Title: Microbial functional diversity and redundancy: moving forward

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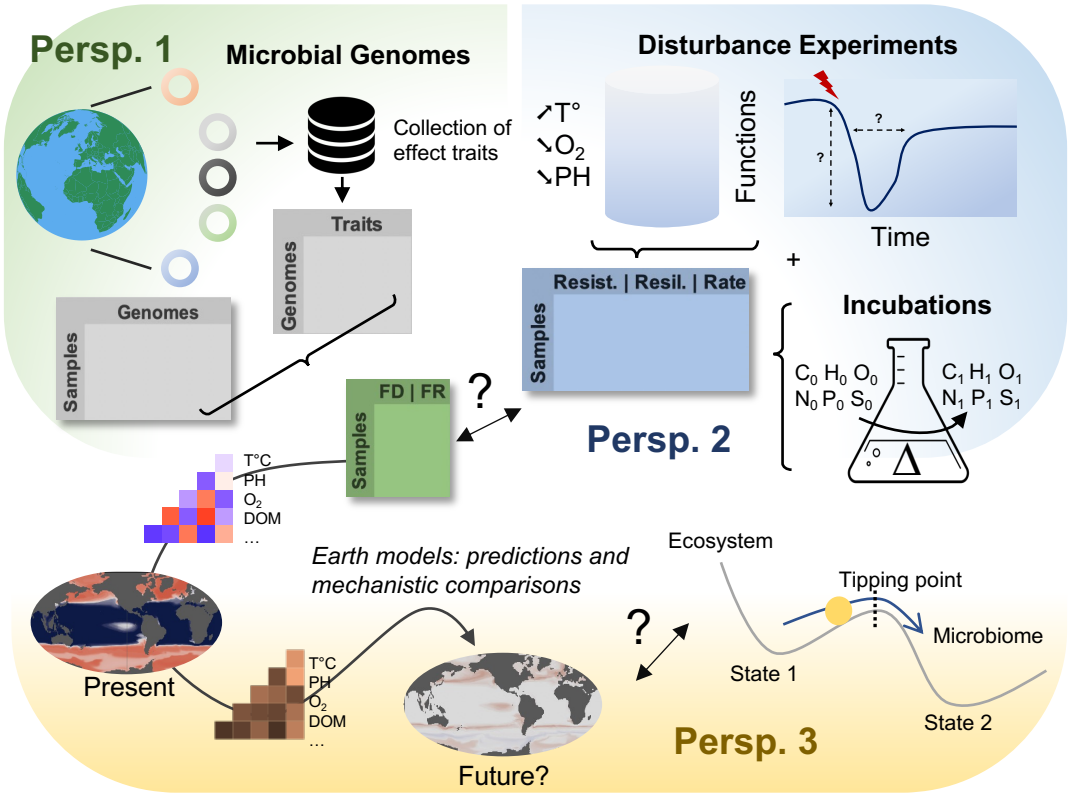

Feature Figure: Summary of our perspectives for studies of microbial functional diversity and redundancy. We outline our first perspective (Persp. 1): retrieving microbial genomes from multiple biomes, studying the presence of a validated set of effect traits across taxa, and computing standardized metrics of their functional diversity and redundancy. Our second perspective (Persp. 2) consists of studying the importance of microbial functional diversity and redundancy for the rate (measured with incubations with labeled elements), resistance, and resilience (measured in disturbance experiments) of microbial ecosystem processes. Our final perspective (Persp. 3) focuses on predicting future patterns of microbial functional diversity and redundancy in order to identify tipping points for microbiomes that could lead to shifts in ecosystems. This work could be based on linking the patterns of microbial functional diversity and redundancy with environmental variables for which future predictions exist.

Abstract: Microbial functional ecology is expanding as we can now measure the traits of wild microbes that affect ecosystem functioning. Here, we review techniques and advances that could be the bedrock for a unified framework to study microbial functions. We then explore the technical, ecological, and evolutionary processes that could explain environmental patterns of microbial functional diversity and redundancy. Next, we suggest reconciling microbiology with biodiversity-ecosystem-functioning studies by testing the significance of microbial functional diversity and redundancy for the efficiency, resistance, and resilience of ecosystem processes. A better understanding of how microbiomes affect ecosystems is crucial to predicting their functioning in a changing planet.

Functional ecology and microbiology

Through functional ecology, it was demonstrated that the relationship between biodiversity and **ecosystem functioning** is generally positive, with biodiversity positively affecting the efficiency of resource uptake, biomass production, decomposition, or nutrient cycling performed by various ecological communities (Cardinale *et al.* 2012). Nevertheless, determining how environmental change, diversity, and ecosystem processes interact remains a great challenge (Loreau 2001). Microbiology has gradually adopted this functional perspective.

Microbes have colonized all habitats on Earth where they drive major ecosystem processes and represent an important part of the standing biomass (Bar-On, Phillips and Milo 2018; Bar-On and Milo 2019). Bacteria and Archaea (prokaryotes) have developed a vast array of metabolisms, that is, enzymatic and metabolic pathways, that directly affect the cycles of hydrogen, carbon, nitrogen, oxygen, sulfur, or iron (Falkowski, Fenchel and Delong 2008). In turn, protists (microbial eukaryotes) influence Earth's biogeochemistry through primary and secondary production (Massana and Logares 2013), participating in many microbial interactions, affecting the availability of organic matter, and its transfer to the rest of the trophic foodweb (Worden *et al.* 2015; Keeling and del Campo 2017). The functioning of ecosystems is therefore closely linked to the microorganisms they contain. In a changing planet, it is crucial to understand the relationship between these two entities. Part of the challenge in relating microbiomes to ecosystem functioning is to integrate the multi-dimensional nature of biodiversity. This includes a taxonomic, phylogenetic (the evolutionary history and relatedness between organisms), and functional dimension (the range of things that organisms do that affect ecosystems) (Petchey and Gaston 2006; Diaz *et al.* 2013), which may change across ecological scales (Ladau and Eloe-Fadrosh 2019).

Functional ecology relies on **traits** (Streit and Bellwood 2023). These are any genetic, morphological, or physiological features that can be measured at the individual, species, or community levels. Ecologists have focused on traits that are proxies of a) an organism's response to environmental change (*response trait*s), or b) of an organism's effects on

ecosystem functioning (*effect traits*) (Lavorel and Garnier 2002; Violle *et al.* 2007). Assembly processes acting upon the *response traits* of organisms (these include biotic and abiotic selection, dispersal, speciation, or ecological drift) (Vellend and Agrawal 2010), and the *effect traits* harbored by the members of the assembled community (Diaz *et al.* 2013), are thought to be responsible for the emergent ecosystem functioning. Relevant traits among microbiomes have been identified (Fierer, Barberán and Laughlin 2014; Litchman, Edwards and Klausmeier 2015; Escalas *et al.* 2019), such as metabolic traits, which are direct indicators of the processes (resource uptake, decomposition, nutrient cycling) that microbes are able to perform (Martiny *et al.* 2015). As is the case for multicellular organisms (Lavorel and Garnier 2002; Diaz *et al.* 2013), evidence was found on the dual nature of resource utilization traits, serving both as response and effect traits (Litchman, Edwards and Klausmeier 2015; Martiny *et al.* 2015). For example, an organism able to degrade cellulose, can be predicted a) to perform poorly in the absence of cellulose (response trait) and b) to degrade cellulose in ecosystems where it is present (effect trait) (Martiny *et al.* 2015).

