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Authors

Long, Philip E
Williams, Kenneth H
Hubbard, Susan S
et al.

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Microbial Metagenomics Reveals Climate-Relevant Subsurface Biogeochemical Processes

Author links open overlay panelPhilip E.Long[1]Kenneth H.Williams[1]Susan S.Hubbard[1]Jillian F.Banfield[12]
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Trends

Datasets from subsurface samples can now be resolved into collections of complete or near-complete microbial genomes, yielding information about biogeochemical roles and mechanisms by which surface- and groundwater quality and atmospheric compositions are impacted.

Deep sequencing reveals extremely high levels of diversity in both the vadose zone and groundwater.

Many novel organisms have an extremely small cell size and small genome size, with restricted metabolic capability. Their growth is likely tightly linked to that of other community members.

Genomic analyses suggest that subsurface geochemical processes reflect the functioning of complex communities as opposed to a few dominant species.

Newly discovered microorganisms catalyze transformations relevant to greenhouse gases and processing of biologically critical elements.

Microorganisms play key roles in terrestrial system processes, including the turnover of natural organic carbon, such as leaf litter and woody debris that accumulate in soils and subsurface sediments. What has emerged from a series of recent DNA sequencing-based studies is recognition of the enormous variety of little known and previously unknown microorganisms that mediate recycling of these vast stores of buried carbon in subsoil compartments of the terrestrial system. More importantly, the genome resolution achieved in these studies has enabled association of specific members of these microbial communitieswith carbon compound transformations and other linked biogeochemical processes–such as the nitrogen cycle–that can impact the quality of groundwater, surface water, and atmospheric trace gas concentrations. The emerging view also emphasizes the importance of organism interactions through exchange of metabolic byproducts (e.g., within the carbon, nitrogen, and sulfur cycles) and

via symbioses since many novel organisms exhibit restricted metabolic capabilities and an associated extremely small cell size. New, genome-resolved information reshapes our view of subsurface microbial communities and provides critical new inputs for advanced reactive transport models. These inputs are needed for accurate prediction of feedbacks in watershed biogeochemical functioning and their influence on the climate via the fluxes of greenhouse gases, CO_2, CH_4, and N_2O.

The Global Importance of Subsurface Microbial Communities

We stand on the cusp of a much deeper grasp of subsurface microbes as agents of biogeochemistry than has ever been achieved before. An understanding of subsurface microbial communities is important at this juncture because the subsurface remains poorly represented in current models of biogeochemical processes, while DNA sequencing and mass spectrometric capabilities now position us to explore this topic comprehensively via metagenomics, metatranscriptomics, and metaproteomics [1], [2], [3], [4], [5], [6]. By 'subsurface' we mean that part of the earth below the surface of the terrestrial landscape. As a practical matter, this paper is focused on the shallow subsurface, which we arbitrarily define as <10 m below the terrestrial surface. The practical and scientific implications of understanding microbial community structure and interactions among species at a mechanistic level, including species present at <0.1% of the community, in this environment are enormous [7], [8]. These include greatly improved ability to (i) estimate greenhouse gas fluxes to and from the subsurface via modeling of microbially driven CO_2 fixation and degradation of organic matter, (ii) environmental management of mining impacts from toxic metals via a complete understanding of microbial metal redox cycles, and (iii) improved design of waste handling from animal confinements via the ability to accurately model nitrogen cycling in the subsurface. A key scientific impact is that we now 'see' all of the species in an environmental sample, including those from previously unknown lineages, and this informs major revisions of the tree of life [9]. Newly acquired data that reveal what specific microbes can and cannot do also impact our understanding of relationships among microbial species. Other benefits are perhaps less predictable but no less real,

for example, the discovery of new biocatalysts from genes in the vast array of new microbial genomes.

