Review

Refocusing the microbial rare biosphere concept through a functional lens

Elena Litchman [1,2,*], Sébastien Villéger [3], Lucie Zinger [4,5], Jean-Christophe Auguet [3], Wilfried Thuiller [6], François Munoz [7], Nathan J.B. Kraft [8], Laurent Philippot [9], and Cyrille Violle [10]

The influential concept of the rare biosphere in microbial ecology has underscored the importance of taxa occurring at low abundances yet potentially playing key roles in communities and ecosystems. Here, we refocus the concept of rare biosphere through a functional trait-based lens and provide a framework to characterize microbial functional rarity, a combination of numerical scarcity across space or time and trait distinctiveness. We demonstrate how this novel interpretation of the rare biosphere, rooted in microbial functions, can enhance our mechanistic understanding of microbial community structure. It also sheds light on functionally distinct microbes, directing conservation efforts towards taxa harboring rare yet ecologically crucial functions.

Let the microbial rare biosphere concept be functional!

The question of rarity in ecological communities has attracted much attention [1,2]. Understanding why some species are rare (occur in low abundance) and how important they may be in ecological communities and ecosystems has always been a central question in ecology [3,4]. Because rare species are also more at risk of extinction, they are often top priority for conservation efforts. These efforts mostly concern large-bodied animals and plants, while the concept of microbial conservation is still nascent [5,6]. In microbial ecology, the importance of rare species goes beyond the need for conservation. The influential 'rare biosphere' concept was proposed almost two decades ago to highlight the prevalence (i.e., high number of species) of rare taxa after observing the long tail of the rank-abundance plots of microbial communities [1,7]. Various approaches have been proposed to define 'rare' taxa, such as quantitative thresholds; for example, <0.1% or 0.01% in relative abundance [8] or species abundance distribution (SAD) [9] models. A related concept of 'conditionally rare' microbial taxa highlights **microbes** (see Glossary) with low densities that occasionally can reach high abundances [10]. The concept of the rare biosphere has fascinated microbial ecologists since its emergence because of its potential links to the functionality of microbial communities. It is often considered a 'seed bank' [8] that can, by including more taxa with the same function, ensure that functions are not lost in a changing environment [11,12]. Members of the rare biosphere can also exhibit **functional rarity** (i.e., possess important and unique functions) despite their low numbers [8,12]. Thus, as for macroorganisms, this functional facet is implicitly central to the microbial rare biosphere, but we currently lack a conceptual framework explicitly focusing on whether the members of the rare biosphere bear unique or common functions. Bridging this gap is especially needed at a time when there is a growing emphasis on the **traits** and functions of organisms, including microbes [13–19]. While the microbial rare biosphere focuses on taxon abundance (low vs. high abundance), it also includes another important aspect of rarity, functional rarity, but only implicitly.

Recently, a novel framework at the intersection of ecology and evolutionary biology has emphasized the importance of functional rarity in macroorganisms. This framework has also highlighted

Highlights

The influential rare biosphere concept in microbial ecology focuses on taxonomic scarcity and treats functional rarity implicitly.

Microbes can possess functions distinct from other taxa in a community and can be present at high or low abundances. We define functionally rare microbes as possessing distinct functions and being numerically scarce.

Functionally rare microbes can contribute disproportionately to ecosystem multifunctionality.

The functional rarity concept helps to mechanistically connect microbial functional diversity, ecosystem functioning, and evolutionary processes. It also highlights the need for conservation of rare functions in microbial communities.

[1]Department of Global Ecology, Carnegie Institution for Science, Stanford, CA, USA
[2]Kellogg Biological Station, Michigan State University, Hickory Corners, MI, USA
[3]MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Montpellier, France
[4]Institut de Biologie de l'École Normale Supérieure (IBENS), École Normale Supérieure, CNRS, INSERM, PSL Université Paris, Paris, France
[5]Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), UMR 5300, CNRS, Institut de Recherche pour le Développement (IRD), Toulouse INP, Université Toulouse 3 Paul Sabatier, Toulouse, France
[6]Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, LECA, F-38000 Grenoble, France
[7]Université Grenoble Alpes, CNRS, LIPhy, F-38000 Grenoble, France
[8]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles, CA, USA

[9]Université Bourgogne Franche-Comté, INRAE, Institut Agro Dijon, Agroecology, Dijon, France
[10]CEFE, Université Montpellier, CNRS, IRD, EPHE, Montpellier, France

*Correspondence:
elitchman@carnegiescience.edu
(E. Litchman).

a disconnect between taxon abundances and **functional distinctiveness** [20,21]. According to this perspective, a taxon may perform a distinct set of functions while being present either at high or low abundance within a community. Functional distinctiveness is conceptually close to the concept of 'narrow' versus 'broad' microbial processes coined by Schimel [22], where broad functions (e.g., respiration, labile C decomposition) are associated with a high **functional redundancy**, where the same function can be present in many taxa [23,24], and narrow ones, such as nitrification, N fixation and methanogenesis, are restricted to particular taxonomic clades. However, the concept of functional distinctiveness comes from a more general framework that also integrates taxon abundances and develops metrics for both functional distinctiveness and rarity [20].

Here we propose to explicitly focus on rare functions by merging the rare biosphere concept with the new functional rarity framework and considering the rarity of both taxa and functions, and to explore how these two dimensions of rarity relate to each other. We examine functional rarity, which combines functional distinctiveness and numerical scarcity (taxonomic or functional), across spatial and temporal dimensions. We propose considering functional rarity both at the level of taxa and at the level of communities (Figure 1) and introduce a conceptual framework to characterize microbial functional rarity.

