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Research Paper

Functional Microbiome Characterization for Assessing Environmental Change Impacts on Microbial Communities

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Abstract: Microorganisms are fundamental to ecosystem functioning, driving essential biogeochemical cycles and sustaining ecosystem services across marine, terrestrial, freshwater, and host-associated environments. Rapid environmental change driven by climate variability, pollution, and anthropogenic disturbance had increasingly altered microbial communities in ways that were not adequately explained by taxonomic composition alone. This limitation prompted a shift in microbial ecology toward function-centered analyses enabled by advances in functional meta-omics. This review examined how metagenomics, metatranscriptomics, metaproteomics, and integrated multi-omics approaches advanced understanding of microbial functional potential, activity, and metabolic output under environmental perturbation. Conceptual frameworks linking genetic potential to realized function were synthesized, highlighting the roles of functional diversity, redundancy, and network organization in community resistance and resilience. Case studies across diverse ecosystems illustrated how environmental stressors reshaped microbial metabolic pathways and ecosystem services, often without corresponding changes in taxonomic structure. The review also evaluated bioinformatic tools and analytical pipelines used for functional annotation, pathway reconstruction, and cross-omics data integration. Key challenges associated with multi-omics research were identified, including high computational demands, lack of methodological standardization, and difficulties in integrating heterogeneous datasets for predictive modeling. Despite these constraints, functional microbiome characterization was shown to provide critical insights for forecasting ecosystem responses to environmental change and for informing applications in environmental management, bioremediation, and sustainable resource use. Overall, the transition from taxonomic inventories to function-based frameworks seems to be essential for advancing predictive microbial ecology and for understanding the role of microbiomes in maintaining planetary health.

Keywords: Functional meta-omics, Microbial ecology, Metagenomics, Environmental perturbations, Functional diversity, Ecosystem resilience.

Introduction:

Microorganisms play a key role in the functioning of ecosystems; they form a continuum of environments, including host-associated and marine and terrestrial environments (Wemheuer et al., 2020). These complex communities of microbes sustain the most vital biogeochemical processes, in particular the conversion of carbon and nitrogen, upon which the survival of all life on Earth relies (Lee et al., 2025). However, these essential microbiomes are now more exposed to environmental disturbances - changes in temperature, pH, moisture, oxygen, and nutrient levels - which arise both naturally and through human actions (Lee et al., 2025). These changes may significantly restructure and reorganize the community of microbes and their functionality, which in turn affects the stability and resilience of the entire ecosystem (Burz et al., 2023). In the last ten years, one of the key research urgency areas has been to define the structure, organization, and functioning of the microbiome, and how it influences and is influenced by its immediate environment (Carrieri et al., 2019).

The Environmental Perturbations and Microbial Ecosystem.

The active interaction between the microbial communities and the environment highlights the continuous adaptation of the consortia to different abiotic and biotic conditions, thus affecting the complex changes in composition and structure, as well as functionalities (Arbas et al., 2021). Lineages of microbes are constantly adapting to fit in different ecological habitats, tuning the metabolic capabilities of the communities they form to have a significant impact on biogeochemical cycles (Delogu et al., 2023). A change in environmental conditions may cause a sudden change in the qualitative and quantitative structure of these microbial habitats, which makes microbial diversity a delicate indicator of the state of environmental health and ecosystem (Kisand et al., 2012). In turn, the most important task is to decipher the mechanisms that regulate the dynamics and functioning of microbiota in the conditions of their environment or host (Burz et al., 2023). Thorough knowledge of such mechanisms is needed to predict the response of microbes to continued environmental changes, including pollution and climate change, and to come up with measures to reduce the negative effects on ecosystem services and functions (Burz et al., 2023; Lee et al., 2025).

Weaknesses of Taxonomic Characterization of Microbial Ecology.