We posit that recently discovered microorganisms from the terrestrial subsurface, now known almost exclusively from metagenomics, play key roles in subsurface biogeochemical reactions that control fluxes of climate-relevant gases (CO_2, CH_4, N_2O, http://cdiac.ornl.gov/pns/current_ghg.html) including breakdown of buried organic carbon (Figure 1). Moreover, to understand the role of subsurface microbial communities in environmental processes it is necessary to genomically analyze a representative set of subsurface environments in a thorough and comprehensive manner such that complete or nearly complete genomes for nearly all community members are obtained. Such genome-resolved information, regardless of whether it uncovers novel organisms or not, is key to understanding the metabolic reactions possible in the subsurface and is a prerequisite for interpreting metatranscriptomic and metaproteomic datasets that provide information about the actual metabolic functions occurring at any time or under specific conditions [10]. In combination, these data represent key microbiologic inputs needed for effective modeling of biogeochemical fluxes. While discussion of modeling of such fluxes is beyond the scope of this paper, it is important to keep that goal in mind because, ultimately, greenhouse gas fluxes from the terrestrial subsurface need to be predicted under conditions of climate change.
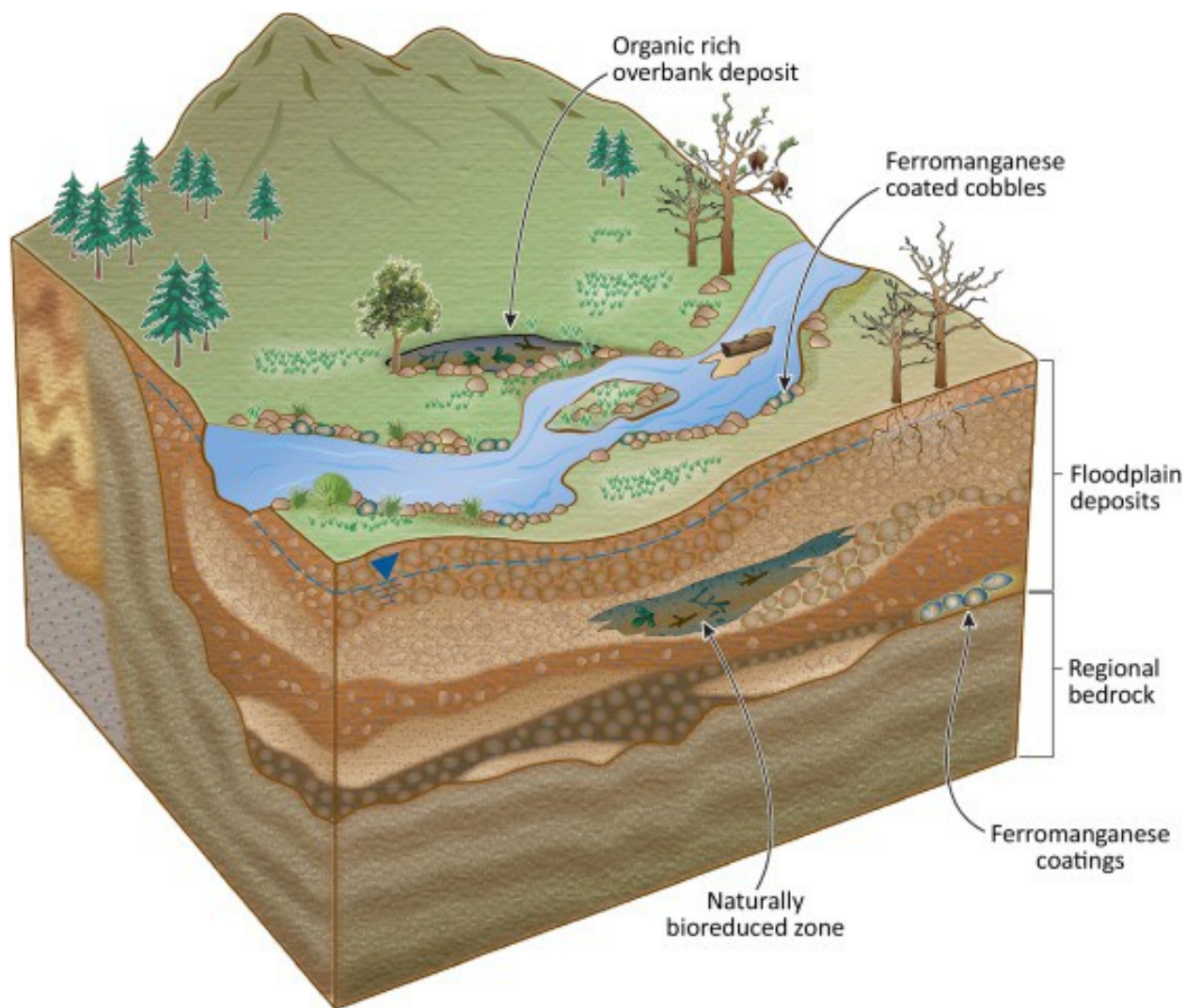
Figure 1. Organic Carbon Deposits and Naturally Bioreduced Zones. These are created when organic matter accumulations, typical of most floodplains, are buried in the shallow subsurface (<10 m below the terrestrial surface of the earth). Genomic information on microorganisms in such zones provides information about their metabolic capacities, enabling modeling of subsurface biogeochemical processes. This will allow accurate prediction of how subsurface processes impact the quality of water and nutrient cycling within watersheds, as well as the flux of greenhouse gases to the atmosphere. The term 'watershed' here refers to a region or area of the terrestrial surface that is bounded by a divide and drains to a particular river or water body, including all the streams or rivers that drain into it. Watersheds are a key functional unit of the earth, governing water resources and mediating biogeochemical processes that support all terrestrial life. For details of the microbially catalyzed biogeochemical reactions that occur in the subsurface, see Figure 4.

New Insights from Genomic Data on Subsurface Ecosystems

Living organisms are classified into three domains: Bacteria, Archaea, and Eukarya, distinguished by their genetic makeup, cellular structure, and metabolic traits [11]. Eukaryotes include complex life forms such as mammals, birds, and fish. Archaea and bacteria dominate life in the subsurface because they fit into soil, sediment, and rock pores and have diverse metabolic capabilities that operate in the subsurface under a wide range of oxygen concentrations. Subsurface microbial communities take advantage of, and also create, biogeochemical gradients that influence fluxes of key biogeochemical species from the subsurface to rivers and the atmosphere. A wide range of metabolisms occur in the subsurface, including heterotrophy (particularly in soils or sediments with buried organic matter), and chemolithoautotrophy, which enables fixation of carbon using energy from redox transitions involving variable-redox elements in the Earth's crust (e.g., 3, 12, 13).

The number of species of single-celled organisms in the Earth is unknown, but it is clear that there is a very large number of rare microorganisms that constitute an important part of the Earth's ecology, catalyzing biogeochemical reactions and serving as a microbial 'bank' from which new consortia can be 'seeded' as conditions change [14]. Only a minute fraction of these species has been grown in the single-organism cultures that have traditionally been used for microbial characterization [15]. It may not be feasible to isolate most species for laboratory study because they require unknown inputs from other organisms or their natural environments. To understand how these organisms function, both individually and collectively, it is therefore necessary to use methods independent of isolation and culturing [16]. DNA sequencing-based means to do this have become available only relatively recently, and with decreasing cost and increasing speed, the approach can now be applied to even the most complex microbial ecosystems. The methods rely upon new bioinformatics approaches that can make use of small snippets of DNA, assembling them into genome fragments and then into near-complete or even complete genomes from which organism functions can be predicted 17, 18.

Recent research on samples of sediment and groundwater from a research site near the town of Rife, Colorado, USA, has used massive amounts of genomic (DNA sequence) data ($\sim$10 gigabases, Gb, where a Gb = 10^9 bases, per sample; a few terabases total, Tb = 10^{12} bases) to reveal previously unrecognized metabolic potential and diversity in the shallow subsurface. A novel aspect of this work is the genome-centric approach, which has yielded thousands of genomes, including the first complete