Conceptual framework: functionally distinct taxa and communities

Before considering how various facets of taxon abundance and functional distinctiveness apply to microbes, we must first establish a clear definition of functional rarity and highlight a related term, 'scarcity', which is commonly used in ecology. It is important to distinguish the two terms to avoid confusion. Scarcity here pertains to a low abundance (or biomass) of taxa (a.k.a. the 'rare biosphere', Figure 1A) or functions (see later). Functionally distinct taxa (i.e., having distinct traits) (a.k.a. taxa with narrow functions, Figure 1B) can be either abundant or scarce in the community (Figure 1A). If functionally distinct species are also scarce, they become part of the rare biosphere in the traditional sense and can be considered functionally rare [20]. While the functional rarity concept overlaps somewhat with the rare biosphere concept, the former complements the latter (and is stricter) by explicitly focusing on rare and distinct functions, hence allowing us to forge mechanistic connections between microbial taxa and ecosystem functioning.
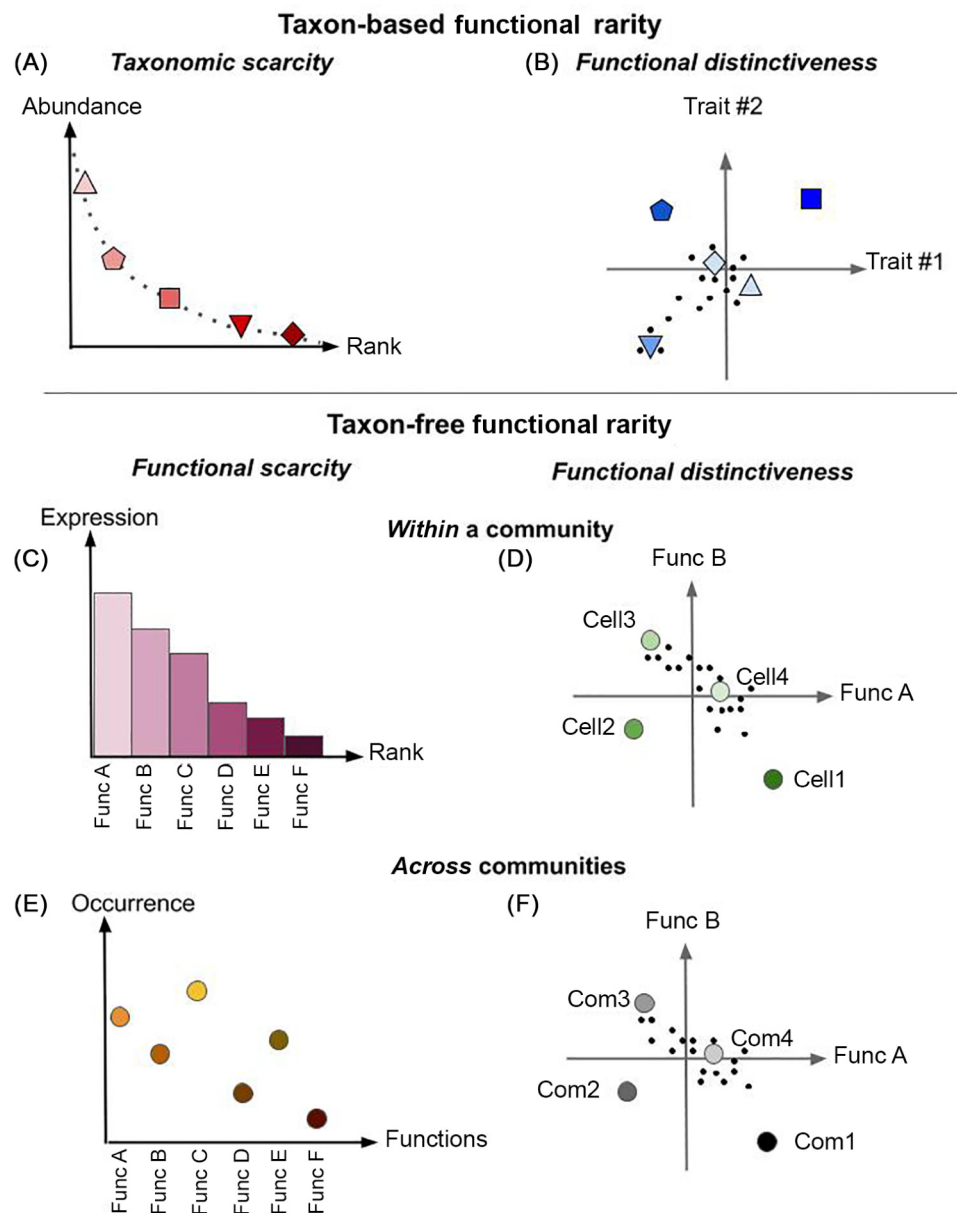
A more direct characterization of the relationship between microbes and ecosystem functioning is possible by adopting a taxon-agnostic approach, focusing solely on the functions/genes/metabolites present in the community without attributing them to specific taxa [25]. This approach is facilitated by the omics methods where the whole genetic/protein/metabolite composition of communities can be analyzed (metagenomes, metatranscriptomes, metaproteomes, metabolomes) and is especially suited for microbial communities [23,26–29]. The abundance of genes/metabolites can be related to ecosystem functions, as genes encoding different enzymes are often used as proxies for functions [30]. For example, by determining the frequency of *nif* genes in a community, we can estimate the potential for nitrogen fixation, a critical biogeochemical function performed only by certain microbes. Moreover, instead of examining individual functional genes, we can investigate metabolic pathways present within the community. In metagenomes, these pathways can be inferred by identifying groups of genes involved in specific pathways [28,31].

