Check for updates

PERSPECTIVE

Microbial biospherics: The experimental study of ecosystem function and evolution

Matthias C. Rillig[a,b,1] and Janis Antonovics[c,1]

Awareness that our planet is a self-supporting biosphere with sunlight as its major source of energy for life has resulted in a long-term historical fascination with the workings of self-supporting ecological systems. However, the studies of such systems have never entered the canon of ecological or evolutionary tools and instead, have led a fringe existence connected to life support system engineering and space travel. We here introduce a framework for a renaissance in biospherics based on the study of matter-closed, energy-open ecosystems at a microbial level (microbial biospherics). Recent progress in genomics, robotics, and sensor technology makes the study of closed systems now much more tractable than in the past, and we argue that the time has come to emancipate the study of closed systems from this fringe context and bring them into a mainstream approach for studying ecosystem processes. By permitting highly replicated long-term studies, especially on predetermined and simplified systems, microbial biospheres offer the opportunity to test and develop strong hypotheses about ecosystem function and the ecological and evolutionary determinants of long-term system failure or persistence. Unlike many sciences, ecosystem ecology has never fully embraced a reductionist approach and has remained focused on the natural world in all its complexity. We argue that a reductionist approach to ecosystem ecology, using microbial biospheres, based on a combination of theory and the replicated study of much simpler self-enclosed microsystems could pay huge dividends.

matter-closed systems | biospherics | biogeochemistry | self-sustaining systems

The acid test of our understanding is not whether we can take ecosystems to bits on paper, however scientifically, but whether we can put them together in practice and make them work.

Bradshaw (1)

The study of enclosed systems has long fascinated biologists. An early example is the aquarium of Warington (2) which, in 1851, achieved an "admirable balance sustained between the animal and vegetable kingdom" and inspired Justus von Liebig to create his "World in a jar" (*Liebigsche Welt im Glase*) (3). More recent pioneers of materially closed systems were Folsome and Hanson (4), who achieved multiple-year stability in laboratory systems based on pond water organisms. Another spectacular example is the Biosphere-2 project, an experiment to create a self-sustaining environment for humans by recreating in miniature the planet's ecosystems (5). A common feature of these systems is that

they are materially closed but energy open in that light energy enters the system, but matter exchange with the surroundings is not permitted; only heat loss is allowed.

Nowadays, studies on completely closed systems are rarely done and only play a minor role in the ecological literature. Even several decades ago, Taub (6) in her review lamented that closed systems are hardly ever used in ecological experimentation. Indeed, some of the more recent examples of self-sustaining communities have been developed by biophysicists with an interest in the stochasticity of population processes (7, 8). There may be many reasons for a lack of interest in closed systems. Most of the literature on matter-closed systems has been a specialized niche field, strongly associated not with understanding ecosystem function in nature but with engineering life support systems for space travel or large projects for self-sustainability of humans in

aInstitut für Biologie, Freie Universität Berlin, D-14195 Berlin, Germany; bBerlin-Brandenburg Institute of Advanced Biodiversity Research, D-14195 Berlin, Germany; and cDepartment of Biology, University of Virginia, Charlottesville, VA 22904
[1]To whom correspondence may be addressed. Email: matthias.rillig@fu-berlin.de or ja8n@virginia.edu.

www.manaraa.com

enclosed spaces (9). While the study of life support systems is crucial to future space travel, in this article we argue that it is important to emancipate the major rationale for closed-ecosystem studies from this restrictive domain.

Within population and community ecology, there has also been a focus away from the study of local systems toward nonequilibrium ideas encapsulated in concepts, such as meta-communities and community coalescence, where there is exchange of species and matter between local habitats. The study of materially closed systems therefore seems to run counter to influential concepts in modern ecological theory. Nevertheless, many feedbacks and interactions in microbial systems are highly local. For example, in the microbial habitat of a biofilm or a soil aggregate, local metabolic products, including gases, are not readily exchanged with the surrounding matrix because of strong limitations on diffusion. An extreme signature of this is that the interiors of soil aggregates tend to be anaerobic, even though a few dozen micrometers away the soil air contains ambient amounts of oxygen (10). Within biofilms, microbial consortia also experience highly localized environments (11).

