Microbial life on a sand grain: from bulk sediment to single grains

报告人：张玲玉

时　间：2018-4-14

International Society for Microbial Ecology

Original Article | OPEN

Microbial life on a sand grain: from bulk sediment to single grains

David Probandt, Thilo Eickhorst, Andreas Ellrott, Rudolf Amann & Katrin Knittel

IF:9.664

The ISME Journal
doi:10.1038/ismej.2017.197
Received: 11 May 2017
Revised: 04 October 2017

David Probandt

Postdoctoral Researcher at Max Planck Institute for Marine Microbiology

Max Planck Institute for Marine Microbiology • International Max Planck Research School of M…

Bremen, Bremen, Germany • 201

目　录

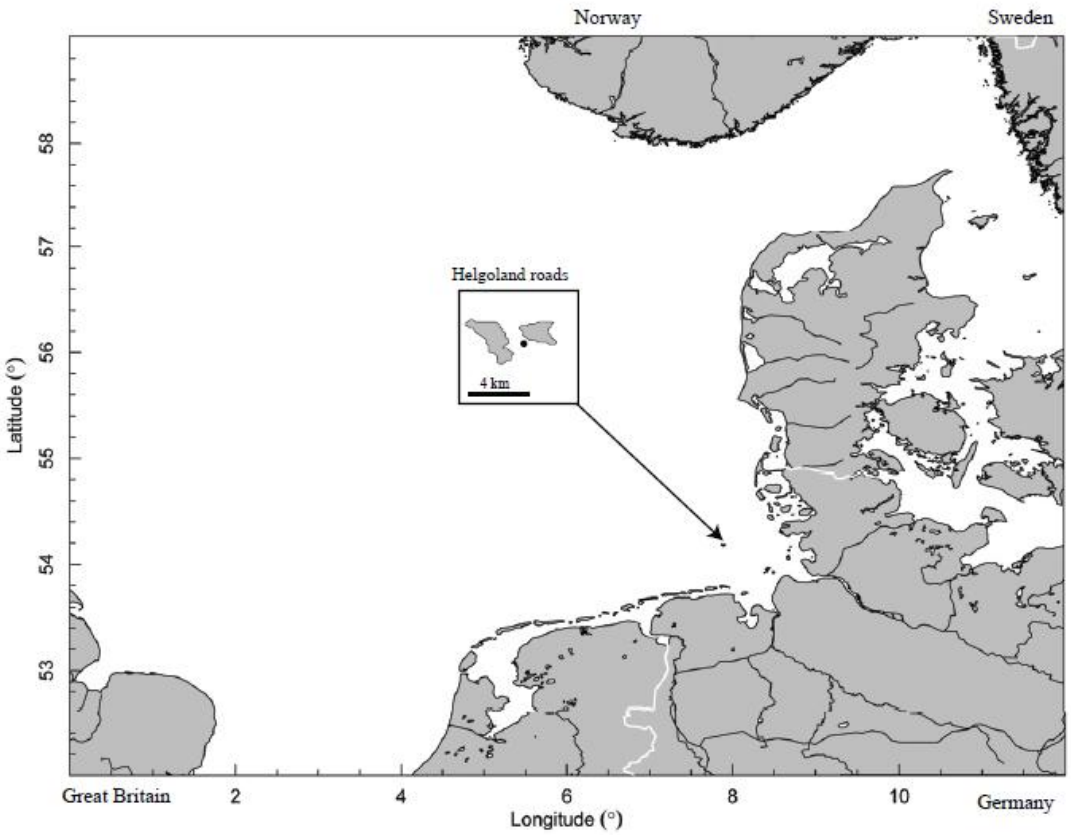
CONTENTS

Introduction

- The top 10 cm of marine sediments constitute a habitat for estimated 1.7×10^{28} bacteria and archaea(Whitman et al., 1998).
- In sandy sediments, ＞99% of the benthic microbial community lives attached to sand grains (Rusch et al., 2003).
- we hypothesize that the diversity and community composition would differ more strongly between sand grains than between replicates of the bulk sediment.
- We established a workflow for (i) bacterial diversity analysis of the sand grain's community using tag sequencing of partial 16S rRNA genes amplified from individual SSGs, and(ii) the direct visualization of microbial communities on native sand grains using catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH).

Materials and Methods

Sampling



Geographic location of sampling site Helgoland Roads in the southern North Sea. The time was 14 June 2016. Sediment push cores were retrieved by scientific divers from a water depth of 8m.

Materials and Methods

Sample treatment:

