Opportunities and challenges for microbiomics in ecosystem restoration

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Abstract

Microbiomics is the science of characterising microbial community structure, function and dynamics. It has great potential to advance our understanding of plant-soil-microbial processes and interaction networks, which can be applied to improve ecosystem restoration. However, microbiomics may be perceived as complicated, and the technology is not accessible to all. The opportunities of microbiomics in restoration ecology are considerable, but so are the practical challenges. Applying microbiomics in restoration must move beyond compositional assessments and incorporate tools to study the complexity of ecosystem recovery. Advances in meta'omics tools provide unprecedented possibilities to aid restoration interventions. Moreover, complementary non-'omics applications, such as microbial inoculants and biopriming, have the potential to improve restoration objectives by enhancing the establishment and health of vegetation communities.

Enhancing ecosystem restoration with microbiomics

The rapid pace and extent of environmental degradation have made **ecosystem restoration (see Glossary)**—the process of assisting the recovery of a degraded ecosystem—a global priority. The United Nations declared 2021-2030 the UN Decade on Ecosystem Restoration to reflect the urgency and scale with which we must restore our damaged ecosystems [**1**]. The scale and challenges involved are immense. Accordingly, restoration ecologists must draw upon the most effective tools available and embrace new opportunities to enhance restoration outcomes [**2**].

Microbiomics is a branch of science providing a detailed characterisation and quantification of microbial communities that inhabit ecosystems, many of which contribute to ecosystem functioning. Microbiomics includes the application of DNA and RNA-based tools along with biochemistry and bioinformatics. It aims to understand the composition, temporal and spatial dynamics, and functional characteristics of microbial communities. Moreover, microbiomics tools are used to scrutinise the functions and biochemical profiles of these microbial communities. Microbiomics is generating new ways of thinking about ecosystems and their recovery. Microbiota play a multitude of functional roles in ecosystems, from nutrient cycling to climate regulation [**3**] and plant [**4**], non-human animal and human health [**5,6,7**]. Gaining a greater understanding of these functions and processes will enhance our ability to restore degraded ecosystems.

Here we start by highlighting the functional importance of microbiota in ecosystem restoration (**Figure 1**). We then provide an overview of the current and emerging technologies used in microbiomics-based restoration studies to tackle testable hypotheses across the restoration continuum (incl. planning, implementation and monitoring). Restoration microbiomics needs to move beyond compositional assessments and towards the functional and longitudinal effects of microbiota on restoration outcomes. We also identify key opportunities and challenges to the integration of microbiomics in restoration ecology. For completeness, our scope includes some non-'omics applications (e.g., inoculations) that ultimately influence or complement microbiota at the community level.

Functional roles of microbiota

Restoring degraded ecosystems relies, in part, on microbiota with desired functional traits, particularly those that play essential roles in enhancing ecosystem resilience and complexity, including network connectivity. As such, microbiota are emerging targets of ecosystem restoration and are receiving increased attention [**8,9**]. Microbiota, directly and indirectly, affect many ecosystem processes (**Figure 1**). Research to understand the mechanistic basis of these effects is increasingly popular. Some of these effects include plant and animal health (e.g., inducing immune responses, outcompeting incoming opportunistic pathogens, producing antibiotic compounds) [**10**], nutrient cycling (e.g., controlling the fate of below-ground carbon by decomposing organic matter or stabilising it in the soil mineral matrix) [**11**], drought stress tolerance (e.g., plant growth-promoting microorganisms can biochemically induce systemic tolerance) [**12**], intra- and inter-kingdom communication (e.g., via quorum sensing and biochemical lures, respectively) [**13**],

hormone production in plants and animals (e.g., microbe-derived auxin as a signalling molecule in plant development) [**14**], climate regulation (e.g., by producing and consuming CO_2, CH_4, N_2O gases) [**15**], and pollination (e.g., by attracting pollinators and inducing pollen bursting) [**16**]. Researchers can use microbiomics to ask important questions and understand the connections between above-ground plant and animal communities (in terrestrial and aquatic systems) and below-ground microbiota. They can use microbiomics to assess the impacts of restoration activities on these inter-kingdom relationships and, thus, ecosystem functionality [**17**]. For instance, does increasing native plant coverage and/or plant species richness enhance the functional diversity of the soil microbiome or vice versa? Or can inoculating degraded soil with locally adapted microbiota improve the resilience and fitness of local, native plants?