Although traditional taxonomic methods have provided some of the basis of understanding microbial diversity, they often do not provide insight into the real metabolic roles and functional responses of the microbial communities to environmental changes (Timmins-Schiffman et al., 2020). As an example, qualitative changes in community composition do not necessarily indicate similar changes in metabolic activity or community function as a whole (Couvillion et al., 2020). This weakness is due to the fact that the presence of a gene does not guarantee its expression, and even transcript levels can not be accurately used to predict protein abundance or functional output (Gómez‑ Varela et al., 2023). In addition, taxonomic surveys, though useful in terms of initial evaluations are like having a vision of the engine components without ensuring that it actually works (Reid and Bergsveinson, 2021). Thus, the functional effects of microbial communities on a given ecosystem may be misrepresented by the mere presence of a community or the gene content (Reid and Bergsveinson, 2021; Timmins-Schiffman et al., 2021). This taxonomic disparity and functional performance highlights the need to adopt strategies that directly assess the active metabolic and ecological functions of microorganisms (Song et al., 2023).

Development of Functional Metagenomics Strategies.

Based on this, sophisticated methodologies, i.e. metagenomics, metatranscriptomics, metaproteomics, and metabolomics, have been developed that allow a more direct assessment of the functional potential, active gene expression, protein synthesis, and metabolic output of microbial communities (Chiriac & Murariu, 2021; Lobanov et al., 2022). They are the so-called meta-omic methods that go beyond taxonomic profiling and investigate what microorganisms are capable of building, what they are currently building, and what they have already built, providing a full picture of microbial community activity (Lobanov et al., 2022). The transition to functional characterization provides a more accurate idea of the contribution of microbial communities to ecosystem processes and reacting to environmental alterations, bypassing the constraints of species identification itself (Zarraonaindia et al., 2012). This is especially relevant, with taxonomic profiles not being as predictive of community functions as functional ones, which can be more resilient and preserved in a variety of microbial communities (Vital-Jacome et al., 2023). Besides, their combination presents a global systems level insight into the complex interactions between microbial genetic potential, active metabolic pathways, and the subsequent biochemical transformations within an environment (Reid and Bergsveinson, 2021). This integration provides a broader view of microbial ecology, going beyond fixed taxonomic catalogs to describe the dynamic interaction of genetic capability, functions implemented, and metabolic phenotypes (Hart et al., 2020).

Aim and Outline of the research paper.

This paper is a review of the shift in the study of microbiomes away from taxonomic to functional characterization tools, and how these tools can be useful in understanding the effects of environmental alterations on microbial communities. It analyses technical principles and uses of the different meta-omic methods with particular focus on their ability to uncover the complex adaptive mechanisms that microbial consortia use in response to environmental stress.

Background: Characterization of the Microbial Community.

In the past, the description of microbial communities was also strongly based on culture-dependent techniques, which inherently predisposed the observations to easily cultivable species and significantly underestimated the real diversity, as well as functional complexity of environmental microbiomes. Sequencing of 16S rRNA gene has transformed the field of microbial ecology by making culture-independent taxonomic surveys possible, which has dramatically increased our understanding of microbial diversity (Alabbosh, 2024). However, this phylogenetic marker primarily can be used to identify taxa, which can offer little information on the metabolic capabilities or functional presence of microbes in their respective ecosystems (Cho, 2021).

Conventional Taxonomic Profiling Technologies.

These techniques include Sanger sequencing and, later on, high-throughput amplicon sequencing of marker genes, including 16S rRNA in bacteria and archaea or ITS in fungi, allowing general scans of microbial diversity in diverse environments.

The strengths and weaknesses of 16S rRNA Gene Sequencing.

Although 16S rRNA gene sequencing is a relatively cheap and fast way to determine the taxon of a microorganism, it has a low level of resolution, which can be as little as genus level, and cannot be used to reliably identify particular metabolic functions or ecological roles (Zhang et al., 2019).

An overview of Metagenomic sequencing technologies.

Metagenomic sequencing, which consists of direct sequencing of all DNA in an environmental sample, overcomes these limitations by providing detailed information on the genetic potential of whole microbial communities, including characterized and uncultivated microorganisms (McDaniel et al., 2021). Such a method allows to recreate metabolic pathways, define functional genes, and even assemble almost entire genomes of complex microbial communities (Yu et al., 2022), which answers the fundamental question, Who is there?

Metatranscriptomics and Metaproteomics to Perform Activity Assessment.

Metagenomics can give an idea of the genetic potential of a community, but metatranscriptomics and metaproteomics can give vital information on actively expressed genes and translated proteins, respectively, which is a dynamic reflection of microbial activity and its response to environmental signals (Franzosa et al., 2015). Collectively, these approaches respond to the central question: What are they doing?