Experimental studies of materially closed systems with known and defined biotic and abiotic components have the potential to address important and fundamental questions in biology. The early closed systems were complex and undefined (e.g., using unsterilized pond water) and often poorly replicated, and their initial states and outcomes were difficult to quantify even in terms of species composition (12). In contrast to studies of completely closed systems, microcosm experiments continue to play a major role in microbial ecology and evolution, including classical designs, such as chemostats (13), Winogradsky columns (14), batch bioreactors (15), or long-term serial transfer experiments (16). It is to these designs that we envision adding completely closed systems. Such systems include a unique dimension in that light is typically the one source of energy, and only dissipative heat loss is allowed; they are completely matter closed. They therefore are crucial to understanding ecosystem processes, such as nutrient cycling, where quantitative, dynamic feedbacks for supporting life cannot be short circuited. Such closed systems are likely to be most tractable with microorganisms, and therefore, we use the collective term "microbial biospherics" for this approach, but designs could include protozoans, nematodes, or even *Drosophila* as the question may demand.

The main advantage of microbial systems is that they can be highly replicated and maintained over many years with a minimum of effort. Historically, almost all highly replicated systems investigated have been relatively small and aquatic (5, 6), but solid substrates would offer opportunities to examine aspects of spatial structure. Likely, such systems would be seeded with known strains and substrates, incubated under defined conditions, and as far as possible, instrumented with sensors and sampling ports. Sampling may violate closure of the system, but this can also be circumvented by replicating such systems and sampling them destructively at predetermined intervals. There are examples of this approach (17) but not on a scale that is now made possible by robotics to set up and seed large numbers of replicate systems (18).

Approaches involving closed ecosystems are likely to be highly diverse, because they should be question driven and can be integrated with and supported by other microcosm experiments. We envisage microbial biospherics as an area of investigation at the intersection of various established disciplines, including community ecology, experimental evolution, ecosystem ecology, and life support system engineering (Fig. 1). The study of such
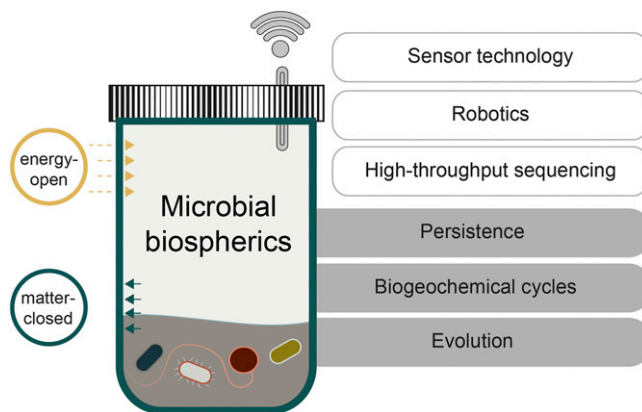
Fig. 1. A framework for "Microbial Biospherics," the study of matter-closed, energy-open microbial ecosystems, which can be viewed as drawing on improved methodologies to experimentally address a range of processes and their interaction in self-sustaining systems.

closed systems also has a place in the teaching of ecology. One simple challenge of "how would you construct a self-sustaining ecosystem?" directly introduces students to the basic principles of ecology and keeps concerns about our planet at center point.

One Species—One Ecosystem?

To highlight the potential value of studying closed systems, we initially posit the simple question of how many species would need to be in such a system for it to show a reasonable level of persistence. Could one species alone (one autotroph, for example) persist in such a system? This is what might be termed the "one species–one ecosystem" hypothesis. To our knowledge, such a simple question seems never to have been asked, even though "one-species ecosystems" have been posited to exist. Thus, Chivian et al. (19) analyzed the encoded proteins and their functions in the sulfate-reducing chemoautotrophic bacterium *Desulforudis audaxviator*. They tentatively suggested that it had "the entire biological component of a simple ecosystem within a single genome" (19), including heterotrophy and recycling of dead cells. Is a one-species ecosystem possible?