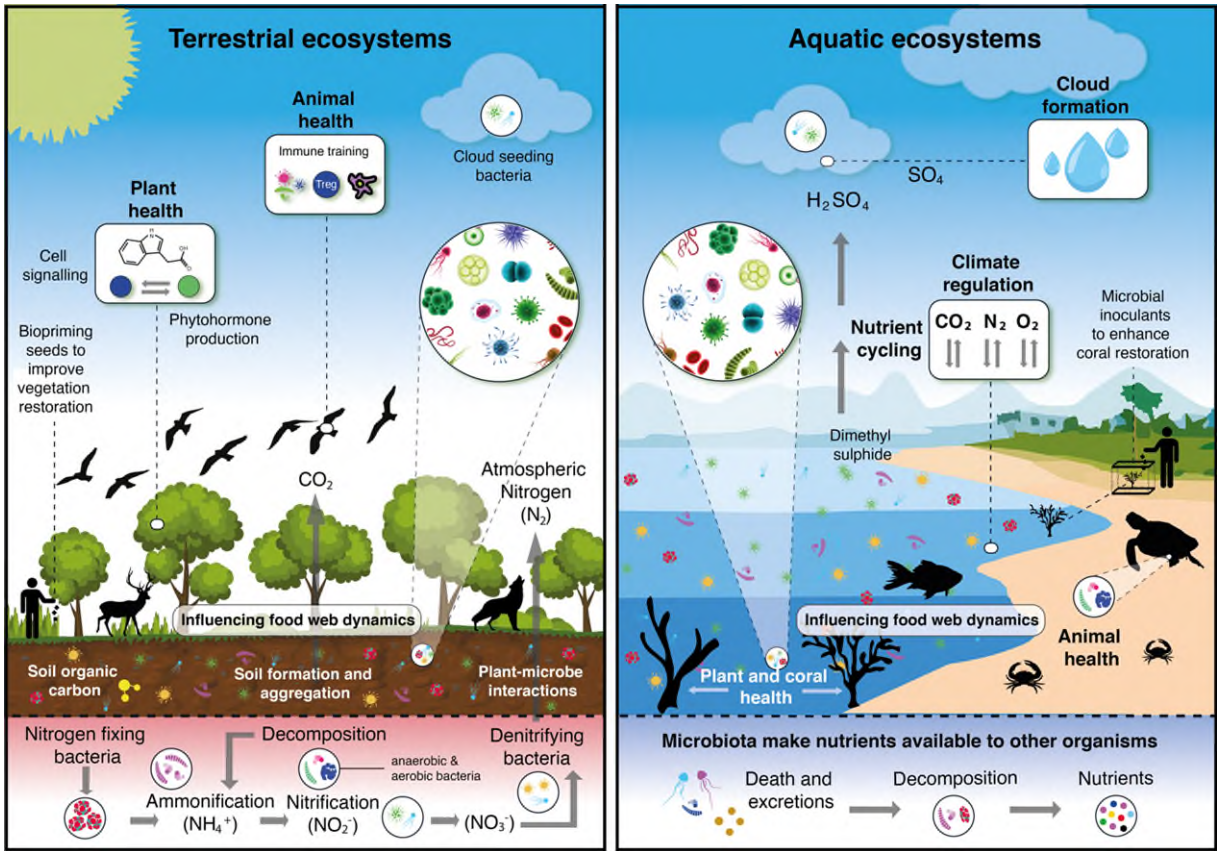

Figure 1 | **The importance of microbial communities to ecosystem functionality.** Microbiota have many functions in terrestrial and aquatic ecosystems, and the full range of functions is far greater than shown here. Microbiota play similar core functional roles in most ecosystems (e.g., nutrient cycling, climate regulation, substrate formation, animal and plant health). We can apply knowledge of these functions to improve ecosystem restoration, for instance, via using biopriming (inoculants to protect seeds) and broader microbial inoculants to optimise vegetation or coral establishment. Figure produced using Adobe Illustrator (v.25.21).

Overall, ecosystem restoration efforts still need to fully operationalise current knowledge of microbiota at the individual, population, community and ecosystem levels. More experimental studies are required to relate microbiomics attributes to restoration outcomes. To this end, microbial ecologists can seek to overcome microbial blindspots (e.g., core gaps in functional knowledge) by employing **meta'omic** approaches in such experiments. Potential blindspots include gaps in knowledge regarding the roles of specific microbial species or groups in nutrient cycling, soil health, plant-microbe interactions, and overall ecosystem functioning. Indeed, scientists still need to improve their understanding of how restoration can bring the structure of microbial taxa to a desired state of functionality [**9**]. However, the uptake of functional enquiry is increasing in restoration studies (**Figure 2**; [**18**]). Advances in microbiomics play a key role in building this knowledge by, for example, allowing ecologists to determine baseline ecosystem conditions (e.g., microbial composition and functional profiles and inter-kingdom network interactions). Such advances also allow us to track changes in microbial communities and their functions and interactions across space and time [**19**]. Manipulative experiments (e.g., micro and mesocosm trials, common garden experiments) are also needed to study interactions between microbes, their metabolites and functions. These experiments should aim to understand how these interactions influence the responses and recovery of target ecosystem components (e.g., vegetation responses) [**20**].

142 The application of microbiomics in ecosystem restoration is still novel, although
143 increasing in recent years (**Figure 2A**). Perhaps unsurprisingly, these technologies
144 and associated data are unequally distributed across the planet—highlighting a data
145 equity issue (**Figure 2B**). Where data are missing, scientists should make efforts to
146 ethically engage with local research and practitioner communities. This is imperative
147 to ensure the communities have opportunities to collect and use microbiomics data
148 to enhance the restoration of their ecosystems.
149