Conceptual Framework: Functional Shifts in Microbiomes.

To obtain insight into the mechanisms behind environmental perturbations that cause changes in the functioning of microbial communities, it is necessary to go beyond the compositional changes and simply measure the expressed genetic potential and the metabolic output of these complex systems. This requires a conceptual framework that combines genomic potential and actual functional expression, which allows one to explain adaptive strategies and resilience of microbial consortia to different environmental pressures (Pereira-Marques et al., 2024). Replacement of taxonomic surveys with functional analyses is the key to building predictive models of ecosystem reactions to environmental change, and in interventions targeted at moderating microbial communities to achieve desired results. Such a strategy is particularly important as functional redundancy in microbial communities frequently allows similar functions to be preserved even when taxonomic changes are significant, so that taxonomic data on its own is not enough to predict ecological outcomes (Clouse & Wagner, 2021). Therefore, microbial functions can be evaluated using metatranscriptomics or metaproteomics, which provide a better and more precise assessment of the manner in which the environmental changes transform the community activity and overall ecosystem processes (Cárcer, 2020; Free et al., 2018).

Microbial Function and Functional Traits.

Functional traits refer to a wide spectrum of cellular activity, such as metabolic routes, responses to stress, and interactions with other species, that altogether define the position of a microbe in an ecosystem. These characteristics are often encoded by particular genes and their combination results in the emergent properties and overall well-being of the entire microbial community (Yang et al., 2025). As a result, the description of such functional features through the use of sophisticated omics technologies enhances the understanding of ecosystem dynamics and sustainability in response to environmental changes. In specific cases, like metatranscriptomics, it is dynamic because it involves tracking the expression of genes, which offers information on which metabolic pathways are actively involved in a response to certain environmental stimuli by microbial communities (Kumar, 2023). This tool will allow researchers to identify up- and down-regulated genes under different conditions, and hence, reveal how microbial communities react functionally to environmental changes (Shade et al., 2012). Besides, the notion of microbial functional traits goes beyond the expression of individual genes to include the ecological network modules, where the highly correlated clusters of genes indicate coordinated microbial responses to environmental changes (Bodelier, 2011). This combined methodology, which goes beyond taxonomic identification to the extensive functional analysis, is imperative in the destruction of the complex interaction between microbial residents and the environment (Galand and Pereira, 2018; Kuang et al., 2016). This functional view, which is described in detail, becomes particularly relevant, as taxonomic diversity does not always correspond to ecosystem performance or well-being, and there are studies that have reported the opposite of these relationships between diversity and disease conditions (Li et al., 2023).

Stressors and Adaptation of Microbes.

Microbial communities are highly adaptable to environmental stressors in multiple ways such as the modulation of metabolic pathways, the development of protective biofilms, and horizontal gene transfer. These modifications play a role in maintaining the equilibrium of the community and the metabolic processes in diverse conditions, sometimes causing the change in the dominance of the functional traits in the community (Elferink et al., 2020). These changes may significantly alter community-aggregated characteristics, which shows the overall reaction to selective pressures (Wood et al., 2023).

Stability and Defiance of Bacterial Communities.

It has been argued that the ability of microbial communities to endure and recover perturbations, which is known as resistance and resilience, respectively, is commonly associated with functional diversity and redundant metabolic functions (Ávila-Jimenez et al., 2020). Such functional redundancy, where many species do the same metabolic job, helps to stabilize and increase the resilience of the ecosystem to environmental changes (Louca et al., 2018; Ramond et al., 2024). High functional diversity communities are highly resilient with phylogenetically diverse taxa, meaning analogous functions, which are less likely to be lost without affecting all potential functions (Jacobson et al., 2021). This flexibility is also supported by modularity in ecological networks, which allows a localized reaction to disruption without affecting the whole community (Sun et al., 2025).

Microbiomes as Ecosystem Services Providers.

