A global perspective on microbial diversity in the terrestrial deep subsurface

Authors:

Soares, A.^{1,2,3}, Edwards, A.^{2,3*}, An, D.⁴, Bagnoud, A.⁵, Bomberg, M.⁶, Budwill, K.⁷, Caffrey, S. M.⁸, Fields, M.⁹, Gralnick, J.¹⁰, Kadnikov, V.^{11,12}, Momper, L.¹³, Osburn, M.¹⁴, Moreau, J.W.¹⁵, Moser, D.¹⁹, Mu, A.^{15,16,17,18}, Purkamo, L.^{6,20,21}, Rassner, S. M.^{1,3}, Sheik, C. S.²², Sherwood Lollar, B.²³, Toner, B. M.²⁴, Voordouw, G.⁴, Wouters, K.²⁶, Mitchell, A. C.^{1,3*}

Affiliations:

1. Department of Geography and Earth Sciences (DGES), Aberystwyth University (AU), Wales, UK
2. Institute of Biology, Environmental and Rural Sciences (IBERS), AU
3. Interdisciplinary Centre for Environmental Microbiology (iCEM), AU
4. Department of Biological Sciences, University of Calgary, Canada
5. Institut de Génie Thermique (IGT), Haute École d'Ingénierie et de Gestion du Canton de Vaud (HEIG-VD), Yverdon-les-Bains, Switzerland
6. VTT Technical Research Centre of Finland, Finland
7. Alberta Innovates, Canada
8. University of Toronto, Canada (UT)
9. Center for Biofilm Engineering (CBE), Montana State University (MSU), USA
10. Department of Plant and Microbial Biology, UM, USA
11. Faculty of Biology, Moscow State University (MoSU), Russia
12. Institute of Bioengineering, Research Center of Biotechnology, Russian Academy of Sciences, Russia
13. Department of Earth, Atmospheric and Planetary Sciences (DEAPS), The Massachusetts Institute of Technology (MIT), United States of America (USA)
14. Department of Earth and Planetary Sciences (DEPS), Northwestern University (NWU), USA
15. School of Earth Sciences, The University of Melbourne (UM), Parkville, Australia
16. Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, UM
17. Doherty Applied Microbial Genomics, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, UM

18. Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, UM
19. Division of Hydrologic Sciences, Desert Research Institute (DRI), Las Vegas, NV, USA
20. School of Earth and Environmental Sciences (SEES), University of St. Andrews (USA), Scotland, UK
21. Geological Survey of Finland (GTK), Finland
22. Large Lakes Observatory, University of Minnesota – Duluth (UMD)
23. Department of Earth Sciences, UT, Canada
24. Department of Soil, Water & Climate, University of Minnesota
25. Institute for Environment, Health and Safety (EHS), Belgian Nuclear Research Centre SCK•CEN, Mol, Belgium

***Corresponding Authors:** AC Mitchell nem@aber.ac.uk; A Edwards aye@aber.ac.uk

Summary

While recent efforts to catalogue Earth's microbial diversity have focused upon surface and marine habitats, 12% to 20% of Earth's bacterial and archaeal biomass is suggested to inhabit the terrestrial deep subsurface, compared to ~1.8% in the deep subseafloor¹⁻³. Metagenomic studies of the terrestrial deep subsurface have yielded a trove of divergent and functionally important microbiomes from a range of localities⁴⁻⁶. However, a wider perspective of microbial diversity and its relationship to environmental conditions within the terrestrial deep subsurface is still required. Here, we show the diversity of bacterial communities in deep subsurface groundwater is controlled by aquifer lithology globally, by using 16S rRNA gene datasets collected across five countries on two continents and from fifteen rock types over the past decade. Furthermore, our meta-analysis reveals that terrestrial deep subsurface microbiota are dominated by Betaproteobacteria, Gammaproteobacteria and Firmicutes, likely as a function of the diverse metabolic strategies of these taxa. Despite this similarity, evidence was found not only for aquifer-specific microbial communities, but also for a common small consortium of prevalent Betaproteobacteria and Gammaproteobacterial OTUs across the localities. This finding implies a core terrestrial deep subsurface community, irrespective of aquifer lithology, that may play an important role in colonising and sustaining microbial habitats in the deep terrestrial subsurface. An *in-silico* contamination-aware approach to analysing this dataset underscores the importance of downstream methods for assuring that robust conclusions can be reached from deep subsurface-derived sequencing data. Understanding the global panorama of microbial diversity and ecological dynamics in the deep terrestrial subsurface provides a first step towards understanding the role of microbes in global subsurface element and nutrient cycling.

Main text

Understanding the distribution of microbial diversity is pivotal for advancing our knowledge of deep subsurface global biogeochemical cycles^{7,8}. Subsurface biomass is suggested to have exceeded that of the Earth's surface by an order of magnitude (~45% of Earth's total biomass) before land plants evolved, at ca. 0.5 billion years ago⁹. Integrative modelling of cell count and quantitative PCR (qPCR) data and geophysical factors indicated in late 2018 that the bacterial and archaeal biomass found in the global deep subsurface may range from 23 to 31 petagrams of carbon (PgC). These values halved previous efforts from earlier that year¹⁰ but maintained the notion that the terrestrial deep subsurface holds ca. 5-fold more bacterial and archaeal biomass than the deep marine

subsurface. Further, it is expected that 20-80% of the possible $2\text{-}6 \times 10^{29}$ prokaryotic cells present in the terrestrial subterranean biome exist as biofilms and play crucial roles in global biogeochemical cycles^{10,11}.

Cataloguing microbial diversity and functionality in the terrestrial deep subsurface has mostly been achieved by means of marker gene and metagenome sequencing in coals, sandstones, carbonates, and clays, as well as deep igneous and metamorphic rocks^{4-6,12-20}. Only recently has the first comprehensive database of 16S rRNA gene-based studies targeting terrestrial subsurface environments been compiled¹⁰. This work focused on updating estimates for bacterial and archaeal biomass, and cell numbers across the terrestrial deep subsurface, but also linked the identified bacterial and archaeal phylum-level compositions to host-rock type, and to 16S rRNA gene region primer targets¹⁰. While highlighting Firmicutes and Proteobacterial dominance in the bacterial component of terrestrial deep subsurface, no further taxonomic insights were gained. However, genus-level identification is critical for understanding community composition, inferred metabolism and hence microbial contributions of distinct community members to biogeochemical cycling in the deep subsurface^{18,21-23}. Indeed, such genus-specific traits have been demonstrated as critical for understanding crucial biological functions in other microbiomes²⁴, and genus-specific functions of relevance for deep subsurface biogeochemistry are clear^{25,26}.

So far, the potential biogeochemical impacts of microbial activity in the deep subsurface have been inferred through shotgun metagenomics, as well as from incubation experiments of primary geological samples amended with molecules or minerals of interest^{13,19,20,27-30}. Recent studies of deep terrestrial subsurface microbial communities further suggest that these are metabolically active, generally associated with novel uncultured phyla, and potentially directly involved in carbon and sulphur cycling³¹⁻³⁶. Concomitant advancements in subsurface drilling, molecular methods and computational techniques have aided the exploration of the subsurface biosphere, but serious challenges remain mostly related to deciphering sample contamination by drilling methods and sample transportation to laboratories for processing^{37,38}. The logistical challenges inherent to accessing and recovering *in situ* samples from hundreds to thousands of metres below surface complicate our view of terrestrial subsurface microbial ecology³⁹.

In this study, we capitalize on the increased availability of 16S rRNA gene amplicon data from multiple studies of the terrestrial deep subsurface conducted over the last decade. We apply bespoke bioinformatic scripts to generate insights into the microbial community structure and controls upon bacterial microbiomes of the terrestrial deep subsurface across a large distribution of habitat types on

multiple continents. The deep biosphere is as-yet undefined as a biome - elevated temperature, anoxic conditions, low levels of organic carbon, and measures of isolation from the surface photosphere are some of the criteria used albeit without a consensus. For this work a more general approach has been taken to define the terrestrial deep subsurface as the zone at least 100 m from the surface^{40,41}.

Meta-analysis of the terrestrial deep subsurface microbiome

Here, we were able to compare datasets encompassing different 16S rRNA gene hyper-variable regions, and derived from different DNA extraction methodologies, facilitated by closed-reference Operational Taxonomic Unit (OTU)-picking of each study individually using the same 16S rRNA gene reference database. This procedure begins to address technically confounding variables by limiting taxonomy assignments to only the archaeal and bacterial diversity listed in the chosen database and precludes the discovery of novel taxa.

The finalized meta-analysis dataset comprised of 16S rRNA data from seventeen aquifers in either sedimentary- or crystalline-host rocks, from depths spanning 94 m to 2300 m below land surface (mbls), targeting mostly groundwater across 5 countries and two continents (**Supplementary Table 3**). Nine DNA extraction techniques were used in these studies, ranging from standard and modified kit protocols (e.g. MOBIO® PowerSoil, see **Table 1**) to phenol-chloroform and CTAB/NaCl based methods^{42–47}. Finally, 6 different primer pair amplified regions of the 16S rRNA gene, in 454 pyrosequencing and Illumina sequencing, were used to generate the datasets.