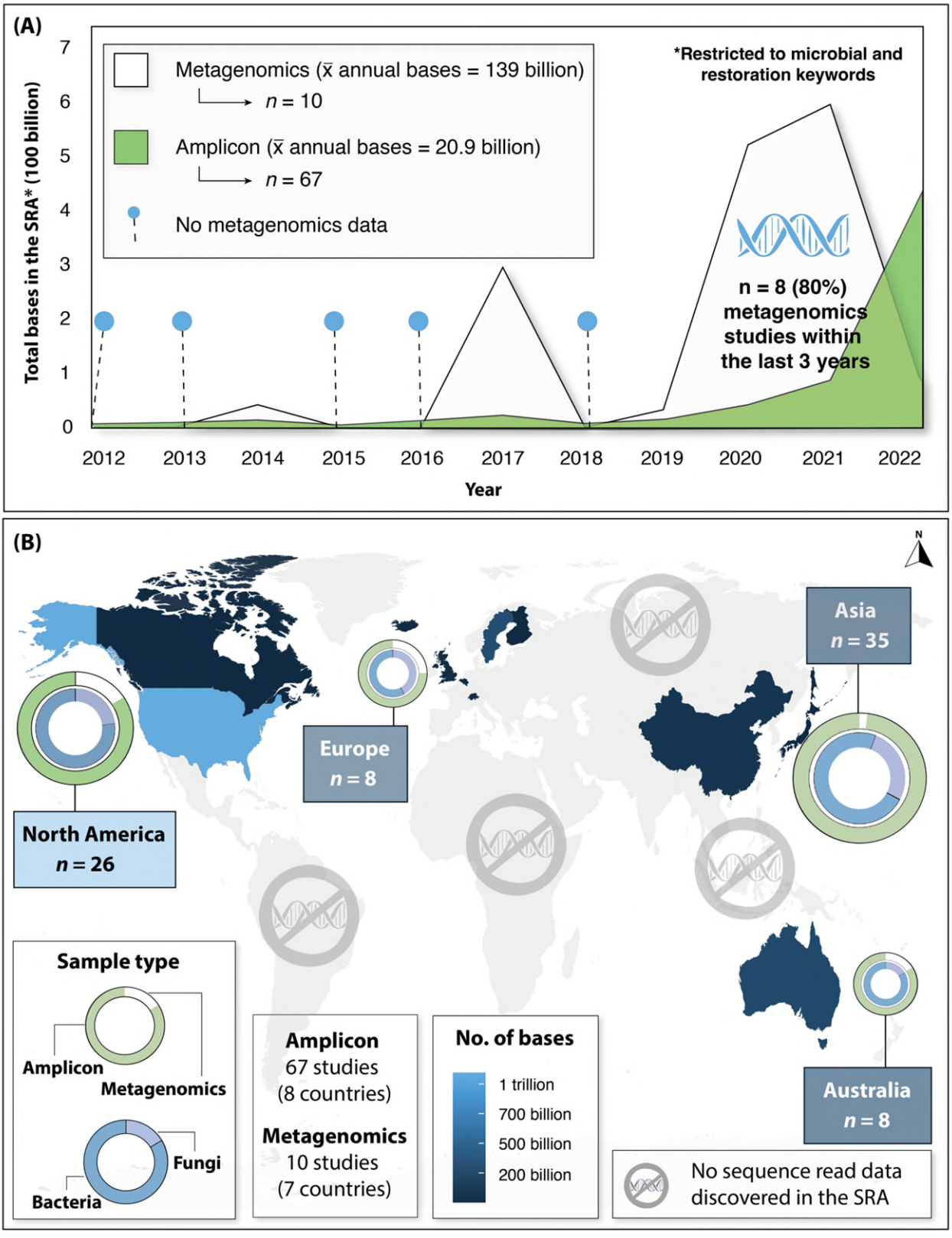

Figure 2 | **Timeline and geography of restoration microbiomics data.** (A) Reads deposited on NCBI's Sequence Read Archive (SRA) for restoration ecology studies over time (until 31st December, 2022) for **amplicon** and metagenomic sequencing. Restoration-centric reads were first deposited in 2012, followed by limited deposits until 2019, when reads for amplicon and metagenomic categories increased

considerably, reaching a peak of ~600 billion reads in 2021. (B) Overview of the geography of deposited reads from restoration studies, plus the proportion of samples that were either amplicon or metagenomics, and bacteria or fungi. The ring diameter represents the number of studies that contributed the reads. The map shows 'data inequity zones' where no reads were found on SRA. Out of the 253 global countries or territories, only 11 countries (=4%) had data on the SRA. The number of restoration studies on the SRA is considerably lower than the number of restoration–microbiomics studies published. This discrepancy suggests that some researchers may not be depositing their data on the SRA and, alternatively, may deposit their data elsewhere. Figure produced using Adobe Illustrator (v.25.21).

Applying microbiomics in restoration

Restoration practice includes planning, implementation, and monitoring phases (**Figure 3A**). Microbiomics offers valuable insights along this continuum (see Table 1 for case studies of the integration of microbiomics in restoration). For instance, successful ecosystem restoration typically requires the return of functional vegetation communities (e.g., forests, meadows, woodlands, grasslands) [**21**]. Microbiomics can be used to better understand how microbiota influence vegetation functioning, including plant immunity [**22**], dispersal, stress tolerance and growth [**23**]. These are directly relevant to vegetation community health and recovery and require considerations at each stage of a restoration project. Nonetheless, there are also some key limitations that need to be addressed (**Figure 3B**) relating to fully integrating microbiomics into restoration.

Restoration planning

Ecosystem restoration requires making informed decisions on interventions and selecting targets to achieve progress towards stated goals [**24**]. Where possible, planning should include thinking about microbiomics and complementary non-'omics approaches. Without these considerations, a restoration project could fail to address

critical ecosystem processes and miss goals accordingly. However, it is also worth noting that restoration projects are often faced with limited budgets. As such, there will be feasibility questions, such as: given the high costs of meta'omics tools, will it be worth investing in such large datasets and complex analyses? An alternative is empirically testing microbial functions using potentially less expensive methods, such as simpler physiological assays or culture-dependent approaches. Practitioners can also embrace soil health thinking and seek informed guidance from microbiome researchers, drawing on current knowledge within a rapidly evolving field. Nonetheless, as sequencing costs continue to fall and bioinformatic expertise continues to rise, the feasibility of incorporating more complex analyses into restoration planning continues. There is also the prospect that, as enough different representative ecosystem types become well-studied, the knowledge gained might be extrapolated over similar environments.

Although knowledge of the relevance of microbiota in ecosystems is rapidly growing, microbiota are rarely considered in restoration planning. Including microbiomics in baseline assessments at the planning stage will help frame the microbial functional, diversity and complexity status of the ecological starting place (e.g., via soil metagenomics). This could, in turn, affect the recovery trajectory as microbiota-plant or microbiota-animal interactions establish [**25**]. A restoration target incorporating soil microbiota at the planning stage should focus on identifying specific microbial compositions found in reference sites (e.g., via amplicon sequencing approaches – where greater sample coverage is needed) and on capturing soil microbiota functionality (e.g., via metagenomic or metatranscriptomic approaches) [**26**]. Such microbiomics is required to assess soil microbiota diversity and functionality, which

has important implications for restoration planning — i.e., determining the approach to measure and monitor interventions.

Traditional metrics used to plan ecosystem restoration assessments often focus on simple attributes, such as taxonomic richness (e.g., plant species richness) or single functions (e.g., carbon accumulation in soil) [**17**]. However, restoration assessments across various ecosystems demonstrate that when these metrics form the basis of restoration interventions, ecosystems may only partially recover their biodiversity, functions and socioeconomic benefits, even after centuries [**17**]. Planning to use traditional restoration approaches and the metrics used to evaluate them may fail to capture the full range of taxa, their interactions, and/or ecosystem functions. For example, high-intensity land use of soils, such as conventional arable farming, often increases microbial taxonomic diversity compared to improved land use practices. However, this does not reflect the system's wider (macro) biodiversity or ecosystem health [**27**]. Thus, more complex restoration metrics are needed to create more rigorous restoration standards. Indeed, recent attempts to update restoration standards to improve restoration planning (e.g., [**28**]) propose that restoration practitioners include attributes such as gene flow, microbial functional roles (trait-based), interkingdom interactions (i.e., between bacteria, fungi, plants and animals), and trophic relationships [**29**]. Each of these recommended attributes can be measured with microbiomics.