archaeal genomes from metagenomicdata. This approach has enabled an in-depth exploration of major segments of both the bacterial and archaeal trees of life, about which little was known previously (Figure 2) [9]. Results from applying these methods to samples of groundwater and sediment yield a tally of the microorganisms present, including species that comprise less than 0.01% of the communities [19]. Moreover, the biogeochemical reactions catalyzed by each organism can be ascertained, as well as how products of one organism may provide resources for another. The dominant reactions can then be incorporated into reaction networks to understand the life strategies of the microorganisms and the overall functioning of the community [20]. The reaction networks, in turn, are used in quantitative models that calculate subsurface properties, such as the concentration of nitrate in groundwater, that have a direct impact on our health when we consume the water, for example [21], or that estimate the fluxes of greenhouse gases that are the focus of this paper 22, 23, 24. A key underlying issue is how much genomic information is needed to effectively define dominant reactions and the response of a subsurface community to perturbations such as climate change. Initial results suggest that genome-resolved data are needed across a broad range of environments, including those in which organic carbon compounds may be released after being sequestered for millennia. Genome-resolved information will make it possible to capture detailed reaction networks, and ascertain the most important mechanisms controlling the dominant biogeochemical pathways [20].
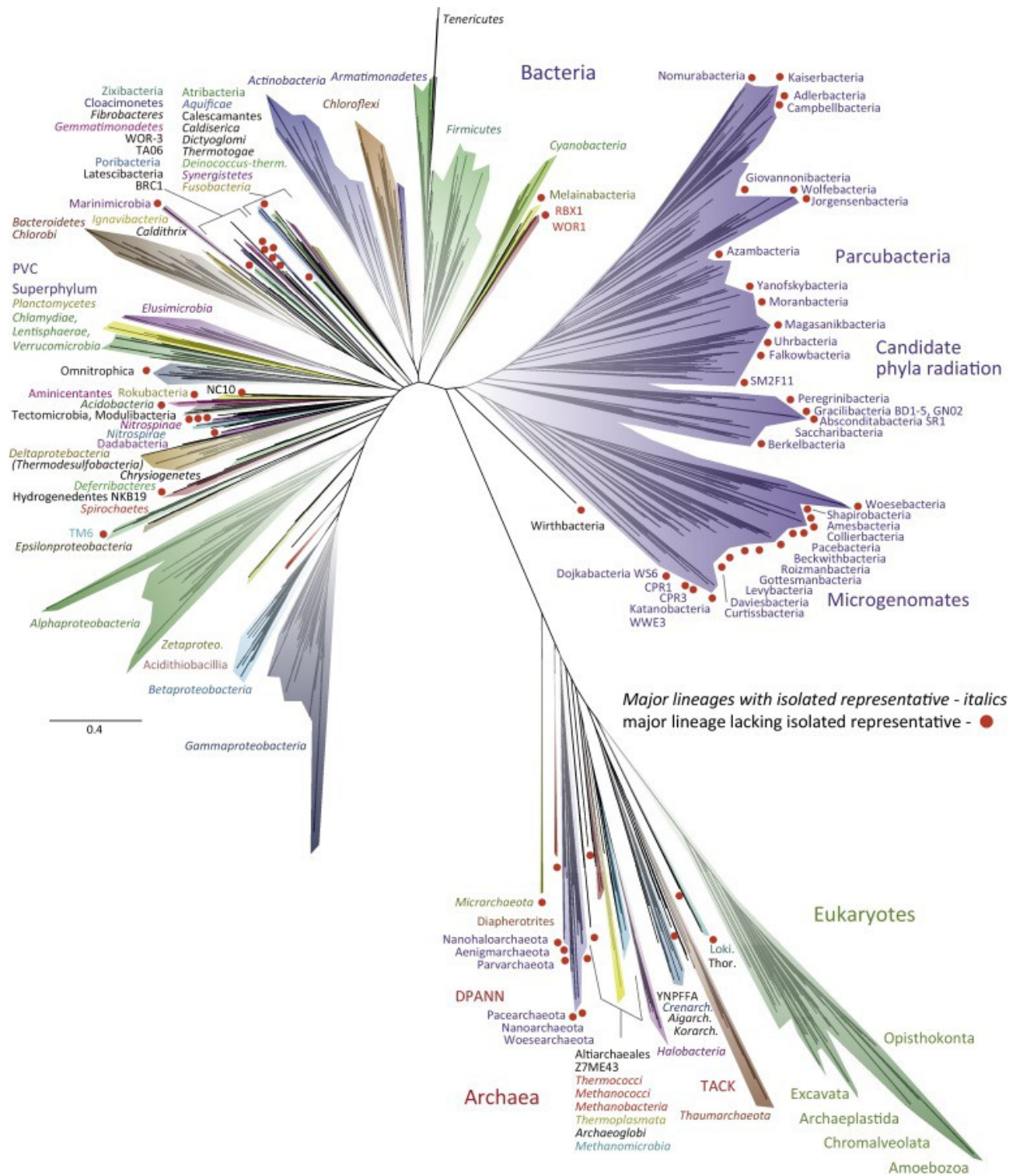
Figure 2. Tree of Life Generated by Hug and Colleagues in 2016 [9]. The total diversity represented by available sequenced genomes, including 100 bacterial phyla, 17 archaeal phyla, and 5 eukaryotic super-groups. Colors and names represent major lineages. Well-characterized lineages are in italic font, whereas lineages lacking a

cultured representative are highlighted in non-italic font and with red circles. DPANN and CPR are assigned single colors as they are without isolated representatives. See [9] for additional information. This figure is reproduced unaltered from [9] under a Creative Commons CC BY license with permission from *Nature Microbiology*.

In addition to reshaping our thinking about carbon, nitrogen, hydrogen, and metal cycling in the subsurface, these recent results from genome-resolved studies are providing the basis for incorporating the metabolic capability of the entire subsurface microbial community into predictive models. Successful use of genomic data to inform watershed-scale models will greatly improve our ability to understand and manage a wide range of environmental impacts, including how nutrients or water quality within a watershed change in response to droughts, floods, and other perturbations that are increasingly attributed to climate change [25] (see the caption of Figure 1 for the definition of the term 'watershed'). The dependence of greenhouse gas fluxes (CO_2, CH_4, and N_2O) from the shallow subsurface on microbially catalyzed reactions linked to water saturation or fluxes is becoming increasing clear (see, e.g., 26, 27). At the same time such fluxes are complex both physically and microbiologically, with field observations differing from study to study, suggesting that a deeper understanding of microbial processes mediating those fluxes is needed [28].

