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Supplementary Materials for

A global comparison of surface and subsurface microbiomes reveals largescale biodiversity gradients, and a marine-terrestrial divide

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Supplementary Text Supplementary Methods Figs. S1 to S24 Legends for datasets S1 to S12 Legends for movies S1 and S2 References

Other Supplementary Material for this manuscript includes the following:

Datasets S1 to S12 Movies S1 and S2

Supplementary Text

The Census of Deep Life synthesis (continued)

Further details concerning the datasets that we used for this global synthesis project can be found on the VAMPS website [\(https://vamps2.mbl.edu/\)](https://vamps2.mbl.edu/) as well as in research studies that have already been published using NCBI project accession numbers (Dataset S1). Datasets that were part of the Census of Deep Life (CoDL) have featured in investigations of volcanoes (*158*, *159*), deep terrestrial subsurface (*7*, *107*, *160*–*162*), methane-rich springs (*163*–*165*), mineral water springs (*166*, *167*), ultradeep bedrock (*108*), rock fracture fluids (*168*–*171*), caves (*121*), marine seafloor (*172*, *173*), marine sediments (*174*–*176*), hydrothermal sediments (*46*, *177*–*179*), hydrothermal vents (*180*), marine hydrocarbon plumes (*181*), and marine mud volcanoes (*33*, *182*). We also included amplicon datasets that were not part of the CoDL, *e.g.*, investigating marine sediments (*183*–*185*), polluted sediments and waters (*186*–*188*).

Due to the large number of datasets, and the very different interests of the researchers leading each project, only a limited set of environmental parameters was available for each sample. In addition to latitude, longitude, and depth (Fig. 1A, B), we have information on the sample material (Fig. S1A), *p*H (Fig. S1B), and temperature (Fig. S1C) for most samples. About half of the samples that qualified based on their physicochemical contextual data, i.e. they originated from a suitable depth layer, were removed during the rigorous quality control of the sequencing datasets, mainly because they had too many contaminants or too little reads and were then considered a failed sequencing run. The remaining 85 archaeal and 122 bacterial sequence datasets in the "subsurface" category originated from $0.2 - 491$ m below the seafloor and $15 -$ 4375 m below ground.

Marine surface conditions may be imprinted in subsurface sediments

We show differences in the archaeal and bacterial communities in marine subseafloor shelf, slope, and abyssal sedimentary environments (Fig. S24), consistent with the differences in cell-specific energy utilization across these different depositional settings (*189*). The differences in archaeal and bacterial richness and evenness between abyssal, slope and shelf subsurface sediments (Fig. S24A, C) are corroborated by differences in the community structure at respective environments (Fig. S24B, D). These findings support earlier studies that show similarities of surface and subsurface sedimentary communities suggesting that subsurface communities are shaped and imprinted by the shallow seafloor communities from which they are derived from (*24*–*26*). However, as the samples are not all from the same margin, and as the number of samples in each category is rather low the trend should be interpreted with caution.

Supplementary Methods

16S rRNA gene amplicon-based community analyses (continued)

We have removed potential contaminant organisms affiliating with the following genera:

Aquabacterium|Pseudopropionibacterium|Cutibacterium|Propioniferax|Afipia|Beijerinckia|Bosea|Brev undimonas|Craurococcus|Methylobacterium|Roseomonas|Sphingomonas|Azoarcus|Acidovorax|Burkho lderia|Comamonas|Cupriavidus|Curvibacter|Duganella|Herbaspirillum|Janthinobacterium|Kingella|Mas

silia|Oxalobacter|Pelomonas|Undibacterium|Acinetobacter|Enhydorbacter|Enterobacter|Escherichia|Pse udoxanthomonas|Stenotrophomonas|Xanthomonas|Aeromicrobium|Brevibacterium|Corynebacterium| Dietzia|Kocuria|Microbacterium|Microlunatus|Propionibacterium|Rhodococcus|Tsukamurella|Abiotrop hia|Brochothrix|Facklamia|Streptococcus|Staphylococcus|Chryseobacterium|Dyadobacter|Wautersiella| Prevotella|Lactobacillus|Erysipelothrix|Rothia|Mycobacterium|Serratia|Staphylococcus|Bacteroides|Bif idobacterium|Shigella|Faecalibacterium|Haemophilus|Klebsiella|Lactobacillus|Listeria|Moraxella|Neiss eria|Porphyromonas|Salmonella|Veillonella|Aeromonas|Pseudarcicella|Romboutsia|Cloacibacterium|C hloroplast'