Complexity (incl. genetic diversity, species and their interactions, traits, and functions) plays a pivotal role in the resilience of ecosystems when facing environmental challenges, such as climate change and biodiversity loss [**30,31,32**].

For instance, plant-microbe-pollinator communities with higher species richness and network connectivity are more resilient to disturbance due, in part, to functional diversity and redundancy [**33**]. An important finding that exemplifies the need to study more complex metrics is that interactions among microbial taxa can change in response to perturbations without significant changes in species composition (a relatively simple metric). Moreover, the functional traits of taxa within a community can remain even if microbial compositions change [**17**]. In addition, the extinction of interactions may negatively affect the provision of ecological functions (e.g., pollination and seed dispersal) more than species extinctions alone [**17**].

Microbiomics can be used to develop a more comprehensive understanding of targets for microbiota reassembly and quantifying gradients of ecosystem complexity in the recovery process [**17**] (**Figure 3A; Table 1**). We emphasise the need to integrate ecological complexity into restoration planning through collaborations with microbial ecologists.

Restoration implementation

An example of an effective microbiome-centric intervention to assist ecosystem restoration is the reintroduction of soil microbiota that may be lacking in disturbed ecosystems. Facilitating the colonisation of appropriate soil microbiota can reshape plant community composition, increase plant growth and establishment, and re-instate fundamental soil ecosystem functions such as nutrient cycling and decomposition [**34,35**].

Culture-independent methods, such as whole soil inoculation (i.e., transferring soil from healthy, remnant or undisturbed areas to degraded ecosystems), offer

considerable benefits in ecosystem restoration [**20,36,37**]. Despite being considered a 'black-box' approach, this microbial-based intervention can be a feasible and cost-effective tool for restoration practitioners [**38**]. These soil-based interventions are generally conducted in restricted areas because they can damage the environment from which soils are sourced. Another major limitation of this approach is that certain soils (e.g., loam) are more conducive to soil translocation treatments than others [**20**]. Moreover, translocated soil is more likely to survive when applied to larger spatial scales. As Gerrits et al. (2023) suggest [**20**], a decision-support framework should be developed to determine appropriate soil translocation techniques in a restoration context. Soil physicochemical characteristics (e.g., texture, pH, organic carbon) are important drivers of microbial community structure and function. Soils are considered to host unparalleled microbial diversity, hence restoring the physicochemical characteristics of soils to a benchmark state has the potential to restore diverse microbiota, their traits and their associated functions. Microbiota can be returned by dispersal events from nearby reference habitat **[39]**, which can be facilitated by minimising habitat fragmentation. Initial restoration activities should address potential physicochemical barriers (e.g., fertiliser and soil compaction legacies of agriculture) that may limit the recovery of target plant-microbiota systems. Addressing legacy issues in soil conditions alongside microbiota is important to overcome barriers in microbial establishment. This can be facilitated either by interventions such as inoculations or by natural processes such as dispersal or regeneration from the seedbank.

Culture-dependent methods, which include isolation and identification of desired key microbiota, offer an alternative approach that should overcome some of the

challenges associated with whole soil translocation. Most culture-based studies that target soil microbiota for microbial inoculants have focused on mycorrhizal fungi and, to a lesser extent, on plant growth-promoting rhizobacteria and N-fixing bacteria [**40,41,42**]. More recently, soil biocrust organisms such as cyanobacteria as bioinoculants in ecosystem restoration have gained momentum. This is because they offer the potential to promote the growth of native plants and increase their tolerance to environmental stressors [**43,44**]. However, several limitations have been identified in biocrust applications. For instance, treating with amendments (e.g., water, nutrients, soil stabilisers, and saline solutions) to facilitate their recovery does not guarantee recovery of soil stability or biocrust performance [**45**]. There is a need to identify the full range of factors that limit biocrust establishment. Microbiomics can be used to help identify these factors. For example, microbiomics can allow researchers to measure responses to experimental changes in soil physicochemical properties alongside biocrust formation.

Overall, there is strong evidence that inoculants composed of indigenous soil microbial communities are more beneficial to restoration outcomes than exogenous strains or consortia that are commercially available [**46,47**]. **Allochthonous microbiota** (i.e., microbiota from non-local sources that cannot typically colonise habitats except under abnormal conditions) are generally not well adapted to local soil and environmental conditions. Moreover, they can reduce the diversity of resident microbiota, resulting in reduced performance of native plants [**48**]. Effective deployment of soil microbiota reintroductions to assist ecosystem restoration has considerable potential but remains challenging because of the complexity of soil-microbiota-plant dynamics and plant-soil feedbacks [**49**].

Symbiotic microbiota living on and in plants can stave off infections by inducing a plant immune response, outcompeting incoming opportunistic pathogens, and producing antibiotic compounds [**10**]. Plant-microbiota experiments utilising microbiomics can potentially aid restoration efforts by providing insights into safeguarding the core microbiota (distinctive microbial communities) of plant communities and the vital microbial metabolites that enhance plant growth, immunity, and stress tolerance. Organisms could be inoculated with microbiota prior to planting a restoration site. For example, aquatic microbiota could be used to inoculate aquatic organisms (e.g., seagrass, coral, macroalgae) prior to transplanting or to seed a restoration site to promote the growth of native beneficial microbiota before planting. Mangrove restoration projects could benefit from the **bioaugmentation** of existing petroleum degraders to mitigate potential impacts from active oil spills, which are a common threat to these ecosystems [**50**].

