

# Insights into Ecological & Evolutionary Processes via Community Metabarcoding

Rosemary Gillespie<sup>1</sup>, Holly Bik<sup>2</sup>, Michael Hickerson<sup>3</sup>, Henrik Krehenwinkel<sup>4</sup>, Isaac Overcast<sup>5</sup>, and Andrew Rominger<sup>6</sup>

<sup>1</sup>University of California Berkeley

<sup>2</sup>University of Georgia

<sup>3</sup>City College of New York

<sup>4</sup>Universität Trier

<sup>5</sup>University of Maine System

<sup>6</sup>University of Maine

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## Abstract

The Special Issue brought together papers that highlighted the power of high-throughput sequencing (HTS) data to address classic questions in ecology and evolution, and/or use models/theory to infer key ecological and evolutionary processes, and make predictions, particularly focused on metabarcoding (amplicon) datasets in conjunction with complementary -omics data types. We highlight key papers that show the power of the new technology to address questions related to: (1) community assembly, and the interplay between competition, environmental filtering, and neutral processes, that can be inferred from the data, and how these change according to environmental conditions, and across successional and extended evolutionary time. (2) Interaction networks, and how these can show predictable changes over similar spatial and temporal gradients, providing insights into questions of biotic resilience. Studies also examined (3) cross scale interactions and those involving hosts and their microbiomes, with the critical development being the ease of comparison and integration across scales of organismic complexity, allowing insights at one scale to inform the other. The approach is also amenable to (4) studies of invasive species and biotic homogenization, providing insights of shifts in alpha and beta diversity across a wide range of spatial scales.

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<sup>1</sup> Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, USA

<sup>2</sup> Department of Marine Sciences and Institute of Bioinformatics, University of Georgia, Athens, GA, USA

<sup>3</sup> Graduate Center of the City University of New York, New York, New York, USA

<sup>4</sup> Department of Biogeography, Trier University, Trier, Germany

<sup>5</sup> School of Biology and Ecology, University of Maine, Orono, ME, USA; Department of Vertebrate Zoology, American Museum of Natural History, New York, New York, USA

<sup>6</sup> School of Biology and Ecology, University of Maine, Orono, ME, USA

Rosemary G Gillespie [gillespie@berkeley.edu](mailto:gillespie@berkeley.edu) ORCID: 0000-0003-0086-7424

Holly M Bik *hbik@uga.edu* ORCID: 0000-0002-4356-3837

Michael J Hickerson *mhickerson@ccny.cuny.edu* ORCID: 0000-0002-5802-406X

Henrik Krehenwinkel *krehenwinkel@uni-trier.de* ORCID: 0000-0001-5069-8601

Isaac Overcast *isaac.overcast@maine.edu* ORCID: 0000-0001-8614-6892

Andrew J Rominger *andrew.rominger@maine.edu* ORCID: 0000-0003-3755-4480

## Abstract :

The Special Issue brought together papers that highlighted the power of high-throughput sequencing (HTS) data to address classic questions in ecology and evolution, and/or use models/theory to infer key ecological and evolutionary processes, and make predictions, particularly focused on metabarcoding (amplicon) datasets in conjunction with complementary -omics data types. We highlight key papers that show the power of the new technology to address questions related to: (1) community assembly, and the interplay between competition, environmental filtering, and neutral processes, that can be inferred from the data, and how these change according to environmental conditions, and across successional and extended evolutionary time. (2) Interaction networks, and how these can show predictable changes over similar spatial and temporal gradients, providing insights into questions of biotic resilience. Studies also examined (3) cross scale interactions and those involving hosts and their microbiomes, with the critical development being the ease of comparison and integration across scales of organismic complexity, allowing insights at one scale to inform the other. The approach is also amenable to (4) studies of invasive species and biotic homogenization, providing insights of shifts in alpha and beta diversity across a wide range of spatial scales.