Within a community, analyzing the relative abundances of different functional genes or metabolic pathways, or their expression levels [28], enables the identification of functions that are scarce (Figure 1C). We propose that analyzing the distribution of all known (and unknown) functions in a community by plotting function abundance distributions (FADs) could offer insights into patterns of community multifunctionality (e.g., changes in FADs along environmental gradients). This novel

Taxon-based functional rarity

(A) *Taxonomic scarcity*

(B) *Functional distinctiveness*

Taxon-free functional rarity

Functional scarcity

Functional distinctiveness

(C)

(D) *Within a community*

(E) *Across communities*

(F)

Trends in Ecology & Evolution

Figure 1. Characterizing taxonomic scarcity, functional distinctiveness, and functional rarity in microbes. (A) Microbial taxon abundance is typically characterized using 16S or 18S rRNA genes, assessing the scarcity of each taxon based on its relative abundance in communities (scarcest taxa being in dark red, corresponding to the concept of 'rare biosphere' [1]). (B) When each taxon is also described using its traits (e.g., using microbial taxonomy to retrieve associated functional information from microbial functional databases such as bactoTraits, from functional inference tools such as PICRUSt, or from cultures), we can calculate its distinctiveness as the average difference from other taxa, illustrated as the distance to other taxa (points) in the multidimensional trait space (most-distinct taxa in dark blue, the symbols in areas of high point density corresponding to taxa with 'broad' functions while those isolated in the functional space correspond to taxa with 'narrow' functions; *sensu* Schimel [22]). Note that only five taxa are illustrated with different large symbols, while others as dots. The functional rarity, as proposed by Violle *et al.* [10], can be calculated by multiplying a taxon's scarcity (inverse of abundance) by its functional distinctiveness. (C) Omics approaches also produce data where individual taxa cannot be distinguished. The scarcity of each function within a community can thus be measured by considering the abundance of

(Figure legend continued at the bottom of the next page.)

Glossary

Functional distinctiveness: characteristic of a taxon (or of a community) having functional traits dissimilar from the whole ensemble of functions present within one or a set of communities. A metric of functional distinctiveness assesses whether a taxon (or a community) is more or less functionally close to the other community members (or to other communities).

Functional rarity: a concept where a taxon of low abundance or occurrence is functionally dissimilar from the rest of the community. It integrates both functional distinctiveness and **taxonomic scarcity**. It can also apply to an ecological community that is scarce and contains functions distinct from other communities.

Functional redundancy: coexistence of organisms that share the same set of functions (strict redundancy) or some specific functions but may nevertheless differ in additional functions or other ecological requirements (partial redundancy) and that can readily replace each other.

Microbes: a taxonomically and phylogenetically diverse group of prokaryotic and eukaryotic free-living or symbiotic microorganisms that includes bacteria, archaea, protists, and fungi.

Taxonomic scarcity: taxa have low relative abundance (in terms of numbers of individuals, biomass, or sequencing reads) in the community.

Trait: any morphological, physiological, phenological, or behavioral feature measurable at the individual level.

metric is similar to a commonly used characterization of ecological communities ranking species according to their abundance (SADs), which allows the identification of common or rare species [32,33]. It is even more similar to the recently introduced concept of trait abundance distributions (TADs) [4]. FADs can provide valuable information about the distribution of functions within a community, facilitating comparisons between communities, and is well suited for the types of data specific to microbial communities. As communities may vary in the number of functions present and in how evenly those functions are distributed, comparing FADs across microbial communities and identifying rare functions may help to focus priorities for conservation and management.

Likewise, we can scale up the concept of functional distinctiveness at the taxon level to entire communities. Combining the multidimensional scaling analysis of the functional composition of communities with methods to identify distinct data points can allow us to pinpoint communities that exhibit distinct functions relative to other communities (Figure 1D). Such communities might be associated with habitats (or hosts for host-associated microbiomes) with some unique characteristics. They could play a pivotal role providing unique services, and thus may require heightened conservation efforts.

The concept of functional rarity may be even more relevant for microbes than for macroorganisms because microbes have many more functional dimensions arising from the astounding diversity of their metabolisms and the broad range of habitats they occupy. Microbes also have faster population dynamics, enabling the exploration of questions that cannot be answered with macroorganisms. Thanks to the development of omics approaches, microbial ecology now possesses a wealth of data on both taxonomic and functional community structures (e.g., see [34]) surpassing the available information for macroorganisms. These various data types can be used to quantify functional distinctiveness and rarity (Figure 1 and Box 1). The concept of rare functions can also be useful in applied microbial fields, such as the development of various therapies (e.g., fecal microbial transplants in the treatment of *Clostridium difficile* infections) or strategies to mitigate the impacts of climate change and pollution (e.g., bioremediation with microorganisms breaking down pollutants such as oil, pesticides, and other xenobiotics [35,36]).

How often do we find species that are abundant in a community but have distinct traits? Among microbes, this can be common. For example, cyanobacterial blooms in freshwater lakes are often nearly monospecific; a single species contributes disproportionately to total biomass [37] (Figure 2A,B). These bloom-forming species can differ functionally from the rest of the phytoplankton community; for instance, some are capable of nitrogen fixation (Figure 2B), a distinct trait of some bloom-forming cyanobacteria. During blooms, such taxa are functionally distinct but not rare. During non-bloom periods, when their abundance is low, they would also be functionally rare, highlighting the importance of temporal scale. Microbes inhabiting hydrothermal vents (Figure 2C) have unique adaptations to withstand extremely high temperatures and pressures [38], which makes them functionally distinct compared with microbes from other habitats that lack such adaptations. Because hydrothermal vent habitats are uncommon globally compared

genes supporting each function (metagenomics) or the level of their expression (i.e., metatranscriptomics) (scarcest function in dark purple). (D) Microbial functional distinctiveness within a community can also be measured at the individual level using gene repertoire (with single-cell metagenomics), gene expression (with single-cell transcriptomics or single-cell proteomics), or traits (e.g., with flow cytometry) (most-distinct individuals in dark green). Such an approach allows accounting for genetic variability, gene expression, or phenotypic diversity in a population or community. (E) Scarcity could also be measured at larger spatial scales across communities as the percentage occurrence of genes across communities (scarcest function in dark brown). (F) Functional distinctiveness could also be computed for each community as its average dissimilarity in function to other communities (most-distinct community in black).

with other habitat types, those microbes are also functionally rare. However, at local scales such microbes could be neither functionally distinct nor rare.