By studying the distribution and patterns of *response traits* ecologists investigate the basis upon which the environment affects the composition of communities. In turn, by studying *effect traits* ecologists have progressively unveiled the mechanistic link between ecosystem processes and a wide range of communities (Petchey and Gaston 2006; Mouillot *et al.* 2013; van der Plas 2019). **Functional diversity** is the breadth of functions that the species are able to perform within an ecosystem (Díaz and Cabido 2001). It can be estimated by identifying the effect traits harbored by the species of a community and measuring their relative abundance (Violle *et al.* 2012). Functional diversity is generally better at predicting ecosystem processes than taxonomic diversity (van der Plas 2019), and the traits harbored by the most abundant organisms are often driving these processes (Grime 1998; Garnier *et al.* 2004). In turn, **functional redundancy**, or functional similarity (Loreau 2004; Nico *et al.* 2023), is the co-existence of species with similar *effect traits* and functional roles within an ecosystem. Functional redundancy may ensure ecosystem functions against disturbance and species loss, maintaining stable ecosystem functioning over time (Yachi and Loreau 1999; Díaz and Cabido

2001; Biggs *et al.* 2020). By accumulating species with the same effects traits, but different ecological strategies, functional redundancy also leads to more efficient resource uptake (Loreau 2001; Loreau M. and Hector A. 2001), and increases the provision of multiple ecosystem functions simultaneously (multi-functionality) (Le Bagousse-Pinguet *et al.* 2019).

Testing the significance of microbial functional diversity and redundancy within ecosystems has been a complex task because of the lack of a unified framework to study microbiomes and their traits (Escalas *et al.* 2019; Lajoie and Kembel 2019). In parallel, several studies indicated an independence between taxonomy and function, suggesting that microbiomes harbored high functional redundancy. For example, a human gut survey showed minimal similarities in the taxonomy of microbiomes among patients, while many microbial **genes** were common and considered essential or core across patients (Turnbaugh *et al.* 2009). In the ocean microbiome, it was found that metabolic functions and taxonomy were driven by different processes (Louca, Wegener Parfrey and Doebeli 2016). Other studies showed high taxonomic variability across spatial scales despite stable patterns of microbial functions (Sunagawa *et al.* 2015; Haggerty and Dinsdale 2017). This suggested that the functions performed by microbes can be carried out by a wide array of taxa, and, thus, that microbes are highly functionally redundant. Nevertheless, other studies found contrasting results. For example, taxonomic and gene compositions displayed a high co-variation in the microbiomes of North-America's prairie soils (Fierer *et al.* 2013). Similarly, the taxonomy and gene content of the marine microbiome in the northwestern Mediterranean Sea showed high covariance over time (Galand *et al.* 2018). For marine protists, the variability in taxonomic composition altered the proportion of protistan **functional groups** across North Atlantic coastal ecosystems (Ramond *et al.* 2019). Altogether, the discrepancy between these results sparked a debate over the extent of functional redundancy in microbiomes.

Understanding how microbial diversity and ecosystem functioning interact is a major goal, especially in the face of global change (Cavicchioli *et al.* 2019). Here, we argue that studying the patterns of functional diversity and redundancy across microbiomes is relevant, timely, and feasible, and will contribute to this goal. We first review the main methods and

results that could be the bedrock for a unified framework of microbial functional diversity. We then focus on the potential drivers of microbial functional diversity and redundancy across biomes. In a final perspective section, we focus on how quantifying microbial functional diversity and redundancy will allow to test their significance for the functioning of current and future ecosystems.

Toward a unified framework of microbial functional diversity

High-throughput DNA and RNA sequencing has provided invaluable information on the genetic content of microbiomes, allowing to infer their taxonomic, phylogenetic, or functional diversity (Bashiardes, Zilberman-Schapira and Elinav 2016; Knight *et al.* 2018). Consequently, various trait-based methods were developed, with the most refined trait level being the gene variant, that is, a nucleotide sequence variant of a gene (Johnson and Pomati 2020). Across surveys, gene variants are first identified and then quantified across microbial species or samples (metagenomes). As not all genes are relevant to the functioning of ecosystems (e.g., housekeeping genes), their function must be defined via annotation with reference databases to determine their ecological relevance (Kanehisa *et al.* 2016; Mistry *et al.* 2021). Specific metabolic processes or other effect traits may also be encoded by multiple genes. Consequently, specific genes can be regrouped per module, chemical reaction, or metabolic step (Kanehisa *et al.* 2016), which represent microbial traits at a coarser resolution. However, the function of many environmental genes still remains unknown (Carradec *et al.* 2018; Salazar *et al.* 2019). As such, genomics has yet to explain the full spectrum of microbial functional diversity (Kysela *et al.* 2016; Lajoie and Kembel 2019). This is especially the case for protists, as linking environmental genes to protistan effect traits remains highly challenging (Keeling and del Campo 2017) (Box 1). Other omics, such as meta-proteomics or metabolomics can also give insights into the abundance of specific traits or substances involved in chemical reactions to complement DNA and RNA-based omics (Johnson C, Ivanisevic J and Siuzdak G 2016; Armengaud 2023). However, these methods have yet to be routinely used and offer little insights onto the microbial units performing these reactions.

Box 1: Protistan functional diversity: further challenges.

The genomes of microbial eukaryotes are larger than those of prokaryotes, have a complex physical structure (including multiple chromosomes), and can contain a huge share of non-coding or repetitive sequences (e.g., telomeres, introns). The reconstruction of microbial eukaryotic genomes directly from the environment is difficult (Caron *et al.* 2016), and biased against lineages with large genomes, low abundance, or high micro-diversity. For these reasons, the genetic potential of microbial eukaryotes has largely been studied with meta-transcriptomics (sequencing of RNA) (Caron *et al.* 2016; Carradec *et al.* 2018), which coupled with RNA polyA-tail isolation, allows to focus on expressed genes (Carradec *et al.* 2018). Even though meta-transcriptomics surveys have shown different ecological strategies among protists (Carradec *et al.* 2018; Zoccarato *et al.* 2022), some authors have argued that the functional roles of marine protists, relying on intricate behaviors and trophic features, can hardly be inferred from genomics (Massana and Logares 2013; Keeling and del Campo 2017). As an illustration, translating genomic information into trophic strategies among phagotrophic taxa has proven particularly complex (Obiol *et al.* 2023). Other authors have discussed using morphological and trophic traits as better descriptors of the functional role of protists in their ecosystem, as these traits are directly related to their trophic strategy, behaviors, and interactions (Litchman and Klausmeier 2008; Ramond *et al.* 2019). This approach is based on annotating morphological and trophic features based on the literature. The selection of traits is motivated by the trait's role in ecological function (Litchman and Klausmeier 2008). A shortcoming of this approach is that it excludes taxa that have never been described, which represent a huge proportion of environmental protistan diversity. In a survey of coastal Atlantic ecosystems, this approach resulted in 47% of annotated taxa from metabarcoding (Ramond *et al.* 2019). Developing a comprehensive trait database compiling morphological and trophic features of all known protists could allow the wider application of such approaches.