Petroleum-degrading bacteria with biosurfactant-producing bacteria have been used to perform bioaugmentation and test its effect on petrochemical degradation in seawater [**51**]. They found that the degradation rate was 72% in 45 days compared with 38% for the treatment without bioaugmentation [**51**]. The impacts on broader microbiota assembly were not tested. However, this could be useful in a restoration context (e.g., understanding whether key microbial components recover due to the intervention). A possible workflow in this scenario would be to sample the water in controlled conditions to assess its physicochemical properties and baseline microbial community composition and functions and sequence the DNA using either amplicon or metagenomics approaches (depending on the level of detail and confidence

335 required). Then the biodegradation and biosurfactant-producing bacteria can be
336 applied, and water re-measured to assess changes. Longitudinal monitoring
337 (discussed below), including the use of microbiomics, would help determine how
338 these interventions affect microbial communities and other ecosystem components
339 and, thus, the progress of ecosystem recovery.
340
341 ***Restoration monitoring***
342 The progress of ecosystem recovery is usually estimated by comparing restoration
343 sites to reference sites using measures such as species richness, community
344 composition and network connectance. To extend this, assessments of microbial
345 functional recovery require going beyond taxonomic information and towards
346 employing whole genome sequencing and other meta'omics techniques (e.g.,
347 metagenomics, metatranscriptomics) that can provide detailed functional information
348 in addition to compositional metrics (e.g., **alpha** and **beta diversity**) (**Figure 3C**). It
349 is worth noting that metagenomic and metatranscriptomic data from the same
350 environment can be poorly correlated. The choice of metatranscriptomics vs
351 metagenomics in restoration monitoring will largely depend on the goals of the
352 monitoring. Metatranscriptomics will provide information about the instantaneous
353 activity of the microbial community, whilst metagenomics will provide information on
354 the genomic potential of the microbial community. Which technique is chosen will
355 also be largely dictated by available budgets and the capabilities of the research
356 team.
357
358 **Amplicon sequencing** is increasingly used to monitor ecosystem restoration due to
359 its capacity for rapidly and reliably generating biodiversity data spanning an

extensive range of organisms [**52**]. Amplicon sequencing is also cost-effective but has important limitations. For instance, taxonomy is generally restricted to the genus level and confident inferences on microbial functional roles and activity levels cannot be made (especially in environments where reference data are lacking). Moreover, true biological abundance of taxa cannot be obtained. Whereas meta'omic approaches, such as metagenomics and metatranscriptomics, offer an improved assessment of taxonomic composition and functionality. They provide a high level of confidence in knowing what communities are present at baseline and post-intervention stages and what functional roles these organisms might possess. However, meta'omic approaches often require a high level of expertise to navigate the complex bioinformatics and analyses involved. Additionally, they are typically more expensive on a per-sample basis than amplicon-based approaches due to increased costs in lab-based library preparation steps and greater sequencing depth required. As analytical costs decrease over the next few decades, these techniques will become more widely available for monitoring restoration performance.

In terrestrial systems, soil microbiota have been targeted for biodiversity monitoring [**52,53**] due to their critical roles in restoring ecosystem functionality [**54**], with various soil microbiota acting as facilitators and/or followers of vegetation recovery [**55**]. For instance, Liddicoat et al. (2022) measured Bray-Curtis similarity to reference ecosystem sites in soil bacterial communities from post-mining rehabilitation chronosequence samples of varying revegetation age to monitor and predict timeframes for ecosystem recovery [**56**]. Barriers to recovery may also be indicated through such approaches. Using similar methods, Lem et al. (2022) studied the effects of native plant revegetation on soil microbial community recovery in a forest

restoration chronosequence by collecting soil samples at two time points that were six years apart [**57**]. Despite showing some indications of microbial recovery, the extent of recovery (again, measured as Bray-Curtis similarity to reference ecosystem sites) was not as much as expected, given an additional six years had passed. However, spatially-dependent factors such as soil chemistry and seasonal variation in sampling could potentially explain the lack of recovery. Thorough collection of environmental metadata, such as pH, soil organic carbon, and water content, is crucial during microbiomics-based restoration activities to establish strong links between environmental factors and changes in microbiota. Monitoring microbial changes alongside comprehensive environmental data enhances the understanding and effectiveness of restoration efforts.

Workflows, pipelines and techniques

A range of **bioinformatics** workflows can be used to generate insights into microbiota in a restoration context. For instance, QIIME2, DADA2 or Phyloseq can be used for amplicon processing, MetaPhlAn3 and HUMAnN4 for metagenomics and metatranscriptomics, MelonnPan for metabolomics, and MetaLab for metaproteomics (**Figure 3C**) with varying levels of detail [**58**]. For instance, shotgun metagenomics provides the opportunity to bioinformatically examine changes in functional genes during the recovery of ecosystem (and microbial) function. Sun and Badgley (2019) analysed soil metagenomes from mine soils spanning 6–31 years since reforestation and used the MG-RAST pipeline to analyse functional genes [**59**]. They found that N cycling groups, ammonia and nitrite-oxidizing bacteria, increased significantly with time since restoration. The authors suggest that their work helps

identify possible mechanisms (e.g., biochemical) linking the soil microbiome to ecosystem recovery.

Different microbiomics techniques can be used to characterise the active microbial community when monitoring restoration projects. For example, metatranscriptomics can be used to study microbial functionality and activity via quantifying RNA transcripts and, thus, gene expression in a given sample. Metatranscriptomics can be paired with transcriptomics of the target host (e.g., target plant species) to gain further insights into the health status of the host and its symbionts and explore ecosystem network linkages. Researchers have recently used metatranscriptomics to investigate whether the taxonomic profile and gene expression activity of microbial communities associated with vine wood was influenced by the health status of the host, which revealed complex multitrophic interactions (e.g., biocontrol activities) that raised “new ecological questions for the exploitation of microbial-assisted sustainable agriculture” [**60**]. This approach is transferable to a restoration context. It may also provide the opportunity to answer important questions on restoration interventions and monitoring activities.

Metabolomic pipelines (**Figure 3C**) can be used to understand microbial metabolic processes and products, thus providing a detailed assessment of active functional roles and interactions between and within organisms. This can enhance monitoring capabilities [**61**]. For example, organisms of restoration interest can be affected by stressors such as xenobiotic (not naturally produced) pollutants—a key cause of ecosystem degradation [**62**]. Metabolomics can facilitate a better understanding of the effects of these perturbations on plant and animal communities, providing

phenotypic and biological information that is vital for effective monitoring [**61**]. Given metabolites can be produced in a time scale from seconds to hours, the application of metabolomics provides an assessment of rapid responses to stress. This can be especially useful in restoration to enable adaptive monitoring. Finally, metaproteomics can be used to study microbial proteins, thereby providing insight into the phenotypes of microbes at the molecular level [**62**]. Both metabolomics and metaproteomics can provide direct assessments of the putative functions ascribed using sequencing approaches. This can aid ecological understanding of the functional response of recovering microbiota at an ecosystem scale, potentially allowing for more targeted restoration interventions and detailed monitoring schemes. For instance, researchers have used metaproteomics to analyse the structure and function of microbial communities, particularly in soils, which provides information on their contribution to ecosystem services [**63**]. Soil proteins can provide information about the biogeochemical potential of soils and pollutant degradation [**64**]. Microbes and their protein metabolites can also act as bioindicators of soil quality—a potentially important attribute in restoration monitoring schemes.