A few simple, obvious ecological considerations of a closed system with one species of autotroph illustrate conditions where this might be plausible. First, resources required for growth and reproduction need to be recyclable. Second, such resources cannot enter into an "unavailable" (completely sequestered) pool; otherwise, resources eventually decline to zero, and the system can no longer maintain life. Simple, well-known theory (20, 21) helps illustrate these issues. Consider N individuals of a species and one resource R. Let c_1 be the uptake rate of the resource and c_2 be the rate of release of the resource back from the organism to the environment via decomposition (of, say, dead individuals). Let b be the conversion rate of the resource into offspring and d be the death rate. Then, the rate of change of these 2 components is given by

$$dN/dt = bc_1 R\, N - d\, N \qquad \textbf{[1a]}$$

$$dR/dt = -c_1 R\, N + c_2 d\, N. \qquad \textbf{[1b]}$$

In such a system, for population increase, R has to be greater than $d/b\, c_1$ (i.e., a minimum initial amount of resources is necessary for the system to function). This equation, analogous to the

Rillig and Antonovics

www.manaraa.com

Lotka–Volterra predator-prey model, predicts "neutral limit cycles" such that resource and population abundance oscillate, eventually (by chance variation) leading to R below the threshold when the system dies out.

Such a system can, however, be stabilized in a number of ways. For example, if light reaching each individual is reduced as the population grows (for example, by self-shading), then modifying Eq. **1**, we get

$$dN/dt = b\, c_1 R(L - c_3 N)N - d\, N \qquad \text{[2a]}$$

$$dR/dt = -c_1 R\, N + c_2 d\, N, \qquad \text{[2b]}$$

where L is the light input and $c_3 N$ is its decline with population size. This is essentially the classic model of population growth under limited resources but with return of the resource to an available pool. Such a system also illustrates the importance of decomposer dynamics in ecosystem cycles, as now the equilibrium R and N increase with increasing rate of return, c_2, of dead material to the decomposer pool.

An assumption of these examples is that the organism has the enzymatic capacities to digest its own excretory or dead material, and while this has been suggested in the case of *Desulforudis* (19), it may be unusual given the high level of specialization in most autotrophs. However, many autotrophs have some heterotroph ability (22), and even classic microbial autotrophs, such as the cyanobacteria, have alternative metabolic activities that have been detected through functional interpretation of genomic data (23).

We give these theoretical examples not to prove that they work in nature but to show that, for even the simplest real-world ecosystem, we do not know the number and kind of species that are needed for system persistence. The equations that we present also emphasize that, even in the simplest ecosystem, determining the reasons for persistence may not be intuitive and certainly cannot usefully be explained by generalizations, such as an ability to recycle resources or biodiversity. Even if decomposers are included in the system, can resources be recycled sufficiently and completely? If they enter any kind of "unavailable" pool, even at a slow rate, the system will eventually fail (in the sense that no living organisms will persist). In some of the longest (multigenerational) closed-system experiments carried out to date (8, 24), the abundance of organisms showed a steady decline over several months. We do not know if such declines will be typical of closed systems or how they might be circumvented.

Methodological Approaches

Early studies of biospheres typically used complex, undefined communities designed to mimic natural environments, but ignorance of species composition and clear measurable parameters limited interpretation of outcomes. The most important changes that now make the study of self-enclosed systems exciting are that they have become highly feasible due to advances in high-throughput sequencing, sensor technology, and robotics.

High-throughput sequencing has opened huge doors in that we can now monitor changes in the composition of microbial communities over time using various tools that were unavailable to the early closed-system pioneers (e.g., barcoding, transcriptomics, proteomics, quantitative PCR). Such tools can be used to study changes in species composition or to confirm species composition and importantly, identify contamination. They can be used to study genetic changes within species, especially in microorganisms such as bacteria where changes can be increasingly interpreted to metabolic function and phenotype.

Advances in sensor technology, particularly optical chemical sensors, now provide nondestructive monitoring of system state, with progressive miniaturization, sensitivity, and reduction in cost (25, 26). Advances in detection are not restricted to environmental components but also include in situ biological monitoring, some of the most striking being the use of fluorescence of

genetically modified strains to detect change in the abundance of the organisms in situ (24).

Robotics can now generate multiple replicate cultures, and while we know of no application of this to closed systems, such robotic approaches now permit the study of processes in experimental microcosms on a large scale and with thousands of replicates (18). None of these approaches are simple, and they will continue to challenge our ingenuity. However, there is a generational difference between what is possible now and what was available in the days of the early biosphere pioneers.