Nature of effect traits and functional diversity

Working at a very fine trait resolution, such as the gene variant, and including uncharacterized genetic diversity, will result in a few species sharing similar traits (Galand *et al.* 2018; Salazar *et al.* 2019) (Box 2). Traits will be more species-specific, leading to lower functional redundancy, and a tight coupling between taxonomic and functional patterns (Dlugosch *et al.* 2022). In turn, when working at a coarse resolution (e.g., KEGG orthologs, modules, or biogeochemical steps), the same traits can be found across various microbial species and

phylogenetic lineages (Martiny *et al.* 2015; Louca *et al.* 2018) (Box 2). At such a resolution, distinct species share traits, resulting in high redundancy, and a potential decoupling between taxonomic and functional composition in communities (Louca *et al.* 2018; Yang 2021). Redundancy is also influenced by the traits investigated, as some traits might be more widespread across microbial clades due to horizontal gene transfers (see next section) or convergent evolution, or in turn, be specific to clades (Martiny *et al.* 2015; Louca *et al.* 2018) (Box 3). Different definitions and resolutions for microbial traits thus result in distinct levels of functional diversity (number of traits) and redundancy (number of traits shared by taxa). The debate over microbial functional redundancy thus raises two main points: 1) trait definition must be standardized to compare functional diversity across microbiomes, and 2) the trait resolution that best predicts ecosystem functioning needs to be identified across biomes.

Box 2: Microbial functional redundancy varies with trait definitions

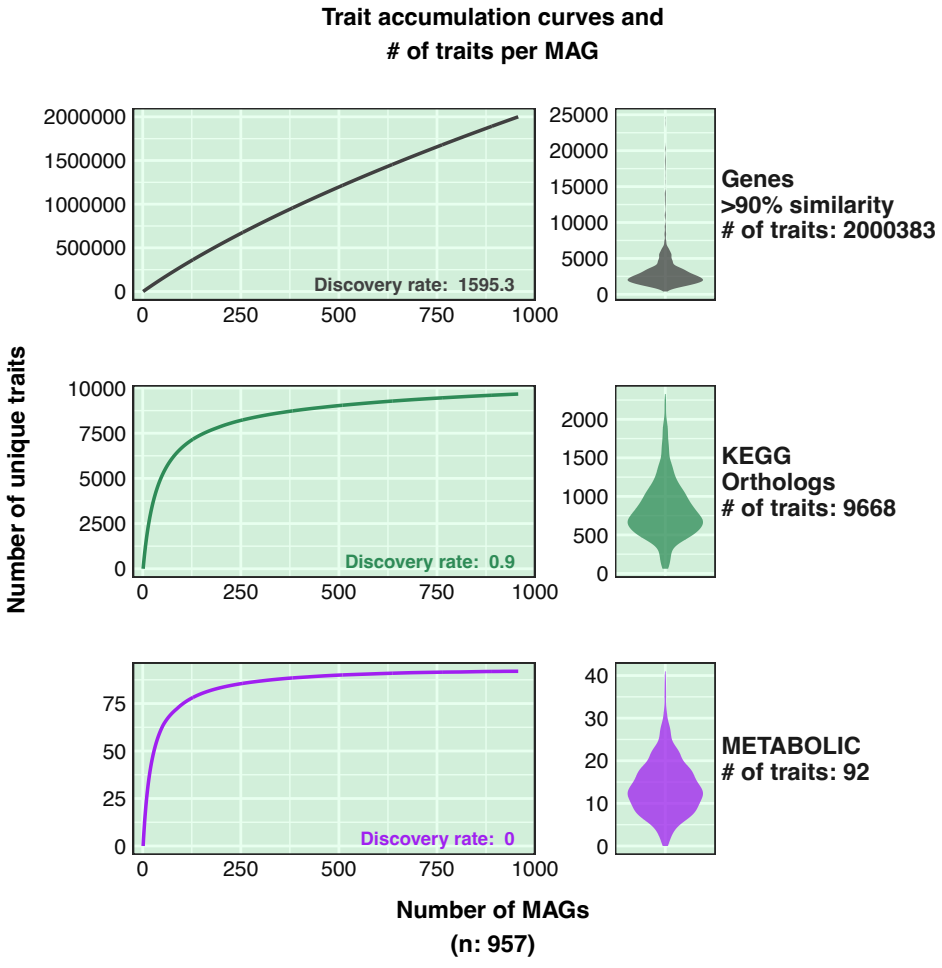

Figure I: Microbial functional redundancy varies with trait definitions. Trait accumulation curves based on 957 Metagenome Assembled Genomes (MAGs), from the surface ocean (Delmont *et al.* 2018). Gene prediction was performed with *Prokka* (Seemann 2014), and *MMseqs2* (Steinegger and Söding 2017) was used to generate the MAGs genes catalog (two steps: 1/ dereplication of the predicted ORFs across all MAGs, and 2/ grouping the ORFs at 90% homology into a catalog. KEGG Orthologs present in each MAG were inferred with *enrichM* (https://github.com/geronimp/enrichM). *METABOLIC* traits (Zhou *et al.* 2022) represent the ability to perform broad-scale biogeochemical processes. Accumulation curves were built using the R package *preseqR* (Daley and Smith 2013). They represent the number of traits (Y axis) retrieved when randomly sampling *n* MAGs (X axis). The number of traits per MAG is represented in the violin plots on the right side of the accumulation curves.

The trait accumulation curves give us insights into (1) the total number of traits observed in the set of 957 prokaryotic draft genomes, or MAGs, (2) whether most traits have been discovered in the set (discovery rate at the end of the curve), and (3) whether traits are generally shared across the MAGs (a straight curve implies that each new MAG brings new traits, in turn, a curve that reaches a plateau suggests that the new MAGs harbor traits already present in the other MAGs), a proxy of functional redundancy.

Among the 957 MAGs, we found about 2 million unique genes (ORFs). At this fine-grained trait resolution, the accumulation curve did not reach a plateau, meaning there was little trait redundancy within the MAGs (Figure I). Grouping genes into KEGG orthologs resulted in fewer traits to describe MAGs (i.e., 9661 unique KEGG Orthologs). With this classification, trait diversity was nearly saturated within the 957 MAGs, suggesting that most KOs existing in this set were found, and that, at this coarse trait resolution, MAGs were more functionally redundant (Figure I). Trait annotation using the pipeline *METABOLIC* (Zhou *et al.* 2022) resulted in 92 traits, e.g., the ability to perform steps in the cycling of nitrogen and sulfur or the degradation of complex organic matter. The curve saturated at a lower number of MAGs (~500) than KOs (~800), and genes (not saturating), suggesting an even higher functional redundancy at this trait resolution.

