Trends in Ecology & Evolution

CellPress
REVIEWS

Opinion

Macroecology to Unite All Life, Large and Small

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Macroecology is the study of the mechanisms underlying general patterns of ecology across scales. Research in microbial ecology and macroecology have long been detached. Here, we argue that it is time to bridge the gap, as they share a common currency of species and individuals, and a common goal of understanding the causes and consequences of changes in biodiversity. Microbial ecology and macroecology will mutually benefit from a unified research agenda and shared datasets that span the entirety of the biodiversity of life and the geographic expanse of the Earth.

Highlights

Macroecology is the study of the mechanisms underlying general patterns of ecology across scales. A major focus of research within macroecology is understanding biodiversity patterns and their underlying processes. The field of macroecology has been biased towards charismatic macroorganisms (also known as macrobes), and has largely ignored insights and breadth that can be gained by considering microorganisms.

We argue that microbial ecology and macroecology are united by common currencies (individuals and species), as well as by comparable challenges of documenting their distributions and abundances.

Future directions that would lead to a unified macroecology include: expansion of spatial and temporal scales to encompass the diversity of microbes; synthesis-driven, systematic comparisons of macrobial and microbial macroecological patterns and processes; and support of interdisciplinary approaches in training, publishing, and funding to equitably value macrobial and microbial insights into understanding the rules and exceptions of life.

It Is Time to Unite

Every individual, be it a mammoth, mule, marmot, or microbe, occupies a particular space and exists at a particular time. The number of marmots varies from place to place, as does the number of any particular microbial taxon. Identifying and counting individuals, regardless of where they reside in the tree of life, is at the crux of understanding **biodiversity** (see Glossary) and the natural world [1]. Decades of research have revealed that variation in the number of individuals of different species in space and time can give rise to a number of patterns, such as **species abundance distributions** and **species–area relationships**. These variables form the foundations of research in **macroecology**, biogeography, and community ecology. From the biodiversity patterns that emerge from counting individuals and species, many of the most general rules of ecology and evolution emerge [2–4].

Until recently, the field of macroecology almost exclusively involved the study of large, multicellular organisms (also known as macroorganisms or macrobes), especially plants, vertebrates, and a few charismatic invertebrate groups like butterflies. However, in the early 2000s, the advent of new (and increasingly less-expensive) molecular tools inspired some ecologists to ask the simple question: do microscopic forms of life play by the same rules as plants and animals? Initially, discussion centered around whether microbes exhibited macroecological patterns that were common in macrobes [5]. For example: do microbes exhibit distance–decay relationships [6,7]? Are there elevational gradients in microbial diversity [8,9]? Do places with high macrobial diversity also have high microbial diversity [10,11]? An especially robust debate commenced around the ideas of dispersal limitation and whether microbial taxa where found everywhere [12] and then selected by the environment, which initiated new research on microbial biogeography (e.g., [13–15]). Despite these initial lines of inquiry, microbial ecology has evolved largely independently from macroecology and the two fields are not yet well integrated. Their continued separation seems to arise for historical and cultural reasons rather than inherent differences.

There is a need to unify microbes and macrobes to ask overarching questions and to test general theories about the rules and mechanisms underpinning patterns in ecology across

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scales. The inclusion of microbial species into macroecological theory will extend and enrich our understanding of ecological patterns, not only to include a far greater range of spatial and temporal scales, evolutionary divergence, and organismal sizes, but also to provide insights into the fundamental processes that govern patterns of diversity and abundance across all types of organisms.

Microbes include the most phylogenetically and functionally diverse and abundant taxa on Earth [16–18]. Large advances in understanding microbial diversity have historically coincided with large advances in the technology used to observe **microorganisms**, from the invention of the microscope to the development of high-throughput DNA sequencing. At the beginning of the high-throughput sequencing revolution, about a decade ago, the technology was expensive. Thus, large datasets to examine microbial diversity in space and time were not common. Calls for the study of microbial biogeography [14,19] would have to wait until there were more empirical data against which to test (and develop) theory. Although many microbial ecologists were using and applying concepts and methods from macroecology [13], there were few calls for microbial macroecology [20]. Meanwhile, macroecology has developed over recent decades with little reference to microbes, although, as discussed above, there are several key references that compare some patterns directly.

The rich data necessary to unify microbes into macroecology are now here. Microbial datasets that consider tens of thousands of microbial taxa observed over hundreds, thousands, or even tens of thousands of samples have become common, and these datasets are often open access. Importantly, high-throughput, deeply sequenced datasets have made it possible to observe the important contribution of rare taxa to microbial community structure and diversity, leading to more precise analysis of biodiversity patterns. Furthermore, ecologists have begun to consider these microbial data in light of macroecological theory [15,21–24], or in direct comparisons to data on macrobes (e.g., [25–28]). As an exemplar case, the metabolic theory of ecology has especially benefited from the inclusion of microbial taxa to generally predict scaling of metabolic rates with body size (Box 1). It is time for macroecology to forge ahead with unified currencies to count the number of individuals of the same or different species, distributed in space and time, for all of life's diversity. This accounting applies to moths, mammoths, and microbes – the bacteria, archaea, fungi, protists, and viruses that are all around us.

