Title: Anergiobiosis: a testable framework for microbial life under extreme power limitation

Authors: Paul Carini[1,2], Roland Hatzenpichler[3], Jennifer F. Biddle[4]

Affiliation:
[1]Department of Environmental Science, University of Arizona, Tucson AZ
[2]BIO5 Institute, University of Arizona, Tucson, AZ
[3]Department of Chemistry and Biochemistry, Department of Microbiology and Cell Biology, Thermal Biology Institute, Center for Biofilm Engineering, Montana State University, Bozeman, MT
[4]School of Marine Science and Policy, University of Delaware, Lewes DE

Abstract:
"Aeonophily" was recently suggested as a new category of extremophily for ultra-slow-growing subsurface microorganisms. This terminology conflates a physiological state with potential extremophilic specialization. We propose "anergiobiosis" to describe life without sufficient power to sustain cell division, separating this state from questions about specialization. Analogous to temperature extremophiles, microbes may exhibit distinct maintenance power optima, with aeonophiles representing low-power specialists. We outline testable hypotheses for establishing whether specific taxa possess such adaptations.

Main Text:
Lloyd and Steen recently proposed that ultra-slow microbial life may persist over geological timescales and represent a distinct extremophile category defined by prolonged survival of ultra-slow-metabolizing organisms, they call “aeonophiles” (long-time-loving)[1]. Their synthesis labels remarkable biology and raises important questions about how we conceptualize extremophily and life at its thermodynamic limits. Here, we propose that distinguishing the physiological state from potential extremophilic specialization requires different terminology that captures the underlying biology.

The challenge with aeonophily as an extremophile category

Terminology shapes how we think about biological phenomena. The words we choose to describe organisms influence our hypotheses, experimental designs, and interpretations. Labeling organisms as “aeonophiles” implies they "love" slow growth in the same way thermophiles "love" heat, suggesting slow growth represents their physiological optimum rather than an ecological constraint they tolerate.

Extremophile nomenclature traditionally reflects a defining feature of these organisms: growth optima under extreme conditions of an independent environmental variable. Thermophiles don't just tolerate high temperature; they grow *better* at high temperature—and become inactive below a threshold—with genomes encoding heat-stable proteins and membrane lipids that confer competitive advantages at high temperature. The ‘-phile’ distinction therefore identifies organisms with specific physiological adaptations to discrete environmental variables (temperature, pH, salinity, pressure) that can be manipulated to demonstrate optimal growth.

Applying this framework to include aeonophiles presents two fundamental challenges. First, time cannot be separated as an independent variable analogous to temperature, pH, salinity, or pressure. Long timescales are the timeframe over which microbes experience environmental limitations, not conditions they adapt to. While organisms can evolve dormancy mechanisms and efficient maintenance strategies, these represent responses to resource scarcity, not to time itself as a selective pressure.

Second, without an independent environmental variable that can be manipulated to show optimal growth, we cannot establish aeonophily as experimentally testable. Unlike the growth optimization apparent in other extremophiles, there is no direct evidence aeonophiles are obligated to ultra-slow rates. For example, subsurface taxa proposed to be aeonophiles—including some members of the Atribacteria, *Thalassospira*, Bathyarchaeia, and *Promethearcheum*—grow orders of magnitude *faster* when grown with increased substrate availability, both in the laboratory[2–5] and during transient high-flux events in sediments[6]. This growth response is the opposite pattern expected for true extremophiles: thermophiles grow poorly when removed from high temperature and halophiles grow poorly at low salinity. Yet, proposed aeonophiles grow *faster* when substrate limitation is relieved. This demonstrates that *in situ* ultra-slow growth rates result from environmental limitations rather than physiological adaptation requiring slow growth. Because time itself cannot be manipulated as an independent variable, we cannot test whether any organism truly optimizes growth at ultra-slow rates; the concept of aeonophily as presented[1] is thus an untestable hypothesis.