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Box 3: Redundancy varies across microbial functions

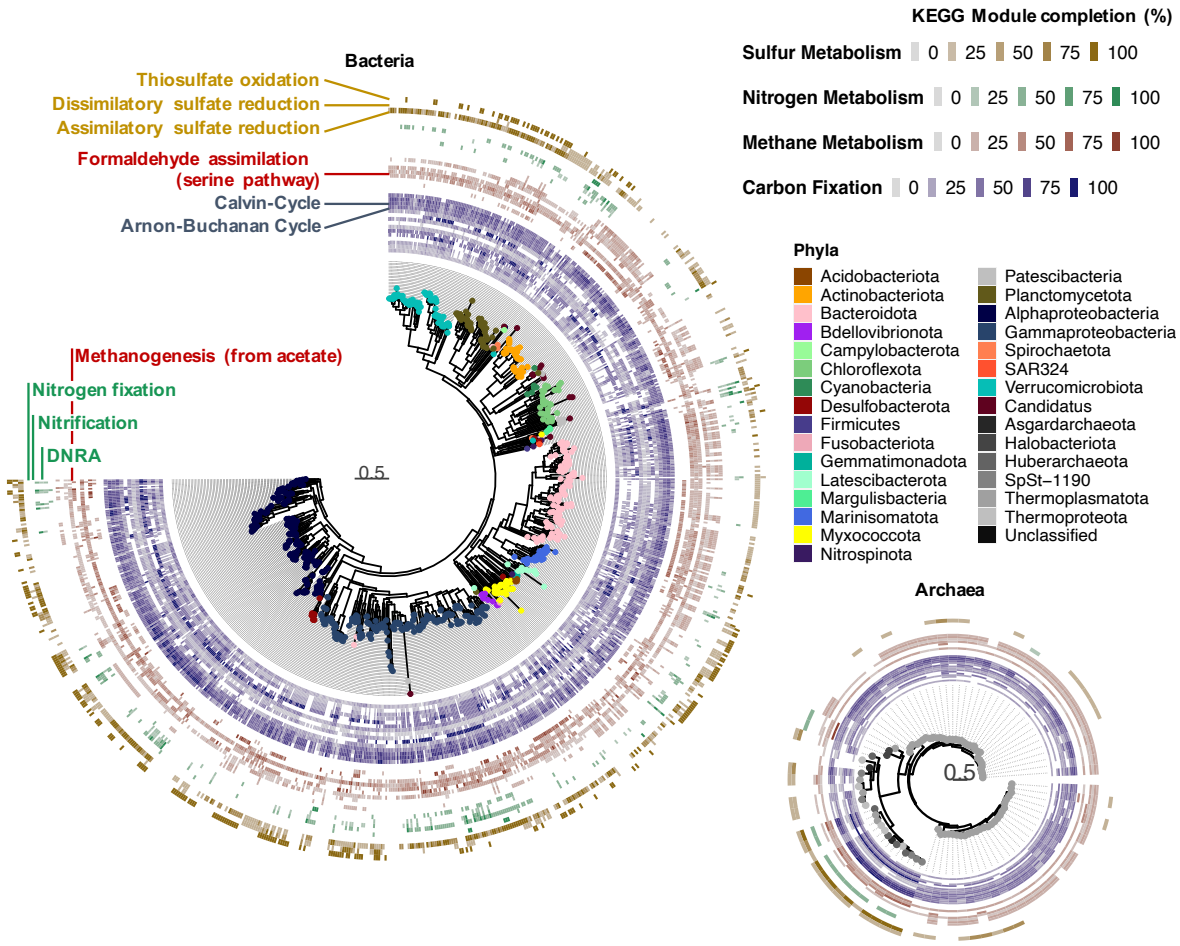

Figure II: Redundancy varies across microbial functions. Bacterial and Archaeal phylogenetic trees of 957 metagenome assembled genomes (MAGs), from the surface ocean (Delmont *et al.* 2018), and the presence of various metabolic steps (carbon fixation, methane, nitrogen, and sulfur metabolisms) in each genome. The taxonomy of each MAG was inferred with *GTDB-Tk* (Chaumeil *et al.* 2022). The bacterial and archaeal phylogenetic trees were built with *FastTree* (Price, Dehal and Arkin 2010) using the alignment of marker genes of each MAG constructed during the computation of *GTDB-Tk*. *FastTree* was run using the generalized time-reversible model and branch lengths were rescaled with a Gamma20-based likelihood (see scale). The tips of the trees' branches are colored by phyla (see color legend). We note mismatches between phylogeny and taxonomy due to low MAG completeness and/or contamination (Delmont *et al.* 2018). The functional annotation of each

MAG was performed with the *METABOLIC* pipeline (Zhou *et al.* 2022). Each concentric line around the tree represents a KEGG module (an ensemble of KEGG orthologs required to perform a reaction). We focused on modules involved in carbon fixation (15 modules), methane (11), nitrogen (6), or sulfur cycling (3, see color code). As modules may require various sets of KOs for the function to be performed, module completeness was computed as the percentage of KOs (involved in a module) observed in each MAGs compared to the total number of KOs required per module.

We studied the distribution of various metabolic traits involved in carbon fixation, methane, nitrogen, and sulfur cycles across the 957 MAGs (Figure II). First, most MAGs harbored carbon fixation traits, such as the KEGG modules for the Calvin (97% of the MAGs) or the Arnon-Buchanan cycles (96% of the MAGs). In turn, steps of nitrogen cycling could only be performed by a narrow set of MAGs: 18% of the MAGs harbored the dissimilatory nitrate reduction module, but only 1.5 and 1.2% of the MAGs harbored the modules for nitrogen fixation and nitrification, respectively. In the cycling of methane, formaldehyde assimilation, also an intermediate step in the cycling of methanol, was harbored by as many as 94% of MAGs, while methanogenesis could be performed by 78% of the MAGs. In the Sulfur cycle, 63, 22, and 20% of the MAGs could perform assimilatory sulfate reduction, thiosulfate oxidation, and dissimilatory sulfate reduction, respectively. These results illustrate the high variability of functional redundancy across various microbial functions in a set of MAGs recovered from the global surface ocean.

A second crucial observation is that most modules were incomplete within single MAGs, suggesting that MAGs did not have the full set of genes required to perform these metabolic reactions by themselves (Figure II). This result should be interpreted with caution as module incompleteness could be a direct effect of the variable genome completeness among the analyzed MAGs. In turn, the previous could also indicate that these functions might rely on metabolic exchanges between microbial taxa to be performed (i.e., cross-feeding).

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Standardized definition of microbial effect traits and phylogenetic units

Recent works have evidenced that variant diversity within functional genes was a great predictor for ecosystem processes (Escalas *et al.* 2019; Beier *et al.* 2020). Databases of genes and modules encoding traits relevant to ecosystem processes and biogeochemical cycles are, therefore, an important standardized resource for investigating functional diversity (Ferrera *et al.* 2015; Karaoz and Brodie 2022; Zhou *et al.* 2022). By determining environmental genes with orthologs to these genes collections, microbial effect traits can now be studied across genomes and biomes (Zhou *et al.* 2022; Auladell *et al.* 2023), and their ecological drivers determined (Dlugosch *et al.* 2022). Further work is required to determine the effect of functional gene variant diversity on a range of ecosystem processes, and to discover more functional genes. Nevertheless, these curated collections of genes and modules relevant to ecosystem processes represent a great avenue for building unified measures of microbial functional diversity allowing comparison across microbiomes.