Microbial traits

Much like macroorganisms, microbes can be characterized by their traits, defined as heritable morphological, physiological, or life-history characteristics [39–41]. Key trait categories in microbes include genomic, metabolic, morphological, physiological, and life-history traits (Box 2; also see Table 1 in [42]).

There are many examples of microbes having distinct metabolic traits or metabolisms unique to a few taxa or even a single taxon in a community. Nitrogen-fixing cyanobacteria (and some heterotrophic bacteria) have an enzyme (nitrogenase) that enables them to convert atmospheric nitrogen to ammonia [43]. The presence of this enzyme and the N_2 reduction metabolic pathway make diazotrophic (i.e., feeding on N_2) cyanobacteria and bacteria functionally distinct. Host-associated microbiomes also have functionally distinct microbes. Some gut bacteria in Tanzanian Hadza hunter–gatherer communities possess unique metabolic pathways that aid in digesting particular dietary components [44]. One hundred and twenty-four microbial taxa were identified that were either absent or rare in gut microbiomes in industrialized societies, making the Hadza gut microbiome functionally distinct [44] (Figure 1D). As hunter–gatherer lifestyles disappear and diets change [45], many of these microbial taxa face extinction, leading to reduced functional distinctiveness of communities. Microbiome conservation, including host-associated

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Box 1. Quantifying the functional distinctiveness of microbes: from taxa to communities

Functional distinctiveness can be assessed using different approaches depending on the question and data availability (Figure I). Here, we present possible metrics for the main types of data in microbial ecology (R code in the supplemental information online).
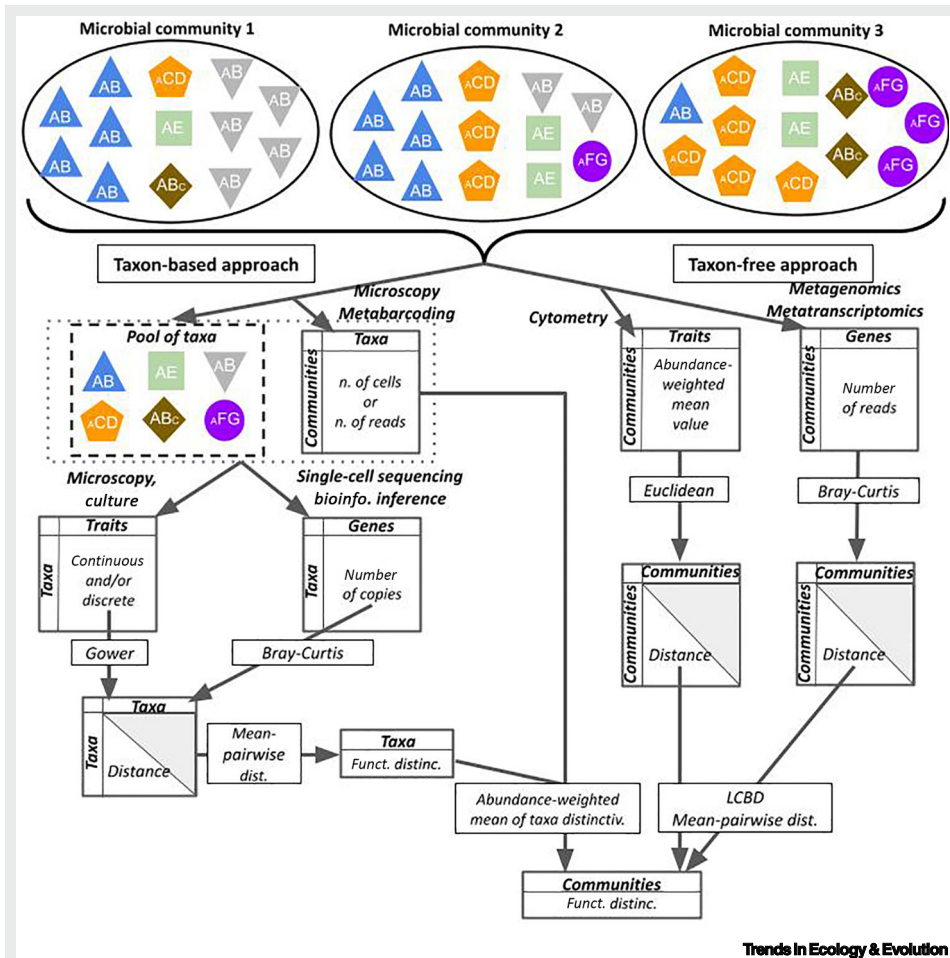
The first approach follows Violle *et al.* [10], originally proposed for macroorganisms. The functional distinctiveness of each taxon is computed from a set of traits. Taxa can be identified to the species level if possible, or to any other relevant level, including operational taxonomic units (OTUs) or amplicon sequence variants (ASVs).

Microbial taxa can be described based on their traits (Box 2), encoded as continuous (e.g., cell size, shape) or categorical (e.g., coloniality, type of reproduction) variables. The functional distance between all taxon pairs can be computed from these trait values, using either the Euclidean or the Gower distance for continuous and discrete traits, respectively. The functional distinctiveness of each taxon is calculated as the mean of all pairwise distances. Taxa with extreme trait values are considered the most distinct (e.g., purple taxa).

Alternatively, taxa can be functionally described using genomics (e.g., shotgun sequencing, single-cell sequencing), retrieving the number of copies of each gene in the genome of each taxon. Gene copy numbers are used to calculate Bray–Curtis dissimilarity for all taxon pairs and then the functional distinctiveness of each taxon is the mean of all pairwise distances. The most distinct taxa have the highest number of copies for genes scarce in the genomes of other taxa (e.g., purple taxa with genes F and G). The functional distinctiveness of a community is the abundance-weighted mean distinctiveness of its constituent taxa. Hence, the higher the abundance of distinct taxa in the pool of taxa, the higher the community distinctiveness (increasing from community 1 to 3).