Box 1. Metabolic Scaling across Macrobes and Microbes

One macroecological pattern that was considered universal across both micro- and macroorganisms is the scaling of metabolic rate (and many other biological rates) with body size. It was generally believed that the relationship is linear when both the body mass and metabolic rate axes are logarithmic, and that this line spans all organisms from microbes to whales with a universal slope $^{3}/_{4}$ (and thus can be represented as a power law with the exponent of 0.75) [69]. However, [70] have shown that a more detailed data analysis provides a different picture. While multicellular organisms indeed reveal $^{3}/_{4}$ scaling, metabolic rate in protists scale proportionally to body size (i.e., the scaling coefficient is close to 1) and bacteria and archaea reveal scaling coefficient close to 2 (i.e., a quadratic increase of metabolic rate with body size). The authors attributed these differences to different constraints on metabolic rate across micro- and macroorganisms. In bacteria the metabolic rate is assumed to be limited by number of genes and proteins involved in metabolism (so that bigger bacteria have a disproportionately higher number of molecules participating in metabolic reactions). In protists it is supposedly limited by the number of mitochondria within the cell, leading to approximate proportionality between cell size and metabolic rate. Multicellular organisms, in contrast, are limited by their ability to provide resources to all metabolically active cells, so that their metabolic rate is constrained by the structure of their transportation system, which leads to sublinear scaling, with coefficient close to $^{3}/_{4}$ [69]. There has been recent work to determine the utility of metabolic scaling in explaining soil microbial community responses to global warming [71], and microbes have been integrated into macroecology energetics (e.g., [72,73]).

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Unified Currency: Individuals and Species

Considering all of life at once, be it macrobial or microbial, expands the breadth and reach of macroecology, if for no other reason than the reality that most individual organisms and species are microbes. The number of individuals of a single bacterial phylum Firmicutes in the guts of a single human, for instance, exceeds the total number of trees on Earth (3×10^{12}, [29]). There are close to 10^{29} or 10^{30} individual prokaryotic organisms (bacterial and archaea) on the Earth [30–32]. These microorganisms derive from an astonishing diversity of taxa. Using scaling laws based on these abundances, Earth could be home to $\sim 10^{12}$ microbial taxa, which far exceeds estimates of plant and animal diversity ($\sim 8 \times 10^{6}$, [33]). This suggests that we have only inventoried one one-thousandth of 1% of all species on the planet [26], and that the majority of these species have yet to contribute to our understanding of macroecology.

The idea that there are common macroecological currencies, individuals and species, that apply to both macrobes and these numerous and diverse microbes has been controversial for several reasons. Here, we argue against each of four challenges cited in support of segregating microbes and macrobes in ecology: defining individuals, identifying individuals, delimiting species, and comparing methods.

Defining an Individual

It is often assumed to be fairly straightforward to identify and enumerate macrobial individuals, but, in practice, this is rarely the case (Box 2). As with some macrobes, some microbes are modular (e.g., filamentous), which make identifying an individual challenging. However, it is no harder to define the individual boundary of an ant supercolony, for instance, than of a clonal or modular bacterium.

Identifying Individuals

For a tiny fraction of microbial biodiversity, there is phenotypic and genomic information that allows for robust identification of the species to which individuals belong. Thus, **genetic barcoding** of **marker genes** [34] can be used to assign names to microbial individuals that can be isolated through culture, or more recently through dilution or physical capture. However, for the vast majority of yet-uncultivated microbial biodiversity, identification of the species to which individuals belong is only possible en masse through **metabarcoding**. This might seem to be a situation different from the case with macrobes, but identifying macrobial individuals to species is not always straightforward or precise (Box 2). For example, many macrobial groups, such as insects, are often named as arbitrary and nonmonophyletic **morphospecies**, especially in highly diverse ecosystems such as the tropics.

Delimiting Species

Identifying the species to which individual organisms belong, assumes that species exist in the first place. It has been argued that the prevalence of **parasexuality** among microbes precludes the use of a common species currency for macrobes and microbes. Because of parasexuality, rates and extents of genetic recombination can vary among microorganisms. The rare but promiscuous exchange of genes among unrelated taxa has the potential to fundamentally alter the species currency for microbes because it can decouple traits and lineages. Traits can spread across unrelated lineages if there is strong selection, as can happen with the spread of antimicrobial resistance genes among pathogens. However, recent studies have provided strong evidence that many ecologically important traits are phylogenetically conserved within microbial lineages (e.g., [35]), suggesting that such genetic exchange is not so widespread or frequent that it reduces the utility of microbial taxa. As a result, while the definition of microbial taxa may depend on the question being asked, they nonetheless represent stable and useful units of study, just as for macrobes.

Glossary

16S rRNA gene: in microbial ecology, the structural gene that encodes the 16S small subunit of the ribosome. It includes both highly conserved and hypervariable regions, which are used for primer design to capture broad phylogenetic diversity and for assessing phylogenetic divergence, respectively.
Abundance–occupancy relationships: generally positive relationship between the mean abundance a species attains at individual sites, and the number or proportion of all sampled sites at which it is found.
Biodiversity: variety of species. Biodiversity can be measured using the currencies of individuals and species. These currencies can be used to estimate biodiversity for local communities, planet Earth, and every scale of spatial observation in between.
Diversity gradients: assessment of how the number of species changes as function of an environmental gradient.
Exact sequence variants: practice of defining highly resolved microbial taxonomic units by identical nucleotide sequences of marker genes. Also called amplicon sequence variants, sequence variants, oligotypes, and zero-radius OTUs.
Fisher's alpha: alpha diversity metric that considers the relationship between the number of species and the number of individuals within species.
Functional redundancy: concept that, within a microbial community, there are several microbial taxa that are capable of performing the same function in the same conditions, and, presumably, at the same rate.
Genetic barcoding: sequencing of taxonomically informative marker genes amplified from individuals.
Housekeeping gene: in microbial ecology, a gene that is present in only one copy within a microbial genome and encodes a function necessary for life (typically involved in central metabolism).
Intergenic spacer (ITS): marker sequence flanked by ribosomal operons that is used to phylogenetically distinguish

Box 2. Primary Currencies of Individuals and Species

Counting theindividual. Even though counting individuals can at first seem straightforward for macrobial biologists, counting of animals or plants relies on simplifying assumptions made within taxonomic subfields (Table I). However, these challenges have not prevented progress in understanding the global patterns in the distribution and diversity of species or the general rules that drive them.

