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Author for correspondence:
Jennifer L. Macalady
e-mail: jlm80@psu.edu

†Present address: Department of Earth Sciences, University of Minnesota, Minneapolis, MN 55455, USA.

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Energy, ecology and the distribution of microbial life

Jennifer L. Macalady[1,2,3], Trinity L. Hamilton[1,2], Christen L. Grettenberger[1,3], Daniel S. Jones[1,2,†], Leah E. Tsao[1,2] and William D. Burgos[4]

[1]Department of Geosciences, Penn State University, University Park, PA 16802, USA
[2]Penn State Astrobiology Research Center (PSARC), Penn State University, University Park, PA 16802, USA
[3]Ecology Program, Penn State University, University Park PA 16802, USA
[4]Department of Civil and Environmental Engineering, Pennsylvania State University, University Park, PA 16802, USA

Mechanisms that govern the coexistence of multiple biological species have been studied intensively by ecologists since the turn of the nineteenth century. Microbial ecologists in the meantime have faced many fundamental challenges, such as the lack of an ecologically coherent species definition, lack of adequate methods for evaluating population sizes and community composition in nature, and enormous taxonomic and functional diversity. The accessibility of powerful, culture-independent molecular microbiology methods offers an opportunity to close the gap between microbial science and the main stream of ecological theory, with the promise of new insights and tools needed to meet the grand challenges humans face as planetary engineers and galactic explorers. We focus specifically on resources related to energy metabolism because of their direct links to elemental cycling in the Earth's history, engineering applications and astrobiology. To what extent does the availability of energy resources structure microbial communities in nature? Our recent work on sulfur- and iron-oxidizing autotrophs suggests that apparently subtle variations in the concentration ratios of external electron donors and acceptors select for different microbial populations. We show that quantitative knowledge of microbial energy niches (population-specific patterns of energy resource use) can be used to predict variations in the abundance of specific taxa in microbial communities. Furthermore, we propose that resource ratio theory applied to micro-organisms will provide a useful framework for identifying how environmental communities are organized in space and time.

1. Introduction

For all or most of the past 4 Gyr, life on Earth has depended on the activity of single-celled micro-organisms that harvest chemical (redox) and light energy from the environment. The evolution of diverse and complex biochemical mechanisms to accomplish this, combined with the revolutionary chemical transformation of the Earth's surface environment and the inexorable trend towards higher levels of biological diversity make for one of the most compelling stories in science. However, many missing pages remain. Among the mysteries are the mechanisms that allow and maintain the enormous taxonomic complexity of contemporary microbial communities. This question is far from academic. Astrobiologists would like to know, for example, whether Earth-like life on other habitable planets would show a similar march towards complexity and diversity, and whether or not this march depends on the types and amounts of energy available for life to harvest. On an even more practical level, environmental engineers use knowledge of relationships between microbial taxa and resource availability to design systems to purify wastewater, produce fuels and feedstocks, harvest ores and remediate polluted environments. Genetic engineers, not to mention politicians and the general public, would like to know under what conditions a genetically engineered microbial strain is competitive or invasive in nature, and why. Last but not least, revenues

from genetically modified 'stuff' including drugs, agriculture, enzymes and plastics exceed 1 per cent of US $ GDP, and as of 2009 were growing at 10–20% per year [1]. Given that most of the genetic and metabolic diversity on Earth lies within the Bacterial and Archaeal domains of life (prokaryotes), microbial machines and their genetic templates represent a rich storehouse of 'bioengineered' solutions optimized over billions of years of evolution.

Our goal in this contribution is to explore the role of environmental selection, specifically selection based on the availability of energy resources, in controlling the distribution of microbial life.

2. Current knowledge of microbial taxonomic and metabolic diversity

Mechanisms that govern the coexistence of multiple biological species have been studied intensively by ecologists since the turn of the nineteenth century. Ecologists have long studied the mechanisms that underpin the biodiversity of plants and animals. By contrast, only since the late twentieth century have microbiologists possessed the tools to adequately determine microbial population sizes and community composition in nature. The methodological limitations faced by microbial ecologists have been further compounded by the lack of an ecologically coherent species definition, the vast taxonomic and functional breadth of micro-organisms as well as their limited morphological diversity. These challenges led to a gap between microbiology and the main stream of ecological theory. The gap is now gradually narrowing due to the accessibility of powerful culture-independent molecular methods. The same methods have sustained an intense and still ongoing period of discovery of new microbial taxonomic diversity (figure 1). Despite the growing potential of environmental genetics and genomics approaches, the number of sequences for which no coherent physiological or metabolic information is available is large and growing ever larger with respect to cultivated micro-organisms. Madsen [3] estimate that the number of uncultured species may be 10 000 times that of the cultured minority. Among prokaryotic phyla (having small subunit (SSU) 16S rRNA sequence divergence equivalent to eukaryotic 'kingdoms' such as fungi, animals, plants, etc.) between one-third and one-half have no cultivated representatives [2,4,5]. These recent statistics offer a staggering vista of the Earth's extant microbial diversity and highlight our relative ignorance about metabolic traits across vast swathes of microbial taxonomic diversity.

Although the array of chemical and physical environments colonized by microbial life on Earth is remarkable, the number of microbial taxa already discovered far outnumber the variety of possible energy-yielding chemical and light-harvesting metabolisms that can be identified based on thermodynamic considerations [6–8]. Thus, future findings that taxa in the uncultivated majority perform existing (known) metabolisms will not be entirely surprising. Many such discoveries are to be expected based on the scarcity of the so-called missing metabolisms versus the vast number of uncultivated microbial taxa. These future discoveries will nonetheless facilitate advances in bioengineering and biotechnology, environmental and agricultural sciences, geomicrobiology and biogeochemistry as well as astrobiology.