Toward Hypothesis Testing

Persistence and System Failure. Investigating the factors that determine the persistence or failure of closed systems is intrinsically challenging to our understanding of how ecosystems function and directly impinges on the design of life support systems. The ability of robotics to create multiple self-enclosed microcosms opens up the exciting possibility of studying survivorship curves of whole ecosystems, analogous to the study of senescence and aging of individuals (Fig. 2). For example, if the ecosystem generates an unavailable pool of an essential resource, we might predict an accelerating ecosystem mortality rate (Type I survivorship curve) as nutrients reach critically low levels. In such systems, transient dynamics should show a steady decline in abundance of the components, with the higher trophic levels failing first. A convex survivorship curve with early failure and subsequent long-term persistence (Type III) would be seen if the dynamics were unstable due to initial conditions far from equilibrium or if they were subsequently stabilized by evolutionary change. Knowledge of component processes, in terms of energy, nutrients, metabolic efficiency, and population dynamics, should make it possible to predict and test the causes of such instability by varying inputs and conditions.

A challenge in studying system persistence is to operationally define "failure" and to design straightforward indicators to
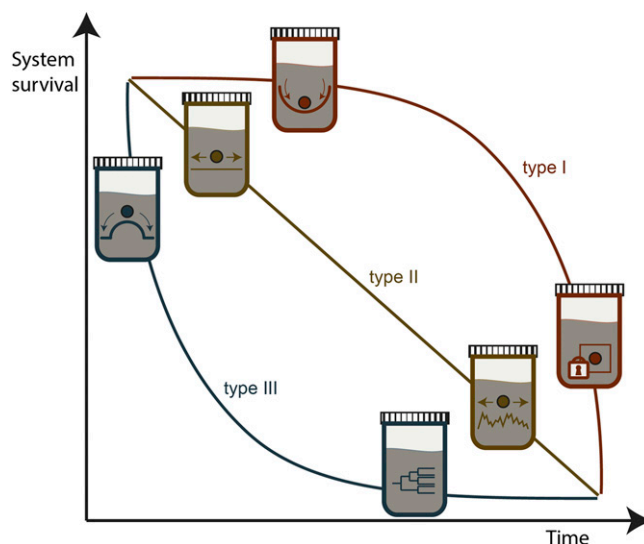
Fig. 2. System failure can be an integral aspect of microbial biospherics, with the system following survivorship curves analogous to those for individuals; estimating such survivorship curves will necessitate extensive replication at the level of the microbial biosphere. The different survivorship curves imply different mechanisms leading to system failure. Type I survivorship curve might result from locking up of nutrients in nonrecycled pools, Type II may result from a dominance of stochasticity throughout system lifetime, and Type III may indicate initial instability, which is then rescued by evolution or by stabilization of community dynamics.

Rillig and Antonovics

www.manaraa.com

measure this. Systems might unexpectedly resuscitate, for example, by recovering from resting spores, and it is difficult to distinguish living from dead microbes (27). Indicators of system failure would have to be adjusted depending on the question asked. For example, if the process to be examined is the nitrogen cycle, losing a signature of phototrophic activity could be considered system failure, perhaps evidenced by a lack of atmospheric carbon dioxide drawdown in light.

After a failure criterion has been met, the microbial biospheres could be destructively harvested to help establish the mechanisms of failure, for example, by analyzing metabolite pools and organismal abundance and activity. In ecology, we do not typically think of inherent system failure; ecologists study system-state changes (e.g., alternative stable states) in response to external drivers, but natural systems usually do not "fail" in the sense that there is complete absence of a functioning, living system. The term failure does appear in restoration ecology, but here, it only implies that some goal of the restoration effort was not reached. The study of microbial biospheres thus introduces a completely new quality of failure, with mechanisms also diverging strongly from those of open systems (Fig. 2).

It may therefore be necessary to develop theory around a "time to failure" as a central value, with system failure defined for the question at hand, and as a continuous variable rather than a binary outcome. Perhaps such a body of theory can take inspiration from failure theory in machine design or material science. For example, against this backdrop, we can ask whether, in systems with a solid phase, filamentous fungi would help nutrient cycles persist by spatially linking different process components. Do diurnal or longer-term cycles favor the persistence of nutrient flow patterns by generating time delays, or do they more likely disrupt them? With all of the knowledge that we have built up about the nitrogen cycle in natural populations and at a global scale, could we produce persistent nitrogen cycles from only known, defined organisms from the ground up?