The aeonophile proposal further redefines extremophile fitness as “who dies the slowest, rather than who grows the fastest.” While this acknowledges distinct selective pressures in power-limited environments, the '-phile' suffix still implies optimal fitness under extreme power limitation. Net growth rate (reproduction rate minus death rate) integrated over geological time determines which lineages persist. The organism that “dies the slowest” is also most likely to survive and reproduce when conditions improve—which is what net growth fitness already measures. Moreover, reframing fitness around survival does not resolve the core problem: time cannot be isolated as a variable to demonstrate that persistence reflects physiological adaptation rather than environmental circumstance. For example, survival over geological timescales may also result from abiotic physical or geochemical protection. Without the ability to disentangle these experimentally, aeonophily describes a pattern of survival rather than a demonstrated adaptive strategy.

Is survival over geological timescales an extremophile trait?

For a trait to qualify as extremophilic, we should demonstrate some organisms possess it while others do not, with demonstrable fitness advantages along a gradient of the relevant environmental variable. The observation that certain taxonomic groups dominate deep subsurface environments is suggestive but does not, by itself, establish extremophily. The key question is not whether organisms can persist at near-zero growth under extreme energy limitation—retentostats demonstrate this is broadly achievable across phylogenetically diverse taxa[7–9]—but whether specific lineages have evolved competitive advantages at maintaining viability under these conditions compared to other organisms held under identical constraints. Phylogenetic clustering in subsurface environments could reflect true specialization (i.e. aeonophily), dispersal limitation, superior dormancy, or historical contingency. The observation that very few taxa are limited to subsurface environments argues against obligate aeonophily and suggests they may tolerate rather than require extreme power limitation.

We agree that evidence from subsurface environments—including lack of genetic recombination[3], minimal mutation accumulation[10], persistent mRNA[11], and active metabolism[12]—supports rare cell division. However, current methods measure community-averaged rates that integrate metabolic states of large cell numbers and cannot distinguish individual cell fates over geological timescales (see below). Distinguishing whether subsurface dominance reflects true aeonophilic specialization, dispersal advantages, superior dormancy, historical contingency, or a combination of these factors requires comparative experiments testing whether subsurface-associated taxa outperform phylogenetically diverse organisms under controlled conditions of extreme power limitation. Current evidence does not yet meet this standard.

Anergiobiosis: Life without work

We propose anergiobiosis (an- = without, ergon = work/energy, bios = life) as a framework for understanding microbial life persisting at thermodynamic limits. This terminology parallels established biological nomenclature like anhydrobiosis (life without water), directly describing the physiological state rather than implying preference or optimization. Unlike aeonophile, 'anergiobiosis' describes what subsurface organisms experience: life without sufficient power to support cell division.

Anergiobiosis describes the state of maintaining cellular viability when energy supply falls below thresholds supporting cell division but allows maintenance metabolism. Power utilization in deep subsurface environments (as low as 1.5×10^{-20} watts per cell[13]) falls orders of magnitude below maintenance power requirements measured in other systems. Anergiobionts are therefore organisms demonstrating capacity to maintain this state.

This framework is mechanistically grounded and testable through energy budgets, ATP turnover, metabolic flux, maintenance power coefficients, and single cell measurements[14]. It separates the physiological state (anergiobiosis) from questions about adaptation versus tolerance and remains accurate regardless of what specifically limits energy availability.

Within the anergiobiosis framework, aeonophily as proposed[1], could represent a specific extremophile designation: organisms that not only tolerate anergiobiotic conditions but are specifically adapted to maintain viability better than other organisms under identical conditions of extreme energy limitation. Just as microbes partition across temperature gradients (psychrophiles, mesophiles, thermophiles), microbes may partition along energy availability gradients based on their maintenance energy optima. We propose three new putative categories of power specialists: paucienergophiles (low power specialists, colloquially “aeonophiles”), mesoenergophiles (intermediate-power specialists), and hyperenergophiles (high-power specialists) (Fig. 1).