The second approach determines functional distinctiveness at the community level, which is novel and well suited for microbes. It has not been previously used, as it is less straightforward for macroorganisms. Often, functions are identified at the community level without characterizing individual taxa. For instance, cytometry provides average cell size and DNA content, while metagenomic (or metatranscriptomic) analysis outputs the total number of reads per gene in each community. Then, the functional distinctiveness of a community is the mean pairwise dissimilarity with other communities (Euclidean and Bray–Curtis dissimilarity metrics). Community distinctiveness is highest when trait values or gene abundances differ from other communities. Another way to measure the distinctiveness of each community is using the local contribution to beta diversity (LCBD) index from Legendre *et al.* [99] showing how much each community contributes to the total variation of traits (or genes) among all communities. Assessing functional distinctiveness at the community level overcomes/integrates the confounding effects of HGT at lower levels of organization (e.g., species/population level).
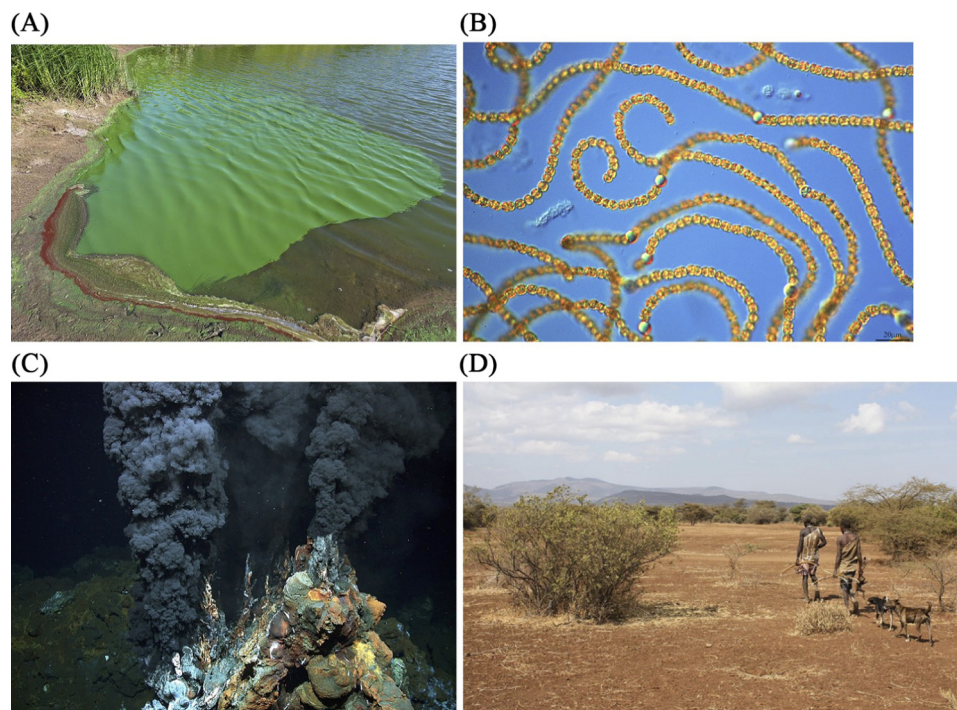
Figure I. Frameworks to assess the functional distinctiveness of microbial communities. Consider a simple case where three communities each have five microbial taxa (illustrated with different colors) with different relative abundances (number of symbols). Symbol shapes summarize morphological, physiological, and/or life-history traits. Letters represent genes present in each taxon, with the size being proportional to the number of gene copies.

ones, is an emerging topic in both wildlife conservation [46] and microbial ecology [47] and could benefit from explicit consideration of functionally rare microbes (Figure 1B) or communities with unique functions (Figure 1C).

Another example of microbes that can be distinct in their traits is pathogens. Understanding what traits make microbial pathogen taxa distinct from the rest of the community may help to predict pathogen invasion success and dynamics, ultimately improving human, animal, and plant health. For example, enteric pathogens are often more oxygen tolerant than the beneficial bacteria in the gut, which helps them survive outside the host [48,49]. Engineering greater oxygen tolerance in key beneficial bacteria (i.e., making them more distinct) can be an important step in effective probiotic applications [50]. Other traits also make microbial pathogens functionally distinct. Sarkar *et al.* [51] found that a pathogenic *Escherichia coli* strain attached to and invaded gut epithelial cells more than nonpathogenic strains. Some viral pathogens have high mutation rates in regions of their genomes involved in antigen drift [52].

Figure 2. Examples of functionally distinct microbial taxa and communities. (A) Cyanobacterial bloom in a water body (photograph credit: Christian Fischer, Creative Commons Attribution-Share Alike 3.0 Unported). (B) Cyanobacterium *Dolichospermum* sp., a toxic genus capable of atmospheric nitrogen fixation that frequently forms almost monospecific harmful algal blooms in freshwaters (photograph credit: Proyecto Agua, licensed by CC BY-NC-SA 2.0 DEED). (C) Deep-sea hydrothermal vent harbors many microbial taxa adapted to high temperatures and pressures, which makes them distinct from microbes in other habitats (photograph credit: MARUM – Zentrum für Marine Umweltwissenschaften, Universität Bremen, Creative Commons Attribution 4.0 International). (D) Gut microbiomes of Hadza hunter–gatherers have unique, functionally distinct microbial taxa [50] (photograph credit: Andreas Lederer, licensed by CC BY-NC-ND 2.0).

Are functionally distinct species generalists or mostly specialists? This question can be addressed by examining the number of metabolic pathways, assuming that specialists possess fewer pathways than generalists. Intriguingly, functionally distinct microbes are not necessarily specialists despite often having unique metabolic pathways. For example, *Nitrospira* sp. occurs in a variety of environments but has a unique ability to perform complete nitrification (complete ammonia oxidation, comammox, converting ammonium to nitrate, thus being a generalist), in contrast to specialist taxa that perform only a part of the nitrification process (either converting ammonium to nitrite or nitrite to nitrate) [53]. *Nitrospira* can be abundant under certain conditions, such as low-oxygen environments [54].