Recent research on ammonia oxidation (figure 2) provides an example of how new links between energy metabolisms

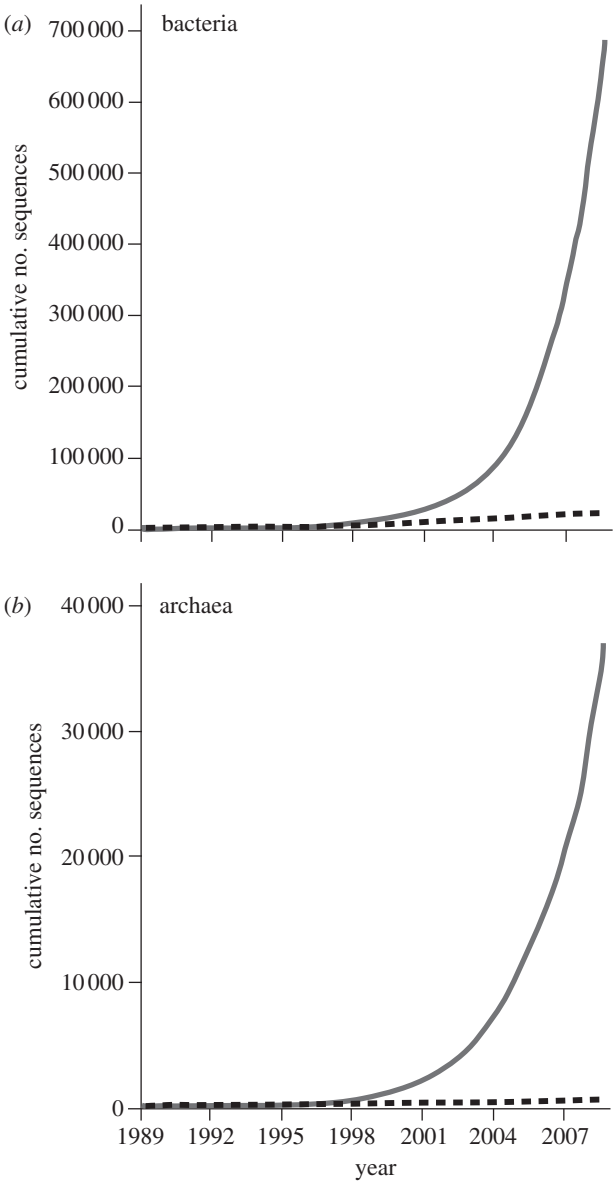

Figure 1. (*a*,*b*) Accumulation of biological sequence data in public databases originating from cultures versus environmental samples. Modified with permission from Pace [2]. Solid grey line denotes environmental; dashed line denotes cultured.

and microbial taxa spur advances in geobiology [10]. Although the fossil record of microbial life is considered poor, 'biosignatures' of past microbial life have been detected in rocks almost 4 Gyr old [10–13]. In addition to fossil cells and microbial biofilms (stromatolites), microbial biosignatures include hydrocarbons derived from cell membrane lipids, isotopic ratios of biologically processed elements such as C and N, and minerals produced as a result of biological activity or under the influence of microbial cells. Given sufficient data from the analysis of rocks, microbial physiology and nucleic acid as well as protein phylogenies, geobiologists have a chance to reconstruct the timing of the evolution of biogeochemical processes such as ammonia oxidation, including implications for the evolution of past Earth's environments. In practice, the scarcity and poor preservation of Archaean (more than 2.5 Ga) and Proterozoic (0.5–2.5 Ga) rocks may place limits on this exercise. Nonetheless, it is clear that an understanding of links between extant microbial diversity and the cycling of redox-active elements is