## Introduction :

Biodiversity - the multiplicity of life, from microbes to macro-organisms and from genes to ecosystems, is in crisis, yet we have little understanding of factors that can sustain biodiversity and enhance resilience to perturbations. Key questions that remain include the interplay between niche and neutral processes in shaping the assembly of communities (Mittelbach & McGill, 2019) and the associated role of stochastic and deterministic processes governing assembly (Menéndez-Serra, Ontiveros, Caliz, Alonso, & Casamayor, 2023); the complexity-stability paradox (Dominguez-Garcia, Dakos, & Kefi, 2019); metacommunity dynamics and the connection between local and regional diversity (Thompson et al., 2020); and the extent to which a given community can exist in equilibrium or steady state (Qian & Akcay, 2020) and concepts of alternative stable states (Van Nes et al., 2016), among others. These questions have been the focus of much theoretical development over the past, but the ability to generate the data needed to validate the theory has been confounded by the difficulty of sampling biological communities at the needed scale. However, without answers to these fundamental questions, we are left with major gaps in our understanding of biodiversity dynamics and questions of biotic resilience, the use of indicator or surrogate species, ecosystem sustainability, and strategies for restoration, which are all so critical for effective conservation and management of ecosystems.

The advance of molecular profiling methods (e.g. metabarcoding, 16S/ITS community profiling, metagenome reconstruction) has recently provided a remarkably effective tool for measuring biodiversity, and presenting the opportunity to answer these outstanding questions. Moreover, because the approach affords a common tool across both macro- and micro-organisms, we have the ability to answer macroecological questions of shifts in community composition across scales (e.g., (Brown, Mihaljevic, Des Marteaux, & Hrček, 2020)). The technology has initiated a dramatic shift in the ability to measure ecological metrics within entire macro- and micro-organismal communities, and how they change over space and time; it also allows comparison of the same data across scales of organismic complexity. In this Special Issue, authors use high throughput technologies to **address classic questions in ecology and evolution, and/or use models/theory to infer key ecological and evolutionary processes, and make predictions.**

Highlighting the promise and importance of metabarcoding for a holistic understanding of entire interacting assemblages of different trophic group, Ficetola and Taberlet (2023) review approaches that can reveal

biodiversity response to global change. Metabarcoding approaches provide information not only on species occurrences, but also on species interactions, with new approaches using species traits, phylogenetic information and machine learning algorithms to infer multitrophic and multitaxa interactions. Moreover, metabarcoding can provide a means for detecting hidden diversity (e.g., (Yin et al., 2022)) and associated cryptic interactions (e.g., (Sow et al., 2019)): **Lu et al.**(2022) focused on cryptic diversity by comparing mycobiomes in marine, gut, and soil samples and found that, while soils have the highest diversity, the gut has the highest number of unknown species, followed by marine sediments.

### **Community Assembly Processes :**

Describing the composition and structure of communities and their responses to perturbations and stressors has been a primary objective of ecological research since its inception. We still struggle to understand and predict the mechanisms shaping the dynamics of biological communities and how these accommodate or collapse in the face of change. Community profiling methods, by providing data on the diversity and abundance of the entire community of taxa across sites of different age, nutrient availability, etc, are now providing unprecedented insights into the processes of assembly. New modeling approaches (Overcast, Emerson, & Hickerson, 2019; Overcast, Ruffley, Rosindell, Harmon, Borges, Emerson, Etienne, Gillespie, Krehenwinkel, & Mahler, 2021) are now being applied to these data to provide insights into the temporal and spatial components that govern the assembly process, and hence the factors that might dictate resilience. In this issue, Overcast et al (2021) have built an eco-evolutionary simulation model that uses community-scale genetic data to study community assembly dynamics and show that there are detectable signatures of neutral and non-neutral processes in simulated biodiversity data axes. Applying the model to soil microarthropod metabarcoding data from Cyprus, they show that widespread low-elevation communities are structured by neutral processes, while isolated high-elevation habitats are shaped by non-neutral processes.