- DNA extraction and PCR

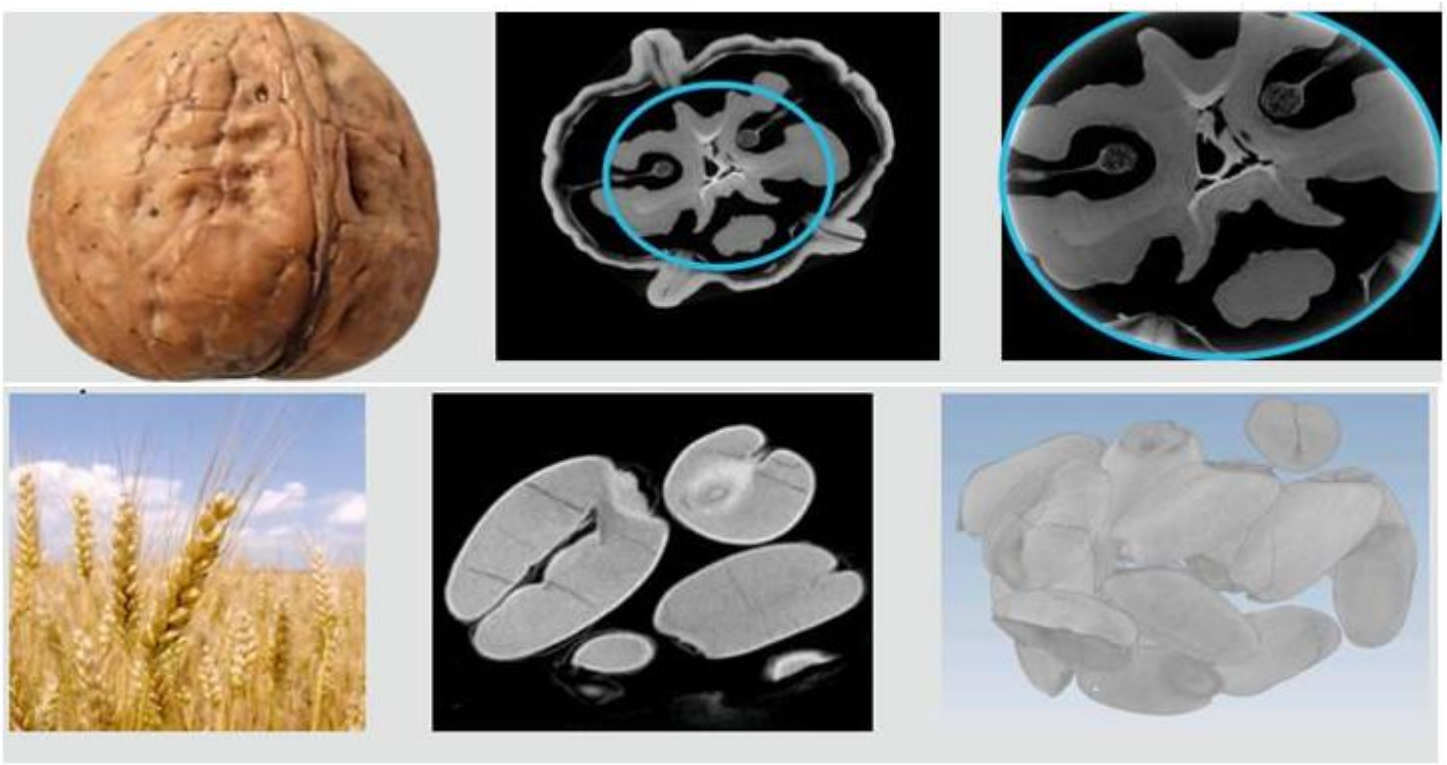
  samples were stored at -20° C.

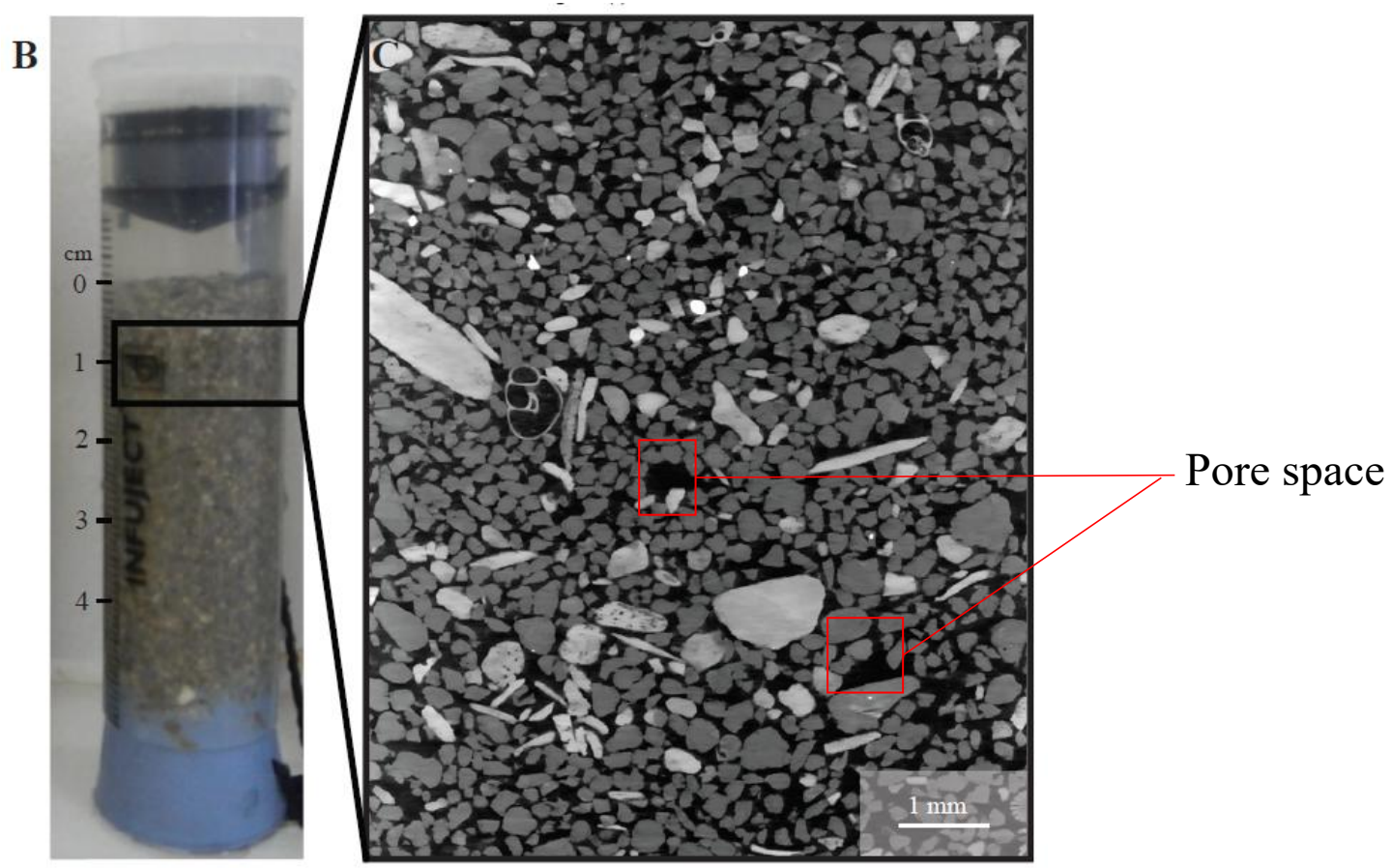
- CARD-FISH

SYBR green I and Acridine Orange staining,surface sediment (0–2 cm) was fixed with 1.5% formaldehyde for 1 h at room temperature, washed in 1 ×PBS /ethanol (1:1, v/v) and stored at − 20 ° C until use.