With meta-omics, microbial functional diversity can be inferred either as community aggregated traits, irrespective of the identity of the organisms bearing the traits, or individually for each species that compose the community (Fierer, Barberán and Laughlin 2014). Studying community aggregated traits has been instrumental in the first descriptions of microbial functional diversity (Turnbaugh *et al.* 2009; Sunagawa *et al.* 2015). However, recent theoretical and technical advances make it feasible to predict effect traits at the microbial species or genome levels, allowing a description of the taxonomic, phylogenetic, and functional dimensions of microbial diversity. Microbial genomes can now be reconstructed from DNA retrieved from cultured isolates, sorted single cells, or directly from the environment, also called Metagenome-Assembled-Genomes (MAGs) (Paoli *et al.* 2022). The functional annotation of microbial genomes and the analysis of their distribution can contribute substantially to our comprehension of traits in uncultured microbes. Yet, they have limitations. For example, MAGs, also called population genomes, do not represent the genome of an individual cell, but that of a population (Grossart *et al.* 2020). Thus, some traits may not be detected as they are present only in a fraction of the cells from a given species. The size range of the

produced DNA fragments in high-throughput sequencing is also a limitation for genome reconstruction, leading to incomplete or contaminated genomes (Parks *et al.* 2015). In addition, current sequencing technologies typically generate MAGs for the most abundant taxa in the community (Nayfach *et al.* 2020; Paoli *et al.* 2022). Long-read sequencing holds great promises in this respect (Haro-Moreno, López-Pérez and Rodriguez-Valera 2021; Liu *et al.* 2022), and should allow the retrieval of large portions or complete microbial genomes from environmental samples, while being less dependent on their abundance (Sun *et al.* 2023). In addition, the continuous progress of single-cell genomics (through advances in microfluidics), may help to investigate the traits of the rarest taxa in complex microbiomes (Pachiadaki *et al.* 2019; Lan *et al.* 2024). Altogether, this means that we are now able to study the phylogenetic units of microbes that carry the effect traits underpinning ecosystem processes.

Developments in numerical ecology

Concomitant with these advances in molecular microbial ecology, advances in numerical ecology have also allowed to standardize metrics to estimate functional redundancy (Johnson and Pomati 2020). In its simplest form, functional diversity is the number of distinct effect traits present in a microbiome, while redundancy is the number of taxa in a community that perform the same functions, i.e., that share effect traits. Information at the single trait level is useful to characterize the functioning of ecosystems (Cheng *et al.* 2022), and also serves to identify the functions with low redundancy that could be threatened by species loss or extinction. But new standardized metrics have been developed to account for multi-functionality at the community scale, which is the diversity and redundancy across multiple effect traits (Miki, Yokokawa and Matsui 2013; Pavoine 2020; Magneville *et al.* 2022). These metrics rely on measuring the similarity between taxa based on the multiple traits they can harbor (Ricotta *et al.* 2016), and do not require that the taxa harbor the exact same set of traits (i.e., be functional equivalents) to infer functional redundancy (Loreau 2004; Magneville *et al.* 2022; Nico *et al.* 2023). Such measures are more adequate to represent the multi-functionality of microbial communities harboring many effect traits, and involved in multiple ecosystem processes (Falkowski,

Fenchel and Delong 2008). They only require 1) a table representing the abundance of species across samples, and 2) a table representing the traits harbored by these same species. For microbes these can now be retrieved using microbial genomes as phylogenetic unit, metagenome read recruitment to study their distribution in space or time (Nayfach and Pollard 2015), and effect-trait prediction through the prediction of a curated collection of functional genes. Meaning that microbiologists now have the ability to estimate and compare indexes of functional diversity and redundancy across biomes.

Limitations and perspectives of this framework

This framework relies heavily on culture-independent approaches, which are likely to include environmental genetic material, genomes, and traits from dead or inactive individuals (Mestre and Höfer 2020). The discrimination of active microbes and traits before trait prediction could, however, allow us to overcome this bias (Emerson *et al.* 2017). Such approaches include stained-based cell sorting before DNA extraction or amplification (Emerson *et al.* 2017), discrimination based on metabolic activity estimated with the uptake of radio-labelled or stable-isotope-probing (Emerson *et al.* 2017; Greenlon *et al.* 2022), or targeting RNA-based gene expression with meta-transcriptomics (although cellular regulation may additionally regulate expression) (Bashiardes, Zilberman-Schapira and Elinav 2016; Emerson *et al.* 2017). Another limitation resides in the potentially lower detectability of functional genes compared with the higher abundances of genes involved in general metabolism or information processing in metagenomes and transcriptomes (Johnson and Pomati 2020). However, this bias could be overcome by increasing the sequencing effort, which seems ever-more feasible due to the decrease in sequencing costs (Duarte *et al.* 2020). Finally, some microbial functions can only be performed entirely through interactions between microbial taxa (Machado *et al.* 2021; Giordano *et al.* 2024). It can be argued that this is no different than for larger organisms, whose functions rely on prey-predator interactions or the presence of engineer species interacting with many members (Byers 2022; Bello, Schleuning and Graham 2023). New methods

developed for larger organisms could be applied to better account for microbial interactions when dealing with their functions (Bello, Schleuning and Graham 2023).

By allowing the high-throughput screening of functionally-characterized effect traits and the species harboring them, meta-omics have made feasible and timely the study of microbial functional diversity and redundancy directly from the environment. Despite these advances, microbiologists have been reluctant to use standard indexes of functional diversity, and interpret their relevance for ecosystem functioning (Johnson and Pomati 2020). Generalizing and standardizing the measurement of functional diversity is needed for a better understanding of the link between microbiomes and the functioning of past, present, and future ecosystems.

Drivers of microbial functional diversity and redundancy

Being now able to estimate and compare functional diversity and redundancy, microbiologists will probably evidence variability across biomes. Which factors explain this variability is thus a crucial question for the field. Theoretical knowledge allows us to hypothesize that microbial functional diversity and redundancy are likely scale-dependent, driven by biological or ecological processes, some of them specific to micro-organisms.

In environmental surveys, the spatiotemporal scale defines the four dimensions (a 3-dimensional space and its change in time) in which microbial species and their functional attributes are studied. The extent of these four dimensions is crucial, as larger spatial and temporal coverage will likely result in the detection of a broader spectrum of functions (i.e., functional diversity) and of species having similar effects on ecosystems (i.e., functional redundancy). Yet these species or functions may have different biotic or abiotic preferences (i.e., niches), or simply not co-occur at smaller spatial and temporal scales (Galand *et al.* 2018). For example, submesoscale oceanic fronts (0.1km to 100km) can delineate subpopulations of microbial species with similar effect traits, but different niches (Clayton *et al.* 2017; Ramond *et al.* 2021). In turn, two communities in close proximity but from distinct water masses can harbor very distinct functional potentials (Galand *et al.* 2009). Microbial communities can also be separated in time. For instance, over a year, different microbial clades can be a) responsible

for the production of the same vitamin (Beauvais *et al.* 2023), or b) the abundance of biogeochemically relevant genes in marine coastal ecosystems (Auladell *et al.* 2023). Also dependent on the spatial or temporal scale is the number of different niches covered by the survey (Cardinale *et al.* 2012). If a habitat has many niches where a function can be performed, it may harbor active taxa with contrasting response traits, but similar effect traits. For instance, the diversity of methane-oxidizing bacteria (MOBs) in wetlands increased at the interface between dry and wet soils after flooding (Bodelier *et al.* 2013). Both MOBs favoring dry or wet soils co-occurred at this interface, leading to a local higher functional redundancy congruent with higher rates of methane oxidation (Bodelier *et al.* 2013).

The phylogenetic scale represents a fifth dimension. A fine phylogenetic resolution may unveil strain-level diversity or micro-diversity (Larkin and Martiny 2017; Needham, Sachdeva and Fuhrman 2017; García-García *et al.* 2019). High micro-diversity may potentially lead to the detection of a higher number of organisms harboring similar effect traits, thus increasing the local redundancy. It still needs to be determined whether micro-diversity is similarly distributed across different microbial clades, specific genes, and ecosystems, see (Fodelianakis *et al.* 2022). The phylogenetic resolution also affects biodiversity surveys and the coverage of the rare biosphere, which could represent a reservoir of high functional redundancy, or taxa with uncharted traits (Caron and Countway 2009; Jousset *et al.* 2017; Ramond *et al.* 2023). Overall, the spatial, temporal, and phylogenetic dimensions should be considered carefully in microbial surveys to determine their influence on estimates of microbes' functional diversity and redundancy (Ladau and Eloe-Fadrosh 2019).

