

Supplementary Information

Diversity and dynamics of rare and of resident bacterial populations in coastal sands

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Materials and Methods for Fig. 1A-B

Acridine Orange staining. The water column and pore water samples were fixed with 1:10 seawater-diluted sterile-filtered (0.2 µm disposable syringe filter) 37% formaldehyde (methanol stabilized) solution. 1 ml of sand was fixed in 9 ml of a particle-free 2% formaldehyde/seawater solution. Pictures of the bacteria in the different samples were done by epifluorescence microscopy after staining with Acridine Orange. The samples of porewater and water column were non-diluted while some sand grains were directly applied onto 0.2 µm black polycarbonate filters, stained with Acridine Orange solution for 3 min and rinsed with 1 ml citrate buffer.

Taxa-environment relationships. As time and depth effects on biological variation may be confounded by the covariation with other measured parameters (pH, water temperature, wind speed, salinity, pigments, nutrients, extra-cellular enzymatic activities and cell properties), we first tested by using multiple regression how much the response variable time or depth could be explained by explanatory environmental variables. Thus, this analysis helps decide whether time and depth should be included or removed from further analyses to reduce collinearity in the ecological models.

Results and Discussion

2. Turnover of the overall microbial community with sediment depth and time: testing data reliability

Several studies have questioned the accuracy of high-throughput sequencing data due to the likely existence of chimeric sequences originating from sequencing or PCR amplification artifacts (*e.g.* (1)). This issue was of particular importance for our study because it may have erroneously inflated the observed community turnover rates. Yet, even after applying the PyroNoise algorithm (2) to remove pyrosequencing and amplification noise from the data and reclustering of the sequences at different levels of sequence similarity, a very large turnover (*i.e.* 40-70% of sequence replacement) of the bacterial community over depth or time could still be observed at various sequence dissimilarity levels used to cluster the data (Figs. 2A-B, S3). If we consider the PyroNoised-corrected data clustered at 3% sequence dissimilarity, turnover patterns were still really high, as observed at the OTU_{all} level (Figs. 2A-B, S3). It may therefore be concluded that the large community turnover present in marine sandy sediments is not due to technical artifacts and is consistently observed at different taxonomic levels, yet to different extent depending on taxonomic resolution.

3. Impact of the rare biosphere on community turnover

We tested several scenarios for temporal fluctuations of the rare biosphere investigating OTU_{0%} and SSO_{rel} occurrence for the top 10 cm sediment layer over the six sampling times (Table 1). For example, less than 1% of the OTU_{0%} appeared at either the first or last three sampling dates only, or had patterns that would skip one sampling date each time. Noticeably, 6-17% of the OTU_{0%} per sample appeared only at one sampling date and mostly consisted of SSO_{abs}, *i.e.* OTU that occurred only in one sample with a sequence abundance of one. The fluctuation in sequence abundance of noise-corrected SSO_{rel} was also explored (Fig. 3): altogether, 94% of SSO_{rel} had a maximum abundance below 10 sequences when all samples were considered, indicating that when an OTU_{0%} was rare it remained rare and was not likely to become abundant within a year, as already observed through a seasonal survey in the Arctic water column (3). Few SSO_{rel} displayed very high fluctuation in abundance (Fig. 3A1-3), which further supports the idea that blindly removing SSO_{rel} or SSO_{abs} from the data set would also remove meaningful patterns of OTU variation (4).

4. Ecological interpretation of overall microbial diversity patterns

Temporal variation significantly explained 70% of nutrients' variation while depth significantly explained the variation of pigments, nutrients, extra-cellular enzymes as well as cell abundances (93%, 74%, 80% and 79% of the variation explained, respectively, using multiple regression analyses, own data not shown), which overall confirms previous observations (5). Thus, in the next step, time and depth were removed from the pool of independent factors to investigate the role of other environmental factors.

Deeper ecological insights may also be gained by the detailed analysis of the correlations between sequence abundances of distinct bacterial taxa and specific environmental parameters (Fig. 4). Contrasting patterns were obtained when phyla were examined individually, with some of them being strongly, but not necessarily identically influenced by depth and related environmental parameters (such as pigments, bacterial properties and some enzymes; Fig. 4). For example, some of the most abundant phyla showed different response patterns with changes in environmental conditions: *Deltaproteobacteria* sequence abundance was positively correlated with depth and negatively correlated with chlorophyll *a*, bacterial abundance, alpha-glucosidase, bacterial productivity and aminopeptidase. About 67% of the *Deltaproteobacteria* were composed of *Desulfobacterales*, an order representing sulfate-reducing bacteria that thrive in anoxic North Sea sand (3). The cyanobacterial group was found to be positively correlated with salinity and negatively correlated with silicate, a factor significantly correlating with depth. The order *Oscillatoriales* accounted for 47% of the *Cyanobacteria* and the main genera represented here was *Oscillatoria*, which can grow in harsh environmental conditions with high seasonal fluctuations of salinity and temperature, and which may play a role in degradation of different hydrocarbon compounds in intertidal oil-polluted sediment (46). In contrast, variations in sequence abundance for other prominent phyla, such as *Bacteroidetes* or *Alphaproteobacteria*, were not significantly correlated with the environmental parameters analyzed here (Fig. 4). Certainly, other environmental parameters such as biological interaction with other bacteria or other organisms, grazing, organic matter composition etc. are also important factors in structuring the diversity of bacterial communities in sand.

5. The rare biosphere responds to environmental drivers

The presence of pathogenic bacteria in the environment is of great interest for public health and for fisheries, and further research concerning regulative factors of pathogen distribution may be of prime importance especially for coastal ecosystems (6). Both the survival and proliferation of pathogens can be influenced by the variation of environmental factors. Potential pathogenic genera in our data set were selected according to the literature. A combination of environmental parameters could explain 55% of their overall biological variation (Table S3, Fig. S4). Noticeably, such pathogens are not usually observed in marine sediments and occurred at low sequence abundance in our samples, thus they could be dispersed by various ways of human interaction with coastal habitats, and occur in a latent stage in sands, further supporting the idea of a “seed bank” of rare organisms as previously proposed by several authors (7, 8).

References

1. Quinlan, A. R., Stewart, D. A., Stromberg, M. P., & Marth, G. T. (2008) *Nature Methods* **5**, 179-181.
2. Quince, C., Lanzen, A., Curtis, T. P., Davenport, R. J., Hall, N., Head, I. M., Read, L. F., & Sloan, W. T. (2009) *Nature Methods* **6**, 639-641.
3. Kirchman, D. L., Cottrell, M. T., & Lovejoy, C. (2010) *Environmental Microbiology* **12**, 1132-1143.
4. Huse, S. M., Welch, D. M., Morrison, H. G., & Sogin, M. L. (2010) *Environmental Microbiology* **12**, 1889-1898.
5. Böer, S. I., Hedtkamp, S. I. C., van Beusekom, J. E. E., Fuhrman, J. A., Boetius, A., & Ramette, A. (2009) *The ISME Journal* **3**, 780-791.
6. Stewart, J. R., Gast, R. J., Fujioka, R. S., Solo-Gabriele, H. M., Meschke, J. S., Amaral-Zettler, L. A., del Castillo, E., Polz, M. F., Collier, T. K., Strom, M. S., *et al.* (2008) *Environmental Health* **7**, S3.
7. Finlay, B. J. (2002) *Science* **296**, 1061-1063.
8. Pedrós-Alió, C. (2006) *Trends Microbiol* **14**, 257-263.