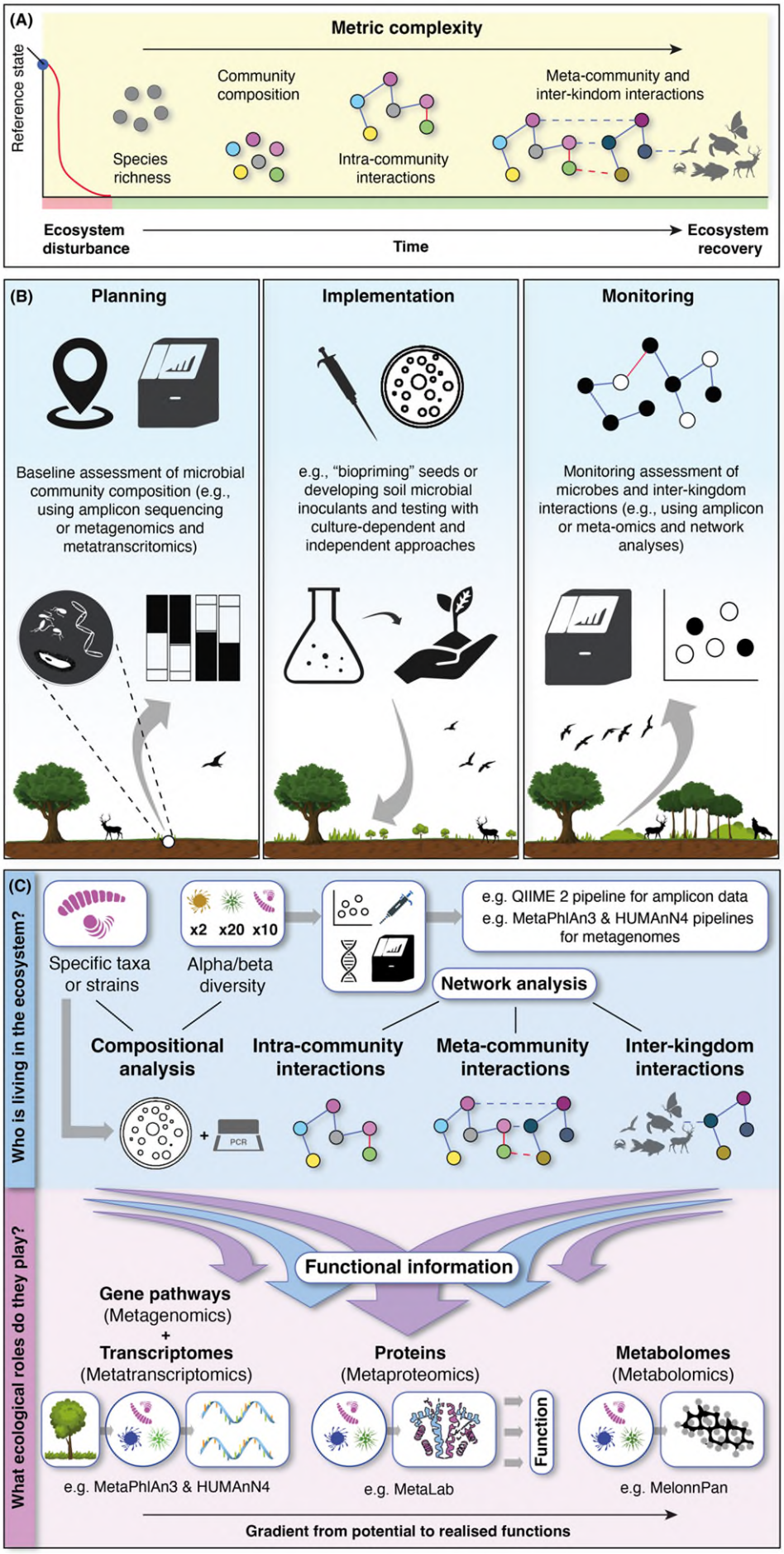
(A)
Metric complexity
Reference state
Species richness
Community composition
Intra-community interactions
Meta-community and inter-kindom interactions
Ecosystem disturbance
Time
Ecosystem recovery
(B)
Planning
Implementation
Monitoring
Baseline assessment of microbial community composition (e.g., using amplicon sequencing or metagenomics and metatranscritomics)
e.g., "biopriming" seeds or developing soil microbial inoculants and testing with culture-dependent and independent approaches
Monitoring assessment of microbes and inter-kingdom interactions (e.g., using amplicon or meta-omics and network analyses)
(C)
Who is living in the ecosystem?
Specific taxa or strains
x2 x20 x10
Alpha/beta diversity
e.g. QIIME 2 pipeline for amplicon data
e.g. MetaPhlAn3 & HUMAnN4 pipelines for metagenomes
Network analysis
Compositional analysis
Intra-community interactions
Meta-community interactions
Inter-kingdom interactions
PCR
What ecological roles do they play?
Functional information
Gene pathways
(Metagenomics)
+
Transcriptomes
(Metatranscriptomics)
Proteins
(Metaproteomics)
Metabolomes
(Metabolomics)
Function
e.g. MetaPhlAn3 & HUMAnN4
e.g. MetaLab
e.g. MelonnPan
Gradient from potential to realised functions

Figure 3 | **Applications of microbiomics and complementary non-'omics approaches along the restoration continuum**. (A) Where, across the restoration continuum, microbiomics can measure ecosystem complexity and its long-term recovery. Higher complexity (from species composition to inter-kingdom interactions) may require longer recovery periods. (B) Representation of how microbiota surveys (via next-generation sequencing) are important in the initial stages of gathering baseline ecological information, determining interventions based on this baseline information, and monitoring post-intervention. (C) Culture-dependent techniques can be used to identify and study individual taxa, whereas next-generation sequencing and bioinformatics pipelines for either amplicon or metagenomics data can be used to study whole communities. Microbial gene pathways and metabolites can be studied to understand the functional roles of the microbes using metatranscriptomics, metabolomics, and metaproteomics pipelines. Figure produced using Adobe Illustrator (v25.21).