Micro-computed tomography (μCT)

Micro computed tomography(Micro-CT,微计算机断层扫描技术)，又称微型CT、显微CT，是一种非破坏性的3D成像技术，可以在不破坏样本的情况下清楚了解样本的内部显微结构。它与普通临床的CT最大的差别在于分辨率极高，可以达到微米（μm）级别，目前国内一家自主研发Micro-CT的公司已经将分辨率提高到0.5μm，具有良好的“显微”作用。Micro-CT可用于医学、药学、生物、考古、材料、电子、地质学等领域的研究。





B:Sediment push core; C: reconstruction of sediment vertical section using μCT images.

- DNA extraction from bulk sediments

  PowerSoil DNA isolation kit

- Amplification of partial 16S rRNA genes

- Quality trimming and sequence processing

  software package BBmap v36.92

  MiSeq SOP

- Diversity analysis

The alpha diversity was studied by phylotype-based Chao1 (Chao, 1984) and inverse Simpson (Simpson,1949)，The beta diversity was studied by phylotype-based comparative $OTU_{0.97}$ presence/absence and phylogenetic measure of weighted and unweighted UniFrac.

- Total cell counts

cells collection (ultrasonication)—filter—staining(Acridine Orange)—observition and count

- Glass slides for microscopy of sand grains

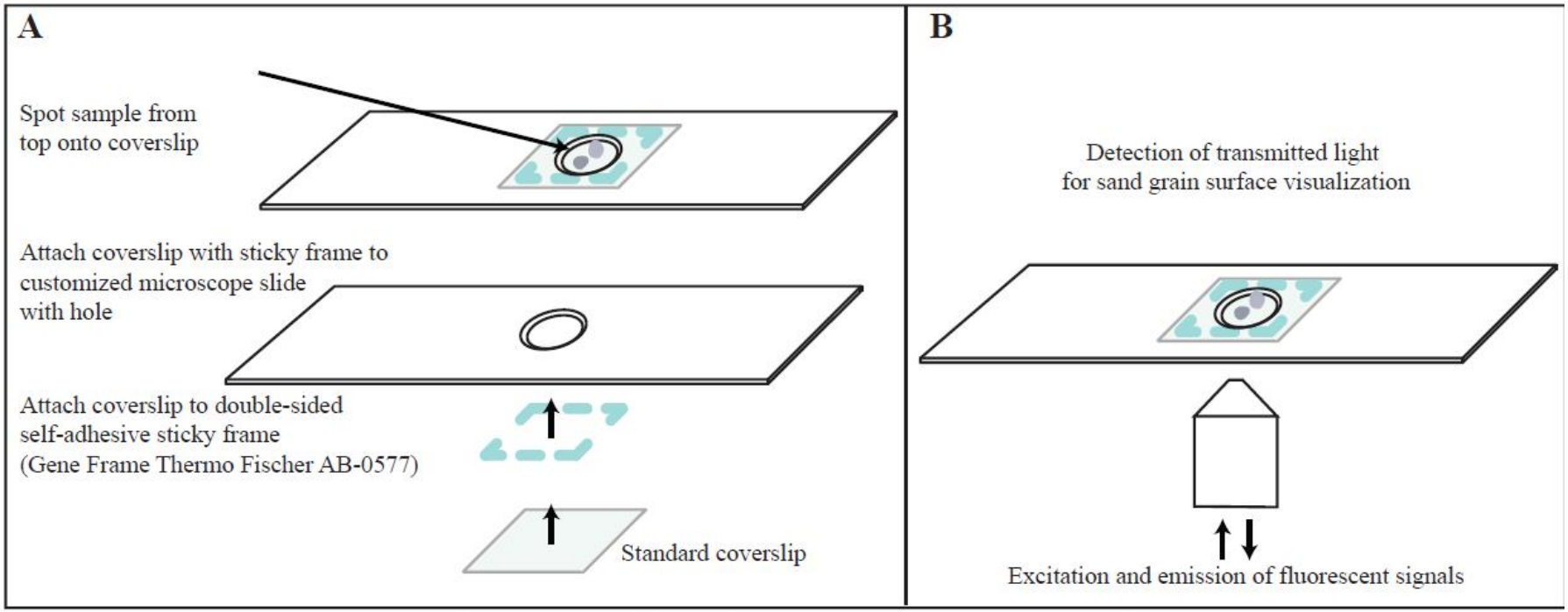


Figure S2: Schematic drawing of customized glass slide for visualization of microbial cells on sand grains using inverse confocal laser scanning microscopy.

A, slide preparation; B, sample visualization using the inverse microscope.

- SYBR green I staining
- Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH)
- Image acquisition using inverse confocal laser scanning microscopy and cell–cell distance measurements
- Calculations of cell density, colonized surface area and cells per sand grain

Results and Discussions

1 Microbial colonization density on sand grains

- Microbial cell numbers in surface sediments (0–2 cm depth) from site Helgoland Roads were $1.1 \pm 0.3 \times 10^9$ cm^{-3} and thereby in the upper range as reported for other sandy sediments(Dale, 1974; Meyer-Reil et al., 1978; Llobet-Brossa et al., 1998; Rusch et al., 2003).
- The colonization density was 0.09 cells μm^{-2} and a theoretical average distance between two cells of 3.3 μm.
- Based on the footprint of 0.43 μm^2 for an average cell and the colonization density of 0.09 cells μm^{-2}, 4% of the grain's' surface is colonized.Each sand grain is populated by 1.2×10^4–1.1×10^5 cells (according to Eq. I; grain size 202–635 μm).