This list is based on several papers of the recent years (*64*, *79*, *80*). However, the original contaminant list by Salter et al. (*79*) contains numerous lineages that are widespread in soil and sediment environments, hence the following organisms that originally were flagged as contaminants of DNA extraction kits were retained in our analyses:

Asticcacaulis|Aurantimonas|Azospira|Pseudomonas|Psychrobacter|Caulobacter|Devosia|Hoeflea|Novo sphingobium|Paracoccus|Pedomicrobium|Sphingobium|Sphingopyxis|Delftia|Leptothrix|Limnobacter| Methylophilus|Methyloversatilis|Polaromonas|Schlegelella|Sulfuritalea|Nevskia|Beutenbergia|Janibact er|Micrococcus|Patulibacter|Bacillus|Brevibacillus|Paenibacillus|Flavobacterium|Hydrotalea|Niastella| Deinococcus|Ralstonia|Bradyrhizobium|Mesorhizobium|Ochrobactrum|Phyllobacterium|Rhizobium|Va riovorax|Arthrobacter|Curtobacterium|Geodermatophilus|Olivibacter|Pedobacter

Based on recent literature searches and the focus of the study we added the following lineages as potential contaminants or eukaryotic remnants that were excluded from this analysis: Aeromonas|Pseudarcicella|Romboutsia|Cloacibacterium|Chloroplast|Mitochondria

Fig. S1: Maps of sampling sites. Maps show the environmental material (A), *p*H (B), and temperature (C) of the collected samples.

 introducing a bias caused by the type of sampled material. Sediment samples for example comprise a very high diversity, both in the marine and terrestrial biome and thus could bias the global trends if sediments were only analyzed for one of the biomes. Marine archaeal diversity in each of the materials is higher or similar to that found in the terrestrial biome (alpha

 diversity – boxplots), while community composition is very different (beta diversity – NMDS ordinations). Note: The ordination of rock samples is not shown due to a stress value close to zero, which is most often caused by insufficient data,

yielding more than one possible solution.

Figure S3: Bacterial diversity in different sample materials grouped based on biomes. We analyzed four sample materials in this study. We included comparable numbers of samples of each material from both biomes, to avoid introducing a bias caused by the type of sampled material. Sediment samples for example comprise a very high diversity, both in the marine and terrestrial biome and thus could bias the global trends if sediments were only analyzed for one of the biomes. Marine bacterial diversity in each of the materials is higher or similar to that found in the terrestrial biome (alpha diversity – boxplots), while community composition is very different (beta diversity – NMDS ordinations), except for rocks, where community composition was largely overlapping.

Figure S4: Archaeal and bacterial diversity in water and sediment samples grouped based on biomes and depth realms. The analyses of different sample materials from the depth realms shows similar trends as the overall analysis of depth realms, e.g., a very high archaeal diversity in subsurface samples, indicating the global trends highlighted in this work were not caused by a potential bias due to the sample materials. Here too community composition was very different between marine and terrestrial samples supporting a great divide, while composition often overlapped between surface interface and subsurface samples, supporting diversity gradients with depth. Note: Not all materials were available for all depth realms, and that boxplots are shown for consistency even for groups with very small sample numbers n.

Figure S5: Archaeal and bacterial diversity in biofilm and rock samples grouped based on biomes and depth realms. The analyses of different sample materials from the depth realms shows similar trends as the overall analysis of depth realms. Note: Not all materials were available for all depth realms, boxplots are shown for consistency even for groups with very small sample numbers n, and an NMDS ordination for archaeal communities on rocks was omitted due to a stress value of near zero.