The microbial communities are essential in the provision of numerous tangible goods and services, the ecological regulators of which are more and more being understood (Shah et al., 2021). These functions include but are not limited to biogeochemical cycling, nutrient mineralization, and organic matter decomposition, which are directly associated with the functional characteristics and metabolic processes of the microorganisms that comprise them (Zhou et al., 2022). The operational diversity and redundancy of microbial communities are essential to the sustained delivery of these key ecosystem services, allowing them to maintain key processes during changes in the environment (Ramond et al., 2024; Wagg et al., 2019). As an example, the soil microbiomes are essential in carbon and nitrogen cycling, whereas the gut microbiomes affect the host metabolism and immunity (Alabbosh, 2024; Martiny et al., 2023). Lighting up these functional functions is essential in understanding the overall effects of environmental changes on ecosystem well-being and sustainability because functional redundancy has the capacity to cushion against taxonomic changes (George et al., 2017).

Functional Microbiome Characterization Methodologies.

The recent advances in technology in the fields of sequencing and bioinformatics have greatly increased our capacity to interrogate microbial functional potential and activity, beyond the previous culture-dependent methods. These technologies allow a detailed study of microbial genomes, transcriptomes and proteomes, and they provide a resolution to an unprecedented level of how these microbial communities act in terms of metabolic functioning and active pathways. Specifically, metagenomics has offered an effective system of cataloguing genetic potential of whole microbial communities, revealing genes that encode a range of functional attributes even in microbes that are uncultivable (Chen et al., 2022).

Assemblies and Metagenomic Sequencing.

Metagenomics provides both phylogenetic and functional information, reconstituting near-complete genomes of environmental matrices to understand the metabolic potential and ecological functions of members of a community. This integrative genomic approach has enabled the identification of new enzymes, metabolic pathways, and antibiotic resistance determinants, all of which are required to comprehend microbial ecosystem services and biotechnological applications (Using Genomics, Metagenomics and Other 'Omics' to Assess Valuable Microbial Ecosystem Services and Novel Biotechnological Applications, 2019). Moreover, the workings of omics-based methods rebuild the functional networks of communities, illuminating the metabolic interdependences and biogeochemical cycling (Zhou et al., 2022). These genomic insights are immensely useful in predicting the behavior of microbial communities to environmental disturbances, hence guiding predictive ecological models (Ramond et al., 2024).

Gene Prediction Pipelines and Annotation Pipelines.

After obtaining the metagenomic sequences, the most important step that follows is the prediction and annotation of genes to deduce the functional potential that is encoded in the community. Such pipelines usually use high-level algorithms and large databases to determine open reading frames and provide functional annotations using homology to characterized genes (Leeming et al., 2021). This can be used to unlock the metabolic capabilities, virulence factors, and other functional features that are found in the metagenome even in uncultured organisms (Xiong et al., 2015). This information is critical to understanding how ecosystems work and predicting the reaction of communities to environmental change (Schiml et al., 2023). The development of metagenomics has had a significant impact on the field of microbial ecology by providing the ability to directly sequence the environmental DNA, which has provided an invaluable understanding of the microbial life (Tas et al., 2021).

Functional Pathway Reconstruction.

The process includes the application of such tools as FragGeneScan to predict genes and InterProScan to extract functional annotations of protein families with the assistance of KEGG to analyze the pathways (Sime et al., 2024). These predicted genes are then combined into contigs and undergo ORF prediction to identify coding regions which in the end produces a full non-redundant set of genes in the metagenome (Klair et al., 2023). This catalog can be used as a critical resource to measure the abundance of individual functional genes and pathways in different samples and compare metabolic capabilities under different environmental conditions (Zhou et al., 2022). Such bioinformatic methods are of special use when studying the biogeochemistry of microbiomes containing uncultivated members, to provide information on their metabolic processes and roles in community- and population-level functions (Kosmopoulos & Anantharaman, 2024).

Data Analysis of Metatranscriptomics.

Unlike metagenomics, metatranscriptomics can give a real-time picture of gene expression activity in a microbial community showing how organisms react to environmental signals (He et al., 2025). This method, using RNA sequencing, does not only define the taxonomic composition, but also provides a clear picture of the biochemical pathways that are actively expressed in complex microbial communities, thus providing a dynamic view of the functional state (Jiang et al., 2016). Therefore, it provides a more immediate measure of the activity and adaptation of microbes than would be derived using metagenomics data alone.

Metaproteomic Profiling Methodologies.