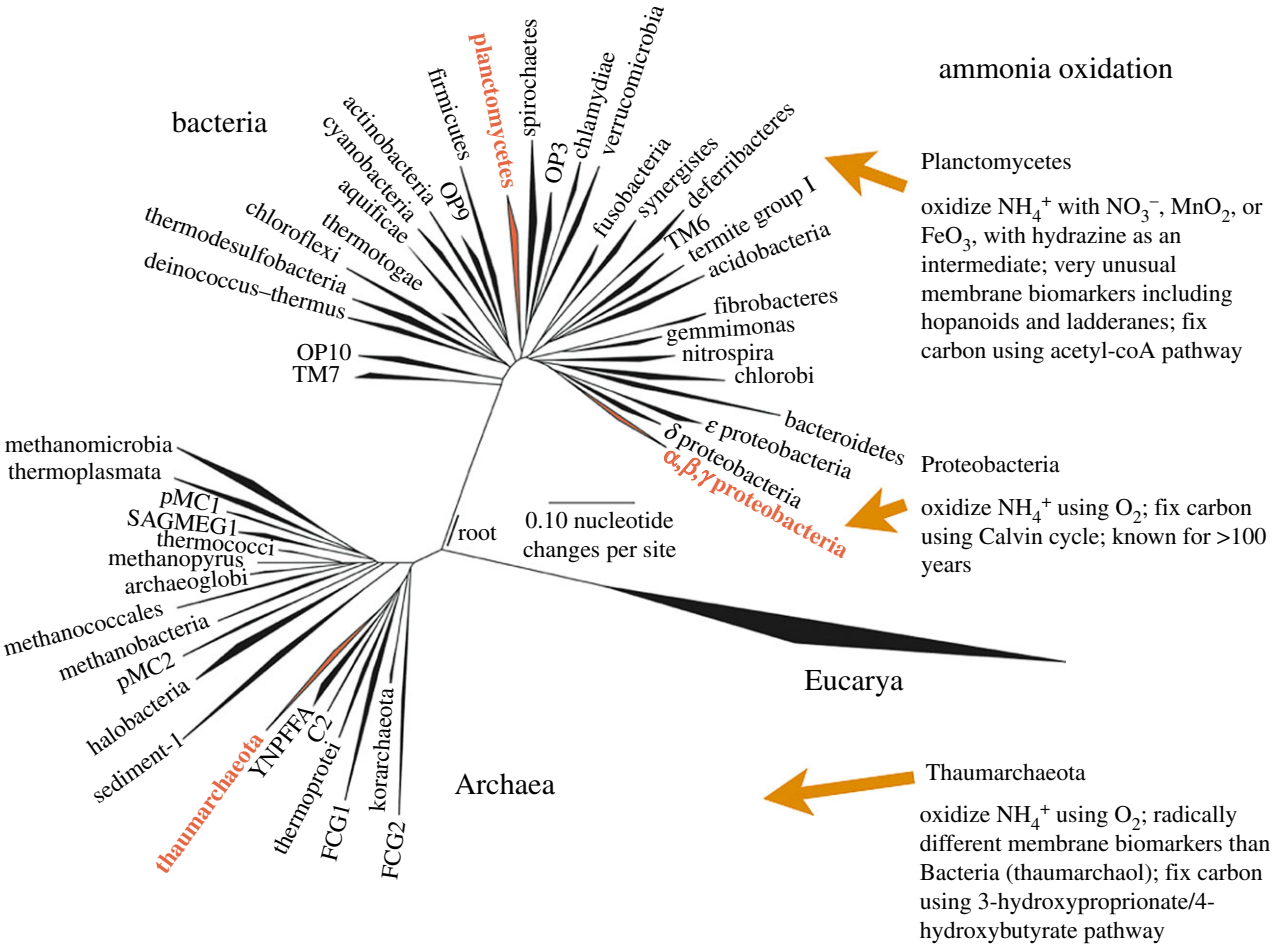

Figure 2. 16S rRNA gene phylogenetic tree illustrating lineages that contain canonical and recently discovered ammonia oxidizing prokaryotes. Highly divergent biosignatures associated with each group are shown, including organic biomarkers and carbon fixation pathways resulting in organic matter with different C isotopic ratios. Although the relative dominance of ammonia oxidizers from these lineages in the environment is still under investigation, one of the principal factors controlling the distribution of Thaumarchaeota versus Proteobacteria appears to be ammonium (electron donor) concentrations [9]. The relative abundance of Planctomycetes that carry out anaerobic ammonia oxidation, or 'annamox', is strongly governed by the availability of oxygen (electron acceptor). C2, FCG1, FCG2, OP3, OP9, OP10, pMC1, pMC2, SAGMEG1, sediment-1, TM6, TM7, termite group I, and YNPFFA represent candidate divisions with few or no cultured representatives [2,4,5]. (Online version in colour.)

essential to reconstructing Earth's history, and at present far from complete.

A more quantitative understanding of microbial distributions also has clear relevance for biotechnology and environmental engineering. Two examples related to iron oxidation serve to illustrate why. First, iron-oxidizing extreme acidophiles are used in metal extraction from low-grade ores (biomining), and have been heralded in biotechnology circles as the future of metal mining owing to the growing scarcity of high-grade ores that can be processed using conventional methods [14,15]. Members of the genera *Acidithiobacillus* and *Leptospirillum* (figure 3) are distantly related iron-oxidizing autotrophs with similar energy metabolisms but different evolutionary origins and physiological characteristics. Although *Acidithiobacillus* strains have served as model acidophiles for decades, *Leptospirillum* species have been studied only more recently. Genomic and genetic approaches including environmental omics [17–21] have begun to identify relationships between environmental parameters and *Acidithiobacillus* and *Leptospirillum* population distributions in natural and engineered systems [16,22]. These investigations are a first step toward engineering more efficient biomining operations.

Second, iron sulfides that are a major component of many metal ores are present in lower concentrations in coal deposits. They are also the source of persistent and globally significant environmental degradation in the form of acid mine drainage (AMD). The northern Appalachian Plateau of the eastern United States contains more than 8000 km of streams affected by acidic and heavy-metal laden drainage from abandoned coal mines (figure 4; [23]). Cost-effective treatment technologies for the remediation of these geographically dispersed and often remote sites are desperately needed. Conventional passive limestone treatment beds neutralize acidic water but promote the precipitation of Fe(III) oxides that coat the limestone surfaces ('armouring') and hydraulically clog the bed, requiring costly and frequent interventions to maintain treatment efficiency. A promising solution to this dilemma is to promote biological Fe oxidation at low pH, so that Fe(III) minerals precipitate before AMD reaches the limestone bed. At the relevant pH values (1.5–4.5), abiotic iron oxidation is kinetically inhibited and catalysed by acidophilic Fe-oxidizing bacteria [24–27].

Naturally occurring and engineered low-pH iron oxidation sites display a wide variety of physical, chemical and microbiological characteristics and have variable iron-removal efficiencies. For example, Heinzel *et al.* [28] found that specific iron oxidation rates in AMD treatment systems varied with the dominant microbial population, and Brown *et al.* [29] found that AMD sediment-hosted microbial communities