Table 1. Metadata table for the studies utilized in this meta-analysis (*cf.* **Supplementary Table 3** and **Supplementary Figure 3** for more details). NA is used as an acronym for “not available”. The dataset unavailable through SRA is available through <http://hmp.ucalgary.ca/HMP/>.

SRA Accession	Originator	DOI	Year of Sampling	Final no. of samples	Location	Depth gradient (m)	Host-rock	Final no. of sequences	Final no. of OTUs
PRJNA262938	Magdalena Osburn, Lily Momper	10.3389/fmicb.2014.00610	2013/2014	6	South Dakota, USA	243.84-1478.28	Sulphide-rich schists	170364	1367
PRJNA268940	Duane Moser	NA	2007-2011	8	California, USA; Ontario, Canada	94-2383	Dolomite, Tuff, Rhyolite/tuff-breccia	68071	741
PRJNA248749	Jeffrey Gralnick	NA	2011	6	Minnesota, USA	730	Hematite	69757	511
PRJNA251746	Rick Colwell	NA	2009	6	Washington, USA	393-1135	Basalt	30121	613
PRJNA375701	Elliot Barnhart	10.1016/j.coal.2016.05.001	2013	5	Montana, USA	109-114.7	Sub-bituminous coal, Shale, Sandstone, Siltstone	35926	154
NA	Karen Budwill	10.1128/AEM.01737-15	2009	38	Alberta, Canada	140-1064.4	Sub-bituminous coal, Volatile bituminous coal	100618	5110
PRJEB1468	Katinka Wouters	10.1111/1574-6941.12171		6	Mol, Belgium	217-232	Kaolinite/illite and smectite	47123	497
PRJEB10822	Lotta Purkamo	10.5194/bg-13-3091-2016	2009-2011	7	Outokumpu, Finland	180-2300	Mica-schist, Biotite-gneiss, Chlorite-sericite schist	12290	177

141

142 Initial processing of 187 retrieved samples revealed 24,632,035 chimera-checked sequences
 143 17,28,43,44,48–50. SILVA 123-aided closed-reference OTU-picking yielded 6,975 OTUs associated to
 144 598,341 sequences following exclusion of singleton OTUs and samples containing 2 or less OTUs.
 145 The final dataset following stricter contamination-aware filtering (*cf.* **Methodology**) was comprised
 146 of 70 samples and 2,207 OTUs (513,929 sequences, 2.54% of the initial sequences), where Archaeal
 147 reads comprised 1.5% of the total number of reads.

148

149 *Trends in taxonomic diversity*

150 Among a total of 45 detected bacterial phyla, Proteobacteria were seen to dominate most community
 151 profiles in this dataset (**Figure 1**). The most abundant proteobacterial classes (Alpha-,
 152 Betaproteobacteria, Delta-, Gammaproteobacteria) represented 57.2% of the total number of reads,
 153 with 13.4% of these assigned to class Clostridia (Firmicutes). A general prevalence of
 154 Betaproteobacteria and Gammaproteobacteria in the deep biosphere may be explained by the diverse
 155 metabolic capabilities of taxa within these clades. Families Gallionellaceae, Pseudomonadaceae,
 156 Rhodocyclaceae and Hydrogeniphillaceae within Betaproteobacteria and Gammaproteobacteria are
 157 suggested to play crucial roles in deep subsurface iron, nitrogen, sulphur and carbon cycling across
 158 the world^{43,51,52}. The relative abundance of order Burkholderiales (Betaproteobacteria) in surficial
 159 soils has previously been correlated ($R^2=0.92$, ANOVA p-value <0.005) with mineral dissolution
 160 rates, while genus *Pseudomonas* (Gammaproteobacteria) is widely known to playing a key role in
 161 hydrocarbon-degradation, denitrification and coal solubilisation in different locations^{53–55}.

162

163 **Figure 1.** Mean relative abundances (% , y-axis) of the most abundant taxonomic groups across the
 164 dataset across all analysed aquifer lithologies (x-axis). Vertical dashed lines divide crystalline and

sedimentary rocks. Coals ranks are also separated due to their higher sample contribution to the dataset.

Mean grouped proportion values indicated that Betaproteobacteria were the most abundant proteobacterial class in most host rocks, representing 26.1% of all reads in the dataset. While Betaproteobacteria accounted for 53.96% of the community profile for sub-bituminous coals, Gammaproteobacteria dominated volatile bituminous coals (49.1% of the profile, **Figure 1**). The dominance of Betaproteobacteria and Gammaproteobacteria in coals builds on culture-based evidence of widespread degradation of coal-associated complex organic compounds by these classes^{56–59}.

Firmicutes were represented in large part by class Clostridia and mostly associated with sedimentary aquifers (i.e. sandstone, dolomite, siltstone, shale – **Figure 1**). This class includes ubiquitous anaerobic hydrogen-driven sulphate reducers also known to sporulate and metabolize a wide range of organic carbon compounds that have been found to dominate extremely deep subsurface ecosystems beneath South Africa and likely globally given the pervasive high levels of H₂ in similar geologic settings^{13,60–62}. Clostridia from metagenomes have been detected from the terrestrial deep subsurface and inferred to have the physiological capabilities needed to thrive in these environments²⁰. Adaptation to extreme environments in Clostridia is posed to be driven by varied metabolic potential, sporulation ability, and capacity for CO₂- or sulphur-based autotrophic H₂-dependent growth^{22,63}.

Lithological controls on community structure

Microbial community structure and composition in soils depend on fine-tuned geochemical, physical and hydrogeological conditions that influence microbial presence and metabolism⁶⁴. This relationship also appears to be reflected in the global subsurface, where host-rock lithology is evident as a primary control on community structure (**Figures 2 and 3**). Indeed, most host-rocks (10 out of 15 in this dataset) have, on average, more unique OTUs than they share with other host-rocks (**Figure 2**). Particularly, in sulphide-rich schists, 73% of the OTUs are, on average, unique to the host-rock. The role of host-rock lithology is further evidenced (**Figure 3**) as some of the host-rocks clustered at a 95% confidence interval, suggesting closely related microbial communities within similar lithologies, despite other environmental factors such as depth or location. Further, 50.6% of Jensen-Shannon distances ordinated (**Figure 3**) were significantly explained by aquifer lithology (ADONIS/PERMANOVA, F-statistic=4.65, p-value <0.001, adjusted Bonferroni correction p-value <0.001); thus showing that rock type was the primary variable defining microbial community

200 structure. Other environmental features such as absolute sample depth and medium-scale location
201 (i.e. state, region of the sampling site) explained only 3.08% and 2.78% of the significant metadata-
202 driven variance in microbial community structure, respectively (ADONIS/PERMANOVA, F-
203 statistic=3.95, 3.57 p-value <0.001, adjusted Bonferroni correction p-value <0.001). This suggests
204 that depth-related changes in temperature and pressure are not significant controls on community
205 structure. The relationship of community structure to hydrogeochemical parameters remains an area
206 for future investigation – since hydrogeology and fluid geochemistry are also strongly controlled by
207 lithology via water-rock reactions.

208 **Figure 2.** UpsetR plot of mean numbers of OTU interactions among rock types. Only interactions
209 involving 25 or more OTUs on average are shown. Coloured matrix rows correspond to host-rocks,
210 and are coloured according to rock type: blue for crystalline, green for sedimentary rocks and pink
211 for coals, which were highlighted due to their higher sample contribution to the dataset. Columns
212 depict OTU interactions: blue dots mark independent (mean number of non-shared OTUs)
213 interactions and black dots connected by black lines mark shared OTUs between two or more host-
214 rocks. Shared interactions are composed of only the host-rocks marked by dots. Vertical bars on top
215 of the coloured matrix correspond to mean OTU numbers present in the described interactions and
216 are coloured black or blue if depicting shared or non-shared interactions, respectively. Horizontal
217 bars by the left of the coloured matrix depict mean total numbers of OTUs per host-rock.

Figure 3. Non-metric Multidimensional Scaling (nMDS) of Jensen-Shannon distances between samples (A). Shapes correspond to different locations, whereas colours depict host-rocks targeted in this study. B depicts a Shepard's stress plot of observed (original) dissimilarities and ordination distances. An R^2 measure of stress is further shown for the non-metric fit of the variables. Confidence interval ellipses were plotted at a level of 95% according to host-rock.