Assessment of individuals is similarly challenging for microbiologists. Counting individual cells was traditionally performed with microscopy, which does not accurately reveal taxonomic identity. Individual microbes and their taxonomic identity are often estimated using molecular approaches like marker gene studies, such as those amplifying and sequencing of bacterial and archaeal **16S rRNA genes**. Quantitative PCR of 16S rRNA genes is used as an estimate of community size, though this value is imprecise because different taxa can have different numbers of 16S rRNA operons. A recent meta-analysis similarly estimated a mean community 16S rRNA gene copy number of 2.2 among free-living bacteria and archaea [17], which supports a trend towards low 16S rRNA gene operon copies per the average cell. Although not widely applied, there are bioinformatics methods to correct for the number of operons per genome (e.g., [74]), however some argue that the information is still too limited to apply such corrections accurately [75]. Alternatively, quantification of a single-copy **housekeeping gene** can be used to enumerate community size.

Despite the limitations of using 16S rRNA genes or similar to count individuals [76,77], macroecological patterns emerge from these types of data. However, with new tools for counting individuals from shotgun metagenomes [78–80], improvements in coverage and quality of high-throughput sequencing and analysis [81] and the use of single-copy marker genes for diversity [82,83], microbial ecologists are poised to increase precision. It is time to no longer be distracted by the limitations of present-day methods [84], adopt standard best practices in sequence analysis, and move forward in using the best quantifications currently available to boldly count individual microbes within their communities.

Counting the species. Species has historically been chosen as the primary unit in studies of plant and animal communities because it is believed to be the smallest consistent unit of variety representing important ecological differences (in life history, optimal growth conditions, resource use, etc.), although these assumptions have been challenged for plants and animals. For macroorganisms, species are often based on morphological characteristics and mating capacity, but still, there are many cryptic species.

Defining a microbial species is also challenging [85,86]. Therefore, microbial ecologists that use molecular approaches, such as sequencing of the 16S rRNA gene, apply an **operational taxonomic unit (OTU)** definition in lieu of species. OTUs are just that: operational, and so they can be defined using whatever method is biologically or statistically defensible. There are examples in which OTU definitions matter for microbial macroecology (e.g., [44]), and others in which they do not (e.g., [26]). In addition, although the 16S rRNA gene is the most common target for bacteria and archaea, microbial functional genes [82], such as the nitrogen fixation gene, *nifH* [87], are also used in microbial ecology to count taxa in terms of their functional traits. For fungi, **intergenic spacer** regions (ITS) are often used to define OTUs. In summary, OTUs can be created from any gene that has nucleotide variation.

There are different methods employed to cluster similar sequences together into an OTU. Most require that a sequence identity cut-off be chosen for the OTU (97% is standard, but 98%, 99%, and 100% cut-offs – **exact sequence variants** – have also been applied). There are a variety of clustering methods available, from those that rely on a well-curated reference database to those that define OTUs *de novo* for every study [88], and it is beyond our scope to discuss them all here, except to say that it has important consequence for OTU definitions [88–90]. Regardless of which OTU definition is applied, a consistent OTU definition is necessary in comparative or meta-analyses among datasets.

Notably, if a 97% sequence identity definition was applied to a similar gene in mammals, it would result in grouping all of the primates (from lemurs to humans) into one taxon. However, we disagree that this suggests that the species currency is fundamentally different for microbes. Macroecological processes function at multiple taxonomic scales and macroecological patterns have been documented for macrobes at various taxonomic [91] and phylogenetic levels [92], including genera and families. As mentioned above, changing the sequence similarity cut-off (essentially sliding from subspecies through species to genera and families), can provide important macroecological information. Macroecologists should view this example set by microbial ecologists as an encouragement towards taxonomic agnosticism. Such agnosticism would support integration around patterns (instead of unmatched species definitions), inform as to which resolution of taxonomic units are most ecologically meaningful, and provide a full understanding of biodiversity patterns across phylogenetic scales.

eukaryotic microorganisms, especially fungi.

Macroecology: study of the rules and mechanisms (processes) underpinning general patterns of ecology across scales [2].

Marker genes: in microbial ecology, genes and their sequences that have been used as a signature of microbial diversity. An example is the 16S rRNA gene for bacteria and archaea and the **ITS** region for fungi.

Mesocosm: small container containing organisms and substrate that can be replicated and manipulated in the laboratory. Microbial mesocosms can have natural or artificial substrate, like soil or microbiological medium, respectively, and can be seeded with wild communities from a particular habitat or inoculated with specified cultivable members. It is expected that the influences of captivity away from nature (sometimes called container effects) can be minimized in microbial mesocosms. This is because microbial individuals, and their expected effective ranges for interactions with each other and with their environment, are small relative to the volume of the container.

