



Improving the assessment of ecosystem and wildlife health: microbiome as an early indicator

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Human activities are causing dramatic declines in ecosystem health, compromising the functioning of the life-support system, economic activity, and animal and human health. In this context, monitoring the health of ecosystems and wildlife populations is crucial for determining ecological dynamics and assessing management interventions. A growing body of evidence indicates that microbiome provides a meaningful early indicator of ecosystem and wildlife health. Microbiome is ubiquitous and both environmental and host-associated microbiomes rapidly reflect anthropogenic disturbances. However, we still need to overcome current limitations such as nucleic acid degradation, sequencing depth, and the establishment of baseline data to maximize the potential of microbiome studies.

Addresses

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Introduction

Earth's ecosystems are largely and increasingly dominated by humans through land transformation, resource exploitation, modifications in biodiversity, climate change, and alterations in biogeochemical cycles [1]. Particularly, the loss of biodiversity has been identified as one of the main threats and challenges for the coming decades [2]. The current rate of species extinction is

estimated to be 100–1000 times higher than historical rates and it is estimated to further increase by 10–100 times over the next 50 years [3]. These human impacts are causing dramatic declines in ecosystem health, compromising the functioning of the life-support system, the sustainment of economic activity, and animal and human health [4]. It is now clear that we cannot escape the responsibility and necessity of restoring and managing our planet. In this context, monitoring the health of ecosystems and wildlife populations is crucial for determining ecological dynamics, prioritizing active management, and assessing the efficacy of management interventions. Nevertheless, the majority of health assessments and symptoms of ecosystem dysfunction are retrospective. Despite years of research, we still rely heavily on alterations that manifest late in the disturbance process such as changes in animal abundance and richness [5], loss of interactions and functional diversity [6,7], decline in ecosystem services [8], or pathogen emergence [9,10]. We urgently need to develop reliable prospective indicators of wildlife and ecosystem health with early-warning capabilities. One such promising indicator is the microbiome, described as the interacting community of commensal, symbiotic, and pathogenic microorganisms within a body space or other environment, together with the whole spectrum of molecules that they produce and mobile genetic elements (i.e. phages, viruses, and extracellular DNA) [11].

Microbiome reflects ecosystem disturbance

A growing body of research points out that microbiome provides a meaningful early indicator of ecosystem disturbance [12]. First, microbiome is ubiquitous in terrestrial, freshwater, and marine habitats, as well as in the animals and plants living in them. Therefore, unlike most traditional indicators (e.g. macroinvertebrate communities), microbiome approaches are potentially applicable to any ecosystem, including extreme or highly degraded environments. In ecosystems, environmental microbiome is a key component performing essential ecological processes such as primary production, nutrient cycling, and retention, and influencing plant diversity and host-associated microbiomes [13–15].

Crucial for signaling ecosystem health, microbiome is highly sensitive to environmental disturbances, including anthropogenic impacts [16]. Recently, Rillig et al. [17]

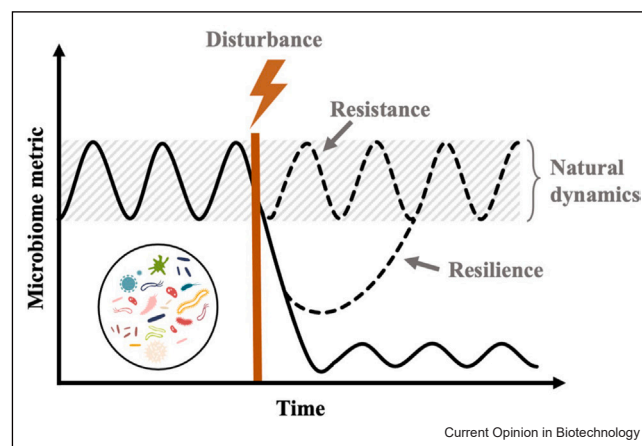
showed that the complexity, composition, and overall abundance of soil microbiomes declined along with the number of global change factors, including resource availability, abiotic factors, toxic compounds, and microplastics. Soil microbiomes have also shown to shift in response to anticipated consequences of climate change, including elevated atmospheric carbon dioxide, elevated temperature, permafrost thawing, drought, floods, and wildfires [18]. One of the primary anthropogenic stressors driving ecosystem degradation worldwide is habitat loss as a consequence of land use change [19], which also influences the soil microbiome [20]. Detectable changes in environmental microbiomes of terrestrial, freshwater, and marine ecosystems are also induced by pollution through chemicals and light [21], mine drainage [22], oil spills [23], heavy metals [24], microplastics [25], and sewage waters from urban centers [26]. The latter is not only an example of pollution but also illustrates how the encroachment of human and domestic animal microbiomes and pathogens can alter environmental microbiomes. Biological invasions, by microorganisms, plants, or animals, are one of the main drivers of global change and generate, as well, shifts in the microbiome of native ecosystems [27].