Biogeochemical Cycles. By serving as models of biogeochemical processes in miniature, microbial biospheres can provide new avenues to answer long-standing questions in ecosystem ecology. For example, how and to what degree are process rates coconstrained by multiple processes or just one main limiting process (Liebig's Law of the Minimum)? This has been mostly studied for primary production and nutrient colimitation (28, 29), but closed systems extend such questions of constraints to system persistence.

Highly replicated experiments can provide a wide range of starting conditions (nutrient pools, organismal traits, abiotic conditions) that can dissect the nature of such constraints on persistence for a range of ecosystem processes. Such experiments could also be used to explore biogeochemical tipping points (30) and the assembly of microbial communities that lead to the initiation of nutrient cycles within a given set of conditions (31). Hypotheses that only particular mixtures can function adequately could perhaps be tested by coalescence (32) of successful and unsuccessful systems to establish system resilience in the face of invasion. Moreover, microbes are known to physiologically respond to the size and geometry of their habitat (33), and ecological dynamics unfold differently in containers of different size (34). One compelling question could therefore be if is there a minimum volume for biogeochemical cycles to persist. Is there a lower limit to the degree of spatial heterogeneity to still permit system persistence?

Just as the study of microbial symbiosis has profited from inclusion of ideas from economics (35), one might also expect that there would be synergies between the study of matter-closed systems and current interest in circular economies, a concept based on eliminating waste and keeping products and materials in use. Learning more about how cycles persist or how they fail (for example, due to incomplete recycling) could help sharpen concepts for circular economies (36).

Evolution. With large eukaryotes, it may be theoretically and experimentally convenient to separate ecological and evolutionary processes, but it is harder to do so in the microbial world where generation times are much shorter and population sizes are larger. In any closed microbial system, evolutionary processes are likely to be ongoing, even rampant, just as they are in "open" microbial microcosms. There is also a sustained interest in integrating ecosystem ideas with evolutionary biology as evidenced by selection directly on ecosystem functions (37) and by a growing interest in the impact of genetic change in populations on ecosystem functions (38) and niche construction (39). However, we know of no study of evolutionary changes in matter-closed systems, even though they have a huge advantage for long-term studies in that the effort needed to maintain them is far lower relative to serial transfer or even chemostats.

Important and intriguing questions are how evolution shapes ecosystem properties (40), whether evolutionary processes will stabilize or destabilize ecosystem processes, or more specifically, whether they maximize certain ecosystem functions (e.g., energy transfer rates, nitrogen cycling). For example, there might be an expectation that selection at the individual level might maximize rates of resource acquisition. While we know that microbes evolve in terms of nutrient uptake and excretion (41), we do not know whether evolutionary change would disrupt nutrient cycles or enhance them, and if so, what mechanisms might be involved, such as more effective use of existing resources or exploitation of new resources (42). For example, would resource partitioning be more likely to evolve in systems with more interacting species, or would alternative competitive strategies, such as resource sequestering, evolve? Would ecosystem functions evolve differently when there are different resource or energy constraints? Is evolution likely to lead to more or fewer trophic levels relative to some initial state?

One controversial theory that could to be explored using microbial biospheres is the "It's the song not the singer" idea of Doolittle and Inkpen (42). This theory posits that ecosystem processes ("songs") can be regarded as units of evolution, with the currency being differential persistence rather than reproduction. Comparing nutrient cycles with different defined components ("singers") in terms of their persistence time in closed systems would reflect these ideas.

Some genetic "control" over the evolutionary process could be achieved by inclusion of sexual vs. sexual lineages, by addition of mutagens, or by use of strains defective in genetic repair. Microbial eukaryotes, such as *Chlamydomonas* and *Tetrahymena*, have alternative mating types, and the inclusion of only one vs. both mating types can determine whether there is genetic recombination or not. Sexual reproduction can also have effects in quite different ways. It may speed the rate of evolution, and it may provide escape from extinction of small populations from Muller's ratchet or mutational "melt down" due to random fixation of deleterious mutations. In serial transfer experiment, where a finite, often very small number of individuals are transferred every few

Rillig and Antonovics

generations, sexual reproduction is critical for strain longevity by providing recombinational escape (43). Such processes may determine the persistence of ecosystem components and thus, determine functioning of microbial biospheres.