Studies in this category included terrestrial and marine systems, macro- and micro-organism assembly, and comparisons of community assembly processes across scales of organismic complexity. For terrestrial communities, several papers focused on the respective roles of environmental filtering, niche conservatism/lability, and spatial isolation in shaping animal species diversity at a given site? In the paper by Noguerales et al (2023), they use whole organism community DNA (Creedy et al., 2022) metabarcoding at both OTU- and ASV-levels to tease apart the role of environmental filtering and spatial isolation in metacommunity dynamics of soil microarthropods. The study showed that OTU (species) richness follows an altitudinal gradient, presumably associated with filtering and niche-based processes; the ASV diversity showed a contrasting pattern of decline in genetic diversity associated with anthropogenic disturbance. The paper by Andujar et al. (2022) used the soil mesofauna in the Canary Islands to highlight the importance of environmental filtering and niche conservatism as a driver of insular community assembly with little evidence of niche lability, with strong geographic structure. Likewise, the paper by Arjona et al (2022) focused on soil arthropod communities at different depths, highlighting the diversity of species (many new species records), with the results supporting the hypothesis that deeper soil beetle communities are much more dispersal limited compared to those closer to the surface. The overall promise of the approach is highlighted in Emerson et al. 2022 (2022) who highlight the potential to complement high throughput barcode sequencing with deep learning computational workflows (of images) to advance the way we study terrestrial arthropod biodiversity as a whole.

Considering marine systems, Kiemel et al. (2022) used DNA metabarcoding (COI and 18S) to ask (i) how the community is spatially and temporally connected, (ii) what are the environmental factors influencing local communities, and (iii) what are the underlying metacommunity dynamics in this system. There was no difference between ephemeral and permanent kettle holes, and overall the results suggest that communities are mainly structured by environmental filtering based on pH, water temperature, kettle hole size and hydroperiod. Species sorting is a dominant driver in community assembly in the studied kettle hole zooplankton metacommunity. Likewise Govender et al. (2022) used a metabarcoding approach to highlight the point that, while sheltered bight areas have lower pelagic zooplankton diversity due to structurally homogeneity, they actually represent important fish spawning grounds (with important ramifications for fisheries

and higher-level consumers). In this case, diversity measures could thus not be used as a proxy for ecological importance.

A number of these studies addressed similar questions in microbial communities. Thus, Pino et al. (2023) addressed used 16S rRNA and ITS metabarcoding of soil microbiomes (bacteria and fungi) across large scale edaphic and climatic gradients in Australia to address classic questions in soil science and macroecology: Are broad soil classifications sufficient to capture biological soil function, and what large-scale factors determine turnover in community composition? The authors found that soil classes are predictive of bacterial and fungal community composition regardless of spatial proximity, natural and cultivated soils are reliably distinct in their microbiomes, and the primary drivers of these microbiome community differences are soil pH and temperature cycles. Van der Loos et al. (2022) explored the interplay between environment and host genotype in shaping the stability and variability of microbial composition. Using seaweed-associated bacterial communities along a salinity gradient, they were able to identify a small group of core microbes possibly involved in salinity adaptation of the host. The experimental study by Nappi et al. 2022 (2022) tested the effects of two bacterial strains on the assembly and succession of microbial communities associated with the green macroalga *Ulva australis* . Both bacterial strains exert a priority effect, with one strain (D2) causing initially strong but temporary taxonomic changes, and the second strain (D323) causing weaker but consistent changes that were predominantly facilitatory and included taxa that may benefit the algal host. Priority effects do not appear to be a simple replacement of functionally equivalent taxa, but result in distinct differences in the functional potential of the community. Besides the implications for community ecology, this work provides insights on the development of new probiotics (e.g. for human health or agriculture)