Functional distinctiveness and rarity across scales

Most phenomena in ecology are scale dependent [55]. Whether a species can be classified as functionally rare is also contingent on the spatial or temporal scale under consideration [56], as well as the data availability and sampling biases. Species can be functionally distinct at all scales, from local to regional and global, or only at specific scales. Similarly, species can be distinct from the rest of the community within one habitat (Figure 2A,B) or across different habitats or patches. Unique or marginal habitats can contain species functionally distinct from those in other habitats (Figure 2C,D). The extent of functional rarity and distinctiveness also varies across different temporal scales. Here we provide some examples.

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Box 2. Microbial traits

Microbes have a plethora of traits that can render them functionally distinct and rare. These include genomic traits, such as 16S copy number, GC content, genome size, and the number of genes, and physiological traits like maximum growth rate, environmental tolerances (oxygen tolerance, pH and temperature optima and ranges), nutrient utilization (carbohydrate utilization, amino acid degradation, lipid degradation), resistance to inhibitory substances (high salt, antibiotics, etc.), enzyme production (catalase, coagulase, hemolysins, etc.), sporulation, and motility [17,100,101].

Morphological traits such as cell size, length, width, and shape and cell-wall type can also contribute to functional distinctiveness [16,41,42,101]. Cell size is a fundamental trait that influences many ecological and physiological functions. Some bacteria are true giants in the microbial realm and thus functionally distinct based on their size. A mangrove-dwelling sulfur-oxidizing bacterium, *Candidatus Thiomargarita magnifica* is more than 1 cm long, surpassing previously known large-celled bacteria by 50-fold, making it the largest bacterium known to date [102]. Other species of *Thiomargarita* are also exceptionally large, reaching 750 μm [102]. Giant bacteria (600 μm) are also found in fish gut where they play key roles in digesting algae [77].

Metabolic traits are particularly significant for microbes due to the vast diversity of metabolic pathways present. Attempting to differentiate microbial taxa or communities within an ordination space by examining their genetic makeup or metabolic pathways can be challenging because the concept of functional redundancy, the coexistence of diverse taxa with the same metabolic processes and, thus, interchangeable within a community, has been a longstanding assumption. For example, using genes annotated from databases [103] or inferred functional profiles [31] unveiled substantial functional redundancy among marine microorganisms worldwide. This redundancy paradigm remains, however, debatable, as shown in marine [23] and soil bacterial communities [104]. To circumvent the masking effect of universal housekeeping genes and pathways that may increase functional redundancy, we could focus on a subset of relevant genes or metabolic pathways, such as N metabolism genes/pathways. Because many metabolic and other traits in microbes are still unknown, future discoveries of novel metabolic pathways/functional genes may greatly increase the number of functionally distinct taxa and expand the functional multidimensionality of microbial communities [105,106]. Many of those unknown functional genes are enriched in rare taxa [107].

What we call a trait and how aggregate it is (e.g., a single gene, a gene cluster, or a metabolic pathway) will have a significant influence on taxon distinctiveness [25,108]. This sensitivity can be reduced through systematically deriving phenotypic traits from genotypes using standardized methods; for example, inferring maximum growth rates from genomic information (codon usage bias) [109], identifying the presence of proteins from nucleotide sequences [110], etc.

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Different spatial scales

At local spatial scales, species can have distinct traits within a given community or within a given habitat. For example, a nitrogen-fixer in a microbial community is locally distinct because of its ability to fix atmospheric nitrogen. Nitrogen fixers are still distinct at large scales because there are only a few taxa capable of nitrogen fixation. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are widespread but may still be functionally distinct at a local scale [53].

Conversely, some taxa in marginal or unique habitats that are not distinct locally can be distinct at a regional or global scale. For example, microbial taxa in hydrothermal vents have traits allowing them to withstand extremely high temperatures and pressures [38,57]. While these traits are common in such habitats, the microbial taxa inhabiting these extreme environments, along with their traits, are globally distinct because hydrothermal vents are scarce relative to other habitats [58]. Similarly, microbes from the oceanic oxygen minimum zones (OMZs) may be abundant in those habitats but scarce in other marine habitats [59]. Changes in the frequency of rare habitats may impact the global functional rarity of microbes from those habitats. For instance, the global scarcity of microbes associated with rare habitats may decrease if these habitats become more widespread, such as the expansion of OMZs due to global warming and changes in land use [60].

Different temporal scales

Functional distinctiveness can change in periodic, fluctuating environments. Species can be functionally rare at specific times but not when averaged over longer periods (e.g., 1 year). An example of such a dynamic relationship between functional distinctiveness and rarity is the seasonal

occurrence of the often-monospecific blooms of toxic cyanobacteria in aquatic ecosystems, typically in the summer. These cyanobacteria are functionally distinct due to their ability to produce toxins, setting them apart from the rest of the phytoplankton community [37,61]. While toxic cyanobacteria may be present year round in aquatic ecosystems at low densities, making them functionally distinct and rare, during blooms they remain functionally distinct but are no longer rare. In microbial communities, some taxa may be scarce for most of the time but experience transient increases in abundance [10].

The emergence and maintenance of functional distinctiveness and rarity

When considering functional distinctiveness and rarity and their importance in microbes, a key question is how such distinctiveness arises [21]. Answering this question would help to connect microbial ecology, evolutionary biology, and biogeochemistry.