The examples above demonstrate that environmental microbiomes are generally not resistant to anthropogenic disturbances. However, microbiomes could be resilient to environmental perturbations, quickly recovering their initial composition and hampering our ability to detect transient shifts (Figure 1). Encouragingly, Allison and Martiny [28] showed that, within a few years, microbial composition still differed from that of undisturbed communities, thus allowing realistic sampling intervals for ecosystem monitoring. Furthermore, microbial communities are probably not functionally redundant and disturbances often impact the rates of ecosystem processes [28]. This suggests that we can detect shifts in community composition and function as two complementary axes of variation [29]. Overall, conditions altering the composition of environmental microbiomes may severely impact the structure, function, and natural balance of ecosystems, and increase human and animal exposure to pathogens. Despite the evidence that microbiome can be used to detect change processes in ecosystems, its use as an indicator has mainly been evaluated for marine ecosystems, particularly for coral reef ecosystems [30–32]. Therefore, microbiome still holds an unexplored diagnostic capacity to predict ecosystem alterations and guide management interventions, especially for terrestrial ecosystems.

Wildlife population health and bioindicator species

Tracking the microbiome of wildlife populations has potential human, animal, and ecosystem health applications. In an era of global change, we are witnessing

Figure 1



Graphical representation of microbiome dynamics. The horizontal streaky bar represents normal oscillations or baseline data over time for a particular microbiome metric (e.g. diversity). Disturbance events (orange vertical bar) can alter environmental conditions and trigger shifts in microbiome (solid black line), leading to alternative stable states that significantly deviate from the natural state. Resistance is the ability of a community to withstand a disturbance without change. Resilience is the rate at which the original state is regained after a disturbance event. Only after the establishment of these baseline intervals of normality, we can begin to understand microbiome responses to disturbance or stress.

major outbreaks of emerging diseases in humans [33]. The majority of emerging infectious disease threats originate from wildlife, and outbreak risk is increased by ecological degradation [9,34]. Over recent years, some progress has been made in monitoring wildlife microbiomes in order to predict, prevent, and control zoonotic disease emergence. For example, in 2009, the U.S Agency for International Development launched the Emerging Pandemic Threats Program (EPT-1), which has been followed by a new generation of investments (EPT-2, <https://www.usaid.gov/cpt2>). Nevertheless, important steps still need to be taken to strengthen the monitoring of population trends and health for wild reservoir species, to identify novel infectious agents, and to better understand the links between ecosystem degradation and zoonotic spillover.

Concurrently, infectious diseases have been identified as a growing threat to wildlife conservation, contributing to the global loss of biodiversity [35,36]. Amphibian chytridiomycosis is perhaps the most striking example of the impact of diseases on biodiversity. Since its discovery in the 1990s, chytridiomycosis has been directly linked to the decline of over 500 amphibian species, the extinction of 90, and continues to threaten amphibian diversity globally [37]. Disease threats to biodiversity share common anthropogenic drivers with disease outbreaks in humans [35]. Despite the need to monitor the health of declining wild populations, little progress has been made

on the use of microbiome as an indicator of wildlife population health and most pathogen threats still remain undiscovered.