Finally, there were several studies in which the authors examined processes across scales (macro- and micro-organisms). Thus, Wang et al. 2022 (2022) were able to compare community assembly processes across scales of organismic complexity showing that (i) small soil microorganisms (bacteria, fungi) were mostly influenced by stochastic processes while larger soil organisms (nematodes) were more deterministic; (ii) the independent effects of habitat (including soil and topographic variables) and its interaction with plant attributes for community structure significantly decreased with increasing body size; and (iii) plant leaf phosphorus directly influenced the spatial distribution of soil-available phosphorus, which indicates their indirect impact on the assembly of the soil communities. Data suggest that the assembly of multitrophic soil communities can be explained to some extent by changes in above-ground plant attributes. Likewise, Guerrieri et al. (2022) looked at the development of successional communities in recently deglaciated soils, focusing on six groups (Eukaryota, Bacteria, Mycota, Collembola, Insecta, and Oligochaeta) and asking how soil communities change through time following deglaciation, and how this change differs between different soil layers. They were able to show increasing diversity within, but also increasing biotic homogenization between, soil layers, with increasing time since deglaciation. The shifts were likely associated with the development of plant communities during succession.

### **Interaction Networks :**

Another major area of study examined interaction networks, and how the properties of the networks might reflect the health, and functioning of both macro-organismal (Banerjee et al., 2022) and micro-organismal (Peixoto et al., 2022) communities. Metabarcoding provides an ideal opportunity to examine questions relating to interaction networks and can provide quantitative assessment of resilience to perturbation. Recent developments in high throughput approaches have revealed entirely novel insights into plant-pollinator interactions. Bell et al. (2022) review the opportunities provided by these approaches to examine how plant-pollinator interactions change as a result of land-use change. They consider how the approach can be applied to understanding key questions in global change ecology, in particular, how interactions change through space and time, including the impacts of climate and other anthropogenic stressors. Similar studies have shown how environmental DNA (eDNA) from flowers can be used to identify the community of pollinating bumblebees and has the potential to reveal complex networks (Harper et al., 2023). The paper by Lowe et al. (2022) provides an empirical example in which they used pollen DNA metabarcoding metabarcoding of honey samples in the honeybee (*Apis mellifera* ) to reveal seasonal changes in diet specialization accord-

ing to resource availability. Because the degree of specialization are linked to network resilience, the study highlighted seasonal changes in network vulnerability. Along similar lines, the paper by Encinas-Viso et al (2022) focused on factors that might drive beta diversity in alpine plant-pollinator communities. By analyzing insect pollen loads they showed that metabarcoding data generated networks that were more diverse but much less specialized compared to observational data. The results supported their hypothesis that niche specialisation of alpine taxa lead to fine-scale spatial turnover of phylogenetic diversity, species and interactions of alpine plant-pollinator networks compared to low-elevation ecosystems. Finally, Tommasi et al. (2022) tested the impact of anthropogenic habitat fragmentation on the complexity of plant-pollinator interaction networks. Using pollen metabarcoding, they analyzed pollinator richness, plant-pollinator interactions, and pollination efficiency in landscapes of different fragmentation levels on the Maldives Islands. Contrary to their expectations, they find that moderate levels of habitat fragmentation increase the local richness of pollinators, consistent with the intermediate disturbance hypothesis. Despite harboring a high pollinator richness, fragmented landscapes resulted in less complex plant-pollinator networks, with detrimental affects on the pollination ecosystem service. A particularly concerning finding is a preference of native pollinators for invasive plant species, possibly additionally speeding up their spread.