Metaproteomics, by studying the whole protein repertoire of a microbial community, also contributes to our understanding of active metabolic activities and enzyme activities, providing beyond reasonable doubt physiological reaction to changes in the environment (Carcer,‘2020; Chaudhari et al.,‘2023). The technique generates important information on protein abundance, post-translational changes and enzyme actions that are directly involved in causing biogeochemical changes in ecosystems (Zarraonaindia et al., 2012). These sophisticated methods can be used together with other omics to explain complex microbial interactions and their functional input, beyond compositional inventories to achieve a mechanistic view of microbial ecosystems (Jain et al., "Using Genomics, Metagenomics and Other omics to Assess Valuable microbial ecosystem services and Novel biotechnological applications, 2019). This type of integrative meta-omics methods is essential to understand the microbial communities comprehensively and can be used to reconstruct the metabolic pathway and have the ability to quantify the active transcripts and proteins (Schiml et al., 2023).

Bioinformatics Resources and Databases of Functional Analysis.

Such resources include taxonomic profiling and co-occurrence networks, up to keystone species and functional patterning in a microbial community (Pohl, 2022; Setubal et al., 2020). They play a critical role in the conversion of raw omics data into biologically informative data and uncovering the complex interactions between the community structure and community functioning (Arikan and Muth, 2023). As an example, scientists use these resources to process transcriptomic data, outlining active metabolic reactions and protein expression to environmental disturbances (George et al., 2017; Jurburg et al., 2022). By combining various omics data, such as metagenomics, metatranscriptomics, and metaproteomics, the community dynamics can be examined in a holistic manner, allowing to connect the genetic potential with the expressed functionality and active metabolism (Arikan and Muth, 2023; Zhang et al., 2019). This multi-omics approach provides us with a panoramic perspective of microbial communities, enhancing our knowledge on their contribution to processes in the ecosystem, as well as on how they react to environmental changes (Ascandari et al., 2023).

Case Studies: Environmental Change and Functional Microbiome Response.

Recent studies have taken advantage of more sophisticated multi-omics approaches to query the effect of a range of environmental manipulations, such as thawing permafrost and changes in nutrient availability, on the potential and ongoing metabolic activities of microbial communities (Reid & Bergsveinson, 2021; Terron-Camero et al., 2022).

Ocean Acidification Effect on Marine Microbiomes

Ongoing acidification of oceanic waters is a significant menace to marine ecosystems since the changes in pH directly affect metabolic processes and gene expression patterns of marine microorganisms which play a central role in the global biogeochemical cycling. Metatranscriptomics and metaproteomics studies have reported carbon fixation pathway reconfigurations, nutrient-cycling gene reconfigurations, and highlighted a fundamental reorganization of ecosystem services (Iyer, 2016). This type of work is impossible without anticipating the overall ecological implications of the climate change on the marine microbial communities and their elemental cycles. In addition to the pH variations, increased sea-surface temperatures and deoxygenation are other forms of stressors that induce changes in the metabolic approaches and community composition, which can be further explained using the combined multi-omics techniques. The interaction of these environmental stressors is dynamic hence requiring a multi-faceted, holistic approach to the study of the adaptive abilities and susceptibility of marine microbial consortia (Marco & Abram, 2019; Yang et al., 2025).

Exposure to pollutants and Soil Microbial Processe.

The addition of a variety of pollutants, such as heavy metals and organic pollutants, changes the functional landscapes of the soil microbiomes significantly, thus, influencing nutrient cycling and the stability of the ecosystems. Meta-omics in its entirety, including metagenomics and metatranscriptomics, is especially beneficial in the discovery of new pathways and enzymes that degrade contaminants and can be missed when the organism is assessed by other conventional taxonomic methods (Ridley et al., 2024; Tas et al., 2021). These methods allow breaking down microbial mechanisms of detoxification or biotransformation of pollutants, which gives important information about the resilience and remediation capacity of polluted soils (Weiman et al., 2021). As an example, systemic studies of compositional heavy-metal exposures have revealed the effects of this exposure on soil microbial processes, particularly elemental cycling and adaptive responses (Zhao et al., 2025).