Metabarcoding: sequencing of taxonomically informative marker genes amplified from an environmental sample that contains mixed populations or communities. General primers that target a conserved nucleotide sequence are used to amplify the signal of marker genes from a mixed microbial community. These sequences are typically multiplexed for sequencing, and then they can be used with databases of known sequences to build phylogeny, assign taxonomy, assess alpha diversity, and create an species-by-sample table (OTU table, as in Figure 1A) for community analysis.

Metagenomics: sequencing of all nucleic acid extracted from an environmental sample, without targeted amplification. Also known as shotgun metagenome sequencing, this method is commonly applied to microbial communities to assess functional potential by annotating sequences against a database of known functional genes.

Microorganisms: broadly defined as those organisms too small to be

Table I. Examples of Biases in Counting Macrobial Individuals

Macrobial community	Challenge in counting the individual
Trees	Seed banks and seedlings less than an arbitrary diameter excluded from surveys; clonal or modular individuals are difficult to distinguish (e.g., *Populus*)
Birds	Arbitrary decisions are made about when and where to count migratory birds
Social insects (e.g., ants and bees)	Trade-off in deciding to practically count individuals versus more precisely count colonies, which are the biological unit on which natural selection acts
Benthic invertebrates	Arbitrary decisions made about mesh size for sieving prior to counting individuals (e.g., all individuals under a certain size are excluded)

Comparable Methods

Some have suggested that contemporary microbial community methods, which typically rely on sequencing from the environment, are fundamentally different from those approaches used to observe individuals and species for macrobes. However, there also are biases in approaches to observe macrobial communities (Box 2). Furthermore, macrobial communities increasingly are observed with metabarcoding methods as sequencing prices plummet. This approach is essentially identical to that used by microbial ecologists.

In short, although there are real challenges in counting both macrobes and microbes, the challenges are more similar between these groups than they are different. As more biologists studying macrobes use molecular (and, particularly, **metagenomic**) approaches, the differences between them will shrink further.

Unified Accounting: Understanding Patterns in Diversity over Space and Time

Regardless of real and perceived differences in tallying macrobes and microbes, there is a primary data structure that is universal to the analysis of biodiversity: a site-by-species matrix, (including presence–absence or abundances; Figure 1A). From this matrix, we can assess patterns of diversity and ask how these patterns scale over space or time [36]. Below, we consider six common patterns in macroecology that can be assessed using the site-by-species matrix. We selected examples from our collective works and the published literature to illustrate how these macroecological patterns of microbes and macrobes can be similar. These datasets (Table S1 in the supplemental information online) are intended to serve as examples of the kinds of patterns that can be discovered, and are not representative of all macrobial and microbial communities. Later, we will discuss how these patterns are interconnected.

Species Abundance Distributions

One of the most fundamental patterns in community ecology and macroecology is the species abundance distribution (SAD). Typical SADs describe communities that have a few species that are highly abundant and many species that are rare; indeed, this has been suggested as one of the true universal laws in ecology [37,104]. Notably, every SAD represents a sampled subset of the true SAD for the whole community. There is some indication that spatial aggregation of species can inflate the representation of rare taxa in the sampled SADs [34]. Although we do not expect any aggregation bias to be different between microbes and macrobes, understanding

visible with the naked eye, including viruses, bacteria, archaea, protists, a subset of fungi, or even the smallest arthropods (such as face mites). When evolutionarily defined, microorganisms include the domains of bacteria and archaea (previously, prokaryotes), which were the first evolved lineages that through endosymbiosis gave rise to eukarya.

Morphospecies: species concept that is based on morphology, and is commonly used in the fields of entomology and botany. Unidentifiable individuals with shared physical characteristics are grouped artificially into an operational taxonomic unit without reference to other distinguishing traits.

Occupancy: number or proportion of sites in which a species is detected.

Operational taxonomic unit (OTU): approximations of species that are commonly used in the field of microbial ecology, arbitrarily defined as informed by the technology used to observe the microorganisms. For example, 16S rRNA gene amplicon sequencing datasets often define OTUs at 97% gene sequence identity. Thus, all sequences that are 97% similar would be counted towards a single OTU.