Genomic underpinnings of functional distinctiveness

Distinct traits can emerge through several mechanisms, ranging from point mutations to substantial genome reorganizations, including the acquisition or loss of multiple genes and their reshuffling. These processes have the potential to lead to major evolutionary innovations giving rise to functionally distinct microbes [62]. Moreover, functional distinctiveness at the intraspecific level can be seen as a driving force for evolutionary adaptation to novel environments, diversification, and colonization of novel ecological niches. For instance, the long-term evolution experiment with *E. coli* revealed that a series of mutations gave rise to the novel ability to aerobically utilize citrate, enabling mutants to exploit a previously unoccupied niche [62]. Similarly, a single mutation allowed *E. coli* to become an insect mutualist [63]. In a marine cyanobacterium, *Synechococcus*, genome methylation lead to divergent thermal ecotypes [64].

If a microbe loses a function or several functions (e.g., through gene loss), it can become functionally distinct. Genome streamlining, either due to a parasitic lifestyle where some functions are lost due to reliance on the host or in oligotrophic or extremely stable conditions where it is too costly or unnecessary to maintain large genomes, could also lead to functional distinctiveness [65–67]. In general, if the benefits of being functionally distinct outweigh the costs, there could be selection for functionally distinct strategies. In oligotrophic oceans, phytoplankton replace phospholipids in their cell membranes with non-phosphorus-containing lipids [68]. This functionally distinct strategy may be widespread in low-phosphorus environments but is rare in other environments [68].

Horizontal transfer of genetic material [horizontal gene transfer (HGT)], where organisms can receive genetic material from other individuals of the same or different species, is common in microbes, although it can also be found, albeit less frequently, in larger organisms as well [69,70]. HGT can affect functional distinctiveness. For example, the acquisition of advantageous genes from other taxonomic groups under a given selective pressure is likely to spread across the community and hence decrease local functional distinctiveness. The spread of antibiotic resistance genes in a microbial community is an example of a loss of functional distinctiveness [71]. In many cases, however, despite widespread HGT and high dispersal rates, microbes still frequently possess unique functions and traits.

Ecological interactions as drivers of functional distinctiveness

Microbial cooperation, such as cross-feeding, can promote functional distinctiveness. Species engaged in an exchange of metabolites (cross-feeding) may lose some functions (i.e., the ability to synthesize some essential compounds) and thus become functionally distinct from other members of the community, including their cross-feeding partners [72,73]. Competition

can also promote functional diversity and distinctiveness, where niche separation due to competition occurs through the evolution of novel traits/functions (e.g., an evolved ability to consume citrate under aerobic conditions that increases fitness [21,74,75]). Coevolution with the host for host-associated microbes could also be a driver of functional distinctiveness [76,77] (Box 3).

Human influence on functional distinctiveness and rarity

Human activities may have contrasting effects on microbial functional distinctiveness and rarity. Humans may promote functional distinctiveness in microbial communities by creating novel selective pressures that give rise to novel strategies. For example, polluted lakes may have distinct microbes that are tolerant to heavy metals [78]. Interestingly, heavy-metal pollution may also lead to increased antibiotic resistance due to similarity of mechanisms of resistance to heavy metals and antibiotics [78,79]. Humans can also decrease functional distinctiveness by homogenizing microbial communities, spreading antibiotic resistance, such as in aquaculture settings with high antibiotic use [80].

Functional distinctiveness and ecosystem functioning

Quantifying the biodiversity effects on ecosystem functioning is a long-term goal in ecology [81]. Three decades of research have highlighted a major role of species diversity in driving ecosystem functions [82]. Subsequently, trait-based ecology has examined the underlying mechanisms of the biodiversity–ecosystem functioning (BEF) relationship through a comprehensive analysis of the trait values of the organisms in the ecosystem [15]. The findings appear contrasted between macro- and microorganisms. In macroorganisms, at least in plants, the BEF relationship seems primarily driven by the traits of the most abundant species present rather than by among-species functional diversity [83], even if functional diversity and functional distinctiveness matter too [84–86]. By contrast, in microorganisms most studies have reported a pivotal role of (functional) diversity [87,88]. Given that greater functional diversity is expected to reflect resource-use complementarity among species and niche dimensionality [89], this discrepancy could reflect the higher number of (limited) resources that microorganisms depend on compared with macroorganisms. Thus, the peripheral position of a species in phenotypic space, as revealed by higher functional distinctiveness, should be a marker of a unique resource-use strategy within the ecosystem. This can have implications for both short-term and long-term functioning of the ecosystem. In trees, using a virtual experiment, Delalandre *et al.* [90] demonstrated that the extinction of functionally distinct species can have a greater impact on ecosystem productivity than the extinction of common species. The effect of functionally distinct species on ecosystem functioning can also be indirect via changing species interactions [91].

Unique metabolic pathways of functionally distinct microbes can be disproportionately important for ecosystem functioning, possibly more so than in macroorganisms, due to a higher diversity of metabolisms. There are many examples in both aquatic and terrestrial ecosystems, as well as in host-associated microbiomes, that highlight the importance of functionally distinct microbial taxa for carbon and nutrient cycling, pollution remediation, providing functions essential to the host, and others. Rare, metabolically distinct microbes in the deep ocean may be more efficient in making refractory organic matter and thus could have a positive effect on carbon sequestration [92]. The functionally distinct and biogeochemically important nitrogen-fixing cyanobacterium *Trichodesmium* spp. is an important source of nitrogen in the oligotrophic ocean [93]. A novel bacterium isolated outside a bottle recycling facility is capable of growing on the common form of plastic PET, through possessing two unique enzymes that degrade PET into environmentally benign compounds [94].