Functional diversity and redundancy are also affected by ecological processes. Functional diversity is generally driven by the availability of the resources and substrates for ecosystem processes. For microbes, it is hypothesized that the redox disequilibria available for energy and cellular uptake is the main driver of functional composition (Louca *et al.* 2018). This explains for instance the rather homogeneous composition of microbial community aggregated traits in the surface open ocean (Sunagawa *et al.* 2015; Haggerty and Dinsdale 2017; Dlugosch *et al.* 2022), where the energy sources (light for photosynthesis and oxygen

for the degradation of organic matter) are uniformly distributed. In turn, functional redundancy is driven by mechanisms allowing the coexistence of species with similar effect traits (Chesson 2000). Processes that lower competitive exclusion, that is, the extinction of the less competitive species within a niche or a resource overlap, are major drivers of species co-existence. Microbial taxa share many effect traits (Box 3), but greatly differ in their niche or in other effect traits. This means that species potentially using the same resource avoid competition by having different niches. This reduced resource overlap allows the co-existence of taxa with the same effect traits, thus increasing microbiomes' functional redundancy (Yu *et al.* 2024). Environmental and biotic variability may also change the identity of the most competitive species at a rate that allows the coexistence of more microbial taxa with similar effect traits (Hutchinson 1961; Ramond *et al.* 2021). If competitive exclusion finally takes place, dispersal and mixing from ecosystems dominated by different competitors could maintain a higher functional redundancy (i.e., mass effects) (Ramond *et al.* 2021). Similarly, positive interactions, e.g., cross-feeding, may favor the survival of less competitive species or generate new niches that allow functional redundancy to be maintained over time (Machado *et al.* 2021; Zoccarato *et al.* 2022).

Other features of microbial ecology and evolution may play a role in their functional diversity and redundancy. Horizontal gene transfer (HGT) represents any mechanism allowing gene transfers between lineages (Soucy, Huang and Gogarten 2015). The reach of HGTs seems unlimited (Smillie *et al.* 2011; Petersen *et al.* 2019), whereas it remains unknown whether HGTs are more frequent in specific clades (both prokaryotic and eukaryotic), genes, or habitats (Redondo-Salvo *et al.* 2020; Pallares-Vega *et al.* 2021; Keeling 2024). HGTs have a major role in functional ecology, as they can confer new traits to microbes, such as the catabolism of specific aromatic hydrocarbons (Yin and Stotzky 1997), or nutrient acquisition and metabolic genes (Palomino *et al.* 2022; Hackl *et al.* 2023). Through transfers of response or effect traits, HGTs may also favor microbes co-existence by shifting the identity of the best competitor (Zhu, Hong and Wang 2024), and therefore contribute to microbial functional redundancy. Microbes might also share traits through symbioses. These symbioses may range

from obligate, such as the interaction between N_2 fixing bacterial symbionts and their eukaryotic hosts (Cornejo-Castillo *et al.* 2016; Tschitschko *et al.* 2024), to facultative, as in kleptoplastidic ciliates which may acquire photosynthesis by retaining preys or their chloroplasts (Stoecker *et al.* 2017). Exchanges of metabolites via cross-feeding may also be required to fulfill microbial functions (Oña *et al.* 2021), such as chitin degradation in marine systems (Raimundo *et al.* 2021). This suggests that not only the effect traits of species are relevant for the functioning of ecosystems but also the interactions between taxa with different traits (Bello, Schleuning and Graham 2023).

Another relevant property when considering microbial functional diversity is genome size. Larger microbial genomes may harbor more effect traits with multiple potential impacts on the functional similarity and redundancy of microbiomes. Habitat and phylogeny are generally the best predictors of microbial genome size (Maistrenko *et al.* 2020), suggesting that the number of traits per taxa could change with biomes and clade abundance. Microbial genomes are smaller in oligotrophic and undisturbed biomes (Swan *et al.* 2013; Bentkowski, Van Oosterhout and Mock 2015), potentially leading to fewer traits (Giovannoni, Cameron Thrash and Temperton 2014). In turn, environments with a wider availability, e.g., marine snow (Leu *et al.* 2022), and diversity of resources, e.g., the deep ocean (Ngugi *et al.* 2023), usually harbor microbes with larger genomes. Microbial genome size could also be driven by the balance between positive (e.g., mutualism) and negative interactions (e.g., competition), which may change across biomes (Machado *et al.* 2021).

Overall, microbial functional diversity and redundancy may be affected by multiple ecological and evolutionary factors acting at different scales which for the most part, remain partially understood (Figure III). Recent advances may help us address this knowledge gap. For example, the influence of ecological processes can be quantified (Stegen *et al.* 2013; Ladau and Eloe-Fadrosh 2019). Furthermore, the growing access to microbial population genomes from environmental microbiomes contributes to linking these effect traits to phylogenetic units, allowing to infer the influence of eco-evolutionary processes on microbes functional diversity (Brennan and Logares 2023; Martiny *et al.* 2023).