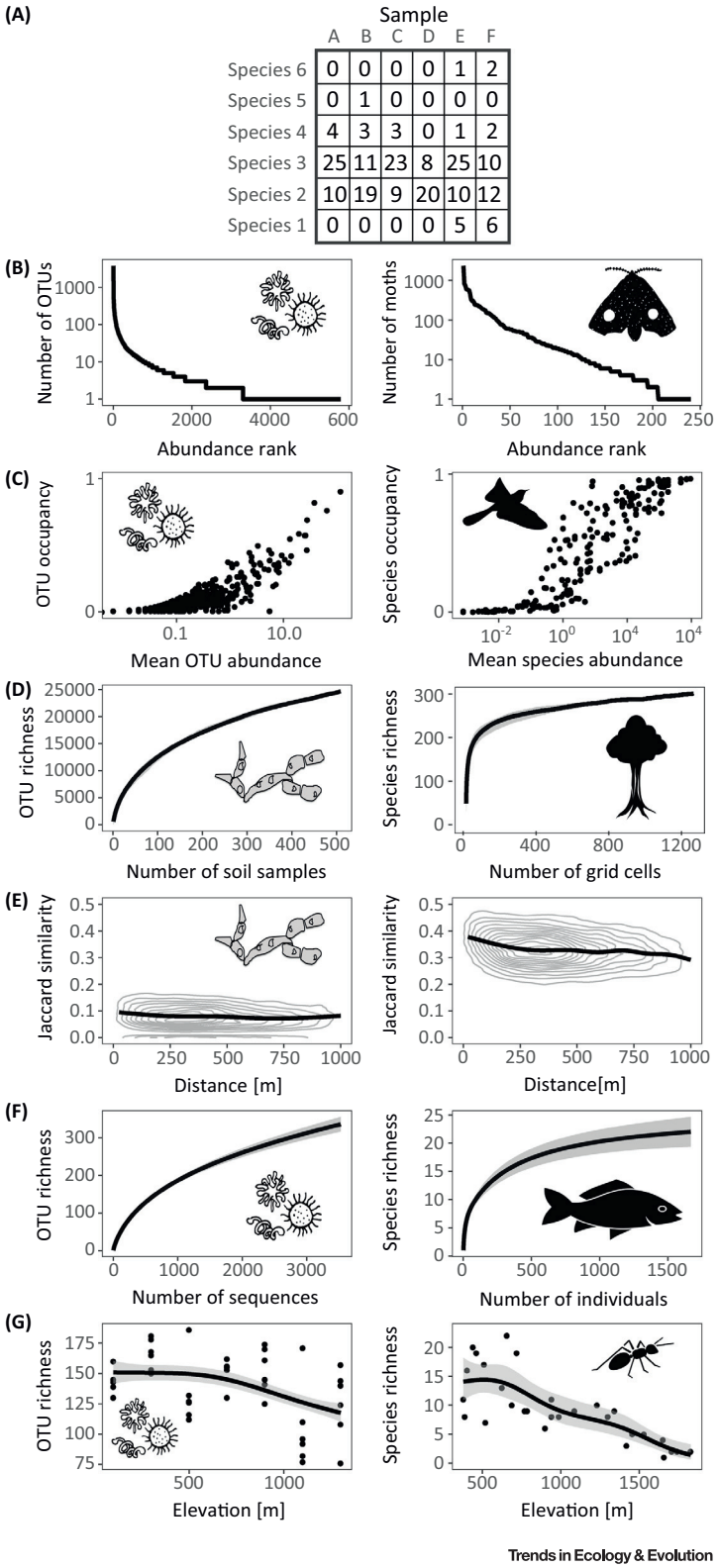
Parasexual: nonsexual mechanisms for transferring genetic material, common among single-celled organisms like bacteria, archaea, protists, and fungi.

Singletons: within a dataset, taxa that are observed only once and in an abundance of one individual. In microbial ecology, this often refers to a singly observed unique sequence of a marker gene.

Species abundance distribution: depicts the number of individuals (N) of each species in a sample, and is often expressed as a relationship between the logarithm of N plotted against species rank (from the most to the least abundant species).

Species–area relationship: relates the number of species (S) to the area of the plot (gray squares) in which species richness is sampled (A). In the nested SAR, larger areas should be therefore contiguous and should encompass all the smaller areas. However, empirical SARs are often constructed based on much smaller

samples, which are assumed to be representative of the whole contiguous and mutually adjacent areas.



(See figure legend on the bottom of the next page.)

Box 3. Microbial Systems in Macroecology: Advantages, Contributions, and Frontiers

Microbial systems, which include *in situ* communities and controlled laboratory models, boast an often-understated legacy of providing foundational insights into ecology and evolution. Microbial systems have contributed to our understanding of, among other topics, long-term evolutionary processes [93], island biogeography [94,95], and dispersal limitation and metacommunities [96]. The utility of microbial systems for ecology has been detailed previously [97]. They offer several advantages, including: efficient observations at temporal and spatial scales that are compressed relative to their macrobial equivalents; molecular tools for characterizing population dynamics; and controlled manipulations of experimental treatments and community biodiversity. Microbial laboratory models include synthetic or simplified microbial communities and **mesocosms**, and have been suggested as an important tool for advancing macroecology [98]. There is an especially rich legacy of using microbial mesocosms in community and population ecology (e.g., [99–101]). The capability to complement *in situ* observations and reductionist models can provide a rich understanding of macroecological patterns and their underlying processes [102]. In addition, because related lineages or similar functional guilds of microorganisms are found across otherwise disparate habitats, microbial systems also offer a common denominator that can be leveraged for cross-ecosystem comparisons and in support of a unified macroecology (e.g., [103]). In summary, microbial systems continue to offer exciting methods that yield insights for macroecology.

differences in aggregation among taxa (be they microbes and macrobes or just different kinds of microbes) will be key to truly generalizing SAD relationships. Here, we show examples of SADs for groundwater bacterial communities and moths, both of which show the characteristic pattern, albeit with some structural differences in the distributions of rarity which we discuss in more detail below (Figure 1B).

Abundance–Occupancy

Another macroecological pattern is revealed when considering the relationship between species abundance and **occupancy** (Figure 1C). Here, we provide examples of **abundance–occupancy relationships** for microbiota sampled from human umbilici and for birds observed in the Czech Republic. Both datasets show that species that tend to have high abundance within one site also tend to occupy many sites, while those that are locally rare tend to not be detected in many sites [4]. Abundance–occupancy patterns have been applied in microbial ecology to create null or neutral expectations about the drivers of community structure [38]. There are many factors that can influence abundance–occupancy relationships. Microbial laboratory models (Box 3) offer a useful approach to assessing the specific influences of biotic interactions and habitat heterogeneity in microbial abundance–occupancy patterns [39]. In the microbial ecology literature, some have argued that deviations from a null hypothesis are suggestive of deterministic drivers of community structure [21,38,40,41]. For example, taxa that are abundant only in a few sites or rare taxa that are consistently observed in many sites would be exceptions to the neutral expectation.