2 Visualization of microbial populations on sand grains

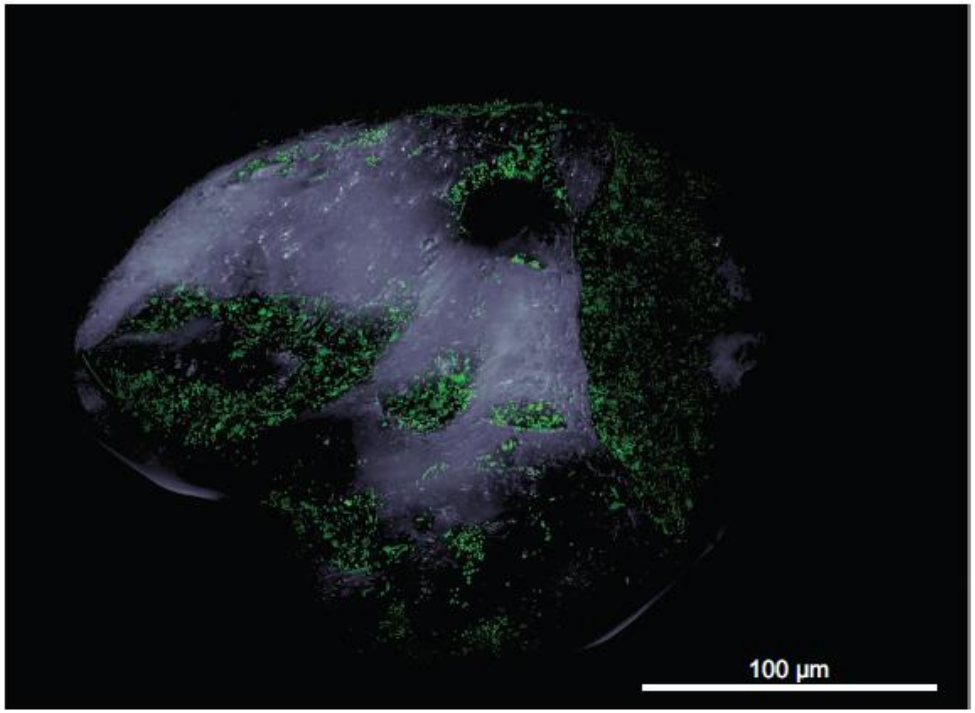
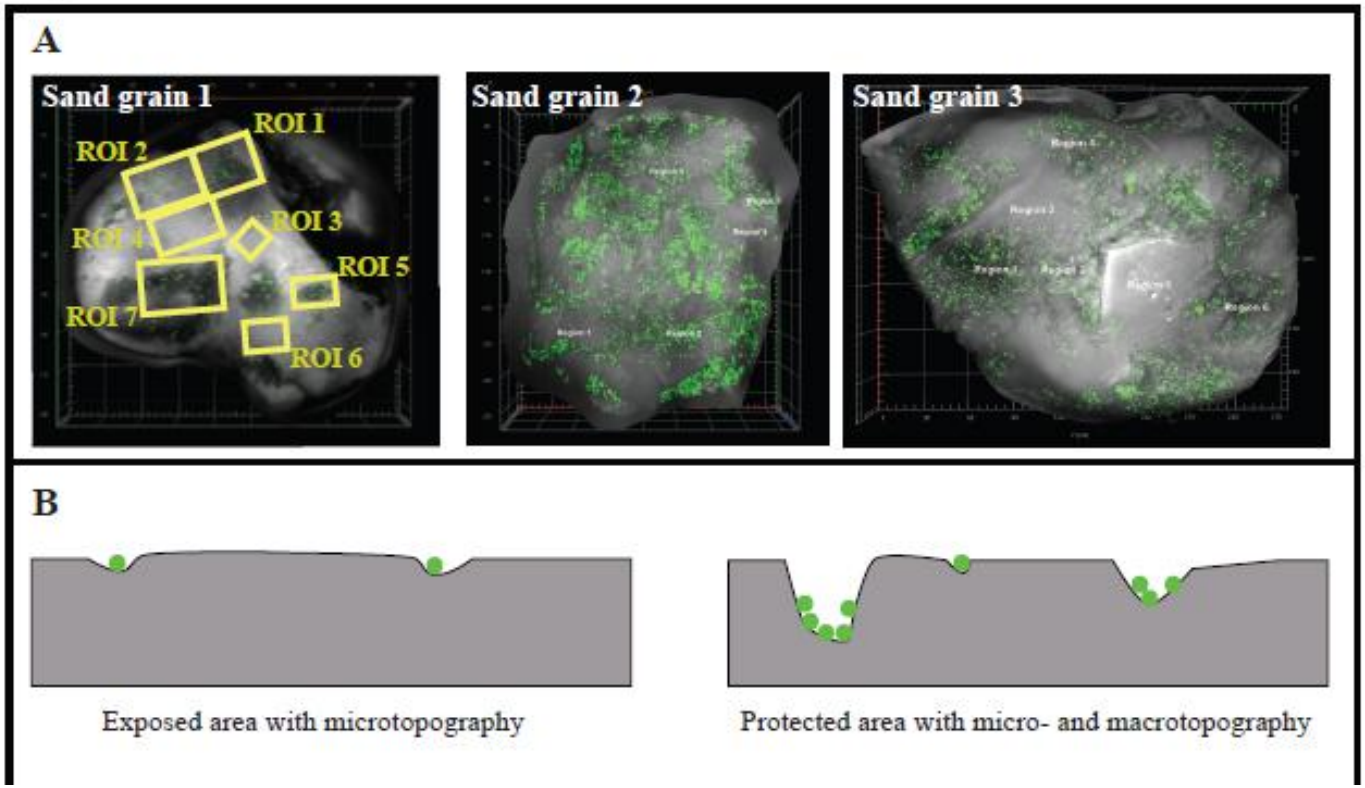
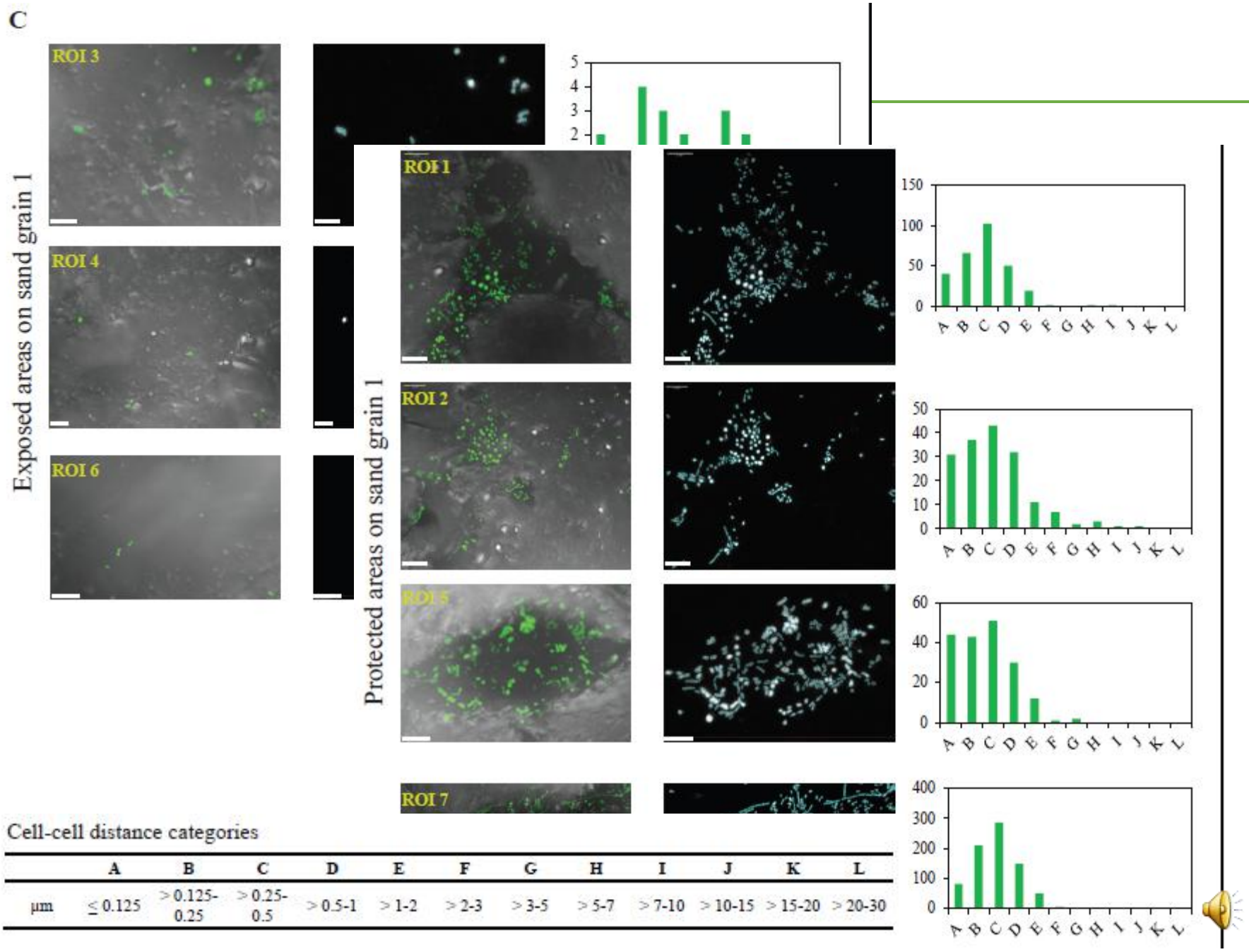