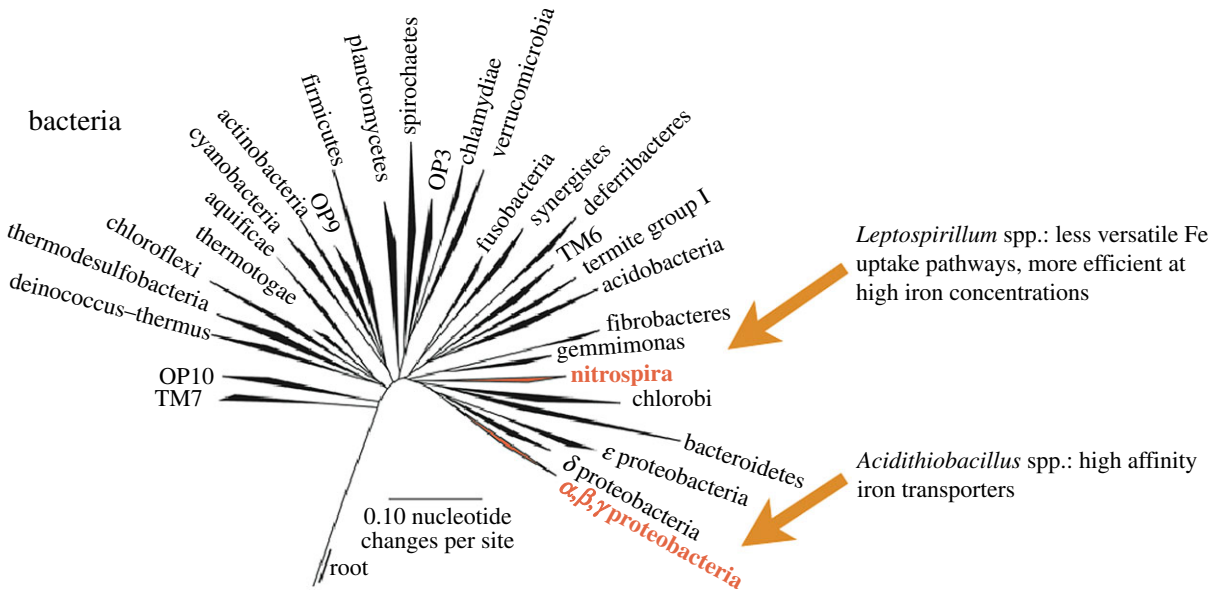

Figure 3. 16S rRNA gene phylogenetic tree illustrating two lineages that contain extremely acidophilic iron-oxidizing lithoautotrophs. Niches and ecological successions between biomining and bioleaching micro-organisms can be at least partially explained by changes in iron availability [16]. OP3, OP9, OP10, TM6, TM7, and termite group I represent candidate divisions with few or no cultured representatives [2,4,5]. (Online version in colour.)

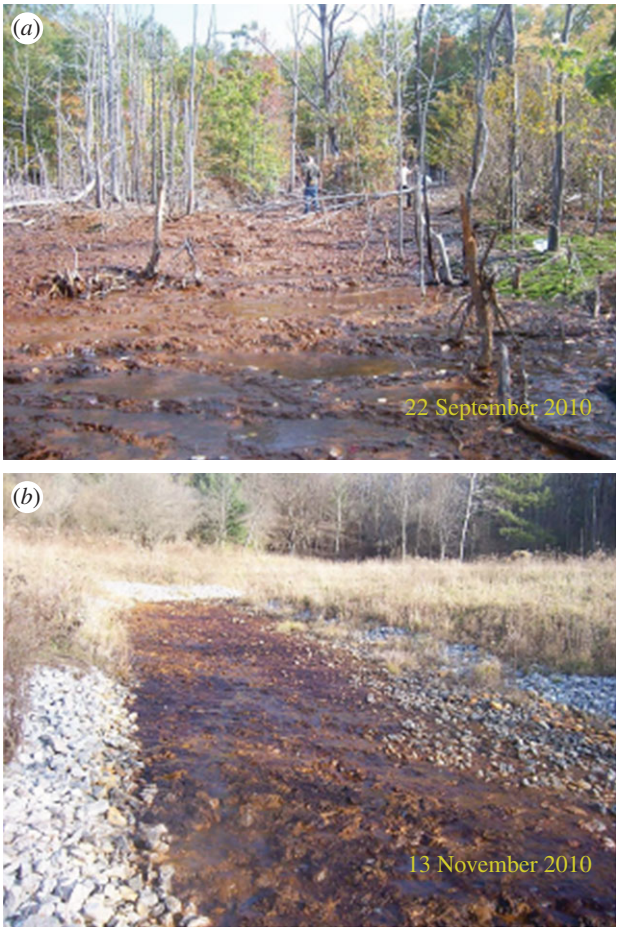

Figure 4. A natural iron oxide mound at Brubaker Run, Cambria County, PA (*a*), and an engineered 'oxidation/precipitation channel' at Dents Run, Elk County, PA (*b*). In both systems, the accumulation of Fe(III) minerals is due to low-pH Fe(II) oxidation by micro-organisms. (Online version in colour.)

dominated by *Ferrovum* spp. exhibited iron oxidation rates roughly twice that of sediments dominated by *Acidithiobacillus* spp. located nearby. Identifying the parameters that control the distribution of organisms with similar but ecologically differentiated patterns of resource use (in this case iron) is the first step in engineering AMD treatment systems that select for the desired microbial populations. These data underscore the need for both phylogenetic and ecological information to understand environmental controls on microbial distribution, especially among phylogenetically diverse and physiologically distinct coexisting populations that use the same core energy metabolic reactions.

3. Microbial distributions in space and time

Predicting the distribution of microbial life in space and time is a fundamental goal of microbial ecology. This endeavour, called microbial biogeography, is informed by a long tradition of research on macroscopic organisms in animal and plant communities [30–33], and more recently by a stimulating decade of biogeographic research specifically targeting microbial communities. According to current theory, a variety of contemporary and historical processes contribute to observed patterns in the spatial and temporal distributions of microbial populations. These processes include mutation, selection, dispersal and drift, and are expertly reviewed from a microbiology point of view by Martiny *et al.* [34] and Hanson *et al.* [35]. Historical processes include lingering effects of past environmental selection, distinct local histories of colonization and speciation, and dispersal barriers that restrict the flow of propagules to otherwise suitable habitats. To what degree does contemporary environmental selection drive distribution of contemporary microbial life? Although it is abundantly clear that historical processes are relevant for micro-organisms as well as macro-organisms [36,37], environmental selection appears to be more important over small spatial scales, and possibly overall [35,37,38]. Nonetheless, we stress that environmental selection is one of several independently operating contemporary and historical processes that potentially contribute to observed microbial biogeographic patterns.