Metadata variables that were unavailable for all samples in the dataset were excluded from the statistical analyses, thus further insights into the significance of other environmental variables was not possible. Nevertheless, this is the first large-scale evidence that deep subsurface microbial community taxonomy appears host-rock-specific. Given the importance of chemolithotrophic metabolisms in dark, subsurface environments, the unique chemical and mineralogical compositions within different aquifer lithologies impart strong controls over microbiomes associated with mineral surfaces and porewaters^{43,62,65–67}. Indeed, direct utilisation of mineral surfaces or dissolved species from minerals for respiration and/or metabolism has been shown to be critical in localised subsurface environments^{30,35,68,69}. Due to low numbers of samples for some host-rock lithologies in this dataset (e.g. one sample each for siltstone, sandstone, shale and chlorite-sericite-schist), it is not possible to ascertain that microbiome specificity is generalisable to all deep subsurface aquifer types on Earth

(see **Figure 3**). Nevertheless, this study provides the first large-scale evidence that, at a global scale, lithology surpasses depth in shaping deep subterranean microbial communities.

A core terrestrial deep subsurface microbial community?

Analysis of prevalence across the dataset revealed that seven OTUs, all affiliated to genus *Pseudomonas*, were present in more than 25 and up to 41 samples (see **Supplementary Figure 1, Supplementary Table 2**). Network analysis (**Table 2**) highlighted a *Pseudomonas* OTU highly connected to other OTUs in the dataset. Further, BLAST⁷⁰ results indicated that recovered sequences for OTUs affiliated to this genus were generally associated to marine and terrestrial soil and sediments (*cf* **Supplementary Table 4, Supplementary Figure 4**). Four OTUs affiliated to Burkholderiales (Betaproteobacteria), the second most prevalent order in the dataset, were also found to be connected to up to 34 other OTUs. Genus *Thauera* (Betaproteobacteria, Rhodocyclales), represented by a single OTU, was the second most central to the dataset. Finally, network and prevalence analysis highlighted the putative importance classes Betaproteobacteria and Gammaproteobacteria may have in the deep subsurface, since taxa affiliated to these were highly connected across the dataset (**Table 2**). These observations suggest genus-level taxonomy may be relevant to shaping subterranean microbial communities irrespective of host-rock lithology.

255 **Table 2.** Top 10 most central OTUs in the Jaccard distances network (as defined by eigenvector centrality scores, or the scored value of the centrality of
 256 each connected neighbour of an OTU) and correspondent closeness centrality (scores of shortest paths to and from an OTU to all the remainder in a
 257 network) and degree (number of directly connected edges, or OTUs) values.

OTU ID	OTU Classification	Centrality	Closeness	Degree
EF554871.1.1486	Proteobacteria; Gammaproteobacteria ; Pseudomonadales; Pseudomonadaceae; Pseudomonas	1.0000000	2.13e-05	38
HH792638.1.1492	Proteobacteria; Betaproteobacteria ; Rhodocyclales; Rhodocyclaceae; Thauera	0.9753542	2.13e-05	36
HQ681977.1.1496	Proteobacteria; Betaproteobacteria ; Burkholderiales; Comamonadaceae; Diaphorobacter	0.9445053	2.13e-05	34
KF465077.1.1336	Proteobacteria; Betaproteobacteria ; Burkholderiales; Comamonadaceae; Acidovorax	0.8887751	2.13e-05	30
JQ072853.1.1348	Proteobacteria; Betaproteobacteria ; Rhodocyclales; Rhodocyclaceae; Thauera	0.8808435	2.13e-05	30
KM200734.1.1449	Proteobacteria; Alphaproteobacteria ; Rhizobiales; Rhizobiaceae; Rhizobium	0.8716886	2.13e-05	31
KC758926.1.1392	Proteobacteria; Betaproteobacteria ; Burkholderiales; Comamonadaceae; Acidovorax	0.8662805	2.13e-05	29
FJ032194.1.1456	Proteobacteria; Betaproteobacteria ; Burkholderiales; Comamonadaceae; Rhodoferax	0.8662805	2.13e-05	29
EU771645.1.1366	Firmicutes ; Bacilli; Bacillales; Planococcaceae; Planomicrobium	0.8476970	2.13e-05	30
JN245782.1.1433	Proteobacteria; Alphaproteobacteria .; Rhodobacterales; Rhodobacteraceae; Defluviimonas	0.8356655	2.13e-05	29

259 The metabolic plasticity of Pseudomonadales and Burkholderiales orders has been demonstrated ^{71–}
 260 ⁷⁴ and may be a catalyst for their apparent centrality across the terrestrial deep subsurface
 261 microbiomes analysed in this study. These bacterial orders may represent important keystone taxa in
 262 microbial consortia responsible for providing key substrates to other colonizers in deep subsurface
 263 environments ^{75,76}. In particular, given the number of highly central *Pseudomonas*-affiliated OTUs
 264 and the prevalence of this genus in the dataset, we suggest that this genus may be key in establishing
 265 conditions for microbial colonization in many terrestrial subsurface environments. Genus
 266 *Pseudomonas* and possibly several members of Burkholderiales may therefore comprise an important
 267 component of the global core terrestrial deep subsurface microbial community.

268

269 ***Challenges from contamination***

270 16S rRNA gene PCR-based approaches for characterizing microbial diversity in low biomass
 271 environments benefit from the sensitivity afforded by PCR, at the cost of vulnerability to
 272 contamination ⁷⁷. Here, we used the prominence of sequences associated with phototrophic taxa as
 273 an indicator of either ingress of surface microbiota or contamination during sample processing. The
 274 discovery of potentially photosynthetic taxa in the initial dataset, namely 46 OTUs classified as
 275 Chloroplast (Cyanobacteria) was read as a sign that further bioinformatics-driven precautions should
 276 be taken, despite recent evidence of some cyanobacterial presence in some locations within the deep
 277 subsurface ^{78,79}. Specifically, the presence of other phototrophic members of phyla Chloroflexi and
 278 Chlorobi as well as classes Rhodospirillales (Alphaproteobacteria) and Chromatiales
 279 (Gammaproteobacteria) informed the decision to filter the dataset to hold only OTUs represented by
 280 more than 500 sequences and present in at least 10 samples. Recent recommendations for quality
 281 control of 16S rRNA gene datasets also support filtering-based approaches when applied to low
 282 biomass subsurface environments ³⁸. This constraint reduced the dataset to the 70 samples and 2,207
 283 OTUs (513,929 sequences) used for the meta-analysis (**Table 1**), and also reduced the number of
 284 prospective contaminants by half, although only ~26% of the reads associated to Chloroplast-like
 285 sequences were removed (17 OTUs, 1958 reads).

286

287 Collecting contamination-free samples from the deep subsurface is difficult but important for
 288 cataloguing the authentic microbial diversity of the terrestrial subsurface. This study follows recent
 289 recommendations for downstream processing of contaminant-prone samples originated in the deep
 290 subsurface (Census of Deep Life project - <http://codl.coas.oregonstate.edu/>), where physical,
 291 chemical and biological, but also *in-silico* bioinformatics strategies to prevent erroneous conclusions
 292 have been highlighted ^{38,80,81}. This study also follows frequency-based OTU filtration techniques

similar to those recommended in Sheik *et al.* (2018)³⁸ and designed to remove possible contaminants introduced during sampling or during the various steps related to sample processing. The pre-emptive quality control steps hereby undertaken support a non-contaminant origin for taxa analysed in this dataset following careful in-field and laboratorial contamination-aware procedures carried out in each study. As such, the predominance of typically contaminant taxa affiliated *e.g.* to genus *Pseudomonas* was accepted as a true trend in the microbial ecology of the terrestrial deep subsurface.

No evidence was found for DNA extraction and PCR procedures significantly affecting microbial community structure in this meta-analysis (6.01% of the microbial community structure cumulatively [ADONIS/PERMANOVA, F-statistic=3.85, 3.23, p-value <0.01, adjusted Bonferroni correction p-value <0.001] vs. 50.6% from host rock lithology). In spite of this, a general convergence in DNA extraction methods would help further reduce methodology based variation and to standardize downstream analysis of deep subsurface microbial datasets⁸², despite the practical challenges of each host-rock matrix and local geochemical conditions.

In the near future, the advent of recently developed techniques for primer bias-free long read 16S rRNA and 16S rRNA-ITS gene amplicon long-read-based sequencing may initiate a convergence of molecular methods from which the deep subsurface microbiology community would benefit greatly^{83,84}. The future of large-scale, collaborative deep subsurface microbial diversity studies should encompass not only an effort towards standardization of several molecular biology techniques but also the long-term archival of samples⁸⁵. This will permit re-analyses using updated or unified methods after collection, where methodological variations would be controlled, and robust conclusions would more easily be achieved.

Conclusions

A global scale meta-analysis addressing the available 16S rRNA gene-based studies of the deep terrestrial subsurface revealed the dominance of Betaproteobacteria, Gammaproteobacteria and Firmicutes across this biome. Further, aquifer lithology was identified as the main driver of deep subterranean microbial communities. Depth and location were not significant controls of microbial community structure at this scale. Finally, evidence for a core terrestrial deep subsurface microbiome population was recognised through the prevalence and centrality of genus *Pseudomonas* (Gammaproteobacteria) and several other genera affiliated to class Betaproteobacteria. The adaptable metabolic capabilities associated to the above-mentioned taxa may be critical for colonizing the deep subsurface and sustaining communities. The terrestrial deep subsurface is a hard-to-reach complex

ecosystem crucial to global biogeochemical cycles. This study attempts to consolidate a global-scale understanding of taxonomical trends underpinning terrestrial deep subsurface microbial ecology and geomicrobiology.