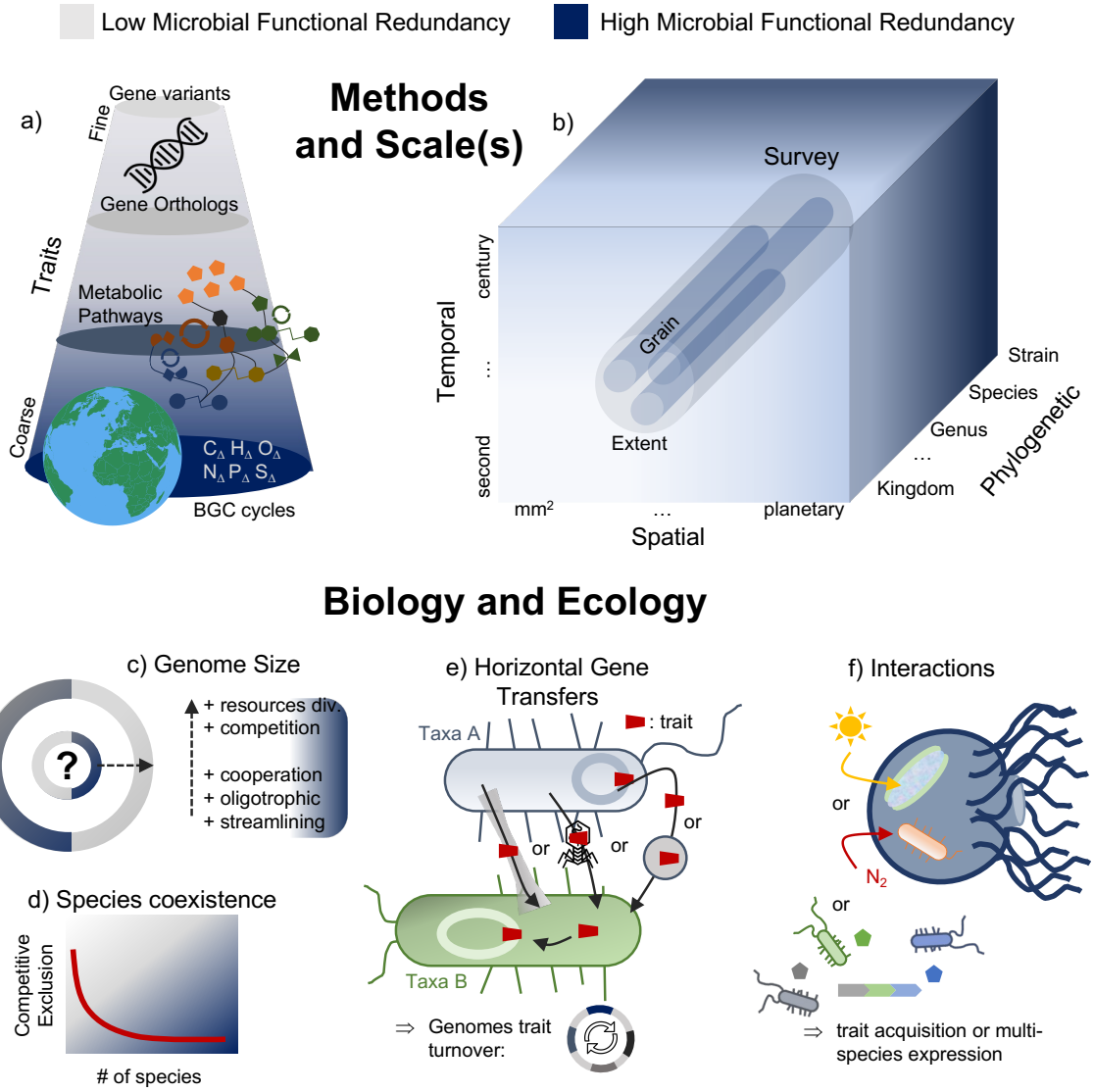

Figure III: Effects of methods, scales, biology, and ecology on the functional redundancy of microbiomes. a) Coarse trait resolution will result in higher redundancy because these traits can be shared by many species. b) Large spatial and temporal scales will group species and ecotypes with similar effect traits that do not co-occur at finer resolutions, resulting in higher redundancy. In turn, a finer phylogenetic resolution will detect micro-diversity and lead to the delineation of various taxa with similar effect traits, resulting in higher redundancy. c) The effect of genome size on redundancy is yet unknown. Larger genomes should increase the number of traits harbored by species. Thus, it could increase the number of traits shared within a microbiome but also decrease genome similarity, with opposite effects on redundancy. d) Higher species coexistence in environments with low competitive exclusion will likely increase

the occurrence of taxa with similar traits, thus increasing redundancy. e) HGTs (conjugation, transduction, vesiduction, or transformation, from left to right) are transfers of traits from one taxon to another, suggesting that the traits harbored by a taxon could vary in space and time, likely affecting the overall functional diversity and redundancy. f) Symbiosis or cross-feeding allows the acquisition or production of new traits.

Perspectives for studies of microbial functional diversity

Even if functional diversity and redundancy can be approximated with community aggregated traits approaches (Finn 2024), moving beyond these approaches is a crucial next step for microbial functional ecology. We detailed three main axes of development. First, working at the genome resolution to allow studying the eco-evolutionary processes influencing microbial functional diversity across spatiotemporal scales and biomes (Brennan and Logares 2023; Martiny *et al.* 2023). Second, to assess the distribution, activity, and trait expression of these genomes across biomes using meta-omics data (Bashiardes, Zilberman-Schapira and Elinav 2016), thus aiming at characterizing expressed functional diversity and redundancy. Third, to use a standardized selection of effect traits (Ferrera *et al.* 2015; Zhou *et al.* 2022) and functional metrics (Ricotta *et al.* 2016) to allow inter-study comparability. By working with microbial genomes and a unified set of methods, we will attain deeper insights on the ecological patterns and evolutionary drivers of microbial functional diversity.

An ensuing perspective is to study the importance of microbial functional diversity and redundancy for ecosystem processes. Microbial taxonomic and phylogenetic diversity positively affect ecosystem multi-functionality. They correlate with broad and narrow ecosystem processes such as nutrient cycling, decomposition, plant primary production, pathogen and antibiotic control in soils (Delgado-Baquerizo *et al.* 2020), but also with microbial production in marine waters (Galand, Salter and Kalenitchenko 2015), or microbial respiration and the degradation of specific toxins in freshwaters (Delgado-Baquerizo *et al.* 2016). Being now able to measure functional diversity and effect traits is likely to yield a better mechanistic understanding of the link between microbes and these processes. For instance, it remains

406 unknown if these processes are mostly driven by abundant microbes (Fierer, Barberán and
407 Laughlin 2014) (mass ratio hypothesis), if a process is enhanced by a larger diversity microbes
408 able to perform it (Escalas *et al.* 2019) (niche complementarity hypothesis), or if the traits of
409 rare "keystone" taxa also matters for ecosystems (Jousset *et al.* 2017). The relationship
410 between functional diversity and ecosystem processes will nevertheless be highly context-
411 dependent, varying with the scale of the survey of the traits included (Delgado-Baquerizo *et*
412 *al.* 2020). The use of standardized functional diversity metrics will help to harmonize microbial
413 biodiversity–ecosystem functioning studies and move the field beyond context dependence
414 (Ricotta *et al.* 2016). Studying microbial functions also means routinely measuring and
415 comparing the rates of the ecosystem processes they perform. This is challenging given the
416 lack of comprehensive methods, the large number of microbial processes and reactions
417 involved in biogeochemical cycles, and the variability in their scale across biomes (Grossart *et*
418 *al.* 2020). We review relevant methods in Box 4. Sampling and incubations of environmental
419 microbiomes required to measure functions are inevitably disruptive, so great care should be
420 taken in recreating natural conditions. Measuring the rate of microbial functions remains a
421 challenge that should be addressed through cross-disciplinary research involving
422 biogeochemists.

Box 4: Measuring microbial functions?

Studying the significance of microbial functional diversity and redundancy for ecosystem functioning starts by measuring the rate of microbial-mediated processes. Various methods might be involved depending on the function under investigation (Kemp *et al.* 1993), but most are based on integrating the concentration of a substrate or a product over time. The concentration of inorganic nutrients is analyzed through auto-analyzers relying on measures of absorbance of the sample mixed with various reagents to make each nutrient fluoresce (Aminot and Kérouel 2007). Similarly, the concentration of total, particulate, or dissolved, organic or inorganic forms of carbon and nitrogen are routinely measured with standard auto-analyzers relying on physical and chemical transformations (acidification and oxidation) and quantification through non-dispersive infrared detection of the gases produced (e.g., CO_2 or NO) (Halewood *et al.* 2022). High-Performance-Liquid-Chromatography (HPLC) or gas chromatography can be used to track the concentration and uptake of specific compounds (e.g., toxic microcystin-LR, or CH_4) in an incubation or a