The relationship between a species or population and contemporary environmental conditions is embodied by the concept of an ecological niche. A niche is defined as

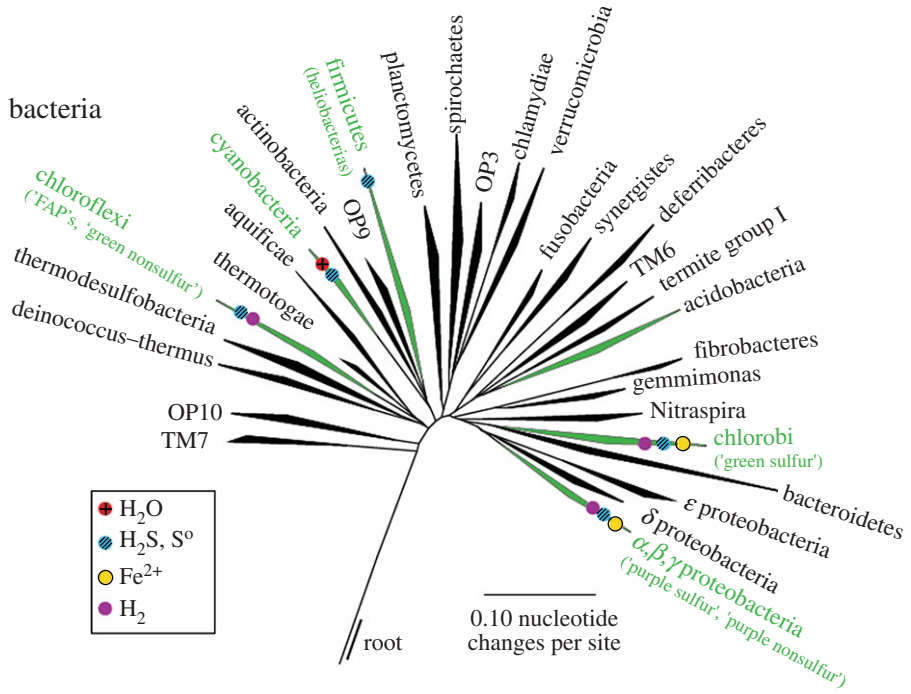

Figure 5. 16S rRNA gene phylogenetic tree illustrating lineages with representatives that harvest light energy using chlorophylls, i.e. chlorophototrophs, in green (not all members of these lineages are phototrophs). Reported photosynthetic electron donors are indicated by coloured dots. Pigment absorption spectra and carbon and nitrogen metabolism vary widely among phototrophs [46,47], indicating differential patterns of energy resource utilization at a variety of taxonomic scales. Notwithstanding significant evidence for lateral gene transfer among phototrophic lineages [48,49], some patterns of resource use are conserved within lineages that likely diverged billions of years ago [50–52]. OP3, OP9, OP10, TM6, TM7 and termite group I represent candidate divisions with few or no cultured representatives [2,4,5]. (Online version in colour.)

'the limits, for all important environmental features, within which individuals of a species can survive, grow and reproduce' [39, p. 31]. There is a notable tendency for coexisting taxa in communities to have different niche requirements, a phenomenon which has been termed variably 'niche separation', 'niche differentiation', 'niche segregation' or 'niche partitioning'. Niche separation is one of the processes commonly invoked to explain the enormous complexity of natural microbial communities, and yet is rarely explored in studies of micro-organisms in nature. It is useful to distinguish between theoretical niches, which conform to the definition above, and 'realized' niches, which correspond to the niche space occupied by a population in a specific environmental context. Inasmuch as environmental features may be said to include predators, phages and competing microbial populations, these definitions largely converge, but may lead to the observation that members of a given taxon occupy slightly different regions of their theoretical niche space in the environment depending on variations in community assembly and the taxonomic resolution of the methods used to gather data on microbial distributions.

The separation of microbial taxa into different niches is assumed to be a result of natural selection that drives competing populations into different patterns of resource use, such that they can coexist. This process has been observed in laboratory experiments [40], as well as in the environment [41,42]. Because relatively minor genetic changes are required to confer new phenotypes [43], small evolutionary steps are ecologically significant but not detectable using methods commonly used in environmental microbiology such as small subunit (SSU) rRNA gene cloning, pyrotags and fluorescence *in situ* hybridization (FISH). Enormous ecological complexity clearly exists within taxa having more than 97 per cent 16S rRNA gene similarity, the most common sequence similarity cut-off for microbial 'species', as well as among populations sharing identical 16S rRNA gene sequences [42,44,45]. The occurrence of ecologically distinct populations within taxa sharing high levels of 16S rRNA gene similarity appears to be the rule rather than the exception. Contemporary environmental selection-driving niche separation among closely related taxa (within-'species') is thus likely to be significant, and requires sensitive genetic and/or genomic methods to resolve.

However, niche separation observed in extant microbial communities is not exclusively due to adaptation and selection on closely related populations after communities are assembled. Environmental selection for differential resource use in the evolutionary past has led in some cases to stable and separate patterns of resource use in descendants of the original populations. Broad taxonomic patterns in energy-harvesting metabolisms are familiar and robust, including sulfate reduction in the *Deltaproteobacteria*, methanogenesis in Archaea and chlorophyll-based phototrophy (shown in figure 5), among others. Species with complementary patterns of energy resource use can thus be recruited from the environment, with the result that populations sharing similar energy resources in a given habitat may be distantly related to each other taxonomically. However, these qualitative patterns are not sufficient to predict the environmental distributions of genera, species, or populations.