Novel Microorganisms and Extreme Diversity of Subsurface Microbial Communities

Several recent research papers report major advances in our understanding of subsurface microbiology. In one such paper [19], researchers reconstructed 8 complete and 789 draft genomes of bacteria from more than 35 phyla, all of which lack isolated representatives and thus are referred to as candidate phyla (CP; phyla are major groups that are as distinct from each other as vertebrates are from mollusks). The organisms from these 35 phyla share features that consistently distinguish them from other bacteria, and they group together in what is referred to as the candidate phyla radiation (CPR), a lineage that represents greater than 15% of the bacterial tree of life. Importantly, bacteria from these phyla mediate hydrogen and organic carbon cycles, and they appear to depend on other organisms for many basic cellular building materials. However, the diversity of the CPR is remarkable. The first complete genomes from one of the candidate phyla (Peregrinibacteria) differ from most organisms in other lineages of the CPR in that they have the capacity for nucleotide synthesis, including biosynthesis of cell-wall and cell-envelope components [29]. However, they also lack biosynthesis capacities for many required cofactors, fatty acids, and most amino acids,

suggesting a symbiotic lifestyle typical of CPR organisms. Further, the CPR organisms generally appear to have different ribosome structures and biogenesis mechanisms than other bacteria. The sequences of the rRNA molecules that form the backbone of the ribosomes of CPR organisms are commonly so unusual that some of these organisms would not be detected in surveys, which routinely use these rRNA sequences [19].

The first extensive genome reconstructions for CPR bacteria indicated that their genomes were very small [30], a feature that suggested that the cell sizes would also be small. This prediction was tested by using cryogenic transmission electron microscopy (cryo-TEM) to study cells that passed through a 0.2 μm filter [31]. Such filters are normally used to sterilize water because no cells should be able to pass through them. The cryo-TEM data showed that cells from three phyla (identified by metagenomic analysis of the same samples) have cell sizes of ~0.009 ± 0.002 mm³ (Figure 3). Characteristics of these cells include tightly packed spirals inferred to be DNA, very few densely packed ribosomes, and a variety of cellular appendages that might enable inter-organism interactions.
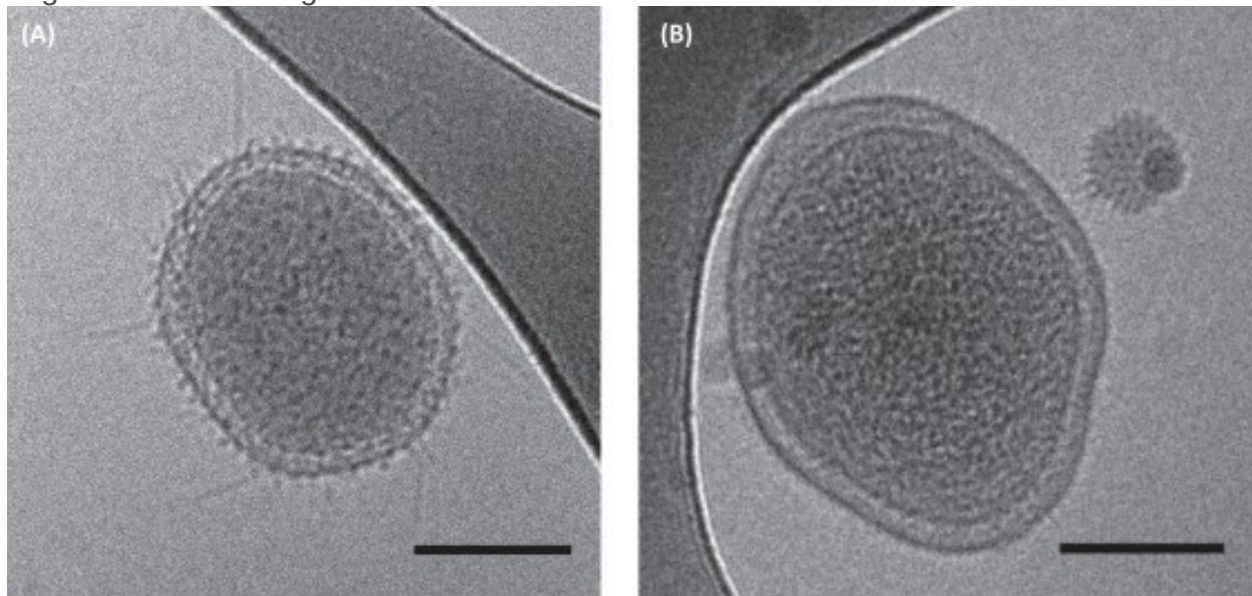
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Figure 3. Cryo-TEM Images Showing the Morphology, Size, and Some Key Features of Ultra-Small Bacteria. The cell envelope includes a distinct outer layer (S-layer). Pili-like structures are clearly visible in the image on the left, and in some cases these pili apparently connect to adjacent bacteria. Scale bar is 100 nanometers or about 1/500th to 1/1000th of the diameter of a human hair. Note that these bacteria are so

small that apparent low resolution of the images reflects the resolution limits of the cryo-TEM technique. See also Figure 3, Figure 4 in [31].

Another recent study investigated archaea that are present in the subsurface of the same alluvial aquifer and reported findings that largely parallel those for the CRP bacteria [32]. The research significantly expanded our understanding of archaeal diversity and metabolism by genomically sampling a major (but little-studied) superphylum from which no organisms have been isolated. Two new phyla were resolved. The members appear to be organisms with small genomes, possibly nanoarchaea with limited metabolic capacities. The primary biogeochemical impact of these archaea appears to be on anaerobic carbon and hydrogen cycles. Metagenomics has also been used to explore microbial diversity in aquifer sediments (as opposed to groundwater). Among the most striking findings are the high degree of biological novelty and vast diversity of little known and unknown organisms present 32, 33, 34. Recently, the utility of long DNA sequence reads for metagenomic analysis to study such communities was also tested [35]. These newly available, accurate long reads enabled distinction of closely related strains that together comprised a significant fraction of sediment communities and enabled identification of very rare microorganisms. The analysis revealed thousands of species whose abundances were <0.1% in all samples. Most of the organisms in this set of rare organisms belong to phyla that are also represented by abundant organisms. Significantly, genes providing the capacity to break down complex organic polymers (glycosyl hydrolases) are more abundant than expected in rare genomes, suggesting that rare species may augment the capability for carbon turnover and confer resilience to changing environmental conditions. Future exploration of community metabolic function and resilience will become increasingly accessible as long-read and nano-pore sequencing are developed, including the likelihood of field-deployable, next-generation sequencing systems 36, 37, 38, 39.