Substantial differences between marine and terrestrial microbiomes (continued)

Fig. S6: Microbial diversity indices. Archaeal (A-C) and bacterial (D-F) alpha diversity (per sample community evenness and estimated richness) in marine (M) and terrestrial (T) microbiomes using 16S rRNA gene amplicon sequence variants (ASV; A, D), as well as metagenome-derived ribosomal protein S3 genes (rpS3; B, E) and 16S rRNA gene sequences detected by phyloFlash (pF16S). Archaeal and bacterial community evenness (inverse Simpson diversity) and estimated richness (Chao1) is similar or higher in marine biomes than in terrestrial biomes in all three methods. Total archaeal (G) and bacterial (H) diversity (gamma diversity) shown as richness and Shannon based on two subsampling approaches. Using 1142 (archaea) or 2271 (bacteria) reads includes all samples, while subsampling with 50000 reads disregards all samples with less than 50k reads. Archaeal gamma diversity is higher in terrestrial biome while bacterial gamma diversity is higher in the marine biome.

Fig. S7: Community overlap. Upset plots of archaeal (A-C) and bacterial communities (D-F) in marine and terrestrial microbiomes using 16S rRNA gene amplicon sequence variants (ASV; A, D), metagenome-derived ribosomal protein S3 genes (rpS3; B, E) and 16S rRNA gene sequences detected by phyloFlash (pF16S). The plots depict the same information as Venn diagrams and show how many unique sequences (ASV, rpS3, or pF16S) exclusively occur in a group or grouping (vertical bars). The total number of unique sequences in a group (gamma diversity) is shown as horizontal bars. For example, in panel A, the total number of archaeal ASV is higher in terrestrial ecosystems (light brown bar) than in marine biomes (purple bar). Within terrestrial biomes 14624 ASV occur exclusively there (first vertical bar), 6565 occur exclusively in marine biomes, and only 553 are shared between both biomes. The ASV data for panel A and D was subsampled, *i.e.*, corrected for the different number of datasets from each biome, to allow direct comparison between biomes.

Fig. S8: Community structure in marine and terrestrial biomes. Plots are based on archaeal (A) and bacterial rpS3 genes (B) as well as on archaeal (C) and bacterial metagenome-derived 16S rRNA genes (pF16S; D). Apart from archaeal pF16S, the biomes show a rather small overlap, corroborating the substantial differences seen with ASV analyses, which is striking given that metagenomes are not ideal in detecting community diversity and structure, but rather useful for investigating the most abundant populations.

Fig. S9: Abundance analyses of marine vs terrestrial lineages. Differential abundance analysis of bacterial (A) and archaeal (B) phyla. The phyla are ordered from top to bottom based on increasing phylum level MaAsLin2 coefficient, *i.e.* likeliness of their occurrence in terrestrial-derived samples ("terrestrial-ness"). Boxplots summarize MaAsLin2 coefficients, i. e., "terrestrial-ness", of orders withing the listed phyla. Note: due to ease of visualization boxplots are also shown for very small number (n) of orders; phyla and orders are based on the SILVA reference tree. The significance of this occurrence is shown in the column denoted "signif". Significance levels are: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: p<0.0001. Phyla in brown are found significantly more often in the terrestrial biome, while phyla in purple are found significantly less often in the terrestrial biome, *i.e.*, occur more often in the marine biome. The analysis was done using MaAsLin2 on phylum and order level (*86*). Relative abundance of archaeal classes (C) and bacterial phyla (D) found in marine and terrestrial biome (C). The 20 most abundant classes are shown, remaining classes are shown as "Other". (D) Total number of unique ASVs found in respective classes (E) and phyla (F).