Methodology

Data acquisition

The Sequence Read Archive database of the National Center for Biotechnology Information (SRA-NCBI) was queried for 16S rRNA-based deep subsurface datasets (excluding marine and ice samples, as well as any human-impacted samples); available studies, were downloaded using the SRA Run Selector. Studies were selected considering the metadata and information on sequencing platform used – i.e., only samples derived from 454 pyrosequencing and Illumina sequencing were considered. Analysis of related literature resulted in the detection of other deposited studies previous search efforts in NCBI-SRA failed to detect. Further private contacts allowed access to unpublished data included in this study. The final list of NCBI accession numbers, totalling 222 samples, was downloaded using *fastq-dump* from the SRA toolkit (<https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft/>).

As seen in **Table 1**, required metadata included host-rock lithology, general and specific geographical locations, depth of sampling, DNA extraction method, sequenced 16S rRNA gene region and sequencing method. Any samples for which the above-mentioned metadata could not be found were discarded and not considered for downstream analyses.

Pre-processing of 16S rRNA gene datasets

A customised pipeline was created in *bash* language making use of *python* scripts developed for QIIME v1.9.1⁸⁶, to facilitate bioinformatic analyses in this study (see https://github.com/GeoMicroSoares/mads_scripts for scripts). Briefly, demultiplexed FASTQ files were processed to create an OTU table. Quality control steps involved trimming, quality-filtering and chimera checking by means of USEARCH 6.1⁸⁷. Sequence data that passed quality control were then subjected to closed-reference (CR) OTU-picking on a per-study basis using UCLUST⁸⁷ and reverse strand matching against the SILVA v123 taxonomic references (<https://www.arb-silva.de/documentation/release-123/>). Closed-reference OTU picking excludes OTUs whose taxonomy has not been found in the 16S rRNA gene database used. Although this limits the recovery of prokaryotic diversity to the recorded in the database, cross-study comparisons of microbial communities generated by different 16S rRNA gene primers are made possible. This conservative

approach classified OTUs in each study individually to the common 16S rRNA gene reference database the merge of all classification outputs. A single BIOM (Biological Observation Matrix) file was generated using QIIME's *merge_otu_tables.py* script. The BIOM file was then filtered to exclude samples represented by less than 2 OTUs using *filter_samples_from_otu_table.py*, as well as OTUs represented by one sequence (singleton OTUs) by using *filter_otus_from_otu_table.py*. In an attempt to reduce the impacts of potential contaminant OTUs from the dataset, the post-singleton filtered dataset was further filtered to include only OTUs represented by at least 500 sequences and present in at least 10 samples overall using *filter_otus_from_otu_table.py*.

Data analysis

All downstream analyses were conducted using the *phyloseq* (<https://github.com/joey711/phyloseq>) package within R, which allowed for simple handling of metadata and taxonomy and abundance data^{88–90}. Merged and filtered BIOM files were imported into R using internal *phyloseq* functions, which allowed further filtering, transformation and plotting of the dataset (see https://github.com/GeoMicroSoares/mads_scripts for scripts).

Briefly, following a general assessment of the number of reads across samples and OTUs, *tax_glom* (*phyloseq*) allowed the agglomeration of the OTU table at phylum-level. For the metadata category-directed analyses, function *merge_samples* (*phyloseq*) created averaged OTU tables, which permitted testing of hypotheses for whether geology or depth had significant impacts on microbial community structure and composition. Computation of a Jensen-Shannon divergence PCoA (Principal Coordinate Analysis) was achieved with *ordinate* (*phyloseq*) which makes use of metaMDS (*vegan*)^{91,92}. All figures were plotted making use of the *ggplot2* R package (<https://github.com/tidyverse/ggplot2>), except for the UpsetR plot in **Figure 2**, which was plotted with package *UpsetR* (<https://github.com/hms-dbmi/UpSetR>).

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617

618 **Author Contributions**

619 ARS developed the methodology, collated and analysed the data, and wrote the manuscript. AE and
 620 AM conceived the study, supervised AS and helped write the manuscript. Other authors provided
 621 data from field sites used in the global meta-analysis. All authors contributed, edited and approved
 622 the final manuscript.

623

624 **Conflict of Interest**

625 We declare no conflict of interest.

1 **Supplementary Information – “A global perspective on** 2 **microbial diversity in the terrestrial deep subsurface”**

3

4 **Authors:**

5 Soares, A.^{1,2,3}, Edwards, A.^{2,3*}, An, D.⁴, Bagnoud, A.⁵, Barnhart, E.^{6,7}, Bomberg, M.⁸, Budwill, K.⁹,
6 Caffrey, S.¹⁰, Fields, M.⁷, Gralnick., J.¹¹, Kadnikov, V.^{12,13}, Momper, L.¹⁴, Osburn, M.¹⁵, Mu,
7 A.^{16,17,18,19}, Moreau, J.W.¹⁶, Moser, D.²⁰, Purkamo, L.^{8,21,22}, Rassner, S. M.^{1,3}, Sheik, C. S.²³,
8 Sherwood Lollar, B.²⁴, Toner, B. M.²⁵, Voordouw, G.⁴, Wouters, K.²⁶, Mitchell, A. C.^{1,3*}

9

Supplementary Text

Depth weakly controls microbial community structure

Life at extreme depths is yet to be analysed at a deep genomic level, but microbial cells have been discovered at depths down to 3,6 km in the terrestrial crust ^{1,2}. Although cell numbers tend to decrease with depth in both crystalline and sedimentary rock in the continental crust, not much is known regarding large-scale taxonomic trends ². No significant correlations were found for the presence of the most abundant clades in the dataset and depth, being Actinobacteria the only major taxonomic group to have a positive, albeit weak, correlation to depth (Pearson's $r = 0.42$, $p < 0.01$, **Figure S2**). Actinobacteria have already been detected at great depths in both the continental and oceanic crusts and some of its members have further been reported to hold ancestral genes for pyruvate oxidoreductase activity, which could potentially propel microbial growth at higher temperatures ^{3,4}. Proportions of Beta- and Gammaproteobacteria decreased with depth (Pearson's $r = -0.29$, Pearson's $r = -0.093$), and no other major clades were shown to correlate. More data needs to be generated to better investigate which, if any, taxonomic groups prefer deeper terrestrial environments. Biochemical limitations to life such as racemization rates of organic acids with depth and temperature will surely select for adaptable taxa and possibly create a mostly depth-defined gradient of representation of adapted extremophilic clades ⁵.

99% closed-reference OTU analysis

A 99% similarity closed-reference OTU-picking strategy to further minimize potential contamination showed a ~10-fold decrease in the number of OTUs and read numbers (1,065 OTUs and 70,527 reads were left). Further, this reduced the number of retrieved samples to 93. Following the previously described filtering steps (hold only OTUs represented by more than 500 sequences and present in at least 10 samples), 335 OTUs (67,151 reads) associated to 14 samples were left. For this reduced dataset, 2 OTUs associated to Chloroplast were found, represented by 115 sequences in total. The very reduced number of samples, OTUs and total reads left in the dataset caused the 99% OTU approach to be discontinued.

Supplementary Methodology

Phylogeny of Pseudomonas representative sequences

Representative 16S rRNA gene sequences for *Pseudomonas* OTUs in the dataset were isolated by retrieving OTU IDs affiliated to this genus in the final dataset using the *subset_taxa* function within *phyloseq*⁶. The SILVA 123 database was then queried against the list of OTU IDs and the results deposited in a FASTA file. Outgroup sequences of genus *Sphingomonas* (Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae) were obtained directly from the SILVA database (<https://www.arb-silva.de/>) and added along with the retrieved *Pseudomonas* 16S rRNA gene sequences to a final FASTA file.

Using MEGA7⁷, an alignment was performed using the MUSCLE⁸ algorithm and 8 iterations of the UPGMB (combines Neighbour-Joining⁹ and UPGMA - Unweighted Pair Group Method with Arithmetic mean) clustering method. An optimal Neighbour-Joining⁹ tree (*cf.* **Supplementary Figure 4**) was then created using 500 bootstrapped replicates and the Maximum Composite Likelihood (MCL)¹⁰ method to calculate evolutionary distances.

Supplementary Figures

Supplementary Figure 1 - Prevalence (number of samples an OTU is present in, x-axis) of OTUs across the dataset and associated reads (y-axis). Colours depict classification of OTUs at order level. Vertical line crosses 20 samples in the x-axis to highlight OTUs present in 20 or more samples.

Supplementary Figure 2 - Correlations between relative abundance of OTUs (% , y-axis) associated to the most abundant taxonomic groups across the dataset and depth (meters below surface, x-axis). Regression lines follow the linear model and shading around lines corresponds to the 95% confidence interval. Annotations in plot facets indicate the associated Pearson correlation coefficient, its corresponding p-value and the fitted linear model equation. Each point represents an OTU associated to the taxonomic group in each facet at a certain depth - a same OTU may be depicted more than one time.