Species–Area Relationships

Species–area relationships (SARs) assess the increase in species richness with increasing spatial area (Figure 1D). The shape and slope of the SAR can be derived from the knowledge of some properties of species distributions [42], such that the SAR can be used to predict and compare changes in diversity over increasing spatial extent. However, there are nuances to its

Figure 1. Examples of Macroecological Patterns from the Microbial (Gray) and Macrobial (Black) Realms. (A) Site-by-species matrix, where samples/communities are provided in columns (sites) and species/taxonomic units (species) in rows. From this table, all subsequent patterns of diversity can be derived, such as (B) rank-abundance curves, (C) occupancy–abundance relationships, (D) species–area curves, (E) distance–decays of similarity, (F) rarefaction curves, and (G) elevational richness gradients. Thick lines in (D) and (F) are means of the simulated species–area and rarefaction curves, and gray ribbons are 95% quantiles of the simulations. Thick lines in (E) and (G) are means modeled by Generalized Additive Model (GAM) splines. Gray contours in (E) show density of the data, gray ribbons in G are 95% confidence intervals of the splines. Data sources for panels (B–G) are in Table S1 in the online supplemental information. For licensing information on the inset icons see Acknowledgments. Abbreviation: OTU, operational taxonomic unit.

application, especially for microbial communities, because of practical challenges in sampling contiguous areas. In the nested SAR, larger areas should be contiguous and encompass all the smaller areas therein. However, empirical SARs are often constructed by a collection of samples from smaller areas (here, we call these piecemeal SARs for clarity), which are assumed to be representative of the whole contiguous and mutually adjacent area. SARs have been extensively examined in many microbial communities [43–46], using the piecemeal approach because of the necessity of destructive sampling for DNA extractions. Such piecemeal SARs are predicted to be more curvilinear in the log–log scale due to the limited total number of individuals at small areas [42,47], and their slope is predicted to be higher due to lower occupancies of individual species [42]. Thus, care is needed when constructing and interpreting nested and piecemeal SARs. Our example shows increases in fungal community richness at Barro Colorado Island (BCI) as compared to tree richness at the same location (but note differences in x and y scales).

Distance–Decay

Distance–decay relationships assess how community similarity or beta diversity [48] changes over space (Figure 1E). Distance–decay is used to address compositional turnover (using unweighted resemblance metrics, like Jaccard) or shifts in relative abundance (using weighted resemblances, like Bray–Curtis) with distance from a reference community. The slope of the distance–decay relationship is interpreted as a rate of change over space, and there are macroecological studies as well as microbial-focused studies that have compared these rates [6,7,49–51]. Our example shows the same BCI fungal and tree communities from Figure 1D, but because the Jaccard metric can be calculated for both, their rates of decay in similarity can be compared directly on the same y-axis scales, although some caution is necessary when comparing trees with microbes, since the area (grain) of the samples differs [6,7,52–54].

Rarefaction

Rarefaction assesses how richness accumulates with the number of individuals or samples observed (Figure 1F). Here, we use individual-based rarefaction curves to compare how species richness accumulates with increasing numbers of individuals (after eliminating spatial structure via randomizations, [55]). We show English Channel bacteria and archaea and Celtic Sea fishes. In microbial ecology, rarefaction is commonly used to assess completeness of sequencing effort for a dataset. The y axis for a rarefaction of microbial sequences reveals the number of taxa observed for each additional sequence collected within a community (increasing sequencing depth – observations of individuals). This is distinct from a sample-based rarefaction analysis that reveals the number of species observed for each additional community observed (increasing sampling – observations of communities).

The first four features of diversity matrices we have described above are intrinsic to the matrices. Each of these features can, as we have shown, be calculated just as readily for microbes as for macrobes. Once these aspects of diversity are estimated, they can be compared along geographic (e.g., latitude and elevation) and environmental (e.g., energy and disturbance) gradients (**diversity gradients**, Figure 1G). Moving forward from these comparative analyses, we can address paramount questions in macroecology: if some patterns in biodiversity are the same for microbes and macrobes, are the underlying processes also the same? Also, do similar processes lead to different patterns?

The abovementioned macroecological patterns are related to each other, and each can be used to inform the others (e.g., [56]). When there is a predictable relationship between abundance and occupancy, there is also a link between the SAD and the probability distribution

of the proportion of available area (or available set of sites). Species richness for a given area can be calculated as the sum of probabilities of occurrence across all species, and the SAR thus can be reconstructed using knowledge of species occupancy patterns in each spatial scale [42]. Therefore, if we know the SAD for some large area and the level of spatial aggregation of individuals of every species (which determines occupancy patterns across spatial scales), we can derive all the other macroecological patterns. Moreover, these links work in all possible directions. For example, it is possible to derive the SAD from scale-dependent patterns of species aggregation [52]. Although these links are complex, the general insight is that patterns of species rarity and occupancy are directly linked to scaling patterns in species richness. Indeed, the rarer the species are on average, the faster the number of species increases with area or number of samples, and the higher are the differences in community composition between neighboring areas or samples (i.e., higher beta diversity). A comprehensive understanding of patterns of diversity, distribution, and abundance (which is one of the main goals of ecology) thus depends on understanding these links among major macroecological patterns.