Metabarcoding has now been used to look at dietary niche and questions of niche partitioning. Ando et al. (2022) used fecal DNA metabarcoding from 7 species of ducks (329 samples) to show strong niche partitioning of plant diet across species but opportunistic foraging when invertebrates were the available food source. Likewise, Boyi et al. (2022) examined niche overlap in a co-occurring predators in the North Sea. Using a new 16S primer to metabarcode gut contents, they showed that the composition of fish prey in the diet of the Eurasian otter overlaps that of both harbour and grey seal diets, highlighting the possibility of interspecies competition where these species sharing foraging ground. Several studies examined how interaction networks change across gradients. The paper by Srivathsan et al. (2022) tested for the impact of human disturbance on fly-vertebrate communities and their interactions to understand whether there is any specialization. They sampled dung and carrion fly communities along a disturbance gradient in a swamp forest remnant in Singapore. While there was no evidence of specialization in the interactions between fly and vertebrate species, they reveal the effect of roads on the presence of native and endangered rainforest vertebrate species, highlighting indirect eDNA monitoring as an important conservation tool. The paper by Pitteloud et al. (2022) used DNA metabarcoding of insect feces to test specific hypotheses regarding factors that might dictate interactions in plant–orthoptera bipartite networks along elevation gradients. The results showed that the structure of the ecological networks was governed by both (i) the phylogenetic position of the plant taxa, where herbivores feed on plants based on their taxonomic identity and (ii) plant abundance, where herbivores feed on the plant species proportional to the cover of the plant species. The results also highlighted other aspects of the environment that shape interactions, in particular leaf nitrogen content in warmer environments, phenolics and terpenoids in colder environments. Dürrbaum et al. (2022) examined the impact of urbanization on diversity and trophic interactions in arthropod communities at two trophic levels. By metabarcoding pollen from herbivorous bees and arthropod prey from wasp nests, they found contrasting responses to urbanization of predator–prey and plant-pollinator interactions. While the available diet is impacted for both trophic levels, the negative effects of urbanization are stronger for predators than herbivores, likely due to their increased requirement for larger, unfragmented habitat. The approach can also be used to address applied questions of biological control interactions as reviewed in Lue et al. (2022) where the approach can allow not only identification of biological control interactions, but also evidence of hyperparasitism or multiparasitism which can disrupt biological control by introduced agents.

High throughput data can also be used to infer changes in the overall set of interactions in a given biological community. Ip et al. (2022) used eDNA in coral reefs to reveal shifts in community composition and trophic structure of coral associated fish species. A key finding was that inversion of the trophic pyramid in reefs was a common response to coral spawning events due to large numbers of predators (secondary and tertiary fish consumers) associated with the high predation on coral eggs by planktivorous fish.

Over evolutionary time, a study by Graham et al. (2022) used the Hawaiian Island geological sequence to show how interactions among arthropod communities become progressively more specialized. Using bipartite

networks of arthropod-plant associations, they showed that the average number of interactions per species (linkage density), ratio of plant to arthropod species (vulnerability), and uniformity of energy flow (interaction evenness) increased significantly with community age, suggesting that the communities show a natural progression towards specialization.

### **Cross Scale Interactions & Microbiomes :**

The widespread adoption of molecular profiling methods has provided unprecedented avenues for comparing processes across scales, with the approaches used for metabarcoding of whole communities of animals or plants sharing the same overall methods, and being amenable to the same analytical tools as microbial community profiling. When applied to the same environmental samples, this suite of sequencing-based methodologies enables deep characterization of organismal communities, ranging from macro-/micro-organismal community structure and ecosystem function down to traits associated with individual taxa. Thus, we now have the opportunity to conduct parallel analyses of macro- and micro-scale community structure across biological communities and the interplay between biotic and abiotic components of entire ecosystems. Highlighting these parallels, Câmara dos Reis et al. (2022) tested the relative importance of stochastic and deterministic processes in shaping bacterial community dynamics associated with a widespread and ecologically important bloom forming phytoplankton species. Through a combination of observational (field sampling) and experimental (microcosm) approaches to assess bacterial community assembly over bloom succession, they found that deterministic processes shape microbial communities within phytoplanktonic bloom conditions, whereas stochastic processes were more prevalent outside of blooms.

Several studies examined questions involved in the interaction between animals and their microbiome, looking at the effects of the microbiome on diet and niche. Michel et al. (2022) used metabarcoding methods to investigate the interplay between diet and gut microbiome in several geographically isolated and genetically differentiated populations of the critically endangered Grauer's gorilla. They showed marked differences in the composition (though not richness or evenness) of the diet and gut microbiome of genetically differentiated populations, associated with social, ecological, and geographic factors. Manthey et al. (2022) tested the hypothesis that the holometabolous insect gut microbiota rapidly remodels during metamorphosis, allowing exploration of novel niches during their ontogenesis. By measuring microbial community turnover during ontogeny, they show that beta-diversity and hence microbiota turnover is much higher in holometabolous insects compared to hemimetabolous insects. The microbial shedding and turnover during ontogenesis of holometabolous insects could open novel ecological niches and explain the evolutionary success of holometabolous insects.