Supplementary Figure 3 – Distribution of samples in the final dataset across depth, coloured by general location. Shapes indicate 16S rRNA gene region utilised for that study.

74 **Supplementary Figure 4** – Optimal Neighbor-Joining tree of representative sequences for
 75 *Pseudomonas* OTUs in the dataset following MUSCLE alignment. OTU IDs are shown in the end of
 76 each branch and outgroup sequences of genus *Sphingomonas* identified by their taxonomic affiliation.
 77 The percentage of bootstrap test replicates are shown next to tree branches and scale for MCL
 78 evolutionary distances is shown in the bottom.

Supplementary Tables

Supplementary Table 1 - Top 10 OTU network interactions ordered by edge betweenness (number of shortest paths going through an edge - OTU/OTU interactions) values as per the calculated Jaccard distances.

OTU 1 Classification	OTU 2 Classification	Betweenness
Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	1686
Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter	1656.17
Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Alishewanella	885.1
Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	749
Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	618
Betaproteobacteria., Rhodocyclales, Rhodocyclaceae, Azoarcus	Betaproteobacteria, Rhodocyclales, Rhodocyclaceae, uncultured	588
Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Peptoclostridium	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Intestinibacter	588
Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae, Paracoccus	563.83
Gammaproteobacteria, Chromatiales, Chromatiaceae, Rheinheimera	Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Alishewanella	499.26
Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter	Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter	495

85 **Supplementary Table 2** - SILVA 123 taxonomic affiliations of OTUs present in 20 or more samples. Prevalence is defined as the number of samples
86 an OTU is present in.

Taxa ID	Taxa Classification	Prevalence
JQ236848.1.1432	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	41
HM142822.1.1501	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	34
JX266364.1.1449	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	31
KJ475025.1.1452	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	27
FJ192249.1.1454	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	26
HQ848110.1.1406	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	25
KC852955.1.1440	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	25
HM773515.1.1498	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae, uncultured	24
AB231158.1.1425	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	24
JX222276.1.1475	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae, Variovorax	23
EU841498.1.1443	Proteobacteria; Gammaproteobacteria; Alteromonadales, Alteromonadaceae, Alishewanella	22
EU305582.1.1496	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	22
FJ900880.1.1395	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	21

88 **Supplementary Table 3** – Metadata table with complete details for all studies utilized.

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SRA Accession	Depth gradient (m)	DNA Extraction method	Sample Type	16S region	Sequencing method	Project
PRJNA262938	243.84-1478.28	Mod. Phenol chloroform	Groundwater	V4	454	
PRJNA268940	94-2383	MoBio UltraClean Microbial DNA isolation kit	Groundwater	V4-V6	454	VAMPS
PRJNA248749	730	MoBio PowerWater	Groundwater	V4-V6	454	VAMPS
PRJNA251746	393-1135	MoBio_PowerMax_Soil	Groundwater	V4-V6	454	VAMPS
PRJNA375701	109-114.7	Mod. MP-Biomedicals FastDNA Spin_Soil ¹¹	Rock + porewater	V1-V3	454	
NA*	140-1064.4	Foght <i>et al.</i> 2004	Rock + porewater	V6-V8	454	HMP
PRJEB1468	217.13-231.83	XS buffer ¹³	Groundwater	V1-V3	454	
PRJEB10822	180-2300	MoBio PowerSoil	Groundwater	V4**, V1-V3	454	

90 * <http://hmp.ucalgary.ca/HMP/>

91 * This primer pair corresponds to archaeal 16S rRNA gene primers (A344,A744, *cf.* 10.5194/bg-13-3091-2016 for further details).

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Supplementary Table 4 – BLAST hits table for representative sequences associated to OTUs affiliated to genus *Pseudomonas*.

OTU ID	Closest BLAST hit	Associated NCBI Title	Associated Environment	Taxonomy ID	NCBI Accession	Percent similarity (%)	E-value	No. Samples Associated	No. Reads Associated	Observations
AB231158.1.1425	<i>Pseudomonas pseudocaligenes</i> gene for 16S rRNA, partial sequence	High nitrogen removal from wastewater with several new aerobic bacteria isolated from diverse ecosystems	Wastewater	330	gi 85539942 dbj AB231158.1	100	0	24	157	
AB294275.1.1475	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: YWB06	Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan	Subsurface	77133	gi 126143387 dbj AB294275.1	100	0	19	135	
AB583907.1.1501	Uncultured bacterium gene for 16S rRNA, clone: RBC-4B	Microbial community in water-rock-microbe interaction systems in subsurface environments	Subsurface	77133	gi 324604950 dbj AB583907.1	100	0	3	137	
AB750584.1.1471	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 23 hydrate.Cas.16S	Novel integrons and gene cassettes from a Cascadian submarine gas hydrate-bearing core	Subsurface	77133	gi 407969505 dbj AB750584.1	100	0	16	69	
AF214645.1.1507	Nitrogen-fixing bacterium MIS 16S ribosomal RNA gene, partial sequence	Molecular characterization of plant associated nitrogen-fixing Bacteria	Subsurface	120486	gi 7542430 gb AF214645.1 AF214645	100	0	4	20	
AF229885.1.1494	<i>Pseudomonas</i> sp. 3CB6 16S ribosomal RNA gene, partial sequence	Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments	Subsurface	126132	gi 9965646 gb AF229885.1	100	0	9	22	
AF275704.1.1463	Unidentified Hailaer soda lake bacterium F5 16S ribosomal RNA gene, partial sequence	China: Inner Mongolia	Lake	148462	gi 12275954 gb AF275704.1 AF275704	100	0	12	47	
AF425998.1.1428	Bacterium UNSW3 16S ribosomal RNA gene, partial sequence	A survey of phylogeny and paralytic shellfish poison production from culturable bacteria associated with a toxic <i>Anabaena circinalis</i> strain	Host-associated	190587	gi 23451685 gb AF425998.1	100	0	14	56	
AGSX0100088.1.1449	<i>Pseudomonas stutzeri</i> SDM-LAC contig000089, whole genome shotgun sequence	Genome Sequence of <i>Pseudomonas stutzeri</i> SDM-LAC, a Typical Strain for Studying the Molecular Mechanism of Lactate Utilization	Lab strain	271420	gi 1093258265 emb LT629970.1	99.83	0	6	28	
AM403206.1.1398	<i>Pseudomonadaceae</i> bacterium D7-21 partial 16S rRNA gene, isolate D7-21	Diversity of Nitrate-reducing and Denitrifying Bacteria in a Marine Aquaculture Biofilter	Seawater	404904	gi 115334066 emb AM403206.1	100	0	8	16	
AY344383.1.1435	Unidentified bacterium clone K2-4-3 16S ribosomal RNA gene, partial sequence	Microbial Communities in the Hawaiian Archipelago: A Microbial Diversity Hotspot	Subsurface	1869227	gi 33391921 gb AY344383.1	100	0	6	10	
AY486384.1.1465	<i>Pseudomonas stutzeri</i> strain AU4823 16S ribosomal RNA gene, partial sequence	PCR-based assay for differentiation of <i>Pseudomonas aeruginosa</i> from other <i>Pseudomonas</i> species recovered from cystic fibrosis patients	Host-associated	316	gi 40019083 gb AY486384.1	100	0	7	17	
AY512621.1.1462	<i>Pseudomonas veronii</i> strain A1YdBTEx2-5 16S ribosomal RNA gene, partial sequence	Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: distribution of the genes in BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site	Subsurface	76761	gi 41056888 gb AY512621.1	100	0	15	91	
AY690673.1.1432	Sulfitebacter sp. GC07 16S ribosomal RNA gene, partial sequence	The diversity of halotolerant heterotrophic bacteria isolated from rhizosphere soil of salt marshes from southwestern coasts in Korea	Subsurface	290364	gi 51243779 gb AY690673.1	100	0	1	33	Latest SILVA database classifies this sequence as genus <i>Pseudomonas</i>
AY770973.1.1523	Uncultured bacterium clone W33 16S ribosomal RNA gene, partial sequence	Molecular analysis of the microbial communities of the oilfield marine sediment and soils	Subsurface	77133	gi 54695050 gb AY770973.1	100	0	10	14	
CU915014.1.1392	<i>Pseudomonas stutzeri</i> partial 16S rRNA gene, strain	Culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona	Subsurface	77133	gi 239913533 emb CU915014.1	100	0	2	13	
DQ205300.1.1384	<i>Pseudomonas</i> sp. HI-G1 16S ribosomal RNA gene, partial sequence	High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment	Subsurface	347772	gi 76365589 gb DQ205300.1	100	0	2	15	
DQ264446.1.1509	Uncultured bacterium clone BANW452 16S ribosomal RNA gene, partial sequence	Isolation of glyphosate-resistant bacterial species M9J918 from glyphosate-polluted wheat soil in China	Subsurface	77133	gi 82393910 gb DQ264446.1	100	0	5	13	
DQ279822.1.1321	<i>Pseudomonas</i> sp. D14 16S ribosomal RNA gene, partial sequence	Direct Submission, strain D14	Lab strain	358759	gi 82697883 gb DQ279822.1	100	0	6	10	
DQ339146.1.1254	<i>Pseudomonas</i> sp. M9J918 16S ribosomal RNA gene, partial sequence	Isolation of glyphosate-resistant bacterial species M9J918 from glyphosate-polluted wheat soil in China	Subsurface	366287	gi 85001608 gb DQ339146.1	100	0	9	13	
DQ497788.1.1344	<i>Pseudomonas</i> sp. SGB396 16S ribosomal RNA gene, partial sequence	Soil, subsurface, bacterial endophytes from <i>Taxus globosa</i>	Subsurface	77133	gi 98975506 gb DQ497788.1	100	0	10	44	
DQ837268.1.1430	<i>Pseudomonas stutzeri</i> strain WWvii23 16S ribosomal RNA gene, partial sequence	wastewater in lagos and ogun states Nigeria	Wastewater	86473	gi 112434217 gb DQ837268.1	100	0	7	16	
DQ837569.1.1446	<i>Pseudomonas</i> sp. K4 16S ribosomal RNA gene, partial sequence	Direct Submission, isolate K4	Lab strain	394945	gi 110704228 gb DQ837569.1	100	0	9	18	
EF179614.1.1501	<i>Pseudomonas</i> sp. WP02-4-9 16S ribosomal RNA gene, partial sequence	Isolation from deep-sea sediments	Subsurface	444156	gi 148251203 gb EF179614.1	100	0	2	4	
EF554871.1.1486	<i>Pseudomonas</i> sp. AB42 16S ribosomal RNA gene, partial sequence	Adaptative Potential of Alkaliphilic Bacteria towards Chloroaromatic Substrates Assessed by a gfp-Tagged 2,4-D Degradation Plasmid	Lake	443000;443001;443002;1740285	gi 146747229 gb EF554868.1	100	0	9	46	
EU101096.1.1498	Uncultured bacterium clone FS0612_B12 16S ribosomal RNA gene, partial sequence	Niche differentiation among sulfur-oxidizing bacterial populations in cave waters	Subsurface	77133	gi 156573149 gb EU101096.1	100	0	5	12	
EU305582.1.1496	Uncultured <i>Pseudomonas</i> sp. clone 3-A 16S ribosomal RNA gene, partial sequence	Nitrite removal performance and community structure of nitrite oxidizing and heterotrophic bacteria suffered with organic Matter	Microcosm	114707	gi 163676410 gb EU305582.1	100	0	22	453	
EU375162.1.1248	Uncultured <i>Pseudomonas</i> sp. clone Sc13 16S ribosomal RNA gene, partial sequence	Bacterial communities from shoreline environments (costa da morte, northwestern Spain) affected by the prestige oil spill	Coastal water	114707	gi 166407785 gb EU375162.1	100	0	2	17	
EU531805.1.1258	<i>Pseudomonas pseudocaligenes</i> 16S ribosomal RNA gene, partial sequence	Bacterial strains isolated from harvested tiger shrimp (<i>Penaeus Monodon</i>)	Host-associated	330	gi 170962995 gb EU531805.1	100	0	7	12	
EU536649.1.1395	Uncultured bacterium clone nbt64e03 16S ribosomal RNA gene, partial sequence	A diversity profile of the human skin microbiota	Host-associated	77133	gi 187964754 gb EU536649.1	100	0	5	503	
FJ005061.1.1494	<i>Pseudomonas</i> sp. enrichment culture clone Guo7 16S ribosomal RNA gene, partial sequence	The bioleaching feasibility for Pb/Zn smelting slag and community characteristics of indigenous moderate-thermophilic bacteria	Subsurface	557865	gi 204342383 gb FJ005061.1	100	0	7	17	
FJ170038.1.1503	<i>Pseudomonas</i> sp. CF14-10 16S ribosomal RNA gene, partial sequence	sediments of the South China Sea	Subsurface	562724	gi 206585088 gb FJ170038.1	100	0	9	568	
FJ192249.1.1454	Uncultured <i>Pseudomonas</i> sp. clone G13-S-5-G03 16S ribosomal RNA gene, partial sequence	Comprehensive census of bacteria in clean rooms by using DNA microarray and cloning methods	Urban	114707	gi 209421869 gb FJ192249.1	100	0	26	712	