Fig. S10: Alpha diversity indices. Alpha diversity of archaeal (A-D) and bacterial communities (E-H) in sequencing datasets from marine (M) and terrestrial (T) surface, interface, and subsurface ecosystems. Columns show four major diversity indices (observed richness, Shannon Entropy, Inverse Simpson Diversity and Chao1 estimated richness), rows show which data was used to calculate diversity indices (metabarcoding-derived 16S amplicon sequence variants – ASVs, metagenome-derived rpS3 genes, and metagenome-derived 16S rRNA gene reads mapped to a reference tree using phyloFlash – pF16S). The number (n) of used samples for each category is shown. Significance was tested using a Wilcoxon rank sum test, significance values are: $*: p<0.05; **: p<0.01; ***: p<0.001; ***: p<0.0001$. p values were corrected using the Bonferroni method. Note: Shannon and Simpson indices are relatively independent of the total number of ASVs analyzed. The presented values derive from an analysis of 2000 subsampled reads per sample but are basically indiscernible from an analysis using 50000 reads per sample (Fig. S11). In contrast, the number of observed ASVs (richness) does change with the number of subsampled reads analyzed, however the relative differences between groups are retained. Almost all pairs that were significantly different were between biomes, very few within biomes, corroborating the great differences between marine and terrestrial communities.

Fig. S11: Alpha diversity indices of subsampled data. To investigate the influence of subsampling on alpha diversity we used a very conservative cutoff using only samples that had at least 50000 archaeal or bacterial randomly chosen reads per sample. The difference in number of samples per group (n) to the ASV rows in Fig. S10 is due to samples that had less than 50000 archaeal or bacterial reads and were thus discarded. Overall trends in alpha diversity and significant differences between groups were very similar to the results obtained by analyzing 1142 archaeal and 2216 bacterial reads, the cutoff that was used for all other analyses.

Fig. S12: Microbial beta diversity. Non-metric multidimensional scaling (NMDS) of archaeal (A, C) and bacterial (B, D) communities based on amplicon sequence variants (A, B) and rpS3 genes (C, D). Each dot represents the microbial community structure of a sample, colors represent six groups – three depths in both biomes. The plots show 469 archaeal amplicon datasets (37 projects, A), and 1105 bacterial amplicon datasets (51 projects, B), 91 archaeal rpS3 (C), and 117 bacterial rpS3 gene datasets (D). In A and B, the groups are overlapping, yet significantly different based on an Analysis of Similarity (ANOSIM). The separation between groups in A and B is easier to visualize in a 3-dimensional space, for this we have calculated and animated a 3D-NMDS (Video S1, S2) visualizing that each of the ecosystem types harbored distinct archaeal (ANOSIM: R=0.63; p=0.001) and bacterial communities (ANOSIM: R=0.54; p=0.001). In C and D, the groups are overall not significantly different, however the clear separation of the centroids, *i.e.* the average within-group distances, and ellipses (depicting 1 standard deviation from the centroid) corroborate the ASV-based trend. Community differences are particularly clear in marine vs terrestrial realms, *e.g.*, the blue (marine interface) and orange (terrestrial interface) ellipses, or the dark blue (marine subsurface) and red (terrestrial subsurface) ellipses hardly overlap, suggesting very distinct communities in these realms.

Total # of ASVs in realm

Fig. S13: Community overlaps between analyzed biomes and depth realms. Upset plots show subsampled archaeal (A) and bacterial communities (B). Subsampling was done to account for different numbers of samples per group and thus resulting unequal sampling effort. The plots depict the same information as Venn diagrams and show how many amplicon sequence variants (ASV) exclusively occur in a group or grouping (vertical bars). The total number of ASV of a group (gamma diversity) is shown as horizontal bars. For example, the highest number of total archaeal ASV in the subsampled dataset is found in marine subsurface environments. This group contains more than 1000 archaeal ASVs in total (gamma diversity, dark blue horizontal bar in A) of which 984 archaeal ASV occur exclusively in this group (1st vertical bar in A). Another example, there are 624 bacterial ASV that occur exclusively in terrestrial surface AND interface datasets, but nowhere else ($7th$ vertical bar in B), this is depicted by the two connected black dots in the $2nd$ and $4th$ row. Archaeal and bacterial richness is decreasing with depth in terrestrial environments, but in marine environments richness is similar across depth for bacteria and even increasing with depth for archaea. Corroborating the trends seen with alpha diversity indices, and NMDS plots, it is clear from the upset plots that more ASVs are shared between realms of the same biome than between biomes.