Tracking Microbial Shifts in Time and Space

Another recent paper applied sequencing methods to study the dynamics of subsurface microbial communities. The 133 most abundant organisms in sediment sampled at 5 meters (16 feet) depth from the Rifle aquifer were tracked across 36 different sediment and groundwater environments [40]. Samples were collected centimeters, meters, and tens of meters apart and on seven occasions over 6 years. Analysis of a massive database of DNA sequences (1400 Gb pairs) showed that these 133 organisms were more consistently detected in saturated sediments than in samples

from above the water table, from widely separated samples, or from groundwater. Abundance profiles across different locations in the aquifer collected at different sampling times identified consistent organism cohorts. The results suggest that these cohorts are at least partly the result of a shared environmental adaptation. Such research demonstrates that we can track subsurface microbial communities in their environmental context in such a way that the objective of predicting key biogeochemical fluxes in the subsurface (e.g., CO_2 or nitrate) is tractable and can be based on the underlying microbially driven processes. This belies the notion that subsurface or even soil microbiology is so heterogeneous and complex that it can never be understood, and supports the idea that using genomic data to inform watershed-scale models is within reach.

## Reaction Pathways, Microbial Diversity, and Functional Resilience

It has been proposed that metabolic function of microbial communities can be modeled based on 16S rRNA data by assuming functions for detected organisms that are based on inferences from available database genomes. Such an approach is clearly inapplicable for subsurface studies, where reference genomes are not available (except from metagenomics) and many organisms (in fact, members of entire phyla) escape detection by rRNA sequencing approaches. Thus, we argue that the level of insight provided by genome-resolved studies is critical to understanding functionality of subsurface systems. A subset of the complex microbial reaction pathways might be expected *a priori* even in the absence of deep metagenomic analysis. For example, reactive transport models can be based on reaction networks inferred from 16S rRNA gene surveys and associated geochemical dynamics of the system 41, 42, 43. Such a model could just incorporate the pathways as individual reaction networks without the need for extensive metagenomic characterization. What limits such an approach is the fact that the unit catalyzing biogeochemical transformations is the individual microbial cell. Specific microbial functions are combined within cells, which operate in their microbial community context. An understanding of which capacities co-occur, and which resources are exchanged among species, is critical to understanding the rate-limiting steps in reaction networks. Given the massive genomic information for sites like the Rifle alluvial aquifer, the genetic capacity and the relative numbers of microorganisms in aquifers can likely be used to infer what underlying metabolic processes are needed to drive the carbon, nitrogen, sulfur, and hydrogen cycles [33]. Detailed evidence for such metabolic processes could reshape the way we construct biogeochemical reactive transport models.

Another facet of detailed metagenomic information is the ability to assess metabolic 'resiliency' within a given system. The 'microbial seed bank' confers an advantage in the face of climate-induced changes in soil/sediment temperature, moisture content, redox conditions, salinity, etc. Understanding of resiliency requires information about very low abundance organisms, and is critical to predicting trajectories of disturbed ecosystems. For example, *Geobacter* sp. may be present at very low abundance levels in sediment, yet relaxation of organic carbon limitation by acetate addition causes this organism to 'bloom' and dominate the active microbial community [44]. Proliferation of *Geobacter* species that also can fix N_2 into a form bioavailable to the larger community has broader implications for the overall ecosystem function 45, 46. It is our thesis, then, that given deep genome-resolved information, we should be able to make robust predictions about how watershed systems, including soils and variably saturated underlying sediments, will respond to environmental changes. Such shifts could be in the form of changes in rainfall (soil moisture and water table excursions) or in vegetation (microbe–plant/root associations). Such shifts likely will have broad, aggregated impacts on cycling of nutrients such as C, N, S, and P [47]. Limited progress is being made toward models that include genomic diversity in prediction of microbial community response to changing environmental conditions [20]. An example of an ecosystem impact arising from changing microbial community composition might be the proliferation of chemolithoautotrophs in response to subsurface nitrate amendment [48], accompanied by increased conversion of CO_2 into biomass. Other examples include release of metal contaminants adsorbed to ferric iron oxyhydroxide mineral surfaces following increased Fe reduction due to stimulation of *Geobacter* sp. by changes in organic carbon availability 41, 44, and an increase in the U (VI) reduction rate in response to amendment with bicarbonate and acetate compared to acetate alone [41]. Ultimately, genomes of the microbial community members encode the capacities that determine the forms of such responses. Looking forward, the challenge will be to validate the consistency and predictability of such responses by experiments and observations that combine deep metagenomics and transcriptomics with detailed biogechemical and isotopic observations capable of revealing the details of reactions that modify key biogeochemical fluxes.

Implications for Subsurface Biogeochemical Processes, Including Greenhouse Gas Fluxes from the Subsurface

Coming into sharper focus is the vast diversity of microorganisms that mediate critically important biogeochemical reactions [29]. In Figure 4 (Key Figure) we illustrate the