Figure 1 Microbial colonization of a sand grain. Confocal laser scanning micrograph showing SYBR green I-stained microbial cells on a sand grain visualized as three-dimensional reconstruction. The grain’s surface was visualized by transmitted light microscopy. Note the bare surfaces of convex and exposed areas in contrast to protected areas dominated by macrotopography, which are densely populated by microbes.

Cell–cell distance measurements

Figure S4





Cell-cell distance categories

	A	B	C	D	E	F	G	H	I	J	K	L
µm	≤ 0.125	> 0.125-0.25	> 0.25-0.5	> 0.5-1	> 1-2	> 2-3	> 3-5	> 5-7	> 7-10	> 10-15	> 15-20	> 20-30

3 Bacterial diversity on SSGs versus diversity in bulk sediment

Table S2: Alpha diversity parameters for single sand grains and bulk sediments based on 16S rRNA gene Illumina tag sequencing. Depicted diversity values show the mean of 25 independent calculations. $OTU_{0.97}$ only represented by one or two sequences in the total dataset (SSO_{abs} and DSO_{abs}; ~0.000001% relative sequence abundance) were excluded from analysis. SSO_{abs}, absolute single sequence $OTU_{0.97}$; DSO_{abs}, absolute double sequence $OTU_{0.97}$ Relative single sequence $OTU_{0.97}$ (SSO_{rel}) are $OTU_{0.97}$ that occur only once in the respective sample but are more sequence-abundant in other samples of the entire data set.

	Quality reads	Subsampled to 44,901 reads each sample						
sample	[No.]	observed $OTU_{0.97}$	Chao1	inverse Simpson	SSOabs [%]	SSOrel [%]	DSOabs [%]	Faith's PD
SSG01	45,980	5,446	9,231	69	1.5	48.1	8.1	295
SSG02	46,555	5,032	7,977	87	1.8	41.6	7.5	284
SSG03	53,143	5,235	8,673	85	2.3	44.7	7.6	297
SSG04	55,507	3,426	5,260	68	2.2	37.7	9.0	222
SSG05	58,205	5,007	8,764	114	2.1	47.2	7.5	280
SSG06	53,230	4,373	7,070	112	2.1	43.3	8.7	253
SSG07	56,972	4,369	7,327	103	1.8	44.8	6.4	253
SSG08	55,758	4,088	7,716	49	1.9	52.3	7.7	236
SSG09	52,930	5,470	9,742	128	1.9	48.2	7.9	292
SSG10	47,116	5,198	8,888	63	1.3	48.5	7.9	277
SSG11	50,657	4,407	7,180	92	2.2	42.6	8.2	253
SSG12	58,769	4,126	7,293	44	1.9	49.8	8.4	227
SSG13	46,215	5,359	9,787	82	1.8	49.9	7.0	288
SSG14	44,901	5,160	9,008	136	1.8	46.2	7.4	290
SSG15	47,901	4,866	8,783	78	1.8	50.4	7.6	265
SSG16	46,828	5,955	9,949	106	1.9	44.0	7.6	326
SSG17	45,131	6,031	10,692	58	1.8	50.8	9.1	317
bulk1	75,134	6,759	13,059	230	3.9	51.2	10.0	348
bulk2	129,394	6,797	13,119	215	4.0	51.4	10.8	354
bulk3	137,585	6,924	14,155	226	4.2	52.3	9.8	358