Fig. S14. Observed archaeal and bacterial diversity in individual CoDL projects. Archaeal and bacterial alpha diversity found in individual marine and terrestrial CoDL projects compared to bulk marine and terrestrial surface and interface realms. Three terrestrial projects with less than 5 samples are grouped as "Other" (n: number of included samples).

Movie S1, S2: Video-animated three-dimensional non-metric multidimensional scaling. Ordination of archaeal (Video S1) and bacterial community structure (Video S2) based on 16S rRNA gene amplicon sequence variants (ASVs). Each dot is one sample, dot size represents Shannon entropy. The spheres depict the volume enclosing one standard deviation from the weighted average mean of within-group distances (centroid). Each sample is connected to the centroid of the group it belongs to.

Figure S15: Species accumulation and rarefaction curves based on 16S rRNA gene amplicon sequence variants (ASV). All archaeal (A) and bacterial (B) accumulation curves, except those of archaeal marine surface samples, show a steep slope. This means that we can expect to find many more species-level clades (ASVs) in all these environments. Global microbial diversity is far from being exhaustively sampled. Using a read-based rarefaction the archaeal (C) and bacterial (D) curves of all groups reach a plateau. Taken together this indicates that the sequencing depth was sufficient to detect all "species" in the presently studied sites/samples, but that more "species" await discovery if more sites are investigated.

Fig. S16: Archaeal class-level composition across all samples and phyloFlash-based community composition in metagenomes. (A) Each bar represents one sample and shows the relative sequence abundance of the top 10 most abundant archaeal classes. All remaining classes are summed and shown as "Other". Relative sequence abundances are based on the subsampled ASV × Sample table that was used for all community analyses. The samples are grouped by depth, biome, and environments (vertical bars above) and ordered analogous to Fig. 3. The bottom panels show the 12 most sequence abundant archaeal classes (B) and bacterial phyla (C) as found in the subsurface metagenomes with a short read mapping approach using phyloFlash.

Fig. S17: Bacterial class-level composition across all samples. Each bar represents one sample and shows the relative sequence abundance of the top 10 most abundant bacterial classes. All remaining classes are summed and shown as "Other". Relative sequence abundances are based on the subsampled ASV × Sample table that was used for all community analyses. The samples are grouped by depth, biome, and environments (vertical bars above) and ordered analogous to Fig. 3.

Fig. S18: Differential sequence abundance analyses. Analyses comparing the occurrence of bacterial (A) and archaeal (B) phyla in subsurface vs surface realms. The phyla are ordered from top to bottom based on increasing phylum level MaAsLin2 coefficient, *i.e.* likeliness of their occurrence in subsurface-derived samples ("subsurfaceness"). Boxplots summarize MaAsLin2 coefficients, i. e., "subsurface-ness", of orders withing the listed phyla. Due to ease of visualization boxplots are even shown for very small number (n) of orders. The significance of this occurrence is shown in the column denoted "signif". Significance levels are: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Phyla in in pink are found significantly more often in the surface realm, while phyla in maroon are found significantly less often in the surface realm, *i.e.*, occur more often in the subsurface. Note: Association with a category does not necessarily mean that respective lineages are sequence abundant in either or both categories. In turn, a preferential occurrence, e.g., in the surface, does not mean a clade is not abundant in the subsurface. *Bacteroidota* and *Crenarchaeota* for example are associated with the surface, yet they are very widespread and abundant also in the subsurface, just not quite as much and as prevalent. Thus, the broad trends suggested by the association analysis are especially useful and informative when interpreted in combination with the actual relative sequence abundances of the respective lineages (Fig. 4, 6, 8).

Fig. S19: Abundance analyses of marine surface vs subsurface lineages. Differential abundance analysis of bacterial phyla. The phyla are ordered from top to bottom based on increasing phylum level MaAsLin2 coefficient, *i.e.* likeliness of their occurrence in marine subsurface-derived samples ("marine subsurface-ness"). Boxplots summarize MaAsLin2 coefficients, i. e., "marine subsurface-ness", of orders withing the listed phyla. Note: due to ease of visualization boxplots are also shown for very small number (n) of orders; phyla and orders are based on the SILVA reference tree. The significance of this occurrence is shown in the column denoted "signif". Significance levels are: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001.