principal capacities predicted from genomic information. Each organism that carries out such processes does so at a rate determined by other aspects of its physiology in concert with the other transformations that it is equipped to mediate. Many of these microbially controlled reactions impact the fluxes of the greenhouse gases CO_2, CH_4, and N_2O through water bodies and into the atmosphere [26] (Figure 4). Of course, no single organism will have all of the catalytic machinery for all of the reactions shown. At one end of a spectrum are the CPR bacteria, which likely cannot to survive on their own because they depend on other community members for critical resources, yet they provide metabolically valuable fermentation products. At another end are the Zixibacteria 35, 47, which have a broad metabolic repertoire that probably explains their dominance in the time-varying environment close to the fluctuating boundary between the water table and vadose zone. Also important, but not depicted in Figure 4, is predation by viral and protist populations 49, 50, 51, 52, 53, 54, 55, 56. While individual microbial species may produce a constituent of interest (e.g., methane from Candidatus 'Methanoflorens stordalenmirensis' or *Methanosarcina* in thawing permafrost 5, 26, 57, 58), other community members (possibly CPR that generate H_2) may be required to support CH_4 production. This is likely emblematic of subsurface ecosystem function: microorganisms growing in a community context drive biogeochemical processes. Thus, understanding the diversity and capacities of these organisms will be critical for predicting greenhouse gas fluxes to and from the subsurface.
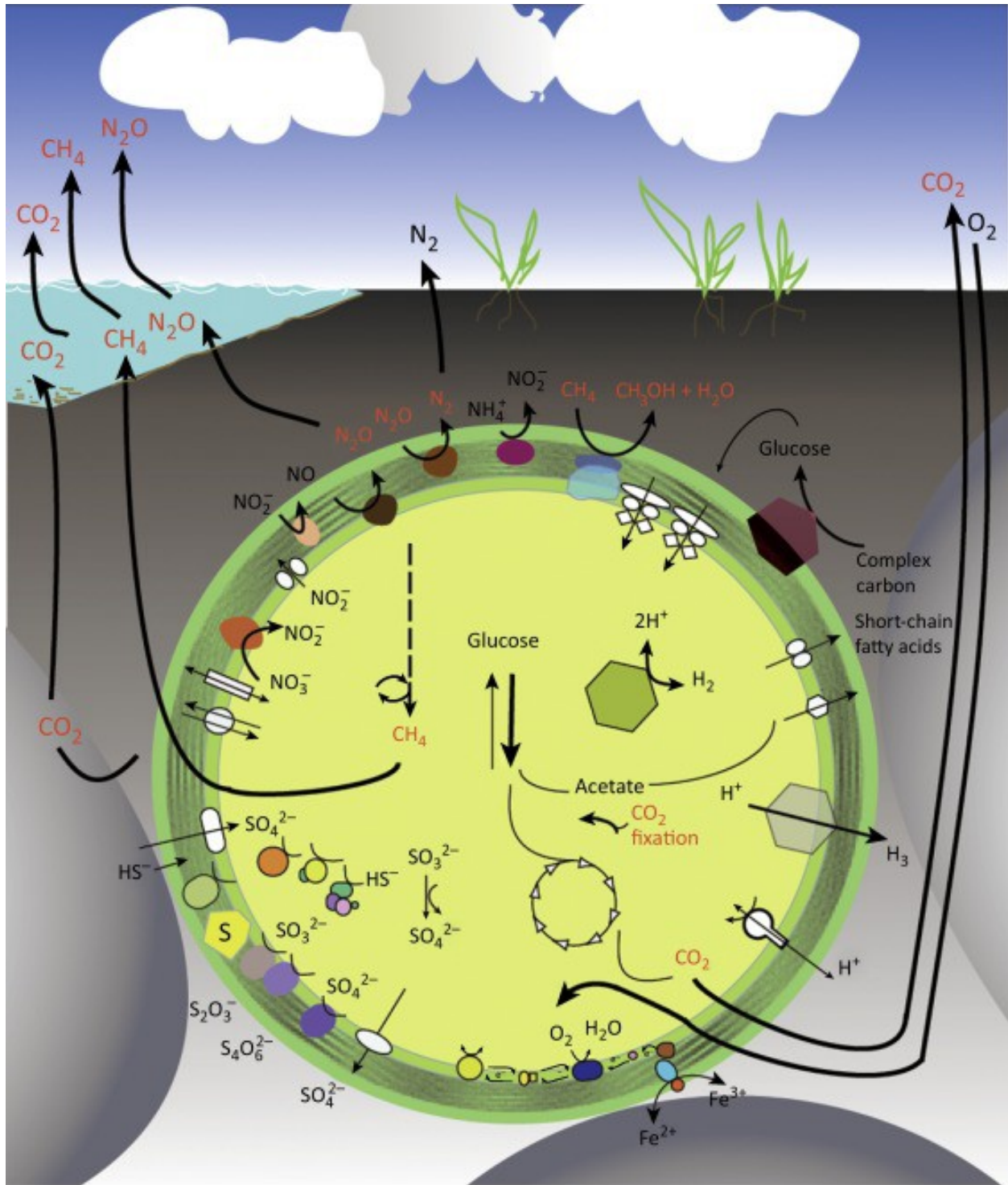
Figure 4. Key Figure: Microbially Mediated Reactions Occurring in the Shallow Terrestrial Subsurface (Diagramatic)

This figure highlights information obtained from genome-resolved studies that inform our understanding of these microbially mediated reactions. The red text highlights pathways

that impact atmospheric greenhouse gas concentrations. Only rarely would any individual cell be expected to have all of the capacities shown in this schematic.

Concluding Remarks

The microbiology of the shallow terrestrial subsurface plays a key role in organic carbon and hydrogen cycles, ultimately contributing to greenhouse gas fluxes to and from the subsurface. Previously little known and unknown archaeal and bacterial species contribute to a variety of interlinked chemical transformations in the nitrogen and sulfur cycles, as well as carbon and hydrogen cycles. But how do these communities change with time, and what are the master controls that influence the microbial community makeup? Or, conversely, what biogeochemical or physical features does the microbial community modify as a result of its underlying emergent properties? While models of microbial community interactions are in development, we do not as yet have the ability to make *de novo* predictions about the response of subsurface microbial communities to perturbations. We argue that a deeper, mechanistic level of understanding of microbial species interactions, competition, and function are required to effectively model subsurface greenhouse gas fluxes at the watershed scale, particularly under the ongoing perturbation of climate change. To address this gap, future research needs to systematically interrogate genomics of representative subsurface biomes, coupled with observations of subsurface genomic, transcriptomic, and proteomic responses to both short-term (human induced, including laboratory-scale) and long-term perturbations (see Outstanding Questions). Such research is consistent with the recently announced microbiomes initiative 7, 8. Our ability in the future to match both aggregate fluxes and detailed microbial observations of perturbations with models will serve as the test of our modeling capabilities. Further, these results will provide a direct measure of the extent to which mechanistic microbial genomics are needed to sustain model fidelity.

Outstanding Questions

Are individual traits, rather than microbial interactions, the main determinant of how a microbial community impacts its physiochemical environment and how (and how fast) the community responds to environmental changes? Prevalent obligate interdependencies (symbiosis, pathogenesis, and virus predation) are likely important in ways we do not yet understand.

Can representative compartments of subsurface ecosystems be identified, and through their detailed study, can we approximate the function of the entire system? Based on what have learned about aquifer microbiology, it is clear that surface microbes are

important and that genome resolution is essential. Just as for other types of ecosystem monitoring, can we measure metogenomics with appropriate sampling density? Genomes will provide direct information on key biogeochemical pathways and insight into coupling of functions, as required for accurate biogeochemical models.