Challenges in the uptake of microbiomics

Scientific disciplines face challenges when introducing innovative tools and perspectives into practice, such as microbiomics into restoration. Different disciplines (e.g., microbiology compared to restoration ecology) tend to have specific lense. These lenses – including normative beliefs and underlying values – guide the research approach through which we view problems [**65**]. These different lenses, based on differences in background, training, and methods, make understanding and using new approaches complicated. These challenges are seen in the uptake of innovations in restoration ecology [**18, 66**]. Disciplinary blinders (or "blinkered professionalism" [**67**]) may limit one's ability to navigate multiple perspectives in analysing complex problems, such as those associated with applying microbiomics in restoration ecology. Blinders may exist due to uncertainty, scepticism, lack of understanding of new methods and approaches, or even individual resistance to innovation and change [**66**]. Various solutions can help facilitate the uptake of microbiomics in restoration. Such solutions include the need to work collaboratively across disciplines [**68**] and build partnerships between academic and non-academic players. This will allow people with different backgrounds and interests to engage in

483 the co-production of knowledge that can bring new solutions to complex problems
484 such as ecological restoration [**69**]. Advances in microbiomics provide an opportunity
485 for restoration ecologists to work across disciplines and develop solutions to
486 restoration problems. This is like the opportunities presented by genomics [**18**],
487 drones [**70**], and ecoacoustics [**71,72**] (to monitor biological sound and
488 anthropogenic noise – the latter affects the biomass and growth rate of microbial
489 communities [**73**]) for restoration. However, the impacts on microbial functions and
490 network interactions are unknown **(Figure 4).** It is worth reiterating here that the
491 complexity of microbiomics analysis requires specialists to engage with restoration
492 practitioners and other researchers.

493 Table 1 **| Case studies of applications of microbiomics in restoration**, including the restoration stage, microbiomics method,
494 bioinformatics and statistics used, and the key restoration insights gained.

Restoration stage	Microbiomics	Bioinformatics & statistics	Key restoration insights	Ref
Planning: New approach that characterises how well soil microbiota match reference ecosystem states post-mining rehabilitation.	16S rRNA amplicon sequencing (bacteria) to characterise the bacterial community composition and diversity in the soil. Illumina Miseq platform.	Secondary data preparation using the phyloseq and dada2 packages in R. Phylogenetic trees were inferred using IQTREE2 in R. The ordiR2step() function from the R vegan package was used to visualise the sample-wise variation in bacterial communities.	A vital element of microbiomics-restoration planning is determining optimal metrics to measure restoration success. This case study approach reduces the complexity of information that often overwhelms ecologically relevant patterns in microbiota studies. The authors used microbiomics data from various restoration sites to assess variation and inform realistic rehabilitation targets.	[**56**]
Implementation: Native bacteria and cyanobacteria can influence seedling emergence and growth of native plants used in dryland restoration.	A combination of culture-dependent methods to isolate 'beneficial' bacteria so they can be inoculated into extruded pellets containing *Acacia inaequilatera* (Fabaceae) and *Triodia epactia* (Poaceae) seeds. 16S rRNA amplicon sequencing (bacteria). Illumina Miseq platform.	Raw sequencing reads processed with OTUreporter v1.0.0-beta pipeline based on Mothur v1.39.5. Sequences were aligned and classified using the SILVA database v132. Linear mixed effect models were done in R.	In this case study, bacterial inoculants increased the emergence of *Acacia inaequilatera* and *Triodia epactia* by 55% and 48%, respectively, indicating that microbial inoculants could be used to successfully aid restoration interventions.	[**74**]

Monitoring: Soil networks become more connected and take up more carbon as restoration progresses.	16S rRNA (bacteria) and ITS2 (fungi) amplicon sequencing. Illumina Miseq platform.	Snakemake workflow in Mothur v 1.33.2 was used. Sequences were aligned and classified with SINA6 against the SILVA 119 database. Network analysis: A correlation network approach was used to visualise the interactions between members of the soil food web. Only the positive correlations between species groups of Spearman's rank ≥0.9 were visualised.	This case study demonstrates that microbiomics network analysis can aid in monitoring the impacts of restoration on the complexity and interactions of the resident microbiota (in addition to inter-kingdom interactions).	[**75**]
Monitoring: Study of how fire intensity effects soil microbiomes and associated biogeochemical processes, deducing implications for ecosystem recovery.	16S rRNA (bacteria) and ITS2 (fungi) amplicon sequencing; metatranscriptomics for post-fire soil microbiome activity; and metagenomics to study viruses. Illumina Miseq platform for amplicon. NovaSEQ6000 platform for metagenomics and metatranscriptomics.	QIIME2 for processing 16S rRNA reads and assign taxonomy. Ecological guilds of fungi were assigned using FUNGuild v1.2. R packages vegan and phyloseq were used to characterise microbial variation. Metagenomics reads assembled using MEGAHIT v1.2.9 and annotated using	Metagenomics and metatranscriptomics can provide important insights into post-disturbance soil microbial taxonomy and functions and reveal the complexity of post-disturbance soil microbiome activity.	[**76**]

		DRAM (also used for metatranscriptomics annotation).

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Concluding remarks

Integrating microbiota in ecosystem restoration can improve restoration outcomes. Microbiomics offers new insights not available through traditional methods. We show how microbiomics offers new opportunities to improve restoration by enhancing the assessment of ecosystem functions and complexity and the vital roles of microbiota across the restoration continuum. Many questions remain (see **Outstanding Questions and Figure 4**), but microbiomics has already advanced our understanding of microbial species composition and associated functions across space and time.