Fig. S20: Abundance analyses of terrestrial surface vs subsurface lineages. Differential abundance analysis of bacterial phyla. The phyla are ordered from top to bottom based on increasing phylum level MaAsLin2 coefficient, *i.e.* likeliness of their occurrence in terrestrial subsurface-derived samples ("terrestrial subsurface-ness"). Boxplots summarize MaAsLin2 coefficients, i. e., "terrestrial subsurface-ness", of orders withing the listed phyla. Note: due to ease of visualization boxplots are also shown for very small number (n) of orders; phyla and orders are based on the SILVA reference tree. The significance of this occurrence is shown in the column denoted "signif". Significance levels are: *: $p<0.05$, **: $p<0.01$, ***: p<0.001, ****: p<0.0001.

Detection Threshold (Relative Sequence Abundance (%))

Detection Threshold (Relative Sequence Abundance (%))

Fig. S21. Subsurface archaeal genus-level core microbiomes. The heatmaps show potential archaeal genus level core microbiomes in the marine and terrestrial subsurface, regarding their prevalence and relative sequence abundances. Lineages are shown as rows, each column color represents the lineages prevalence at a certain RSA threshold. Uncultured/unclassified lineages are denoted by "unc" and the closest known phylogenetic level or the closest phylogenetic level with an isolated representative is shown. For example, Bathyarchaeia uncl are all uncultured/unclassified/ genus level lineages in the class Bathyarchaeia, and thus likely condense many uncultured genera into one row.

Fig. S22. Subsurface bacterial genus-level core microbiomes. The heatmaps show potential bacterial genus level core microbiomes in the marine and terrestrial subsurface, regarding their prevalence and relative sequence abundances. Lineages are shown as rows, each column color represents the lineages prevalence at a certain RSA threshold. Uncultured/unclassified lineages are denoted by "unc" and the closest known phylogenetic level or the closest phylogenetic level with an isolated representative is shown. For example, Caldatribacteria_JS1 uncl are all uncultured/unclassified/ genus level lineges in the class Caldatribacteria_JS1, and thus likely condense many uncultured genera into one row.

Fig. S23: Phylogenetic novelty. Summary of the percent identity values of n (below each boxplot) archaeal (A) and bacterial (B) amplicon sequence variants (ASVs) for the six studied realms. To determine the percent identity, each 16S rRNA gene amplicon was aligned to a database of cultured isolates. Every pairwise comparison was highly significant using a Wilcoxon rank sum test (p<0.01), except marine and terrestrial surface (archaea) and terrestrial interface and subsurface groups (archaea), significances not shown.

Fig. S24: Microbial diversity in subsurface sediments from different depositional environments. Archaeal (A, B) and bacterial (C, D) alpha diversity and beta diversity are significantly different between shelf and slope subsurface sediments. Significances were calculated with a Wilcoxon Rank sum test. Levels are: *: p<0.05, **: p<0.01, ***: p<0.001. Note: The number of archaeal datasets for abyssal and shelf environments is low providing limited statistical support.

Additional supplementary materials

Dataset S1: Project IDs and Dataset Accession numbers

Dataset S2: Sample IDs and contextual data

Dataset S3: Diversity Indices of Archaea

Dataset S4: Diversity Indices of Bacteria

Dataset S5: List of phyloFlash NTUs and their taxonomy

Dataset S6: List of rpS3 seqs and their taxonomy

Dataset S7: Wilcoxon statistics of diversity index calculations

Dataset S8: VisuaR community analysis log for archaea

Dataset S9: VisuaR community analysis log for bacteria

Dataset S10: Example DADA2 analysis script

Dataset S11: Example VisuaR User Input script

Dataset S12: Example VisuaR Analysis script

Movie S1: 3D NMDS ordination of archaeal ASV grouped based on biomes and depths

Movie S2: 3D NMDS ordination of bacterial ASV grouped based on biomes and depths

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