4. Quantitative understanding of microbial niches

Given the inherent taxonomic and environmental complexity of natural microbial communities, quantitative frameworks to

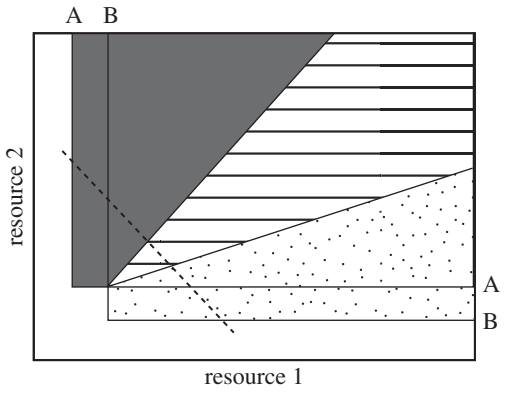

Figure 6. Illustration showing the basic features of resource ratio theory most relevant for autotrophs (after [54,56]). The field represents a range of habitats described by variations in the supply rates of two essential limiting resources (resource 1 and resource 2). Two populations (A and B) are shown that may be selectively enriched as a result of resource availability. Lines A–A and B–B represent zero net growth isoclines (ZNGI) for populations A and B. ZNGIs represent combinations of resources where growth rate is equal to death rate for each population. Below and to the left, population growth is negative. Population sizes are constant on the ZNGI. In the white area, no growth is observed for either population. In the grey area (high ratio of resource 2: resource 1), population A dominates, while in the stippled area (low ratio of resource 2: resource 1), population B is dominant. Populations A and B coexist in the striped area. The dashed line indicates the change in community structure as a function of changes in resource ratios [54,56].

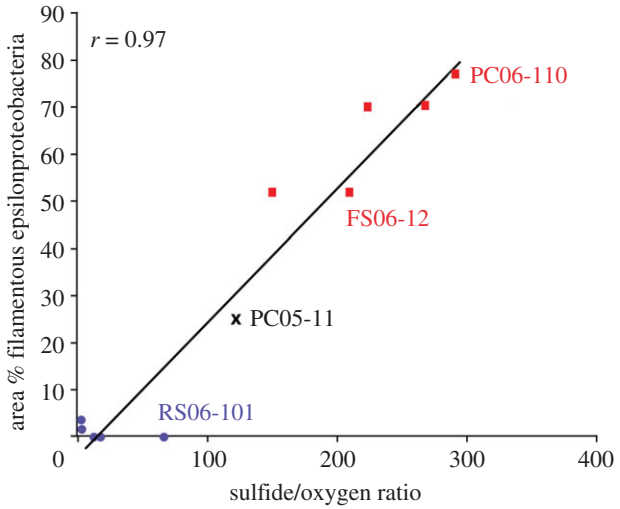

Figure 7. Correlation between dissolved sulfide : oxygen ratios and the abundance of cells hybridizing to a FISH (fluorescence *in situ* hybridization) probe targeting *Sulfurovumales* (filamentous Epsilonproteobacteria) in 12 biofilms from sulfidic cave groundwaters. Squares indicate biofilms with >50% *Sulfurovumales* biomass, whereas circles indicate biofilms dominated by *Thiothrix* populations. The black x (PC05-11) represents a biofilm comprised primarily of as-yet unidentified micro-organisms (not *Thiothrix* or *Sulfurovumales*), which likely have a distinct intermediate energy niche. Modified from Macalady *et al.* [59]. (Online version in colour.)

investigate patterns of microbial diversity and energy resource use are sorely needed. Resource ratio theory (RRT) was elaborated by Tilman [53,54] based on earlier work by MacArthur [55] as an alternative to non-mechanistic models of competition between species. The model is designed to explore patterns of species dominance or coexistence that emerge from competition between species sharing multiple resources (figure 6). Resources in the model have defined supply rates, and increases in their availabilities increase the growth rates of the populations that consume them. A central assumption of the theory is that there are unavoidable trade-offs in the ways that populations meet the challenge of acquiring resources, and therefore that no population can be a superior competitor for all the shared resources.

In the years since RRT was proposed, it has been largely successful at predicting autotrophic (mainly plant and algal) species' dominance based on ratios of limiting resource availabilities [57]. Two decades ago, an excellent review of the applicability of RRT to microbial ecology [58] concluded that the predictions of RRT are consistent with a large body of microbial competition data, including both autotrophs and heterotrophs. Despite these encouraging prospects, the theory has not been significantly explored by other microbiologists, with very few exceptions [56].

Our recent work on sulfide-oxidizing autotrophs in groundwater biofilms is consistent with a central prediction of RRT, namely that population dominance varies with the ratio of resources' availabilities (figure 7). Our data showed that populations belonging to the *Sulfurovumales* clade (*Epsilonproteobacteria*) always dominated in groundwaters with sulfide : oxygen ratios greater than 150, whereas *Thiothrix* populations dominated in groundwaters with sulfide : oxygen ratios less than 100 (figure 7; [59]). Based on this pattern, we successfully predicted the dominance of *Sulfurovumales* populations in high sulfide : oxygen biofilms collected at much higher temperatures (45 °C compared with 13 °C) approximately 100 km distant [60]. Data from the existing literature did not allow further tests of the pattern, because previous studies did not incorporate both sulfide and oxygen concentration data and quantitative measures of population sizes. Phylogenetic analyses of the *Sulfurovumales* populations from our sample sites showed that the populations were distantly related within the clade, suggesting the adaptation to high sulfide : oxygen ratios in these organisms occurred in the distant evolutionary past (figure 8).