Does high diversity in subsurface aquifer sediments confer community functional resilience? Knowledge of the presence and metabolic capacities of very low abundance organisms is critical to predicting how ecosystems respond to environmental change because these are part of a 'seed bank' that can provide new capacities as opportunities arise and confer ecosystem resilience. Robust predictions about how a given subsurface system will respond to environmental changes, including changes in vegetation and microbe–plant/root associations, will require understanding of this resilience stemming from diversity and enabling prediction of broad, aggregated impacts on nutrient cycling and nutrient export (especially C, N, S, and P).

Acknowledgments

References

1

J. Raymond, E.B. Alsop**Microbial evolution in extreme environments: microbial migration, genomic highways, and geochemical barriers in hydrothermal ecosystems**
Environ. Systems Res, 4 (2015), pp. 1-12
View Record in Scopus

2

T. Kieft, *et al.* **Workshop to develop deep-life continental scientific drilling projects**

Sci. Dril., 19 (2015), pp. 43-53

CrossRefView Record in Scopus

3

J.B. Emerson, *et al.* **Metagenomic analysis of a high carbon dioxide subsurface microbial community populated by chemolithoautotrophs and bacteria and archaea from candidate phyla**

Environ. Microbiol. (2015), 10.1111/1462-2920.12817

Published online April 8, 2015.

4

J. Tan, *et al.* **Unraveling interactions in microbial communities–from co-cultures to microbiomes**

J. Microbiol., 53 (2015), pp. 295-305

CrossRefView Record in Scopus

5

J. Hultman, *et al.* **Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes**

Nature, 521 (2015), pp. 208-212

CrossRefView Record in Scopus

6

F.-A. Herbst, *et al.* **Enhancing metaproteomics–The value of models and defined environmental microbial systems**

Proteomics, 16 (2016), pp. 783-798

CrossRefView Record in Scopus

7

A.P. Alivisatos, *et al.* **A unified initiative to harness Earth's microbiomes**

Science, 350 (2015), pp. 507-508

CrossRefView Record in Scopus

8

N. Dubilier, *et al.* **Microbiology: Create a global microbiome effort**

Nature, 526 (2015), p. 631

CrossRefView Record in Scopus

9

L.A. Hug, *et al.* **A new view of the tree of life**

Nature Microbiol (2016), 10.1038/nmicrobiol.2016.48

Published online April 11, 2016

10

F. Abram **Systems-based approaches to unravel multi-species microbial community functioning**

Computat. Struct. Biotechnol. J., 13 (2015), pp. 24-32

[ArticleDownload PDFView Record in Scopus]

[11]

C.R. Woese, *et al.***Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya**

Proc. Natl. Acad. Sci. U.S.A., 87 (1990), pp. 4576-4579

[CrossRefView Record in Scopus]

[12]

D.C. Reed, *et al.***Predicting the response of the deep-ocean microbiome to geochemical perturbations by hydrothermal vents**

ISME J., 9 (2015), pp. 1857-1869

[CrossRefView Record in Scopus]

[13]

M.R. Osburn, *et al.***Chemolithotrophy in the continental deep subsurface: Sanford Underground Research Facility (SURF), USA**

Front. Microbiol (2014), [10.3389/fmicb.2014.00610]

Published online November 12, 2014

[1]
[4]

M.D.J. Lynch, J.D. Neufeld**Ecology and exploration of the rare biosphere**

Nat. Rev. Microbiol., 13 (2015), pp. 217-229

[CrossRefView Record in Scopus]

[15]

K.T. Konstantinidis, R. Rosselló-Móra**Classifying the uncultivated microbial majority: A place for metagenomic data in the Candidatus proposal**

Syst. Appl. Microbiol., 38 (2015), pp. 223-230

[ArticleDownload PDFView Record in Scopus]

[16]

F.O.P. Stefani, *et al.***Culture-dependent and -independent methods capture different microbial community fractions in hydrocarbon-contaminated soils**

PLoS ONE, 10 (2015), p. e0128272

[CrossRef]

[17]

D.R. Garza, B.E. Dutilh**From cultured to uncultured genome sequences: metagenomics and modeling microbial ecosystems**

Cell. Mol. Life Sci., 72 (2015), pp. 4287-4308

[CrossRefView Record in Scopus]

[18]

J. Zhou, *et al.***High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats**
mBio, 6 (2015)
e02288-02214

19

C.T. Brown, *et al.***Unusual biology across a group comprising more than 15% of domain Bacteria**
Nature, 523 (2015), pp. 208-211
CrossRefView Record in Scopus

20

D.C. Reed, *et al.***Gene-centric approach to integrating environmental genomics and biogeochemical models**
Proc. Natl. Acad. Sci. U.S.A., 111 (2014), pp. 1879-1884
CrossRefView Record in Scopus

21

B. Arora, *et al.***Influence of hydrological, biogeochemical and temperature transients on subsurface carbon fluxes in a flood plain environment**
Biogeochemistry, 127 (2016), pp. 367-396
CrossRefView Record in Scopus

22

K.M. Jarecke, *et al.***Coupled soil oxygen and greenhouse gas dynamics under variable hydrology**
Soil Biol. Biochem., 95 (2016), pp. 164-172
ArticleDownload PDFView Record in Scopus

23

S. Blagodatsky, P. Smith**Soil physics meets soil biology: towards better mechanistic prediction of greenhouse gas emissions from soil**
Soil Biol. Biochem., 47 (2012), pp. 78-92
ArticleDownload PDFView Record in Scopus

24

B.K. Singh, *et al.***Microorganisms and climate change: terrestrial feedbacks and mitigation options**
Nat. Rev. Microbiol., 8 (2010), pp. 779-790
CrossRefView Record in Scopus

25

N.S. Diffenbaugh, *et al.***Anthropogenic warming has increased drought risk in California**
Proc. Natl. Acad. Sci. U.S.A., 112 (2015), pp. 3931-3936
CrossRefView Record in Scopus

26

C.K. McCalley, *et al.***Methane dynamics regulated by microbial community response to permafrost thaw**
Nature, 514 (2014), pp. 478-481
[CrossRefView Record in Scopus](#)

27

A. Lagomarsino, *et al.***Past water management affected GHG production and microbial community pattern in Italian rice paddy soils**
Soil Biol. Biochem., 93 (2016), pp. 17-27
[ArticleDownload PDFView Record in Scopus](#)

28

S.J. Hall, *et al.***Environmental forcing does not induce diel or synoptic variation in the carbon isotope content of forest soil respiration**
Biogeosciences, 12 (2015), pp. 5143-5160