Supplementary Table 4 – BLAST hits table for representative sequences associated to OTUs affiliated to genus *Pseudomonas*.

FJ557456.1.1399	Uncultured bacterium clone ET_H_1d10 16S ribosomal RNA gene, partial sequence	Endotracheal tube biofilm inoculation of oral flora and subsequent colonization of opportunistic pathogens	Host-associated	77133	gi 224569195 gb FJ557456.1	100	0	6	11	
FJ674339.1.1393	Uncultured bacterium clone LL141-1D15 16S ribosomal RNA gene, partial sequence	Synecology of the primary and secondary feedlot habitats of <i>Escherichia coli</i> O157:H7	Biological sample	77133	gi 223678636 gb FJ674339.1	100	0	7	10	
FJ900861.1.1397	Uncultured bacterium clone C-44 16S ribosomal RNA gene, partial sequence	Comparison of microbial community compositions of injection and production well samples in a long-term water-flooded petroleum Reservoir	Subsurface	77133	gi 229428754 gb FJ900861.1	100	0	7	9	
FJ900872.1.1397	Uncultured bacterium clone C-55 16S ribosomal RNA gene, partial sequence	Comparison of microbial community compositions of injection and production well samples in a long-term water-flooded petroleum Reservoir	Subsurface	77133	gi 229428765 gb FJ900872.1	100	0	9	14	
FJ900880.1.1395	Uncultured bacterium clone C-62 16S ribosomal RNA gene, partial sequence	Comparison of microbial community compositions of injection and production well samples in a long-term water-flooded petroleum Reservoir	Subsurface	77133	gi 229428773 gb FJ900880.1	100	0	21	326	
FN652910.1.1237	<i>Pseudomonas putida</i> partial 16S rRNA gene, isolate P5	Isolation, characterization and screening for antimicrobial activities of <i>Padina pavonica</i> associated bacteria	Host-associated	303	gi 290490824 emb FN652910.1	100	0	3	45	
FN813480.1.1499	<i>Pseudomonas stutzeri</i> partial 16S rRNA gene, isolate Gr20	The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat	Subsurface	316	gi 295810396 emb FN813480.1	100	0	17	30	
GQ033288.1.1360	Uncultured bacterium clone nbw1028h09c1 16S ribosomal RNA gene, partial sequence	Topographical and temporal diversity of the human skin microbiome	Host-associated	77133	gi 238303670 gb GQ033288.1	100	0	4	10	
GQ258636.1.1493	<i>Pseudomonas</i> sp. SR3(2009) 16S ribosomal RNA gene,	Direct Submission, strain SR3	Lab strain	659426	gi 256352433 gb GQ258636.1	100	0	5	933	
GQ358933.1.1498	<i>Pseudomonas</i> sp. BSw21506 16S ribosomal RNA gene,	Direct Submission, strain BSw21506	Lab strain	664427	gi 255689465 gb GQ358933.1	100	0	12	19	
GQ388993.1.1508	Uncultured bacterium clone J41 16S ribosomal RNA gene, partial sequence	Characterization of Bacterial Community Structure in a Drinking Water Distribution System during an Occurrence of Red Water	Subsurface	77133	gi 256593919 gb GQ388993.1	100	0	5	11	
GQ389202.1.1509	Uncultured bacterium clone D75 16S ribosomal RNA gene, partial sequence	Characterization of Bacterial Community Structure in a Drinking Water Distribution System during an Occurrence of Red Water	Subsurface	77133	gi 256594128 gb GQ389202.1	100	0	8	16	
GQ415381.1.1510	uncultured bacterium clone DQB-P183 genomic sequence	Microbial diversity in different production waters of Daqing Oilfield	Subsurface	77133	gi 308097127 gb GQ415381.1	100	0	10	21	
GQ979956.1.1504	Uncultured bacterium clone SRC_NRB027 16S ribosomal RNA gene, partial sequence	Direct Submission, clone SRC_NRB027	Lab strain	77133	gi 261499583 gb GQ979956.1	100	0	15	283	
GU136489.1.1369	<i>Pseudomonas</i> sp. DY1 16S ribosomal RNA gene, partial	Direct Submission, strain DY1	Lab strain	690347	gi 265263160 gb GU136489.1	100	0	7	13	
GU179609.1.1317	Uncultured <i>Pseudomonas</i> sp. clone D006011A15 16S ribosomal RNA gene, partial sequence	Microbial diversity profiles of fluids from low-temperature petroleum reservoirs with and without exogenous water perturbation	Subsurface	114707	gi 306491368 gb GU179609.1	100	0	2	159	
GU179630.1.1499	Uncultured <i>Pseudomonas</i> sp. clone D118231H09 16S ribosomal RNA gene, partial sequence	Microbial diversity profiles of fluids from low-temperature petroleum reservoirs with and without exogenous water perturbation	Subsurface	114707	gi 306491389 gb GU179630.1	100	0	9	17	
GU272270.1.1504	<i>Pseudomonas amygdali</i> pv. <i>lachrymans</i> str. M301315 chromosome, complete genome	Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 <i>Pseudomonas syringae</i> isolates	Subsurface	77133	gi 284025723 gb GU272270.1	100	0	3	648	
GU384267.1.1319	<i>Pseudomonas aeruginosa</i> strain SZH16 16S ribosomal RNA gene, partial sequence	In situ degradation of phenol and promotion of plant growth in contaminated environments by a single <i>Pseudomonas aeruginosa</i> strain	Subsurface	287	gi 288189621 gb GU384267.1	100	0	4	13	
GU390196.1.1300	Uncultured bacterium clone SLE33F 16S ribosomal RNA gene, partial sequence	Shifts in microbial community structure of granular and liquid biomass in response to changes to infed and digester design in anaerobic digesters receiving food-processing wastes	Subsurface	77133	gi 312178659 gb GU390196.1	100	0	14	70	
HE576400.1.1451	uncultured bacterium partial 16S rRNA gene, clone K16.133 AW	A combined cultivation and cultivation-independent approach shows high bacterial diversity in water-miscible metalworking fluids	Urban	77133	gi 377549755 emb HE576400.1	100	0	4	10	
HM066682.1.1514	Uncultured bacterium clone EDW07B006_73 16S ribosomal RNA gene, partial sequence	Microbial diversity and impact on carbonate geochemistry across a changing geochemical gradient in a karst aquifer	Subsurface	77133	gi 313770588 gb HM066682.1	100	0	8	16	
HM128723.1.1454	Uncultured bacterium clone SINN704 16S ribosomal RNA gene, partial sequence	Diversity of bacterioplankton in contrasting Tibetan lakes revealed by high-density microarray and clone library analysis	Lake	77133	gi 295879789 gb HM128723.1	100	0	20	184	
HM142822.1.1501	Proteobacterium WJQ No.5 16S ribosomal RNA gene,	Direct Submission, strain WJQ No. 5	Lab strain	797062	gi 298256144 gb HM142822.1	100	0	34	2472	
HM317009.1.1358	Uncultured bacterium clone ncd315a03c1 16S ribosomal RNA gene, partial sequence	Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis	Host-associated	77133	gi 297010604 gb HM317009.1	100	0	4	12	
HM445212.1.1363	Uncultured bacterium clone BL1289fO5 16S ribosomal RNA gene, partial sequence	Comparison of Bacterial Diversity in Azorean and Hawaiian Lava Cave Microbial Mats	Subsurface	77133	gi 302398051 gb HM445212.1	100	0	7	32	
HQ011396.1.1501	<i>Pseudomonas stutzeri</i> strain NCTC10475 genome	NCTC10475	Lab strain	77133	gi 307776705 gb HQ011396.1	100	0	8	139	
HQ224612.1.1430	<i>Pseudomonas</i> sp. SGB387 16S ribosomal RNA gene, partial sequence	Phylogenetic relationships of bacterial endophytes from <i>Taxus globosa</i>	Host-associated	1248114	gi 411172546 gb JX897952.1	99.15	0	15	73	
HQ246001.90.1551	UNVERIFIED: Uncultured <i>Pseudomonas</i> sp. 16S ribosomal RNA-like sequence	Proteobacteria dominance in the estuarine belt of river Narmada, India as depicted by molecular phylogenetic approaches	Freshwater	471	gi 226430883 gb FJ816052.1	88.52	0	4	19	
HQ323436.1.1502	<i>Pseudomonas stutzeri</i> strain M4 16S ribosomal RNA gene, partial sequence	Diversity of airborne bacteria community in the Mogao Grottoes, Dunhuang, China	Air	316	gi 319658795 gb HQ323436.1	100	0	6	10	