A crucial feature to harness the full potential of microbial biospheres would be to seed them with defined, genome-sequenced isolates. Sampling and strain characterization as well as high-throughput sequencing of outcomes in replicated biospheres would enable the study of parallel evolution, help distinguish random from selected substitutions, and identify genes responsible for changing ecosystem processes.

Conclusion

In suggesting that this is a timely moment for a renaissance of experimental studies of ecosystem function and evolution using microbial biospheres, we do not imply that the challenges seen by the early pioneers in this field have all been solved or that there is a singular preferred approach to such systems. Importantly, the challenges themselves generate novel questions and innovative approaches that can further studies in this rather forgotten field of ecology and evolution. The enthusiasm of early pioneers of closed-systems ecology never transferred itself to mainstream ecology in part because of the intrinsic "black-box" nature of the systems that they were investigating. We argue that a reductionist approach to ecosystem ecology and evolution based on the replicated study of much simpler self-enclosed microsystems could pay huge dividends. Such approaches, while not addressing the immediate threats to our planet, will lead to a greater understanding of system stability and persistence, nutrient and carbon recycling, and ecosystem responses to microevolutionary change. In the applied sphere, such approaches will lead to improved microbial reactors for biosynthesis and to improved strategies for engineering self-sustaining systems for space exploration or colonization of inimical habitats.