chamber (Delgado-Baquerizo *et al.* 2016; Wu *et al.* 2017). Radioisotope tracing coupled with mass spectrometry methods can be used to track a wide array of chemical reactions mediated by microbes (Cresswell *et al.* 2020). For example, radiolabeled thymidine and leucine incorporation are routinely used to estimate microbial biomass production (Kemp *et al.* 1993), while the uptake of radio-labelled N substrates in incubations is often used to determine the rate of different steps in N cycling (Gago and Ramírez 2012; Garrido-Amador *et al.* 2023; Liu *et al.* 2023). Specific reactions can also be measured by inhibition (e.g., acetylene affects nitrification and denitrification) and comparison to a non-inhibited incubation (Groffman *et al.* 2006). The natural abundance of N isotopes variant has also been used to model and quantify the dominant microbial nitrogen transformations across ecosystems (e.g., in soils) (Xu *et al.* 2021). Quantitative Stable-Isotope Probing could additionally be used to track and isolate the DNA of microbes actively incorporating the labelled substrates (Greenlon *et al.* 2022), thus allowing a finer investigation of the mechanistic link between the process, microbes, their activity, and their genomic traits. To simultaneously measure various reactions will require multiple incubation experiments with various substrates. EcoPlates (Biolog) have streamlined the profiling of the degradation of 31 carbon sources by microbes. Integrating changes in the absorbance of a tetrazolium dye released by degradation over time, then allows us to semi-quantitatively measure and compare the rate of carbon source degradation by microbes (Miki, Yokokawa and Matsui 2013; Ruiz-González *et al.* 2015). Attempts at measuring microbes' multi-functionality and combining multiple-methods exist (e.g., soils) (Delgado-Baquerizo *et al.* 2020). Microbial biomass production, respiration, or the ratio between these metrics, are often used as a proxy for microbial multi-functionality (Delgado-Baquerizo *et al.* 2016). Further collaborating with biogeochemists should increase our ability to routinely measure the whole spectrum of microbial functions with high-throughput.

Functional redundancy is a key factor in predicting the resistance and resilience of communities and the processes they perform (Biggs *et al.* 2020). For microbes, it is often assumed that high redundancy represents an insurance for ecosystem processes (Allison and Martiny 2008). As within a pool of organisms that have similar functions, different species can grow in response to environmental disturbances and maintain ecosystem processes at a similar rate (Beauvais *et al.* 2023). However, this is not always the case, as microbial communities and their processes vary in their resistance and resilience to disturbances (Allison and Martiny 2008; Shade *et al.* 2012; Jurburg *et al.* 2024). Studying whether functional

redundancy can be used as a predictor of microbiome and ecosystem resistance, resilience, and health is thus a pressing need (Philippot, Griffiths and Langenheder 2021). Testing such hypotheses also represents an opportunity to study the functional response of microbiomes to climate change (Cavicchioli *et al.* 2019), which will help include microbiomes in future Earth climate scenarios (Gewin 2023). Microbiomes have been shown to respond variably to climate disturbances. For instance, increases in temperature can generate a shift towards smaller organisms (Brown *et al.* 2024), while increases in CO_2 cause homeostatic stresses that lower other bacterial activities and the rates of some of their functions (Bunse *et al.* 2016). Due to their crucial role in regulating biogeochemical cycles, shifts within microbiomes could have feedbacks on ecosystems and the climate (Cavicchioli *et al.* 2019). Further experiments or surveys of microbiomes exposed to disturbances, will help us understand if, and how, redundancy affects their response to climate change. Such information will be crucial to learn how to rescue microbiomes and their functions (Shade 2023), but also whether conditions favoring high microbial functional redundancy should be preserved (Bodelier *et al.* 2013; van der Plas 2019).

If the importance of microbial functional diversity and redundancy for ecosystem functioning and resilience is verified, it will become urgent to predict their future patterns across biomes. To that end, we could rely on earth model data from the Coupled Model Inter-comparison Project (Eyring *et al.* 2016), which provides predictions of environmental and biogeochemical variables across biomes according to different climate change scenarios (Assis *et al.* 2024). Statistical models of microbial diversity and production, coupled with environmental variables for which future predictions are available, have yielded useful projections that could further guide decision-making (Ibarbalz *et al.* 2019; Zhang *et al.* 2024). However, such models might not account for microbial evolution and adaptation (Brennan and Logares 2023). In addition, a lag between environmental changes and their effects on microbiomes has been observed (Ladau *et al.* 2018; Kalenitchenko, Peru and Galand 2021). The variability of this lag in space could be explained by the dominance of taxa more or less sensitive to the changes (Ladau *et al.* 2018; Kalenitchenko, Peru and Galand 2021), but also

by historical contingencies in community composition (Vass and Langenheder 2017; Kalenitchenko, Peru and Galand 2021). In parallel, complex models have been developed to study the evolution of microbial functional diversity (Coles *et al.* 2017; Zakem, Polz and Follows 2020; Zhu, Hong and Wang 2024). Comparing the outputs of multiple-regression models based on predictions of environmental data to theoretical models could allow us to quantify the influence of evolutionary patterns on microbial functions. Importantly, this work should lead to the identification of microbiome tipping points and help predict regime and functioning shifts across ecosystems (Scheffer *et al.* 2015; Shade 2023).

Concluding remarks

Even though microbial functional ecology is still lagging behind the knowledge acquired for animals and plants, the field is growing rapidly due to manifold technical advances. Importantly, a census of microbial effect traits exists and is growing. Furthermore, new genome-centric methods allow to study the distribution of these traits across microbial taxa and ecosystems. This opens the door to robust comparisons of microbial functional diversity and redundancy. Working at the level of population genomes will also help quantify the contribution of various ecological and evolutionary drivers to changes in microbial functions. This outlines a framework to study functional diversity and redundancy in microbiomes. Testing their importance for ecosystem processes, their resistance, and resilience is thus within our reach. Nevertheless, further cross-disciplinary research including biogeochemists and modelers is required to fully apprehend microbial functional diversity. Results from these joint efforts will expand our understanding of ecosystem functioning and could inform decision-makers in the context of global change.

Glossary

Ecosystem functioning: the sum of properties or processes measured at the ecosystem level, e.g., energy flow or chemical cycling (Violle *et al.* 2007; Krause *et al.* 2014). While a **function** represents a single process, e.g., denitrification affects the nitrogen cycle.

Trait: morphological, physiological or phenological feature measurable at the individual, population or community level, e.g., the *nirK* gene (Violle *et al.* 2007), encoding for nitrite reductase that performs denitrification, part of the nitrogen cycle.

Effect trait: any trait that affects ecosystems. They reflect, or can be used as a proxy of, the function a microbe performs in the ecosystem, e.g., the presence of the *nirK* gene in a genome.

Response traits: traits that vary in response to changes in environmental conditions (Violle *et al.* 2007). Response traits are used as proxies of the performance of an individual along an environmental gradient, e.g., cell size and morphology usually correlate with diverse environmental conditions (Litchman and Klausmeier 2008). Confusion can arise from traits that can be used both as *effect* and *response* traits. For example, size in phytoplankton is related to nutrient uptake efficiency. Thus, it is a *response trait*, as it predicts the success of larger phytoplankton cells in resource-replete conditions. But it is also an *effect trait*, as it predicts the rate at which nutrient uptake will be performed.

Functional group: a group of taxa that affect the ecosystem in the same manner, perform the same function, or harbor similar traits. Groups can be defined at different levels, e.g., all denitrifiers or only the individuals possessing the *nirK* gene.

Functional redundancy*:* the fact that different taxa harbor the same effect trait(s) and can thus play the same role in ecosystem functioning. Using this definition, microbial taxa can share some traits, but can differ in their rate, the presence of other traits, or ecological preferences (Nico *et al.* 2023).

Gene: here we use gene as a synonym of Open Reading Frame (ORF). An ORF is a sequence delimited by a start and stop codon and holds the potential to be translated into a protein.

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Declaration of interests

There are no interests to declare.

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