Supplementary Table 4 – BLAST hits table for representative sequences associated to OTUs affiliated to genus *Pseudomonas*.

HQ395163.1.1497	Uncultured bacterium clone OTU51 16S ribosomal RNA gene, partial sequence	Culture-independent and culture-dependent methods reveal diverse bacterial and archaeal communities in a biodegraded Malaysian oil Reservoir	Subsurface	77133	gi 320172034 gb HQ395163.1	100	0	4	18	
HQ438076.1.1492	<i>Pseudomonas</i> sp. TeU 16S ribosomal RNA gene, partial sequence	Isolation and characterization of an environmental cadmium- and tellurite-resistant <i>Pseudomonas</i> strain	Subsurface	944290	gi 318063761 gb HQ438076.1	100	0	8	11	
HQ848110.1.1406	<i>Pseudomonas argentinensis</i> strain PL-40-1 16S ribosomal RNA gene, partial sequence	Phylogenetic diversity of culturable bacteria isolated from the disused ancient Kiyik River	Freshwater	289370	gi 340025389 gb HQ848110.1	100	0	25	378	
JF430817.1.1403	<i>Pseudomonas</i> sp. P56(2011) 16S ribosomal RNA gene, partial sequence	Genetic and functional diversity of fluorescent <i>Pseudomonas</i> from rhizospheric soils of wheat crop	Subsurface	1079824	gi 345132366 gb JF430817.1	100	0	14	76	
JF500179.1.1495	Uncultured bacterium clone 1572_50bact 16S ribosomal RNA gene, partial sequence	The deep biosphere in terrestrial sediments in the chesapeake bay area, virginia, USA	Subsurface	77133	gi 343170179 gb JF500179.1	100	0	11	230	
JF500197.1.1495	Uncultured bacterium clone 1563_48bact 16S ribosomal RNA gene, partial sequence	The deep biosphere in terrestrial sediments in the chesapeake bay area, virginia, USA	Subsurface	77133	gi 343170197 gb JF500197.1	100	0	10	18	
JF741936.1.1498	Uncultured bacterium clone LHJB-8 16S ribosomal RNA gene, partial sequence	Microbial communities in oil reservoirs with different salinities	Subsurface	77133	gi 332712701 gb JF741936.1	100	0	3	10	
JF820098.1.1429	<i>Pseudomonas</i> sp. PG-3-1 16S ribosomal RNA gene, partial sequence	Diversity of Culturable Butane-oxidizing Bacteria in Oil and Gas Field Soil	Subsurface	1036156	gi 334690415 gb JF820098.1	100	0	8	11	
JN609543.1.1229	<i>Pseudomonas fluorescens</i> strain Cantas14 16S ribosomal RNA gene, partial sequence	The culturable intestinal microbiota of triploid and diploid juvenile Atlantic salmon (<i>Salmo salar</i>) - a comparison of composition and drug resistance	Host-associated	294	gi 358440878 gb JN609543.1	100	0	5	38	
JQ236848.1.1432	<i>Pseudomonas stutzeri</i> strain hswx82 16S ribosomal RNA gene, partial sequence	Direct Submission, strain hswx82	Lab strain	316	gi 377830169 gb JQ236848.1	100	0	41	5419	
JQ425963.1.1492	Uncultured bacterium clone CTC1CA03 16S ribosomal RNA gene, partial sequence	Bacterial diversity in an alkaline saline soil spiked with Anthracene	Lake	77133	gi 385760667 gb JQ425953.1	100	0	9	22	
JQ684327.1.1500	Uncultured bacterium clone HWGB-69 16S ribosomal RNA gene, partial sequence	Bacterial and archaeal diversity in permafrost soil from Kunlun Mountains Pass, Tibet Plateau of China	Subsurface	77133	gi 387568220 gb JQ684327.1	100	0	3	12	
JQ723717.1.1405	<i>Pseudomonas</i> sp. B-5-1 16S ribosomal RNA gene, partial sequence	beach sand in the west sea, Korea	Coastal water	1172620	gi 384392471 gb JQ723717.1	100	0	13	68	
JQ894900.1.1514	Uncultured bacterium clone YPMB-G12 16S ribosomal RNA gene, partial sequence	Microbial diversity of endosymbiont bacteria in the spiraling Whitefly	Host-associated	77133	gi 411181008 gb JQ894900.1	100	0	9	21	
JQ900532.1.1441	<i>Pseudomonas mendocina</i> strain B8 16S ribosomal RNA gene, partial sequence	Crude oil degrading bacterial isolates from ecological region of Assam	Subsurface	300	gi 402244732 gb JQ900532.1	100	0	2	126	
JX222557.1.1395	Uncultured bacterium clone EMIRGE_OTU_s2b2b_12537 16S ribosomal RNA gene, partial sequence	Subsurface microbial community response to acetate amendment	Subsurface	77133	gi 395559126 gb JX222557.1	100	0	1	12	
JX222807.1.1498	Uncultured bacterium clone EMIRGE_OTU_s3c2d_3949 16S ribosomal RNA gene, partial sequence	Subsurface microbial community response to acetate amendment	Subsurface	77133	gi 395559376 gb JX222807.1	100	0	4	9	
JX223934.1.1501	Uncultured bacterium clone EMIRGE_OTU_s6b4a_3133 16S ribosomal RNA gene, partial sequence	Subsurface microbial community response to acetate amendment	Subsurface	77133	gi 395560503 gb JX223934.1	100	0	5	10	
JX266364.1.1449	<i>Pseudomonas</i> sp. B2085 16S ribosomal RNA gene, partial sequence	Depth-Related Changes in Community Structure of Culturable Mineral Weathering Bacteria and in Weathering Patterns Caused by Them along Two Contrasting Soil Profiles	Subsurface	1225045	gi 402549839 gb JX266364.1	100	0	31	558	
JX945764.1.1431	<i>Pseudomonas</i> sp. LARP66 16S ribosomal RNA gene, partial sequence	Microbial diversity of Ethiopian soda lakes assessed by cultivation Methods	Lake	1266816	gi 428274238 gb JX945764.1	100	0	8	11	
KC119131.1.1470	<i>Pseudomonas</i> sp. RCC12 16S ribosomal RNA gene, partial sequence	Isolation of CO2 fixing bacteria from dehradun caves	Subsurface	1268824	gi 429841843 gb KC119131.1	100	0	2	9	
KC166766.1.1460	Uncultured <i>Pseudomonas</i> sp. clone BC061 16S ribosomal RNA gene, partial sequence	Bacterial community and groundwater quality changes in an anaerobic aquifer during groundwater recharge with aerobic recycled water	Subsurface	114707	gi 523453881 gb KC166766.1	100	0	5	15	
KC469080.1.1418	<i>Pseudomonas</i> sp. EM174 16S ribosomal RNA gene, partial sequence	Direct Submission, isolate EM174	Lab strain	1282313	gi 452108584 gb KC469080.1	100	0	6	19	