1 A. D. Bradshaw, The reconstruction of ecosystems. Presidential address to the British Ecological Society, December 1982. *J. Appl. Ecol.* **20**, 1–17 (1983).
2 R. Warington, Notice of observations on the adjustment of the relations between the animal and vegetable kingdoms, by which the vital functions of both are permanently maintained. *Q. J. Chem. Soc.* **3**, 52–54 (1851).
3 R. Sachtleben, *Liebigsche Welt im Glase: vom Kreislauf des Lebens* (Ludwigshafen am Rhein, 1958).
4 C. E. Folsome, J. A. Hanson, "The emergence of materially-closed-system ecology" in *Ecosystems Theory and Applications*, N. Polunin, Ed. (John Wiley & Sons, New York, 1986), pp. 269–288.
5 M. Nelson *et al.*, Using a closed ecological system to study Earth's biosphere: Initial results from Biosphere 2. *Bioscience* **43**, 225–236 (1993).
6 F. B. Taub, Closed ecological systems. *Annu. Rev. Ecol. Syst.* **5**, 139–160 (1974).
7 D. R. Hekstra, S. Leibler, Contingency and statistical laws in replicate microbial closed ecosystems. *Cell* **149**, 1164–1173 (2012).
8 D. R. Hekstra, S. Cocco, R. Monasson, S. Leibler, Trend and fluctuations: Analysis and design of population dynamics measurements in replicate ecosystems. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **88**, 062714 (2013).
9 I. I. Gitelson, G. M. Lisovsky, R. D. MacElroy, *Man-Made Closed Ecological Systems* (Taylor & Francis, London, UK, 2003).
10 A. J. Sexstone, N. P. Revsbech, T. B. Parkin, J. M. Tiedje, Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Sci. Soc. Am. J.* **49**, 645–651 (1985).
11 P. S. Stewart, Diffusion in biofilms. *J. Bacteriol.* **185**, 1485–1491 (2003).
12 B. Maguire, L. B. Slobodkin, H. J. Morowitz, B. Moore, D. B. Botkin, "A new paradigm for the examination of closed systems" in *Microcosms in Ecological Research*, J. P. Giesey, Ed. (CONF-781101, Technical Information Center, US Department of Energy, Springfield, VA, 1978), pp. 30–68.
13 J. Adams, F. Rosenzweig, Experimental microbial evolution: History and conceptual underpinnings. *Genomics* **104**, 393–398 (2014).
14 D. J. Esteban, B. Hysa, C. Bartow-McKenney, Temporal and spatial distribution of the microbial community of Winogradsky columns. *PLoS One* **10**, e0134588 (2015).
15 P. Sierocinski *et al.*, A single community dominates structure and function of a mixture of multiple methanogenic communities. *Curr. Biol.* **27**, 3390–3395.e4 (2017).
16 Z. D. Blount, C. Z. Borland, R. E. Lenski, Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7899–7906 (2008).
17 D. H. Wright, C. E. Folsome, D. Obenhuber, Competition and efficiency in closed freshwater algal systems: Tests of ecosystem design principles. *Biosystems* **17**, 233–239 (1985).
18 E. Tekin *et al.*, Prevalence and patterns of higher-order drug interactions in *Escherichia coli*. *NPJ Syst. Biol. Appl.* **4**, 31 (2018).
19 D. Chivian *et al.*, Environmental genomics reveals a single-species ecosystem deep within Earth. *Science* **322**, 275–278 (2008).
20 R. M. May, *Stability and Complexity in Model Ecosystems* (Princeton University Press, Princeton, NJ, 1973).
21 R. A. Parker, Nutrient cycling in closed ecosystem models. *Ecol. Modell.* **4**, 67–70 (1978).
22 S. C. Rittenberg, The obligate autotroph–The demise of a concept. *Antonie van Leeuwenhoek* **38**, 457–478 (1972).
23 M. Monchamp, P. Spaak, F. Pomati, Long term diversity and distribution of non-photosyntheitc cyanobacteria in peri-alpine lakes. *Front Microbiol.* **9**, 3344 (2019).
24 Z. Frentz, S. Kuehn, S. Leibler, Strongly deterministic population dynamics in closed microbial communities. *Phys. Rev. X* **5**, 041014 (2015).
25 P. Gruber, M. P. C. Marques, N. Szita, T. Mayr, Integration and application of optical chemical sensors in microbioreactors. *Lab Chip* **17**, 2693–2712 (2017).
26 P. O'Mara, A. Farrell, J. Bones, K. Twomey, Staying alive! Sensors used for monitoring cell health in bioreactors. *Talanta* **176**, 130–139 (2018).
27 J. B. Emerson *et al.*, Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. *Microbiome* **5**, 86 (2017).
28 J. J. Elser *et al.*, Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* **10**, 1135–1142 (2007).
29 W. S. Harpole *et al.*, Nutrient co-limitation of primary producer communities. *Ecol. Lett.* **14**, 852–862 (2011).
30 T. Bush, I. B. Butler, A. Free, R. J. Allen, Redox regime shifts in microbially mediated biogeochemical cycles. *Biogeosciences* **12**, 3713–3724 (2015).
31 E. Pagaling *et al.*, Assembly of microbial communities in replicate nutrient-cycling model ecosystems follows divergent trajectories, leading to alternate stable states. *Environ. Microbiol.* **19**, 3374–3386 (2017).
32 M. C. Rillig *et al.*, Interchange of entire communities: Microbial community coalescence. *Trends Ecol. Evol. (Amst.)* **30**, 470–476 (2015).
33 L. J. Barkal *et al.*, Microbial metabolomics in open microscale platforms. *Nat. Commun.* **7**, 10610 (2016).
34 T. Fukami Assembly history interacts with ecosystem size to influence species diversity. *Ecology* **85**, 3232–3242 (2004).
35 R. Noë, E. T. Kiers, Mycorrhizal markets, firms, and co-ops. *Trends Ecol. Evol. (Amst.)* **33**, 777–789 (2018).
36 J. Korhonen, A. Honkasalo, J. Seppala, Circular economy: The concept and its limitations. *Ecol. Econ.* **143**, 37–46 (2018).
37 W. Swenson, D. S. Wilson, R. Elias, Artificial ecosystem selection. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9110–9114 (2000).

www.manaraa.com

38 R. D. Bassar *et al.*, Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3616–3621 (2010).

39 J. Odling-Smee, D. H. Erwin, E. P. Palkovacs, M. W. Feldman, K. N. Laland, Niche construction theory: A practical guide for ecologists. *Q. Rev. Biol.* **88**, 4–28 (2013).

40 S. A. Levin, Ecosystems and the biosphere as complex adaptive systems. *Ecosystems (N. Y.)* **1**, 431–436 (1998).

41 H. T. Williams, T. M. Lenton, Artificial selection of simulated microbial ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8918–8923 (2007).

42 W. F. Doolittle, S. A. Inkpen, Processes and patterns of interaction as units of selection: An introduction to ITSNTS thinking. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 4006–4014 (2018).

43 G. Bell, *Sex and Death in Protozoa. The History of an Obsession* (Cambridge University Press, New York, 1989).

www.manaraa.com