Similar work on iron-oxidizing autotrophs in AMD suggests that RRT will prove to be a useful starting point for further investigations into patterns of microbial energy resource use and other ecological niche dimensions. We examined the composition of sediment microbial communities ($n = 61$) from five AMD sites using FISH. At most sites, communities were dominated by either *Acidithiobacillus*, acidophilic *Gallionella*-like, or *Ferrovum* populations. The taxonomy of the dominant populations appears to be largely predictable based on pH and ferrous iron concentrations (figure 9). Like the *Sulfurovumales* populations mentioned earlier, *Gallionella*-like and *Ferrovum* populations at these AMD sites are uncultivated. A greater understanding of their ecology may explain why some naturally occurring AMD sites are efficient at low-pH iron removal while others are not. Future bioengineering systems might manipulate pH, iron concentrations or other factors related to niche separation among these populations to increase the rate of iron removal and treatment efficiency and decrease the cost of AMD treatment. While pH is not a resource and thus this niche model does not fit easily into RRT, pH controls many other chemical variables in AMD systems (thus a proxy for an undiscovered resource dimension), and may be a direct energy tax [62,63]. Future research will be aimed at unravelling these aspects of the model.

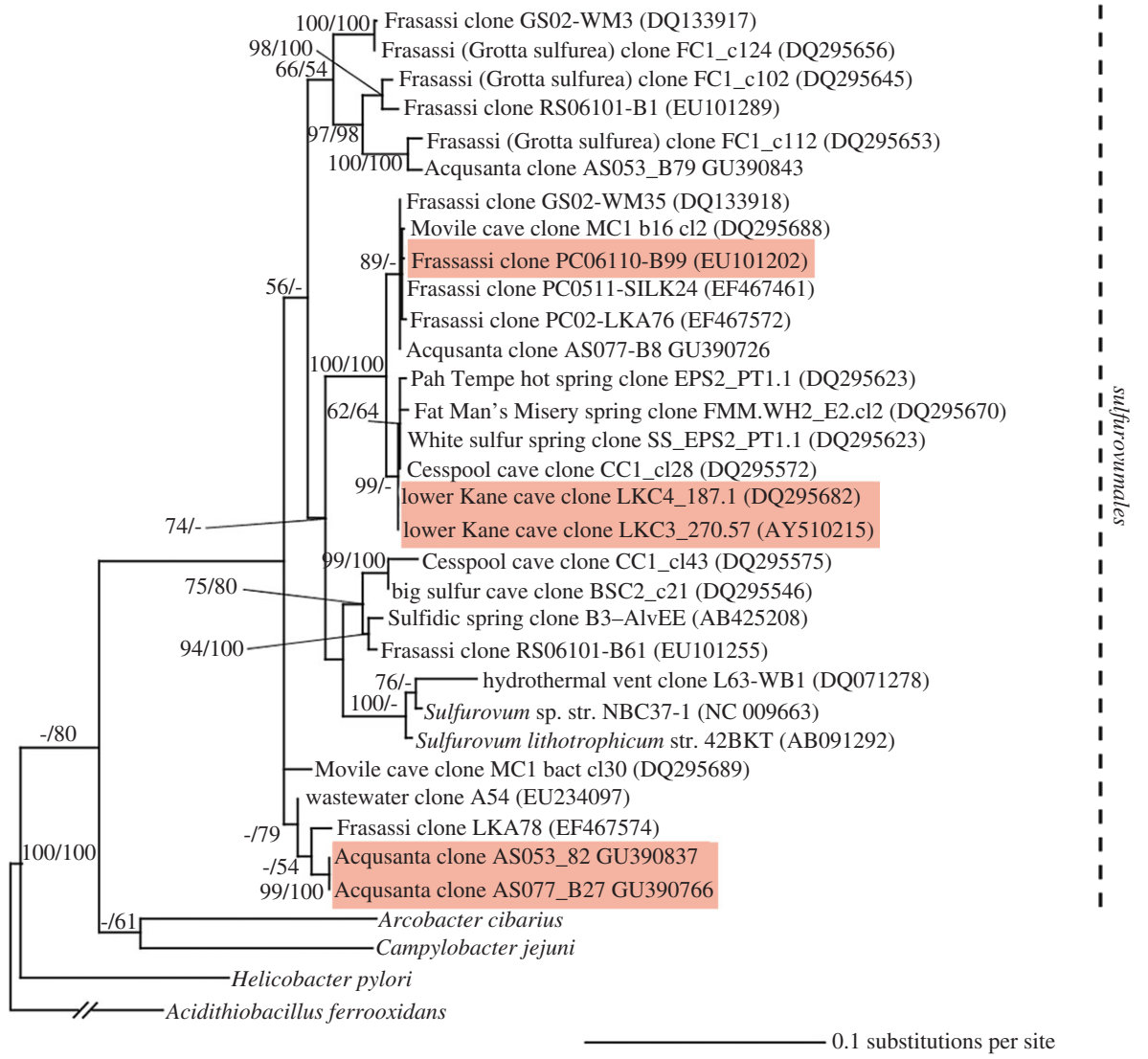

Figure 8. Maximum-likelihood phylogram of 16S rRNA sequences showing relationships among *Sulfurovumales* (*Epsilonproteobacteria*) clones retrieved from geographically distant sulfidic groundwaters in Italy and USA. Populations highlighted in red are known to be the dominant populations in waters with sulfide : oxygen ratios more than 150. Neighbour joining (left) and maximum parsimony (right) bootstrap values greater than 50 are shown for each node. Reprinted from Jones *et al*. [60], with permission. (Online version in colour.)