True paucienergophiles (“aeonophiles”) would possess low basal power requirements, conferring competitive advantages by maintaining lower death rates and resuming cell division at lower energy inputs. However, systematically measuring maintenance power thresholds—the power level at which cell division equals zero—remains technically challenging and is largely unaccomplished. Not all organisms capable of entering anergiobiosis would qualify as paucienergophiles (aeonophiles). The designation requires demonstrating *superior* performance under energy limitation, not merely persistence.

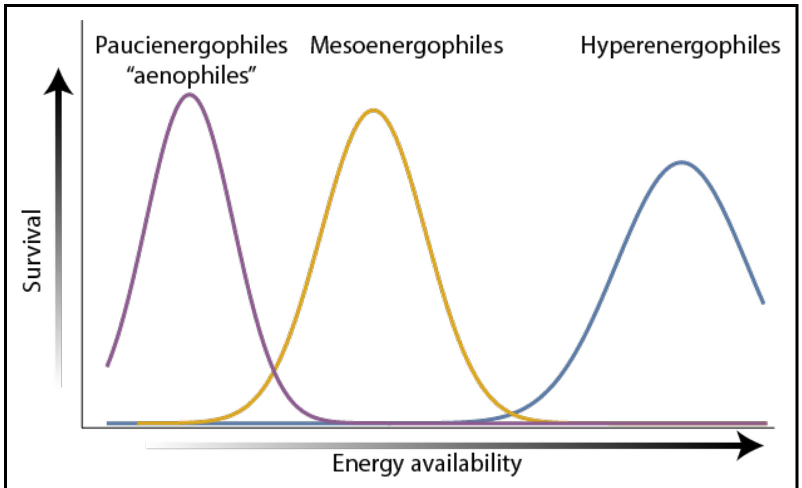

Fig. 1 Microbial survival may partition along energy availability gradients. Proposed framework analogous to specialization in other extremophiles. Paucienergophiles (“aeonophiles”, purple) would exhibit optimal survival performance at very low power availability, possessing lower basal maintenance requirements than other organisms. Mesoenergophiles and Hyperenergophiles are potential specialists at moderate (yellow) and high (blue) power availability, respectively. Measurements of maintenance power thresholds across microbial diversity would be required to validate this framework.

We agree that low energy delivery represents the dominant selective pressure in deep subsurface environments. Importantly, hypotheses about aeonophilic specialization remain testable even in organisms that have been successfully cultivated and grow readily in the laboratory. The question is not whether organisms can grow, but whether they possess specific molecular adaptations enabling extended viability under energy limitation. A thermophile remains a thermophile even when growing at suboptimal temperature—its heat-stable proteins and specialized membrane lipids are demonstrable regardless of culture conditions. Similarly, if subsurface taxa possess adaptations conferring aeonophilic advantages, these should be detectable through comparative molecular and physiological analyses whether organisms are actively growing or not.

Current evidence suggests but does not yet establish energy specialization in this manner as a distinct extremophile category. Subsurface-associated taxa may possess specific adaptations enabling extended persistence: ultra-stable biomolecules resisting degradation, efficient repair systems that minimize damage accumulation, protective compounds preventing protein aggregation, mRNA-stabilizing factors, and specialized enzymes degrading recalcitrant organic matter. These are testable hypotheses about mechanisms enabling superior performance in anergiobiosis. Lloyd and Steen cite slow growth under ideal conditions and specialized enzymes as potential aeonophilic trade-offs[1]. However, establishing aeonophily as an extremophile category requires demonstrating that these taxa maintain viability *better* than other non-aeonophilic organisms under controlled conditions of extreme energy limitation, not just observing that they dominate natural subsurface environments, produce unusual enzymes or biomolecules, or grow slowly in the laboratory.