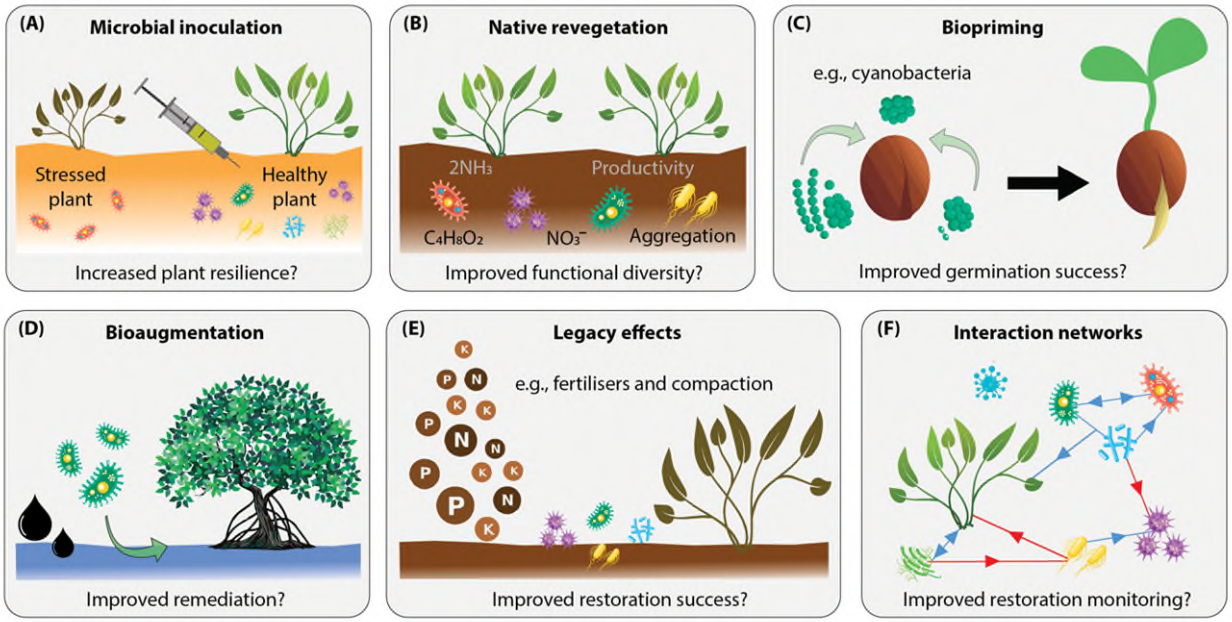

Figure 4 | **Examples of microbiomics-based and restoration-related hypotheses that need further experimental studies.** (A) Can inoculating degraded soils with locally adapted microbiota improve plant resilience in different contexts, as recently demonstrated in a temperate environment **[77]**? (B) Can native revegetation improve the functional diversity of the soil microbiome in various contexts? (C) Does seed

biopriming improve plant germination in degraded landscapes? (D) Can bioaugmentation enhance oil spill remediation in coastal environments? (E) Can a better understanding of agricultural legacy effects improve our knowledge of how to restore the soil microbiome? (F) Can a better integration of interkingdom food webs and network interactions improve restoration monitoring?

Studies still need to make quantitative and predictive linkages of microbiota to ecosystem-level processes, such as changes in carbon and other nutrient pools and fluxes. These processes are essential to ascertain the success of restoration approaches. This requires harnessing a new genome-level understanding of individual microbial taxa, microbe-host and microbe-microbe interactions, and functional capacities that might operate at the level of the entire microbial community. The advancement of meta'omics technologies has opened unprecedented possibilities in restoration ecology. These technologies enable a deeper understanding of ecosystems, allowing for more comprehensive and precise approaches to address the challenges posed by emerging global environmental threats.

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Glossary

Allochthonous microbiota: microorganisms that have been ‘imported’ into an ecosystem.

Alpha diversity: Species richness in an ecosystem system (the number of species in a population) and species evenness (the abundance of each species in a population).

Amplicon sequencing: is targeted, next-generation sequencing of a DNA sequence – ‘amplicon’ – specifically chosen to differentiate fine-scale taxonomic groups. It typically involves extracting DNA from an environmental sample (e.g., soil), and amplifying then sequencing this amplicon. For example, a fragment of the 16S rRNA gene is the most used amplicon to characterise bacterial communities.

Archaea: Single-celled microorganisms with a structure similar to bacteria. They are evolutionarily distinct from bacteria and eukaryotes. They form the third domain of life and are often associated with extreme environmental conditions.

Bacteriophage: A virus that infects bacteria, also known as ‘phages’.

Beta diversity: A measure of differences in community composition from one environment to another.

Bioaugmentation: the addition of microorganisms that can biodegrade recalcitrant molecules in a polluted environment.

Biofilm: a collective of one or more types of microorganisms that can grow on many different surfaces

Bioinformatics: The science and computational study of biological information. An interdisciplinary field that develops methods and tools to understand often large and complex biological data.

Dysbiosis: A term used to describe an imbalance or maladaptation in a microbiome (collection of microbial communities in a given environment), typically with adverse effects on animal health.

Ecosystem restoration: The process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed. Restoration ecology is the corresponding scientific discipline.

Eubiosis: Microbial balance in a given body.

Holobiont: “biomolecular network composed of the host plus its associated microbes [i.e. the Holobiont], and their collective genomes forge a Hologenome”.

Meta'omics: studies aim to identify a panel of microbial organisms, genes, variants, pathways (metagenomics), proteins (metaproteomics) or metabolic functions (metabolomics). In restoration, this approach can (a) determine what species are present, (b) what their functional and activity levels are, and (c) how these microbiota interact with each other plus their environment.

Metaorganism: A host and its entire associated microbial community.

Microbiome: The entire collection of microorganisms (and their genetic material) in a given environment and its ecological theatre of activity.

Microbiomics: the science of characterising and quantifying entire microbial communities, including their structure, spatiotemporal dynamics, genetic makeup, functional potential and biochemical profiles.

Phage: See bacteriophage.

Rhizosphere: The soil region directly influenced by root secretions and associated soil microorganisms, known as the root microbiome.

Symbiont: an organism living in close association with another (often denoting the smaller organism in the partnership).

Transdisciplinary: the inclusion of non-academic stakeholders in the process of knowledge production.