3.2124  1e-04 ***
Residual             18   6.7692 0.84856
Total                19   7.9772 1.00000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Community Heterogeneity

```
Homogeneity of multivariate dispersions

Call: betadisper(d = vegdist(transformed community relative abundances, distance = "bray"), group
= Sand Source)

No. of Positive Eigenvalues: 19
No. of Negative Eigenvalues: 0

Average distance to median:
Artificial Sand      Beach Sand
         0.5173          0.6318

Eigenvalues for PCoA axes:
(Showing 8 of 19 eigenvalues)
 PCoA1  PCoA2  PCoA3  PCoA4  PCoA5  PCoA6  PCoA7  PCoA8
1.4810 0.8001 0.6035 0.5196 0.4806 0.4537 0.4169 0.3975

Analysis of Variance Table

Response: Distances
          Df  Sum Sq  Mean Sq F value   Pr(>F)
Groups     1 0.06549 0.065490  9.6578 0.006076 **
Residuals 18 0.12206 0.006781
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Community Correlation to Organic Matter

```
                      NMDS1    NMDS2     r2 Pr(>r)
Organic matter      0.51663 -0.85621 0.6527  2e-04 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 9999
```



Supplementary Figure 1. Ordination plot of the microbial communities from the artificial (circles) and beach (triangles) sands. Numbers refer to the samples and locations as shown in Fig. 1. Each point refers to the composition of the microbial community in a sample. Points close to each other indicate microbial communities that are more similar than points (samples) further away. Samples are colored according to the organic matter content (%) in the closest sampled sediment from Meesters et al., 2022[1]. As outlined in Meesters et al., 2022[1], artificial sand samples tend to have lower organic matter contents. Consequently they are more darkly colored in this graph. The red arrow indicates the correlation between organic matter content in samples and the community composition. Microbial communities in the direction of the arrow on the plot tend to have higher organic matter contents.

Sphingomonas Relative Abundances

Welch Two Sample t-test

data: (Spingomonas Relative Abundance)^(1/4) by sand source
t = 2.8601, df = 10.403, p-value = 0.01634
alternative hypothesis: true difference in means between group Artificial Sand and group Beach Sand is not equal to 0
95 percent confidence interval:
0.1296612 1.0228043
sample estimates:
mean transformed *Spingomonas* abundance in group Artificial Sand
1.2042382
mean transformed *Spingomonas* abundance in group Beach Sand
0.6280055

Kocuria Relative Abundances

```
        Welch Two Sample t-test

data:  (Kocuria Relative Abundance)^(1/4) by sand source
t = 2.123, df = 17.894, p-value = 0.04797
alternative hypothesis: true difference in means between group Artificial Sand and group Beach Sand is
not equal to 0
95 percent confidence interval:
 0.003712055 0.741104486
sample estimates:
mean transformed Kocuria abundance in group Artificial Sand
                     0.7224958
mean transformed Kocuria abundance in group Beach Sand
                    0.3500875
```

Appendix 2: Materials & Methods

DNA analysis

Sand samples for DNA analysis were stored in ethanol and transported to the Netherlands for analysis. Samples were stored at -20˚C until processing.

During sampling, the coordinates of each sample were collected with a handheld GPS (Garmin 78). Samples were collected on Bonaire on May 10-14, 2022. An overview of the locations sampled is given in Figure 1 and Figure 2.

DNA was extracted from the sediments using the NucleoSpin® Soil kit with a modified protocol. First, sediments were dried at 56˚C overnight to remove ethanol. The dried sediments were homogenized with pestle and mortar. The homogenized sediments were used as substrate for the NucleoSpin® Soil kit, following the manufacturer's protocol. 50µL of Proteinase-K at a concentration of 28.8 mg/ml were added to the lysis buffer, and the lysate was incubated at 37˚C overnight before continuing with the protocol.

The microbial communities were characterized from the DNA extracts at the University of Hawaii. The primers[27] 515F-Y (5′-GTGYCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYMTTTRAGTTT) were used to target the microbial communities. For the bioinformatic processing, primers were removed and forward and reverse reads were merged with a minimum overlap of 20bp. Amplicon Sequence Variants (ASVs) were assigned to reads using the DADA2 package from the statistical software R. Any ASV found in a control was removed from the analysis. ASV taxonomies were assigned to Genus level using the SILVA database nr99 v138.1. ASVs from potentially pathogenic genera were BLASTed against the GenBank 16S ribosomal RNA database, to identify species with 97% identity. The microbial communities of the artificial sand and beach sand were then compared to each other.

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