Rarity: Exception or Statistical Inevitability?

Our illustration of macroecological patterns among microbes and macrobes (Figure 1B–G), reveals similar shapes in general, as expected from major macroecological theories, but notable differences that are all related to higher rarity in the microbial realm. The species abundance distribution has proportionally more **singletons** for microbes from groundwater compared to Fisher's moths (Figure 1B); the occupancy of bacteria in human umbilici is lower than the occupancy of birds among sites in the Czech Republic (Figure 1C); the fungi continue with an appreciable slope as the trees have tapered in their species–area curves of the BCI data (Figure 1D), which is also reflected by the lower similarity in species composition among even nearby fungal samples (Figure 1E); finally, the accumulation of new taxa with increasing numbers of marine microbes has not slowed as appreciably as the marine fishes (Figure 1F).

While the vignettes presented in Figure 1 suggest possible differences in rarity between microbes and macrobes, they are anecdotal. Nevertheless, we illustrate a similar preponderance for rarity in microbes in a systematic comparison of >14 000 macrobial and microbial SADs (Figure 2). As sequencing technologies have improved and coverage of microbial communities has increased, it has often been noted that many microbial communities have a high proportion of rare taxa [53–55]. Subsequently, it was shown that some rare microbial taxa can provide specific and important functions within their communities [57].

To consider a particular aspect of rarity, microbial communities often include a large number of singletons. It has been argued that singletons might not be real individuals (e.g., [58–60]) but an artifact of sequencing methods. As such, singletons are removed prior to analysis [22,61,62]. However, singletons are a general feature of ecological communities (e.g., [63,64]) and provide a potential quantitative point of comparison between microbes and macrobes. We argue that microbial singletons from high-quality sequences should not be arbitrarily removed. Study-to-study variability in whether to include microbial singletons presents a hurdle to the common accounting required for cross-dataset comparisons in macroecology.

Communities become increasingly uneven with increasing numbers of individuals [65], and rarity also increases with more individuals [26]. However, for a given community size, microbial communities have more rarity than macrobial communities [26]. There are ecological reasons to explain rarity, including transiency (vagabonds), recent speciation, local extinction, and negative frequency dependence [63,64,66,67]. Future work should be directed to testing

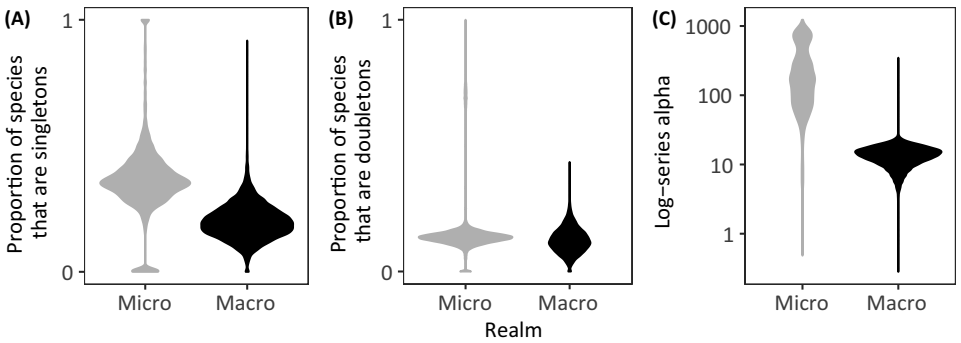


Figure 2. Rarity Is a Distinctive Ecological Feature of Microbial Communities. Microbial data (gray) are from [26]; macrobial data (black) in panels were downloaded using the R data retriever [68] (n = 14 980 for both microbes and macrobes). In general, microbial communities have proportionally more singletons (A) than macrobial communities. Doubletons (B) are more comparable, with a wider observed range and more bias observed in microbial doubletons. Fisher's alpha (C) is notably higher in microbial communities as compared to macrobial communities.

ecological hypotheses concerning the mechanisms supporting rarity and singletons generally, and specifically within microbial communities.

A Call for a Unified Macroecology of All Life, Large and Small

Moving forward from the understanding that species and individuals provide basic units from which a unified macroecology can emerge, we must systematically observe and compare macroecological patterns across macro- and microorganisms. The next steps are to understand the processes that underlie the patterns, determine their generality, and use them to inform a grand, macroecological view of the rules and exceptions of life (Figure 3 and Box 1; see Outstanding Questions). It is important to understand when microbes are distinct from macrobes in pattern, as these distinctions can inform process. There are two particularly intriguing scenarios: one in which divergent patterns result from the same process (Figure 3ii), and one in which convergent patterns mask distinct processes (Figure 3iii). Divergent and convergent scenarios simultaneously offer a challenge and an opportunity towards a unified macroecology. The challenge is that microbial ecologists often struggle with determining processes *in situ* because observations are difficult and methods reliant on available technology and its limitations. The opportunity is that laboratory microbial models offer the ability to manipulate and control systems to explicitly test macroecological hypotheses of processes; an experimental luxury that is uncommon for communities of macrobes because of logistical constraints in scale, expense, and, sometimes, ethics (Box 3). After standardizing language and a conceptual framework, a priority should be to systematically determine which scenario in Figure 3 applies to which macroecological comparison. Microbial ecology especially will benefit from advancement towards synthesis, and macroecology provides a foundation for this pursuit. A unified synthesis of macroecology is needed and imminent.