KC758944.1.1396	Uncultured bacterium clone 12ALLV2e09 16S ribosomal RNA gene, partial sequence	Using DNA-stable isotope probing to identify microorganisms involved in mtbe and the biodegradation	Coastal water	77133	gi 478444906 gb KC758944.1	100	0	2	22	
KC852955.1.1440	<i>Pseudomonas cuatrocieneegasensis</i> strain LEH6_4A 16S ribosomal RNA gene, partial sequence	Midgut Microbial Community of <i>Culex quinquefasciatus</i> Mosquito Populations from India	Host-associated	77133	gi 523453428 gb KC852955.1	100	0	25	293	
KF021824.1.1426	<i>Pseudomonas</i> sp. H-144 16S ribosomal RNA gene, partial sequence	Cultured diversity of marine bacteria	Seawater	1345863	gi 513045820 gb KF021824.1	100	0	2	9	
KF494749.1.1502	Uncultured bacterium clone B24-205 16S ribosomal RNA gene, partial sequence	Vertical changes of the structure of bacterial communities through a permafrost core profile from Qinghai-Tibet Plateau	Subsurface	77133	gi 532529728 gb KF494749.1	100	0	2	8	
KF494759.1.1501	Uncultured bacterium clone B9-456 16S ribosomal RNA gene, partial sequence	Vertical changes of the structure of bacterial communities through a permafrost core profile from Qinghai-Tibet Plateau	Subsurface	77133	gi 532529738 gb KF494759.1	100	0	5	11	
KF657327.1.1480	<i>Pseudomonas mendocina</i> strain 2E 16S ribosomal RNA gene, partial sequence	Alkaline Tolerant Bacteria, water	Lake	300	gi 545599206 gb KF657327.1	100	0	6	10	
KF722300.1.1267	Uncultured <i>Pseudomonas</i> sp. clone DVBSW_J342 16S ribosomal RNA gene, partial sequence	Response of bacterial community structure to seasonal fluctuation and anthropogenic pollution on coastal water of Alang-Sosiya ship breaking yard, Bhavnagar, India	Coastal water	114707	gi 643039831 gb KF722300.1	100	0	5	9	
KF836136.1.1422	<i>Pseudomonas plecoglossicida</i> strain SBADK2 16S ribosomal RNA gene, partial sequence	rhizosphere bacteria for agricultural and environmental use	Subsurface	70775	gi 578003379 gb KF836136.1	100	0	15	46	
KF851140.1.1502	Uncultured <i>Pseudomonas</i> sp. clone BJP8S20-c24 16S ribosomal RNA gene, partial sequence	Lithology-Controlled Bacteria Community in an Ammonium-Rich Aquifer-Aquitarad System in the Pearl River Delta, China	Subsurface	114707	gi 582054428 gb KF851140.1	100	0	10	30	
KF923425.1.1502	<i>Pseudomonas xanthomarina</i> strain 15 16S ribosomal RNA gene, partial sequence	Culturable bacteria from the Qinghai-Tibet Plateau	Soil	271420	gi 594591039 gb KF923425.1	100	0	8	10	
KJ210658.1.1345	<i>Pseudomonas xinjiangensis</i> strain WL-257 16S ribosomal RNA gene, partial sequence	Phylogenetic diversity of eudiphytic bacteria from <i>Populus euphratica</i> in 20-year <i>Populus jube</i> forest ecotone of Chakhikh county of Xinjiang	Subsurface	487184	gi 612340510 gb KJ210658.1	100	0	8	25	
KJ424421.1.1503	<i>Pseudomonas</i> sp. GW28-5 16S ribosomal RNA gene, partial sequence	Cytotoxic bacteria from Antarctica	Host-associated	77133	gi 601035987 gb KJ424421.1	100	0	4	121	

Supplementary Table 4 – BLAST hits table for representative sequences associated to OTUs affiliated to genus *Pseudomonas*.

KJ475025.1.1452	<i>Pseudomonas</i> peli strain IARI-RP26 16S ribosomal RNA gene, partial sequence	Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes	Soil	53406;592361	gi 387285851 gb JQ795777.1	100	0	27	962	
KJ600877.1.1458	Uncultured bacterium clone 83A 16S ribosomal RNA gene, partial sequence	Manipulating the banana rhizosphere microbiome for biological control of Panama disease	Subsurface	77133	gi 646117505 gb KJ600877.1	100	0	9	24	
KJ806232.1.1411	<i>Pseudomonas</i> alcaligenes strain PBR-49 16S ribosomal RNA gene, partial sequence	Phylogenetic characterization of heterotrophic bacteria isolated from photobioreactor (PBR) cultures of <i>Synechocystis</i> sp. PCC6803	Host-associated	43263	gi 669340641 gb KJ806232.1	100	0	10	26	
KJ806252.1.1456	<i>Pseudomonas</i> stutzeri strain PBR-57 16S ribosomal RNA gene, partial sequence	Phylogenetic characterization of heterotrophic bacteria isolated from photobioreactor (PBR) cultures of <i>Synechocystis</i> sp. PCC6803	Host-associated	316	gi 669340661 gb KJ806252.1	100	0	7	10	
KJ809251.1.1512	Uncultured bacterium clone F33GN 16S ribosomal RNA gene, partial sequence	Bacteria associated with arbuscular mycorrhizal fungi within roots of plants growing in a soil highly contaminated with aliphatic and aromatic petroleum hydrocarbons	Subsurface	77133	gi 671777739 gb KJ809251.1	100	0	2	40	
KJ862118.1.1417	Uncultured <i>Pseudomonas</i> sp. clone 35 16S ribosomal RNA gene, partial sequence	16S rDNA gene clone libraries which isolated from biodesulfurization bioreactor	Microcosm	114707	gi 672443372 gb KJ862118.1	100	0	8	79	
KP704423.1.1437	<i>Pseudomonas</i> sp. UYFA113 16S ribosomal RNA gene, partial sequence	Identification and characterization of the part of the bacterial community associated with field-grown tall fescue (<i>Festuca arundinacea</i>) cv. SFRO Don Tom s in Uruguay	Subsurface	1605337	gi 756058359 gb KP704423.1	100	0	7	11	
LN560920.1.1377	Uncultured bacterium partial 16S rRNA gene, clone SIGCS87_N11D1_16S_B	Leaf-cutter ant refuse dumps are nutrient reservoirs harboring diverse microbial assemblages	Microcosm	77133	gi 697256444 emb LN563985.1	100	0	15	200	
LN563984.1.1372	Uncultured bacterium partial 16S rRNA gene, clone SIBS643_N12D0_16S_B	Leaf-cutter ant refuse dumps are nutrient reservoirs harboring diverse microbial assemblages	Microcosm	77133	gi 697256444 emb LN563985.1	100	0	6	16	
LN565700.1.1370	Uncultured bacterium partial 16S rRNA gene, clone SIBG551_N12D2_16S_B	Leaf-cutter ant refuse dumps are nutrient reservoirs harboring diverse microbial assemblages	Microcosm	77133	gi 697264995 emb LN565700.1	100	0	7	123	

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