29

K. Anantharamana, *et al.***Analysis of five complete genome sequences for members of the class Peribacteria in the recently recognized Peregrinibacteria bacterial phylum**
PeerJ, 4 (2016), p. e1607

30

K.C. Wrighton, *et al.***Fermentation, Hydrogen and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla**
Science, 337 (2012), pp. 1661-1665
[CrossRefView Record in Scopus](#)

31

B. Luef, *et al.***Diverse uncultivated ultra-small bacterial cells in groundwater**
Nat. Commun. (2015), [10.1038/ncomms7372](#)
Published online February 27, 2015

32

C.J. Castelle, *et al.***Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling**
Curr. Biol., 25 (2015), pp. 690-701
[ArticleDownload PDFView Record in Scopus](#)

33

L.A. Hug, *et al.***Critical biogeochemical functions in the subsurface are associated with bacteria from new phyla and little studied lineages**
Environ. Microbiol. (2015), [10.1111/1462-2920.12930](#)
Published online July 22, 2015

34

P.N. Evans, *et al.***Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics**

Science, 350 (2015), pp. 434-438

[CrossRef](#)[View Record in Scopus](#)

35

I. Sharon, *et al.***Accurate, multi-kb reads resolve complex populations and detect rare microorganisms**

Genome Res., 25 (2015), pp. 534-543

[CrossRef](#)[View Record in Scopus](#)

36

Y. Feng, *et al.***Nanopore-based Fourth-generation DNA Sequencing Technology**

Genom. Proteom. Bioinform., 13 (2015), pp. 4-16

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37

E. Pennisi**Pocket DNA sequencers make real-time diagnostics a reality**

Science, 351 (2016), pp. 800-801

[CrossRef](#)[View Record in Scopus](#)

38

S. Lindsay**The promises and challenges of solid-state sequencing**

Nat. Nanotechnol., 11 (2016), pp. 109-111

[CrossRef](#)[View Record in Scopus](#)

39

C. Gasc, *et al.***Capturing prokaryotic dark matter genomes**

Res. Microbiol., 166 (2015), pp. 814-830

[Article](#)[Download PDF](#)[View Record in Scopus](#)

40

L.A. Hug, *et al.***Aquifer environment selects for microbial species cohorts in sediment and groundwater**

ISME J., 9 (2015), pp. 1846-1856

[CrossRef](#)[View Record in Scopus](#)

41

P.E. Long, *et al.***Bicarbonate impact on U(VI) bioreduction in a shallow alluvial aquifer**

Geochim. Cosmochim. Acta, 150 (2015), pp. 106-124

[Article](#)[Download PDF](#)[View Record in Scopus](#)

42

S. Yabusaki, *et al.***Coupled-process modeling of a uranium bioremediation field experiment (abstract)**

Mineralog. Magaz., 76 (2012), p. 2562

[View Record in Scopus](#)

43

C. Bao, *et al.***Uranium bioreduction rates across scales: Biogeochemical "hot moments" and "hot spots" during a field biostimulation experiment at Rifle**
Colorado. Environ. Sci. Technol., 48 (2014), pp. 10116-10127
CrossRefView Record in Scopus

44

R.T. Anderson, *et al.***Stimulating the *in situ* activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer**
Appl. Environ. Microbiol., 69 (2003), pp. 5884-5891
CrossRefView Record in Scopus

45

P.J. Mouser, *et al.***Influence of heterogeneous ammonium availability on bacterial community structure and the expression of nitrogen fixation and ammonium transporter genes during *in situ* bioremediation of uranium-contaminated groundwater**
Environ. Sci. Technol., 43 (2009), pp. 4386-4392
CrossRefView Record in Scopus

46

D.A. Bazylinski, *et al.***N_2-dependent growth and nitrogenase activity in the metal-metabolizing bacteria, *Geobacter and Magnetospirillum species***
Environ. Microbiol., 2 (2000), pp. 266-273
CrossRefView Record in Scopus

47

B.J. Baker, *et al.***Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria**
Microbiome, 3 (2015), p. 14
CrossRefView Record in Scopus

48

T.N.M. Jewell, *et al.***Metatranscriptomic evidence of pervasive and diverse chemolithoautotrophy relevant to C, S, N, and Fe cycling in a shallow alluvial aquifer**
ISME J (2016), 10.1038/ismej.2016.25
Published online March 4, 2016

49

A.Z. Worden, *et al.***Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes**
Science, 347 (2015), p. 1257594
CrossRef

50

S. Roux, *et al.***Viral dark matter and virus–host interactions resolved from publicly available microbial genomes**
eLife, 4 (2015), p. e08490

51

D.E. Holmes, *et al.***Enrichment of specific protozoan populations during *in situ* bioremediation of uranium-contaminated groundwater**
ISME J., 7 (2013), pp. 1286-1298
CrossRefView Record in Scopus

52

T. Engelhardt, *et al.***High virus-to-cell ratios indicate ongoing production of viruses in deep subsurface sediments**
ISME J., 8 (2014), pp. 1503-1509
CrossRefView Record in Scopus

53

K. Pedersen**Subterranean microbial populations metabolize hydrogen and acetate under *in situ* conditions in granitic groundwater at 450 m depth in the Äspö Hard Rock Laboratory, Sweden**
FEMS Microbiol. Ecol., 81 (2012), pp. 217-229
CrossRefView Record in Scopus

54

M. Legendre, *et al.***In-depth study of Mollivirus sibericum, a new 30,000-y-old giant virus infecting *Acanthamoeba***
Proc. Natl. Acad. Sci. U.S.A., 112 (2015), pp. E5327-E5335
CrossRefView Record in Scopus

55

D.E. Holmes, *et al.***Evidence of Geobacter-associated phage in a uranium-contaminated aquifer**
ISME J., 9 (2015), pp. 333-346
CrossRefView Record in Scopus

56

D.E. Holmes, *et al.***Methane production from protozoan endosymbionts following stimulation of microbial metabolism within subsurface sediments**
Front. Microbiol., 5 (2014), p. 366

57

R. Mondav, *et al.***Discovery of a novel methanogen in thawing permafrost**
Nat. Commun., 5 (2014), p. 3212

58

J.K. Jansson, N. Taş**The microbial ecology of permafrost**
Nat. Rev. Microbiol., 12 (2014), pp. 414-425
CrossRefView Record in Scopus