Microbiome has been linked to metabolic functions, aiding digestion, nutrition, development, and immune defenses in animal hosts [15,38,39]. Shifts in microbial community composition may thus lead to dysfunction of host machinery, contributing to the development of a broad range of infectious and noninfectious diseases. Animals interact with their environment extensively and environmental characteristics strongly shape microbiome composition and influence microbiome functionality. Anthropogenic disturbances also leave traceable changes in hosts' microbiomes, suggesting that host-associated microbiomes may be realistic indicators of individual and population health in wildlife species. In amphibians, shifts in skin microbiome as a result of habitat degradation and environmental pollutants can increase the susceptibility and the outcomes of disease [40]. Similarly, impacts on the fecal microbiome of polar bears have been detected as a result of global change, suggesting that it could be incorporated into health assessments for conservation practices [41]. In honeybees, changes in the structure and function of the gut microbiome were detected after pesticide exposure, which appeared to reflect the overall health of the colony [42]. Moreover, monitoring microbiomes could significantly improve ethical and practical aspects of wildlife sampling, replacing procedures such as physical and chemical restraint, blood sampling, or tissue samples (e.g. toe clipping in amphibians). Indeed, microbiome studies can be performed in noninvasive samples, and in elusive, protected, too-large, or too-small species [15]. Importantly, these microbiome alterations seem to precede clinical manifestations of disease processes. For example, despite healthy appearance, coral microbiome communities under anthropogenic stress resembled those of diseased individuals, potentially being at a tipping point toward disease [43]. This shows that wildlife microbiomes could serve as a reliable early-warning system, advising management interventions before disease outbreaks or further biodiversity losses.

More broadly, wildlife microbiomes can also be used to signal ecosystem degradation and inform overall ecosystem health. Host-associated microbiomes are influenced by environmental conditions and can reflect stressors and processes of change. It is clear, however, that not all wildlife species are equally capable of signaling ecosystem health. Therefore, we need to identify suitable bioindicator and biomonitor species, which respond predictably to environmental change and quality [44]. Wildlife species that have disproportionate effects on the ecosystem relative to their abundance are interesting targets since they may respond earlier to changes. Coral microbiomes can be one such bioindicator/

biomonitor of the health of coastal marine ecosystems, since corals are the backbone of reef systems, provide food and shelter for many species, and are tied to the changes in reef health [32]. The gut microbiome of several filter-feeding species is especially sensitive to the environment and also holds potential for aquatic ecosystems [45]. In terrestrial ecosystems, honeybee microbiomes have been suggested as good proxies for ecosystem health status [46]. Other promising indicators are top predators or species with narrow ecological niches as they can indicate changes in the trophic network and respond rapidly to minimal shifts in habitat conditions [41]. Bats are another interesting taxon that, despite having received great interest as reservoirs for zoonotic agents, have not been explored in ecosystem health assessments. Their diversity of species, broad distribution, feeding habits, and ability to fly point out their inherent capacity to signal ecosystem alterations. In fact, new studies demonstrate that the fecal bacterial microbiome of insectivorous bats can reflect habitat degradation and propose innovative uses of microbiome monitoring [47].