Each grain harbored a tremendous bacterial diversity as shown by 3426–6031 observed species-level $OTU_{0.97}$.

Table S3: Beta-diversity.

Genetic similarity between single sand grain and bulk sediment communities measured by UniFrac and expressed as shared phylogenetic branch length. Color code corresponds to proportion of shared branch length: low (red) to high proportion (green). Panel A. Unweighted UniFrac, B. Weighted UniFrac. Calculcations performed on $OTU_{0.97}$ representative sequences of subsampled data sets (N=44.901).

A

	SSG 1	SSG 2	SSG 3	SSG 4	SSG 5	SSG 6	SSG 7	SSG 8	SSG 9	SSG 10	SSG 11	SSG 12	SSG 13	SSG 14	SSG 15	SSG 16	SSG 17	Bulk 1	Bulk 2
SSG2	46																		
SSG3	47	49																	
SSG4	42	44	44																
SSG5	47	46	48	43															
SSG6	44	41	44	42	47														
SSG7	46	46	49	45	47	46													
SSG8	42	40	43	40	42	42	45												
SSG9	48	48	48	43	49	44	47	42											
SSG10	44	46	47	42	46	43	48	46	45										
SSG11	45	44	47	43	47	44	48	42	47	45									
SSG12	43	43	44	41	42	42	45	44	44	43	43								
SSG13	46	48	48	43	48	45	49	45	49	48	46	43							
SSG14	49	46	48	42	44	42	47	43	46	46	44	43	46						
SSG15	44	44	46	41	46	44	47	48	46	48	44	43	49	45					
SSG16	50	49	49	42	46	41	46	39	47	45	44	40	46	49	43				
SSG17	46	47	47	41	44	41	44	40	45	46	42	40	45	48	43	50			
Bulk1	44	45	46	40	44	41	44	40	45	44	43	40	46	43	43	46	45		
Bulk2	44	45	46	41	44	41	44	40	46	44	42	41	45	43	43	46	46	54	
Bulk3	45	45	46	40	45	41	44	39	45	44	42	40	45	44	43	46	45	54	55

	SSG to SSG	Bulk to bulk	SSG to bulk
Min	39	54	39
Max	50	55	46
Mean	45	54	44

Unweighted Unifrac showed a genetic similarity of 39–50% (mean 45%) between any sand grain community confirming that these are different.

