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

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Abstract:
"Aeonophily" was recently suggested as a new category of extremophily for ultra-slow-growing subsurface microorganisms. This terminology misdescribes the physiological state of slow growth as potential extremophilic specialization. Unlike temperature or salinity, time cannot be manipulated to demonstrate a growth optimum, making aeonophily untestable as currently framed. We propose "anergiobiosis" to describe life where energy flux is sufficient to prevent death, but insufficient for cell division, separating the state from questions about specialization. Within this framework, microbes may exhibit distinct energy flux optima, with low-energy-flux specialists representing the potential biological basis for aeonophily. 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, representing an 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 at life’s thermodynamic limits from potential specialization to low energy flux conditions requires different terminology than what was proposed to capture 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" long time scales in the same way thermophiles "love" heat, suggesting ultra-slow-growth represents their physiological *optimum* instead of a consequence of extreme energy limitation.

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 demonstrable 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” as a variable cannot be experimentally separated independently like temperature, pH, salinity, or pressure. Time is defined as a measurable period during which an action, process, or condition exists or continues. Thus, long timescales are the frame over which microbes experience environmental conditions. While organisms can evolve dormancy mechanisms and efficient maintenance strategies, these represent responses to resource scarcity, not to time itself as a selective pressure. Although time is implicit in evolutionary and growth rates, it serves as the medium over which selective pressures act, not itself as an ever-changing environmental variable analogous to temperature or salinity. Without an independent environmental variable that can be manipulated to show optimal growth, aeonophily, as presented, is not experimentally testable.

Second, unlike the growth optimization apparent in other extremophiles, there is no direct evidence aeonophiles are obligated to ultra-slow rates. Instead, currently available evidence suggests taxa proposed to be aeonophiles grow faster when substrate limitation is relieved. 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]. These examples show that at least for some taxa, *in situ* ultra-slow growth rates result from environmental limitations rather than an obligate requirement for slow growth. While there is undoubtedly much to learn about the physiology of microbes at their thermodynamic limits, sufficient evidence pointing to a requirement for slow growth does not exist at this time.

We recognize some taxa may be limited to slow maximal growth rates due to low ribosome copy number, codon usage constraints, membrane biophysics, or inefficient replication machinery. Slow growth in this sense is an adaptation to a specific molecular or bioenergetic constraint, not to time itself. A chemostat can manipulate energy flux delivery—not time—and would therefore test whether organisms have low maintenance energy thresholds or low maximum growth rates. Even granting slow growth rate as a testable variable, the aeonophily hypothesis is practically untestable at the scales proposed. If generation times of proposed aeonophiles reach 1,000 years, no feasible experiment can establish whether ultra-slow growth represents a physiological optimum instead of an environmental constraint. Finally, growth rates across diverse microbes almost certainly fall on a continuum shaped by ribosome biology, codon usage, membrane composition, and energy availability. There is no evidence this variability is the result of adaptation to time as a resource. Furthermore, no principled growth rate threshold separates aeonophiles from other organisms persisting under other limitations, and as such the designation loses operational meaning.

The aeonophile proposal further redefines extremophile fitness as “who dies the slowest, rather than who grows the fastest.” While this acknowledges distinct selective pressures under low energy availability, the '-phile' suffix still implies optimal fitness under extreme energy limitation. Yet, 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. Laboratory work on the Growth Advantage in Stationary Phase (GASP) phenotype showed that cells aged in long-term batch cultures outcompete younger cells by improving utilization of compounds released from dying cells[7]. Such adaptation under starvation reflects exploitation of alternative energy sources, not a ‘love’ for slow growth. Moreover, reframing fitness around survival does not resolve the fundamental testability problem: persistence over geological timescales may reflect abiotic physical or geochemical protection as much as physiological adaptation. 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, there should be demonstrated variability where some organisms possess the trait while others do not, with testable 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. Whether organisms can persist at near-zero growth under low energy flux is well established—retentostats demonstrate this is broadly achievable across phylogenetically diverse taxa[8,9]. However, it is unknown if 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, dormancy, or historical contingency. The observation that very few taxa are exclusively limited to subsurface environments argues against obligate aeonophily and suggests they may tolerate rather than require extremely low energy fluxes.