Although microbiologists rarely use RRT, aspects of the theory are inherent to microbial ecology and recent studies do ask whether coexisting populations have different niches with respect to energy resources. For example, Rocap *et al*. [64] found that *Prochlorococcus* genotypes have different optimum light intensities. Similarly, *Synechococcus* A/B types at Yellowstone hot springs are differently adapted to light quality and quantity [65]. Hatzenpichler [66] reviews literature suggesting that competition between bacterial and archaeal ammonia oxidizers is controlled by ammonium : oxygen ratios. Closely related sulfur-oxidizing autotroph populations within the genus *Achromatium* occupy different depth-defined sediment redox zones [67], which although not reported, would be expected to correlate with sulfide : oxygen ratios, iron availability and/or organic acid concentrations that would be expected to be essential resources for *Achromatium* growth. Based on these reports and our own research, we suggest that resources related to energy metabolism are a good place to begin mapping microbial niches, and that RRT should be explored in future studies.

We note in closing that other resources or even niche dimensions that may be difficult to assume within the framework of RRT are clearly also important for environmental selection. In our studies of sulfur-oxidizing autotrophs in sulfidic groundwater, *Beggiatoa* populations inhabited waters with a variety of sulfide : oxygen ratios (figure 7), but only in microenvironments with significantly slower water flow than *Thiothrix* and *Sulfurovumales* populations [59]. Fluid dynamical niche dimensions are often overlooked but have recently shown to be important for phytoplankton distributions in the ocean [68]. Temperature and pH have been shown to play an important role in niche separation in environments ranging from soils to hot springs. For example, although Yellowstone hot spring cyanobacteria show niche separation based on differential light adaptation (i.e. energy resource), temperature and presence/absence of phosphonate and phycocyanin (organic nutrient) acquisition pathways were also important niche dimensions controlling the distributions of *Synechococcus* strains and other phototrophs within microbial mats [65,69].

5. Conclusions

Continuing rapid taxonomic discovery enabled by molecular methods has revealed many new microbial phyla with no

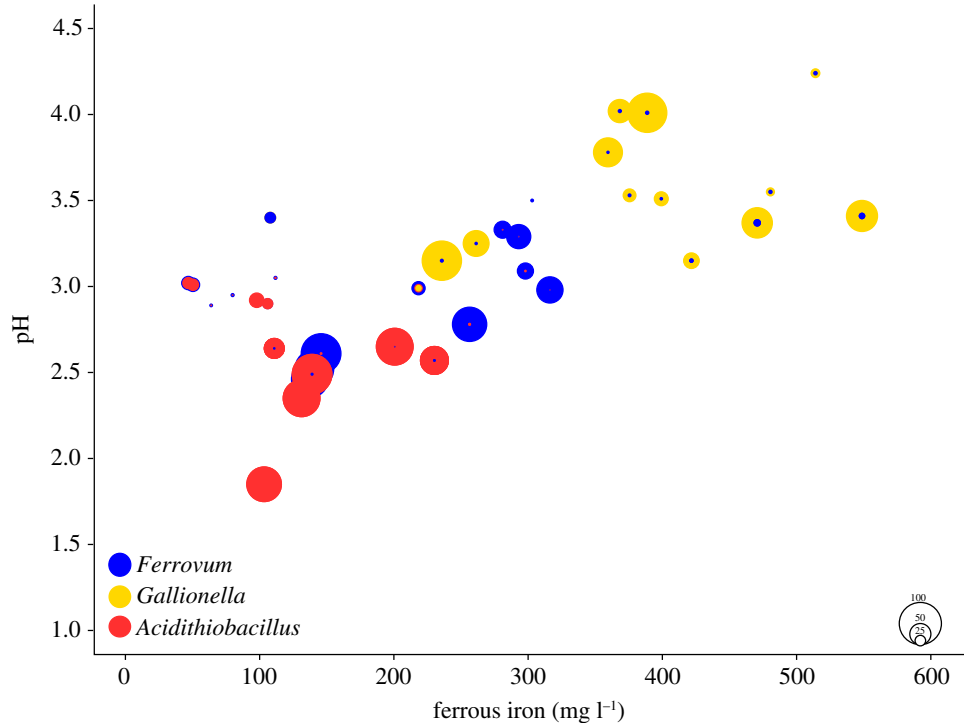

Figure 9. Dominant iron-oxidizing acidophiles in $n = 61$ microbial communities from five AMD sites. Colours indicate three genus-level taxa common in the communities: *Ferrovum*, *Gallionella*-like and *Acidithiobacillus*. Symbol diameters are scaled to the percentage of the community represented by each genus as indicated by the key at lower right. Community compositions were determined using fluorescence *in situ* hybridization (FISH), informed by 16S rRNA gene cloning. Methods follow those described in Brown *et al*. [29]. (Online version in colour.)

cultivated representatives. Environmental omics approaches enhance our ability to learn about the physiology of these unfamiliar taxa, and will play an increasing role in the discovery of their metabolic potential. It is likely that most of these enigmatic micro-organisms will make use of light and redox energy resources similar to those used by better-known micro-organisms, because there are far more novel taxa than 'missing' energy-yielding redox or light-harvesting reactions. There are nonetheless strong scientific and societal motivations to explore the ecological niches of the uncultivated majority, which may be novel even if their energy resources, broadly speaking, are not. Future discoveries of the metabolic capabilities of novel microbial groups will have important implications for engineering, environmental science, biotechnology and interpreting organic, mineral, and isotopic biosignatures in the rock record of Earth history.

In addition to revealing metabolic traits in the uncultivated majority, genetic data are gradually closing gaps between ecological theory and microbiology, and enable testing of ecological models and hypotheses in the absence of a defined microbial species concept. We suggest that RRT provides a useful and quantitative framework for exploring how energy resource availability in the environment controls the distributions of microbial populations and groups. One of the main predictions of RRT, that population dominance varies with the ratio of the availability of two or more essential limiting resources, is supported by our recent work on sulfur- and iron-oxidizing lithoautotrophs.

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