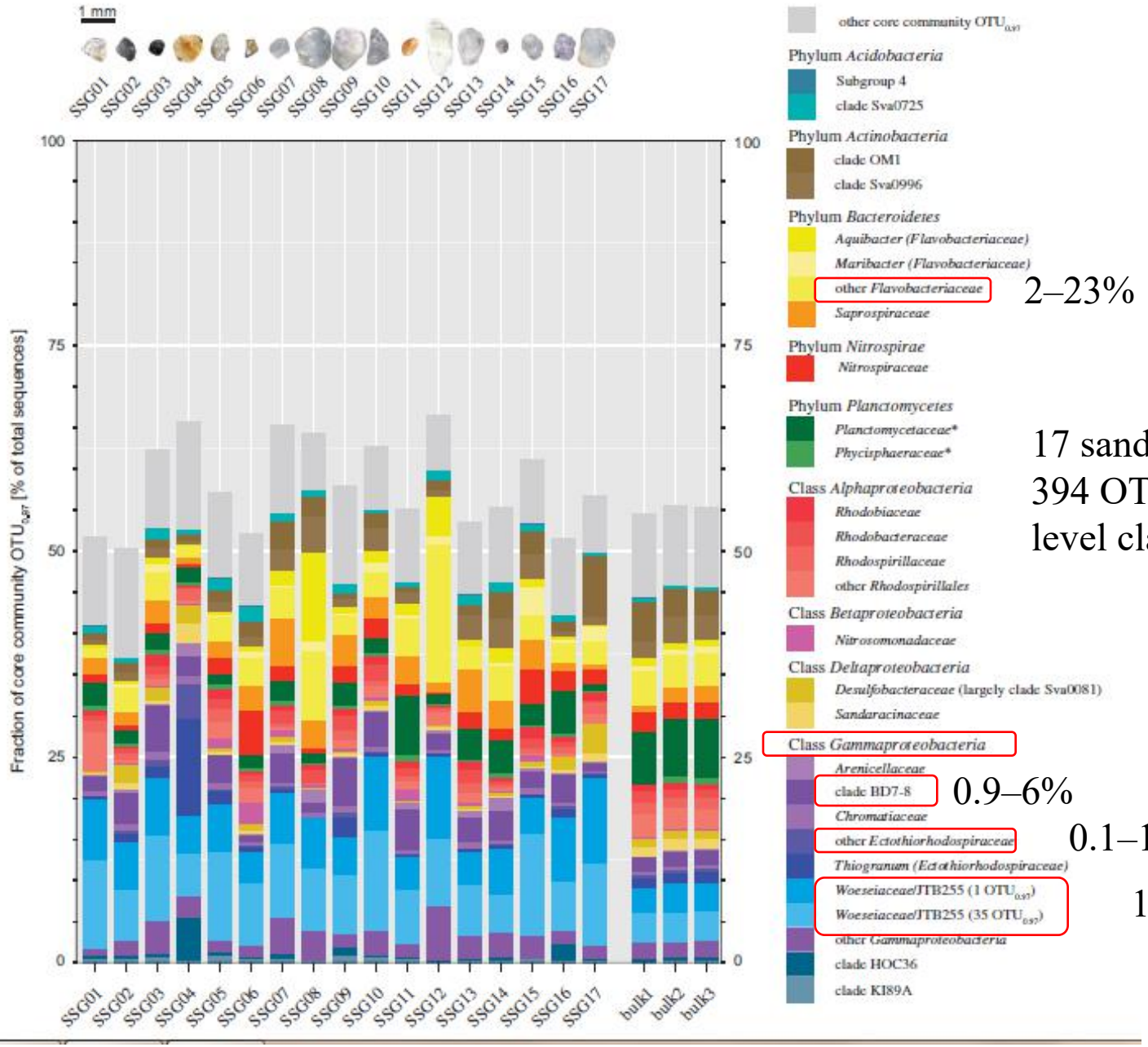
B

	SSG 1	SSG 2	SSG 3	SSG 4	SSG 5	SSG 6	SSG 7	SSG 8	SSG 9	SSG 10	SSG 11	SSG 12	SSG 13	SSG 14	SSG 15	SSG 16	SSG 17	Bulk 1	Bulk 2
SSG2	70																		
SSG3	76	78																	
SSG4	68	69	72																
SSG5	78	71	82	68															
SSG6	75	71	78	62	82														
SSG7	71	75	81	63	77	77													
SSG8	55	62	64	50	60	63	72												
SSG9	80	75	82	69	81	76	79	61											
SSG10	71	72	84	66	81	78	82	66	79										
SSG11	69	71	75	58	71	71	75	65	76	71									
SSG12	54	61	61	50	56	59	63	80	58	60	63								
SSG13	70	72	75	60	75	74	81	75	78	78	76	69							
SSG14	70	76	75	60	70	71	85	72	76	75	77	65	79						
SSG15	67	70	76	58	73	76	83	74	75	79	72	64	81	79					
SSG16	75	78	78	68	74	72	75	60	80	73	78	59	74	79	70				
SSG17	66	67	74	66	74	70	71	59	69	77	61	53	66	67	69	68			
Bulk1	71	65	71	59	72	72	74	61	73	73	70	54	70	71	71	74	73		
Bulk2	71	68	73	60	72	73	76	62	74	74	73	56	72	74	72	76	71	93	
Bulk3	71	68	73	60	72	74	76	63	74	74	74	57	72	74	73	76	71	93	96

	SSG to SSG	Bulk to bulk	SSG to bulk
Min	50	93	54
Max	85	96	76
Mean	71	94	70

Weighted UniFrac analysis considering $OTU_{0.97}$ abundances resulted in a much higher genetic similarity with 50–85% (mean 71%) indicating that less abundant and rare $OTU_{0.97}$ are mainly responsible for the observed genetic differences between sand grains.

4 Core community on sand grains



2–23%

17 sand grains comprising 394 $OTU_{0.97}$, 82 family-level clades.

0.9–6%

0.1–16%

10–21%

5 In situ identification of microbial communities on sand grains

Table S1. Oligonucleotide probes used for CARD-FISH and FISH.

Probe name	Target	Sequence (5' - 3')	FA [%][1]	Target	Reference
EUB338 I		GCT GCC TCC CGT AGG AGT	35	16S rRNA	Amann et al., 1990
EUB338 II	Most *Bacteria*	GCA GCC ACC CGT AGG TGT	35	16S rRNA	Daims et al., 1999
EUB338 II		GCT GCC ACC CGT AGG TGT	35	16S rRNA	Daims et al., 1999
ARCH915a	*Archaea*	GTG CTC CCC CGC CAA TTC CT	35	16S rRNA	Stahl and Amann, 1991
CREN537	Marine Group I *Thaumarcheota*	TGA CCA CTT GAG GTG CTG	20	16S rRNA	Teira et al, 2004
EUK516	*Eukarya*	ACC AGA CTT GCC CTCC	0	18S rRNA	Amann et al., 1990
NON338	nonsense probe	ACT CCT ACG GGA GGC AGC	35	16S rRNA	Wallner et al., 1993
GAM42a[2]	*Gammaproteobacteria*	GCC TTC CCA CAT CGT TT	35	23S rRNA	Manz et al., 1992
GAM42a_T1038_G1031[2]	*Xanthomonadaceae*	GCC TTT CCA CAT GGT TT	35	23S rRNA	Siyambalapitiya and Blackall, 2005
GAM42a_T1038[2]	*Xanthomonadaceae*	GCC TTT CCA CAT CGT TT	35	23S rRNA	Siyambalapitiya and Blackall, 2005
BET42a[2]	*Betaproteobacteria*	GCC TTC CCA CTT CGT TT	35	23S rRNA	Manz et al., 1992
JTB1270	*Woeseiaceae*/JTB255	GAG CTT TAA GGG ATT AGC GCA CCA	40	16S rRNA	Dyksma et al., 2016a
hJTB1270	Unlabeled helper oligo, used with JTB1270	TTG CTG GTT GGC AAC CCT CTG TAT	40	16S rRNA	Dyksma et al., 2016a
CF968	*Bacteroidetes*	GGT AAG GTT CCT CGC GTA	30	16S rRNA	Acinas et al., 2015
NTSPA712	*Nitrospirae*	CGC CTT CGC CAC CGG CCT TCC	50	16S rRNA	Daims et al., 2001
cNTSPA712	Unlabeled competitor used with NTSPA712	CGC CTT CGC CAC CGG TGT TCC		16S rRNA	Daims et al., 2001
NM645[3]	*Nitrosospira, Nitrosovibrio,* some *Nitrosomonas,* uncultured *Nitrosomonadaceae*	GCC ACA CTC TAG YCT TGT	20-30	16S rRNA	This study
c1NM645	Unlabeled competitor used with NM645	GCC ACA CTC TAG CCT TGC		16S rRNA	This study
c2NM645	Unlabeled competitor used with NM645	GCC ACA CTC CAG CCT TGC		16S rRNA	This study
NM478[3]	*Nitrosospira, Nitrosovibrio,* uncultured *Nitrosomonadaceae,* some *Acidobacteria*	TCT TCC GGT ACC GTC AGT A	20-30	16S rRNA	This study
cNM478	Unlabeled competitor used with NM478	TCT TCC GGT ACC GTC AGM A		16S rRNA	This study
PLA46[4]	*Planctomycetes* except *Phycisphaerae*	GAC TTG CAT GCC TAA TCC	30	16S rRNA	Neef et al., 1998
PHYC309	*Phycisphaerae*	AGT GTC TCA GTC CCG ATG CGG CG	35	16S rRNA	Probandt et al., 2017