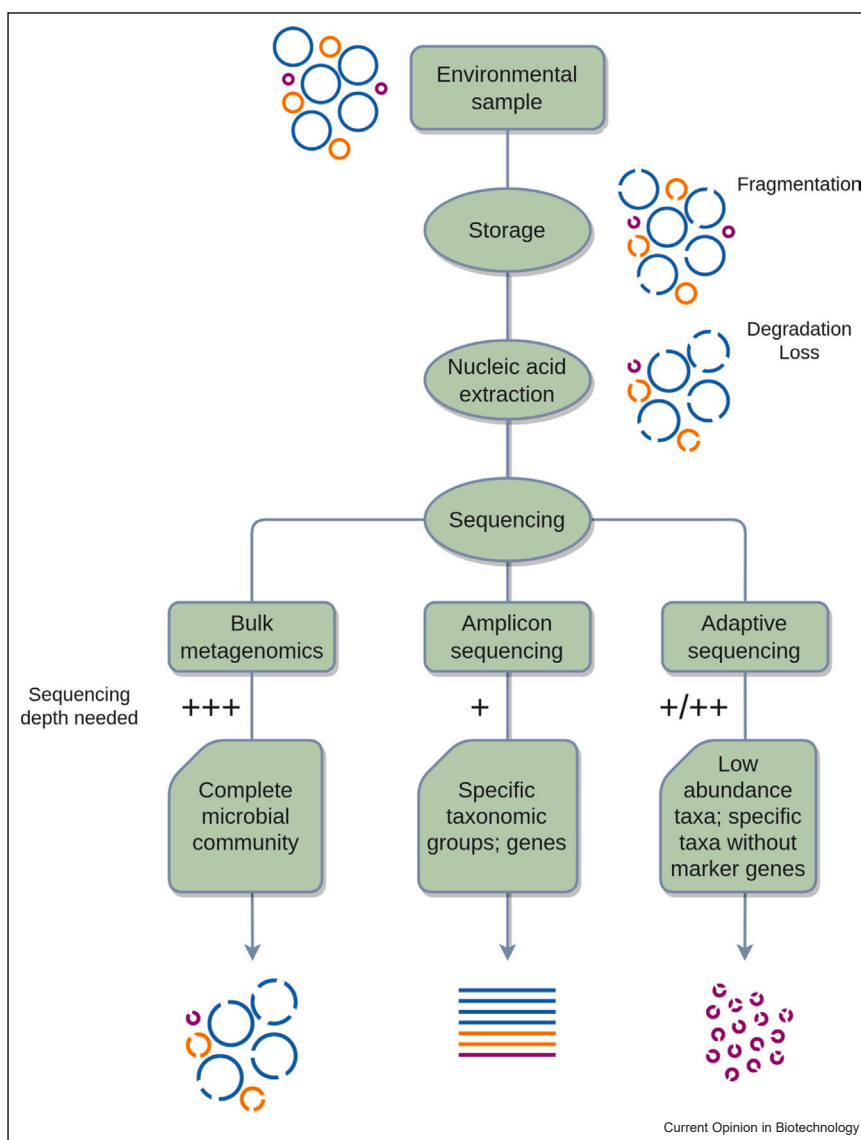
Future needs for microbiome studies

Since the advent of next-generation sequencing technologies, microbiome studies rely on metagenomics to collect taxonomic and functional data from microbial communities [48]. These molecular technologies make studying microbial communities a cost-effective alternative to traditional methods and enable a more integrative approach. However, a careful balancing act between resource allocation, sample size, and sequencing depth must be performed in order to achieve meaningful results. First, a large amount of genetic material should be recovered in order to achieve a complete community sampling. With variable results, multiple buffer solutions and rapid freezing, often in liquid nitrogen, are the two most common methods implemented in order to avoid high degrees of nucleic acid degradation in fresh samples [49,50]. Even in the best-case preservation scenario, partial fragmentation and loss of genetic material are inevitable at the nucleic acid extraction step regardless of the method [51,52]; commercial kits are fairly standardized and scalable but provide moderate-to-low yields, while phase separation methods such as phenol–chloroform generate high yields but require manual precision and often carry contaminants to downstream processes. Second, sufficiently high sequencing depth must be met to provide a full recovery of the community, a need that rapidly increases with the amount and diversity of the target sequences [53]. The question of how much sequencing depth is enough is often difficult to answer, particularly for exploratory studies targeting low-diversity taxa and those involving nonmodel organisms, given the lack of reference studies. Thus, sequencing the totality of the

genetic material may only be suitable for select cases of high-quality, low-diversity samples with previous references. Despite its known methodological biases [54], researchers have turned to selectively sequence a gene or small subset of genes that serve as a proxy for the entire community composition or a subset of its functionality. For instance, most taxonomic studies focus on deep amplicon sequencing of the 16S rRNA gene from prokaryotes, which nowadays can easily be recovered in its entirety through long-read sequencing technologies [55,56]. Contrastingly, viral diversity is often difficult to

study, since viruses do not have a universal marker gene and are often found in low abundance in healthy individuals [57]. Targeted sequencing of functional genes is also tricky, as there are not many standardized kits available and the scope of the intended scientific questions should be rather narrow by design. Recently, Nanopore adaptive sequencing emerged as a promising tool for the study of low-abundance sequences through various enrichment approaches [58], although its full potential is still under study and development [59,60] (Figure 2).

Figure 2



Common drawbacks and constraints in the collection of nucleic acid data from environmental samples. The composition of microbial community samples becomes altered when subjected to laboratory procedures. For instance, nucleic acid material often gets fragmented when stored, and further degraded and partially lost during the extraction step. In exploratory studies, the decision of which type of sequencing to use is strongly constrained by the complexity of the sample and the resources available to afford the necessary sequencing depth to achieve meaningful results.