The anergiobiosis framework clarifies experimental approaches needed to test aeonophily. Current methods measure community-averaged rates and cannot resolve individual cell fates; a measured community doubling time of thousands of years could represent all cells dividing slowly, a fraction dividing while most remain dormant, or turnover balancing sporadic divisions. Testing requires resolving individual cell behaviors through single-cell measurements, identifying molecular signatures of specialized maintenance or repair machinery, and comparing performance across taxa under controlled energy limitation. Such approaches need not depend on cultivation and could leverage *in situ* single-cell techniques, comparative genomics, and experimental manipulations of natural communities to determine whether subsurface-associated taxa represent true extremophiles or simply persist in power-limited habitats.

Conclusion:

Lloyd and Steen's synthesis highlights biology that represents a dominant mode of microbial life on Earth. We extend their work to propose anergiobiosis as terminology that captures this biology while maintaining experimental testability and mechanistic precision. This framework distinguishes the physiological state all organisms can enter from potential extremophilic specialization some may possess. We suggest microbes may partition along power availability gradients based on maintenance power optima, with “aeonophiles” representing potential specialists at very low power. This focuses research on testable hypotheses about survival mechanisms, maintenance power minimization, and damage repair under extreme power limitation. Whether subsurface-associated taxa represent true aeonophilic specialists remains an open question that the anergiobiosis framework provides clear experimental pathways to resolve.

Acknowledgements:

P.C. was supported through an Early Career in Aquatic and Marine Ecology and Evolution award from the Simons Foundation. R.H. was supported through NSF award OCE-2049445. J.F.B. was supported by an award from the WM Keck Foundation.

References:

1. Lloyd, K. G. & Steen, A. D. Defining ultra-slow-growing extremophilic microorganisms as aeonophiles. *Nat. Microbiol.* **10**, 1555–1557 (2025).
2. Katayama, T. *et al.* Isolation of a member of the candidate phylum “Atribacteria” reveals a unique cell membrane structure. *Nat. Commun.* **11**, 6381 (2020).
3. Orsi, P. C. W. *et al.* Genome Evolution in Bacteria Isolated from Million-Year-Old Subseafloor Sediment. *mBio* **12**, e01150-21 (2021).
4. Yu, T. *et al.* Widespread Bathyarchaeia encode a novel methyltransferase utilizing lignin-derived aromatics. *mLife* **2**, 272–282 (2023).
5. Imachi, H. *et al.* Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* **577**, 519–525 (2020).
6. Vuillemin, A. *et al.* Atribacteria reproducing over millions of years in the Atlantic abyssal subseafloor. *MBio* **11**, (2020).
7. Vos, T. *et al.* Maintenance-energy requirements and robustness of Saccharomyces cerevisiae at aerobic near-zero specific growth rates. *Microb. Cell Fact.* **15**, 111 (2016).
8. Tappe, W. *et al.* Maintenance energy demand and starvation recovery dynamics of Nitrosomonas europaea and Nitrobacter winogradskyi cultivated in a retentostat with complete biomass retention. *Appl. Environ. Microbiol.* **65**, 2471–2477 (1999).
9. Ercan, O., den Besten, H. M. W., Smid, E. J. & Kleerebezem, M. The growth-survival trade-off is hard-wired in the Lactococcus lactis gene regulation network. *Environ. Microbiol. Rep.* (2022) doi:10.1111/1758-2229.13073.
10. Starnawski, P. *et al.* Microbial community assembly and evolution in subseafloor sediment. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 2940–2945 (2017).
11. Orsi, W. D., Edgcomb, V. P., Christman, G. D. & Biddle, J. F. Gene expression in the deep

biosphere. *Nature* **499**, 205–208 (2013).

12. Morono, Y. *et al.* Aerobic microbial life persists in oxic marine sediment as old as 101.5 million years. *Nat. Commun.* **11**, 3626 (2020).

13. Bradley, J. A. *et al.* Widespread energy limitation to life in global subseafloor sediments. *Sci Adv* **6**, eaba0697 (2020).

14. Hatzenpichler, R., Krukenberg, V., Spietz, R. L. & Jay, Z. J. Next-generation physiology approaches to study microbiome function at single cell level. *Nat. Rev. Microbiol.* **18**, 241–256 (2020).