There are also cultural and infrastructural silos to overcome before a truly unified macroecology can be achieved. Patterns and processes typical of microbial communities provide value and insights for macroecology, even when they are distinct from the patterns and processes of macrobial communities. In publication and funding, microbial ecology should be considered equitably and not as a subspecialty with limited scope or utility. Collaborations between macrobial and microbial ecologists are key for advancing a unified macroecology, first to understand jargon, culture, and methods and limitations, and as a next step to tackle together

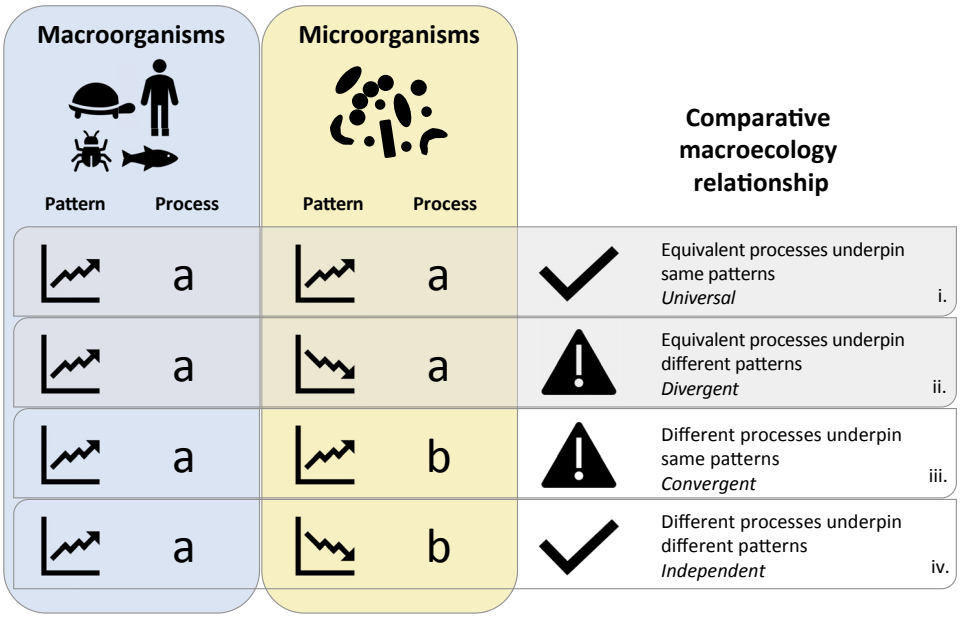


Figure 3. Conceptual Framework for Comparing the Relationships between Patterns and Processes across Macro- and Microorganisms. Different relationships are represented by different letters. (i) Universal is when macroecological patterns agree between microbes and macrobes, and result from the same processes despite nuances or variability in exact mathematical properties, like the exponents of species–area relationships and metabolic scaling (Box 1). (ii) Divergent is when equivalent underlying processes result in different patterns for microbes and macrobes. (iii) Convergent is when microbes and macrobes exhibit the same patterns, but the patterns are attributable to fundamentally different mechanisms. Divergent and convergent relationships are difficult to characterize without measurement of potential processes. Convergent patterns in particular can be overlooked because similar patterns are often assumed to be underpinned by similar processes when the processes are yet uncharacterized. (iv) Independent is when microbes and macrobes exhibit distinct patterns that are also underpinned by distinct processes. In independent relationships, both sets of patterns and processes are equally valuable in informing a unified macroecology.

Outstanding Questions

How does the inclusion of microbial metabolic and phylogenetic diversity impact our understanding of macroecological patterns of biodiversity?

How does the SAR change at microbially relevant spatial scales?

How does the inclusion of microbial spatial scales impact the overall shape of the SAR?

Are patterns of microbial rarity, or the mechanisms that underpin them, distinctive from those in plant and animals systems?

What is the relationship in the scaling of functional and taxonomic biodiversity, and how does microbial **functional redundancy** contribute?

How does biodiversity change over time, and how does the inclusion of microbially relevant temporal scales impact these patterns?

How do macroecological patterns in space relate to and intersect with those in time?

select questions. Long-term working groups, focused workshops, and integrated sections in professional societies can provide infrastructure for research efforts, and these should include opportunities for trainees to contribute. Collaborative mentoring of students and post-docs, who can bridge micro- and macro- advisers and move forward working group research initiatives, is another mechanism by which macroecology can aim to unify with the next generation of inspired ecologists.

Let us move forward together, away from the artificial delineation in the ecological study of micro- and macroorganisms and towards an encompassing macroecology, inclusive of all biodiversity.

Acknowledgments

This piece is the product of a Micro–Macro Working Group initiated by J.C. and K.K. that met at, and was supported by, the German Centre for Integrative Biodiversity Research (iDiv) in September 2017. J.C. acknowledges financial support from the Deutsche Forschungsgemeinschaft (DFG) FZT 118. A.S. acknowledges support in part from the National Science Foundation under Grants DEB #1655425 and DEB #1749544, from the USDA National Institute of Food and Michigan State AgBioResearch, and from the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018409. N.J.S. acknowledges

support from a Semper Ardens grant from the Carlsberg Foundation. D.S. acknowledges support from the Czech Science Foundation (grant no. 16-26369S). With the exception of the fungi in Figure 1D,E, all inset icons in Figure 1 are from the Noun Project under CC license: Microbes by Dima Lagunov, moth by Carpe Diem, bird by Ian Graham, fish by Andy Mc, tree by Rayhan Maulana Rikzan, and ant by Cédric Stéphane Touati.

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tree.2018.08.005.

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