Current evidence—including lack of genetic recombination[3], minimal mutation accumulation[10], persistent mRNA[11], and active metabolism[12]—supports the notion that cell division is rare in subsurface environments. However, most methods measure community-averaged rates that cannot distinguish individual cell fates over geological timescales. Distinguishing whether subsurface abundance reflects true aeonophilic specialization, dispersal advantages, effective dormancy or historical contingency requires comparative experiments testing whether subsurface-associated taxa outperform phylogenetically diverse organisms under controlled conditions of energy limitation. Much remains to be learned about life at thermodynamic limits, and dedicated funding and exploration of subsurface environments will be essential to making progress.

Anergiobiosis: Life at the cusp of work

We propose anergiobiosis (an- = without, ergon = energy, bios = life) as a framework for understanding microbial life persisting at thermodynamic limits. Anergiobiosis describes the physiological state in which energy flux is sufficient to sustain maintenance metabolism (and stave off death) but insufficient to support net positive cell division (Fig. 1a). Thus, anergiobiosis is a state accessible to phylogenetically diverse organisms and is separate from adaptation to low energy flux. Analogous to anhydrobiosis, which describes survival under severe desiccation, not the literal absence of water[13], anergiobiosis describes survival when energy flux falls below a threshold supporting cell division, not absolute absence of metabolic activity, and does not imply optimization to low energy flux. Unlike aeonophily, this terminology directly describes what subsurface organisms experience without presupposing specialization.

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

Within the anergiobiosis framework, organisms that tolerate low energy flux and maintain viability better than others under identical conditions of extreme energy limitation could represent a specific extremophile designation. Just as microbes partition across temperature gradients (psychrophiles, mesophiles, thermophiles), they may partition along energy flux gradients based on maintenance energy optima. We propose three operational categories of energy flux specialists: paucienergophiles (low energy flux specialists, colloquially “aeonophiles”), mesoenergophiles (intermediate), and hyperenergophiles (high) (Fig. 1b). These proposed categories anchor what may be a continuum of maintenance energy optima, instead of discrete physiological classes. Whether meaningful variation exists is a testable prediction of the framework.

True paucienergophiles would possess low basal energy flux requirements, conferring competitive advantages by maintaining lower death rates and resuming cell division at lower energy delivery rates. Energy fluxes in deep subsurface environments reach as low as 1.5×10^{-20} W cell^{-1} ([15]), orders of magnitude below maintenance requirements measured in other systems. However, systematically measuring maintenance energy flux thresholds is technically challenging and largely unaccomplished. Thus, not all organisms capable of entering anergiobiosis would qualify as paucienergophiles. The designation requires demonstrably higher performance or survival under controlled energy flux limitation, not merely persistence.

We agree that low energy delivery represents the dominant selective pressure in deep subsurface environments. Importantly, hypotheses about energy flux specialization remain testable even in organisms that have been successfully cultivated and grow readily in the laboratory. The relevant evidence is whether they possess specific molecular adaptations enabling extended viability under low energy fluxes, not their ability to grow. A thermophile remains a thermophile even when growing at suboptimal temperature, and its heat-stable proteins and specialized membrane lipids are present 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[6,10] but does not yet demonstrate energy flux 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 higher survival 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 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 these ideas. 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, advanced or fluorescent microscopy approaches, identifying molecular signatures of specialized maintenance or repair machinery, and comparing survival across taxa under controlled energy limitation in chemostats or retentostats. Many of these approaches do not depend on cultivation and could leverage *in situ* single-cell techniques[14], comparative genomics, and experimental manipulations of natural communities to determine whether subsurface-associated taxa represent true extremophiles or simply persist in energy flux-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 a term that captures this biology while maintaining experimental testability and mechanistic precision. The anergiobiosis framework distinguishes the physiological state all organisms can enter from potential extremophilic specialization some may possess. We suggest microbes may partition along energy flux gradients based on maintenance flux optima, with paucienergophiles (aeonophiles) representing potential specialists at very low fluxes. This focuses research on testable hypotheses about survival mechanisms, maintenance flux minimization, and damage repair under extreme energy limitation. Whether subsurface-associated taxa harbor these adaptations remains an open question that the anergiobiosis framework provides experimental pathways to resolve.