[1]: formamide concentration in the hybridization buffer.
[2]: Probes GAM42a, GAM42a_T1038_G1031 and GAM42a_T1038 were used as "GAM42a-mix" at a molar ratio of 1:1:1 together with Bet42a as competitor.
[3]: Probe NM645 is recommended to use. It gives brighter signals than NM478 and has no not-target hits.
[4]: Probe Pla46 was used HRP-labeled or directly labeled with four Alexa594 dye molecules using CLICK chemistry

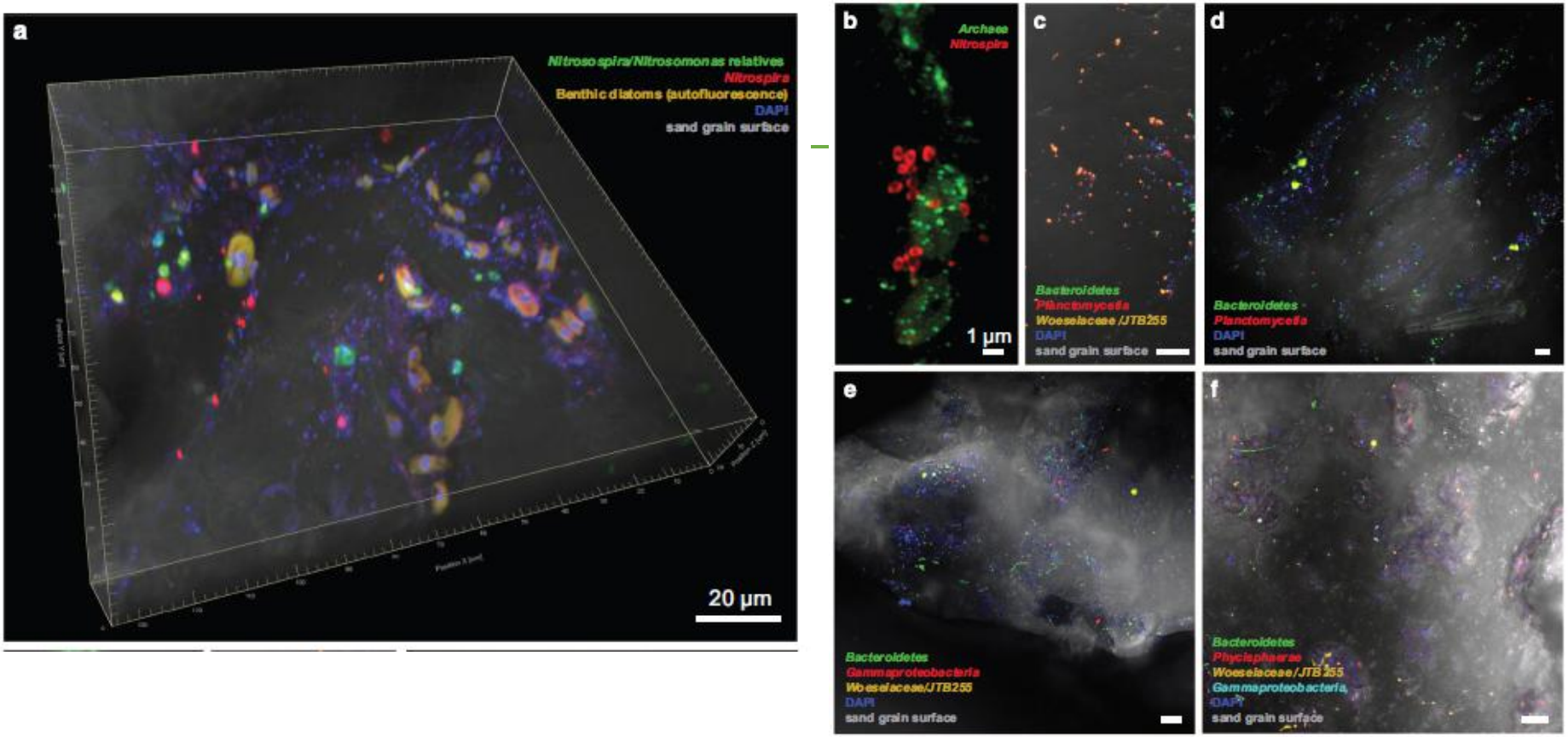


Figure 4 Direct visualization of taxa on sand grains using CARD-FISH and confocal laser scanning microscopy.

Gammaproteobacteria (including Woeseiaceae/JTB255), Planctomycetes and Bacteroidetes (Figures 4c–f) were most abundant.

conclusions

- each sand grain investigated in this study was the habitat for around 10^5 cells representing several thousand species.
- The average distance between any two cells on a sand grain in protected areas was 0.5 ± 0.7 μm and therefore 100-fold shorter than the average distance between cells in the water column.
- Confirming our original hypothesis, the diversity and community composition differ more strongly between sand grains than between replicates of the bulk sediment.

The Reasons for Choosing This Paper

- the diversity of meathods
- CARD-FISH and confocal laser scanning microscopy

Thanks for your attention!