Besides methodological constraints, the use of microbiome indicators still entails some other relevant limitations. Microbiome responses to stress do not have a straightforward interpretation and we cannot infer health or disease based solely on the direction of the change (i.e. increases or reductions in abundance, diversity, and function). There is a growing consensus that occurrence and in some cases even degree of anthropogenic disturbance often manifests as one of the following microbiome alterations: 1) overall decrease in alpha-diversity, 2) increase in beta-diversity, 3) decrease in beneficial and/or increase in pathogenic or disease-associated bacteria (sometimes can occur along an increase in alpha-diversity), 4) increase in the dominance of generalist over specialist bacteria, and 5) increase in the abundance of microbial resistance genes [16,40,61–69]. These parameters and changes may represent useful microbiome indicators of wildlife and ecosystem health. However, the magnitude and direction of these shifts are difficult to generalize across different ecosystems, resulting in both increases and reductions of particular species, microbial biomass, and activity [18]. A clear example is seen in arctic and subarctic ecosystems experiencing permafrost thawing where increases in alpha-diversity are commonly reported [18,70]. Most probably, this means that we need to develop specific microbiome indicators and be aware of their particular responses in each ecosystem and species, and probably for each threatening process. Local changes imply that local authorities and environmental agencies should become more involved in microbiome studies, whereas now, they mostly attract the interest of international institutions.

Microbiomes naturally are dynamic, their composition and function oscillate within certain intervals, even in undisturbed ecosystems and in healthy hosts [71]. Therefore, we need comprehensive analyses of the temporal and spatial variation of each unique microbial community and the subsequent identification of baseline reference data in order to correctly interpret microbiome changes. Only after the establishment of these intervals of normality, we can begin to timely detect microbiome responses to disturbance or stress (Figure 1). Considering that human impacts are widespread throughout the planet, are we too late to establish baseline data for the microbiomes of wildlife hosts and ecosystems? Probably. However, we can define health based on reference data by comparing different ecosystems and wildlife populations. Systems capable of maintaining ecological integrity, ecological processes, and services can act as proxies of pristine and unperturbed communities, and will constitute our best-guess estimations for baseline microbiomes. Importantly, the dynamic nature of microbiomes also allows for signaling positive changes in ecosystem and wildlife health. Most scientific attention has focused on studying microbiome responses to disturbance, however, it may be

equally valuable as an indicator of improvement in health parameters. Particularly, microbiome could help monitor the outcome of management interventions to restore ecosystem functionality or re-establish healthy wild animal populations. Finally, further challenges remain for the broadscale application of microbiome as a health indicator, particularly in remote areas or developing countries. Metagenomic tools require advanced technical and computational skills and, despite they have become cheaper over the past years, they still entail significant costs. Therefore, these methods may not be readily available in remote areas and can be prohibitively expensive in low-income countries [72]. Moreover, in order to detect changes in microbiome indicators, it is likely that long-term datasets are needed, requiring long-term commitment and funding, and being even more difficult to achieve in such regions. Unfortunately, most biodiversity hotspots and the highest rates of environmental change occur in such remote or economically poorer regions, hampering many real-world applications for microbiome studies.

Conclusion

Human activities are having dramatic impacts throughout the planet and we urgently need reliable early-warning indicators for monitoring the health of wildlife populations and ecosystems. Microbiome, both environmental and host-associated, can be one such promising indicator since it is ubiquitous, performs key processes in ecosystems and hosts, and reflects anthropogenic disturbances. Changes in the microbiome tend to occur earlier in the disturbance process compared with common health indicators, allowing effective monitoring of wildlife and ecosystem health as well as the outcomes of management interventions. Nevertheless, microbiome potential as a health indicator remains largely unexplored, mostly in terrestrial ecosystems. Important limitations still remain for the widespread application of microbiome as a health indicator, including biotechnological tools, interpretation of shifts in selected parameters, understanding baseline and altered microbiome dynamics, and logistical constraints. Overcoming current limitations will require establishing clear methods and best-guess baselines, increasing funding, and fostering international interdisciplinary collaboration. Future efforts in this direction will maximize the potential of microbiome for monitoring wildlife and ecosystem health.

CRedit authorship contribution statement

Maria Puig Ribas: Conceptualization; Data curation; Writing – original draft; Writing – review & editing. **Manuel García-Ulloa:** Writing – original draft. **Johan Espunyes:** Methodology; Writing – original draft; Writing – review & editing. **Oscar Cabezon:** Conceptualization; Supervision; Validation; Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Conflict of interest statement

The authors of the paper have no conflicts of interest to disclose. All authors read, revised, and approved the final paper. We confirm that the submitted paper has not been published or neither submitted for publication elsewhere.

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