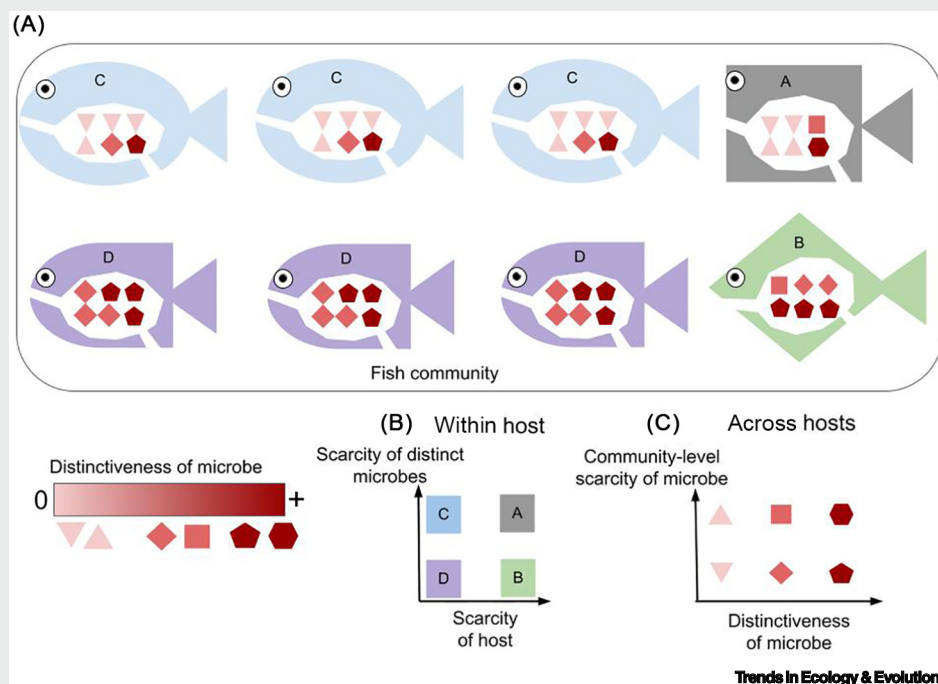## Box 3. Functional rarity of microbes associated with macroorganisms

The framework to measure microbial functional rarity has the same two dimensions (scarcity and distinctiveness) as the framework initially proposed for the measurement of functional rarity in macroorganisms. Over the past decade, there has been a growing emphasis on the study of microbes closely associated with macroorganisms due to their importance for host health.

The functional rarity framework helps to explore critical questions that bridge ecoevolutionary processes and conservation applications. Here are some example questions.

- Do the most distinct hosts harbor the most distinct microbiomes (Figure I)?
- Do the scarcest hosts have the scarcest and/or the most distinct microbes (Figure I)?

In the cases where the scarcest microbes also happen to be the most distinct, and if their sole host species is scarce, these functionally rare microbes may face a high risk of extinction. Conversely, some distinct microbes could be abundant within their host while the host itself is abundant as well, ensuring that these distinct microbes have a lower risk of extinction.

All of these questions could be addressed at different spatial levels, from local communities to global floras and faunas, taking advantage of the growing number of available trait and microbiome datasets.

Figure I. Assessing congruence between the rarity of microbial taxa and their hosts. The figure illustrates how microbial functional rarity could vary with the scarcity of the host. (A) The community of eight fish individuals comprises four fish species (shapes and colors) whose microbiomes are shown in white ellipses, with the shapes of the symbols depicting the microbial taxa (with some taxa being shared across hosts). The functional distinctiveness of each microbial taxon is illustrated as the fill color (scale at bottom of panel). The relative abundance of microbial taxa in each microbiome is illustrated by the number of symbols (assuming no intraspecific variability). (B) Host species A is scarce and the most-distinct bacteria of its microbiome are scarce as well (e.g., dark-red hexagon, being functionally rare). Host species B is scarce and the most functionally distinct bacteria of its microbiome are abundant (dark-red pentagon, not functionally rare). Host species C is common and the most-distinct bacteria of its microbiome are scarce (e.g., dark-red pentagon; it is functionally rare within the host but not across hosts). Host species D is common and the most-distinct bacteria of its microbiome are dominant (not functionally rare). (C) Microbial taxa with similar distinctiveness within the microbiome can have contrasting total abundances (i.e., among all hosts) depending on both their abundance within each host and the abundance of each host.

Concluding remarks

The concept of functional rarity and distinctiveness in microbes introduced here develops the influential rare biosphere concept [1] by explicitly anchoring it in traits and functions. Functionally distinct taxa, defined by their distinct traits, may hold particular importance for ecosystem functioning and thus could be the focus of conservation efforts. Isolating those taxa into culture and characterizing their traits would help such efforts.

Furthermore, we propose examining functional distinctiveness and rarity not only at the individual taxon level but also at the community level. This allows the comparison of (multi)functionality across communities and could potentially guide conservation. A greater number of distinct functions and more even distributions of those functions in a community (FADs) would render such communities more multifunctional. Future directions could include explicit characterization and comparison of a wide range of microbial communities based on their functions.

There are research challenges to implementing the functional rarity conceptual framework, as we lack information on many traits and functions of microbial taxa. Given the rapid progress of methods for genomic analyses, focusing on genomic traits may be the most promising approach to characterize the functional distinctiveness and rarity of individual taxa and communities.

Microbial systems provide powerful opportunities to experimentally assess many ecological and evolutionary theories, from resource competition, ecological stoichiometry, and trait-based ecology to evolutionary adaptation and speciation [39,95–98]. Similarly, microbes could be a good experimental system to assess different hypotheses for the role of functional rarity in ecosystem functioning. It may be possible to test different scenarios of how functional rarity affects ecosystem functioning proposed recently [20]. Several outstanding questions (see Outstanding questions) can be addressed empirically and theoretically.

Declaration of interests

The authors declare no competing interests.

Supplemental information

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Outstanding questions

Are there traits or trait combinations that make microbial taxa functionally rare?

How does the potential to transfer functions laterally across microbial taxa (e.g., through HGT) affect functional distinctiveness and rarity?

How do different environments compare with respect to microbial functional diversity and evenness (can we compare FADs)? Do some environments harbor more distinct microbial communities?

How much of the rare biosphere is also functionally rare? Do rare microbes usually possess distinct functions?

What are the rarest microbial functions, and the most redundant? Which functions should we prioritize protecting?

What are the environmental or host factors leading to the emergence and maintenance of rare functions?

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