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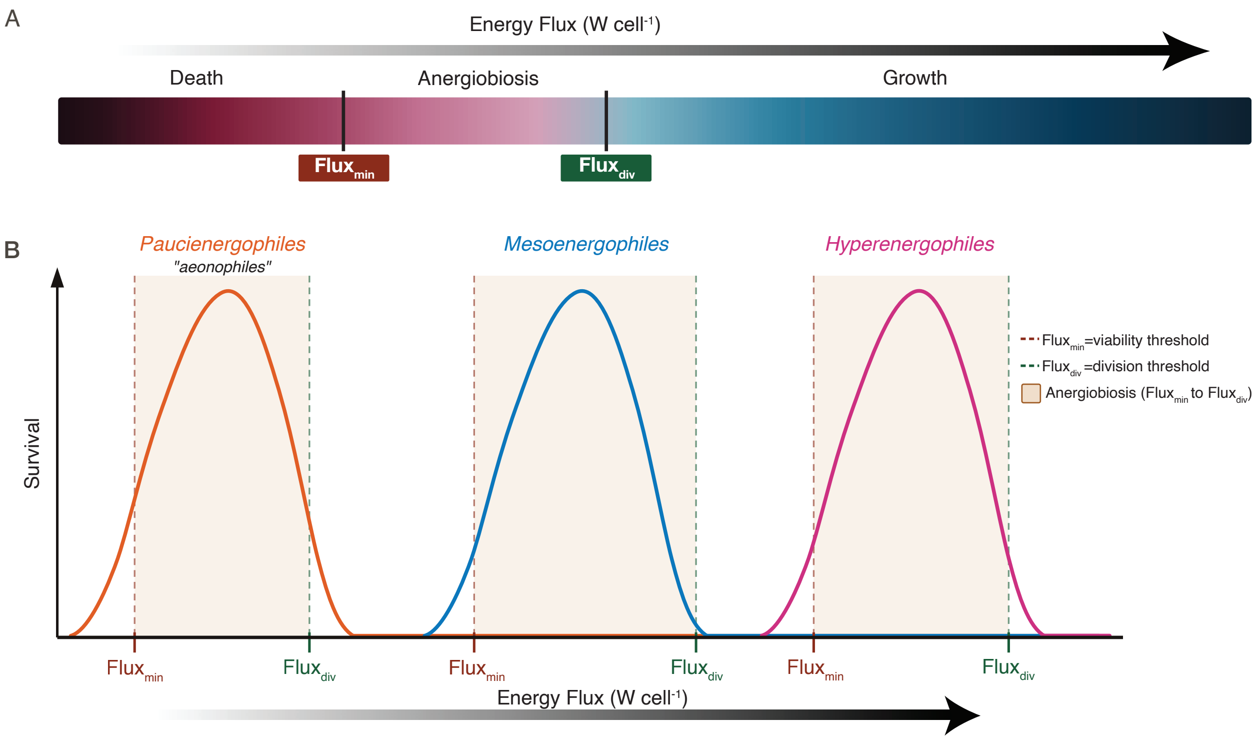
FIGURE CAPTIONS

Figure 1: Defining anergiobiosis and proposed framework for microbial specialization along energy flux gradients. (A) Energy flux (W cell^{-1}) defines three physiological regimes. Below Flux$_{min}$ (viability threshold) energy flux is insufficient to prevent cell death. Between Flux$_{min}$ and Flux$_{div}$ (division threshold), cells maintain viability, but energy flux is quantitatively consumed to support maintenance processes rather than growth. We define this region as anergiobiosis. Above Flux$_{div}$, energy flux supports net growth. (B) Within the anergiobiosis framework, microbes may partition along energy flux gradients based on maintenance energy optima, analogous to temperature specialists. Paucienergophiles ("aeonophiles"), mesoenergophiles, and hyperenergophiles are hypothetical specialist categories with peak survival at low, intermediate, and high energy fluxes, respectively. For each category, the anergiobiotic zone (Flux$_{min}$ to Flux$_{div}$) shifts along the energy flux axis, reflecting differences in basal maintenance requirements. Paucienergophiles would possess the lowest Flux$_{min}$ and Flux$_{div}$ values, conferring competitive advantages under extreme energy limitation.

FIGURE


A
Energy Flux (W cell⁻¹)
Death
Anergiobiosis
Growth
Flux min
Flux div
B
Paucienergophiles
"aeonophiles"
Mesoenergophiles
Hyperenergophiles
Survival
Flux min=viability threshold
Flux div =division threshold
Anergiobiosis (Flux min to Flux div)
Flux min
Flux div
Flux min
Flux div
Flux min
Flux div
Energy Flux (W cell⁻¹)