The impact of Climate Change on Gut Microbiomes

The effects of the climate change are not limited to external environments, as it affects host-linked microbiomes as well. Temperature changes and changes in food availability restructure the structure and metabolism of the gut microbiota (Microbes and Climate Change -Science, People and Impacts, 2022). Such changes further affect the health of hosts and resilience, hence the complex nexus between the environment, microbial physiology, and host adaptation (Shree��DED, 2022). In fact, it has been demonstrated that dietary shifts due to climate have significant alterations on the gut microbiome with downstream effects on host metabolism and immune functions. This literature highlights the urgency to use omics technologies to perform detailed meta-data processing and trend analysis in environmental microbiology and especially the understanding of the interactions between uncultivable organisms and their in-vivo environment (Bilal, 2024).

Man-made perturbations in Freshwater Systems

Several factors contribute to the pollution of freshwater systems; industrial discharge and agricultural runoff are anthropogenic inputs that add a plethora of pollutants into the system, thus fundamentally changing the structure and functional capacity of the microbial community (Reid & Bergsveinson, 2021; SEE et al., 2024). Such disruptions may trigger major changes in biogeochemical processes and ecosystem services because microbes either evolve or are overcome by pollutant-tolerant species (Kumar et al., 2024). These perturbations normally favor microorganisms with a specific set of detoxification mechanisms or resistance mechanisms, which are also delineated by detailed metatranscriptomic and metaproteomic studies (Kumar et al., 2022). Pioneering omics approaches are therefore critical in explaining the ecological forces behind the formation of a community, as well as in identifying sentinel taxa that can be used as early warning signs of ecosystem change (Jungblut et al., 2022; Yu et al., 2023).

Difficulties and Future Projections.

Although these advanced multi-omics techniques provide a previously unseen level of understanding about the functioning of microbes, they come with substantial technical challenges and underlying constraints (Reid and Bergsveinson, 2021).

Computational Requirement of Functional Metagenomics

The large volumes of data produced by such methods require complex bioinformatics pipelines and high-performance computing power, and present an insurmountable challenge to many studies.

Protocol and Data Data Analysis Standardization.

One of the most frequent and unsolved issues is that there are no standardized techniques of sample collection, nucleic acid extraction, sequencing, and downstream bioinformatic analyses, which often produce different and incomparable results across studies even considering similar environments (Rodriguez, 2023).

The Multi-Omics Data Integration.

The scale and diversity of data generated during multi-omics experiments create significant challenges in creating integrative analytical models that can synthesize information across the different levels of molecules coherently and identify important microbial activities and interactions (Arikan and Muth, 2023).

Predictive Microbiome Function Modeling.

It is still a challenging goal to develop powerful predictive models capable of predicting microbiome responses to environmental perturbations due to the complexity of microbial interactions and the dynamism of ecosystems. In addition, defining the specific functional roles of uncultivated microbial taxa those that generally prevail in environmental microbiomes remain a daunting challenge even with the advances in single-cell genomics and cultivation-independent methods. To overcome these obstacles, further development of computational tools and machine-learning algorithms is urgently required, which will make it possible to consider complex multi-omics data more fully and comprehensively to predict the behavior of microbial communities in conditions of environmental change (Jurburg et al., 2022).

Environmental Management and Biotechnology.

Information obtained through the characterization of functional microbiomes is useful in the development of specific biotechnological solutions, including bioremediation of polluted environments or the improvement of nutrient cycling in agricultural systems. These methods are also critical in shaping environmental policies and management practices to reduce the negative effects of environmental change on the microbial ecosystems (Arikan and Muth, 2023; Hallin and Bodelier, 2020).

Conclusion:

The shift in the approaches in taxonomic towards functional analyses, which is supported by the development of multi 2 -omics technologies, is fundamentally redefining our understanding of the microbial ecology, as well as its critical importance to the health of the planet. The paradigm shift also incorporates a more in-depth investigation of the metabolic potential and functional stability of microbial communities in different ecosystems, and it is possible to produce the entire biomaps that combine microbial taxonomy and activity (Jansson et al., 2011). Future studies should, thus, apply these combined omics methods to solve the complex systems through which microbial communities react to environmental stresses, thus leading to the establishment of effective restoration technologies and sustainable resource management (Cho, 2021). Breaking the current restrictions in analytical sensitivity and spatial resolution will be essential to define microbial activities at ecologically viable scales (Blaser et al., 2016). Lastly, it will also be necessary to include non-bacterial components of the microbiome, including viruses, fungi, and protists, in order to have a comprehensive picture of their fundamental roles in host-microbe interactions and ecosystem functioning (Fountain-Jones et al., 2023)

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