Several approaches considered the importance of the high throughput sequencing approaches for understanding how microbial communities can affect biogeochemical cycling.

Considering microbes and their viral infection dynamics, Merges et al. (2022) tested the hypothesis that the activity of bacteria and bacteriophages co-declines across an elevational gradient. Using an elevational transect in the Swiss Alps they used transcriptome levels to show that metabolic activity of bacteria declined with increasing elevation, but activity of bacteriophages did not, highlighting a gap in our understanding of microbial predator-prey relationships and associated viral contributions to carbon, nitrogen and phosphorus cycling. The paper by Pereira et al. (2022) examined the microbiome of a pelagic tunicate and the potential role of the microbiomes in pelagic biogeochemical cycling and nutrient remineralization. They showed that the trophic activity of the tunicates affects the structure of pelagic food webs and biogeochemical nitrogen, sulfur, and organic cycling.

The interactions between microbiomes and their host species can change across gradients, allowing fine scale adaptation. To understand these relationships, Molina et al. (2022) tested the role of climate, site, and host variables in structuring sapwood-inhabiting fungal communities across a gradient of climatic, seasonal and site factors in the North Patagonian *Nothofagus* forests. The results supported their hypothesis that host identity and site were the major drivers of fungal community structure. Remarkable insights are now showing the tight relationship between hosts and the different components of their microbiome. Rolshausen

et al. (2022) tested the predictability in the structuring of the different components of a multi taxon holobiont across environmental gradients. Using a combination of whole genome analysis and metabarcoding in fungal, algal and bacterial components of lichen holobionts along elevation gradients they showed that, while chemically and morphologically indistinguishable, these lichen holobionts show pronounced compositional turnover with elevation. The turnover happens in a concerted fashion for the three taxonomic components, highlighting the importance of coadaptation of different components in complex holobiont in evolutionary diversification. The paper by Kivistik et al. (2022) examined the combined impact of diet and environmental disturbance (salinity and antibiotics) on the gastrointestinal microbiome of aquatic gastropods. The results showed that a transition to salinity led to lower gut community richness and higher host viability, but only when there was an increase in bacterial generalists in the gut. Brinker et al. (2022) tested the interplay between host population structure, environmental conditions and the presence of an endosymbiont on the bacterial community of an insect host. They simultaneously investigated the population structure of a parasitic wasp host and the spatial turnover in its microbiome, with high similarity among microbial communities in *Wolbachia* infected (asexually reproducing) hosts and marked host population structure in uninfected (sexually reproducing) hosts.

High throughput approaches have also provided insights into the role of microbiomes in imparting disease resilience. Navine et al. (2022) tested the effect of microbiome communities on resistance to avian malaria by comparing two birds species in Hawaii, one native, one introduced. Neither microbial alpha nor beta diversity covaried with infection, but 149 microbes showed positive associations with malaria survivors, highlighting possible candidates for probiotics to facilitate immunity to malaria in endangered birds.

A critical component in microbiome studies is to tease apart the relative importance of the host and the environment in shaping observed patterns, something that can be difficult. Perez-Lamarque & Morlon (2022) evaluate several widely used methods for inferring host-microbiome cophylogenetic processes that aim to differentiate between vertical transmission and host-switching. They use simulations to measure power and type-I error rate and find that there are trade-offs between computational and statistical performance among the methods. They conclude that no one current method is optimal and make recommendations for the scenarios under which different methods are most appropriate.

### **Invasive Species/ Homogenization :**

Homogenization of landscapes and seascapes through the arrival of non native species leads to loss of resilience, with subsequent erosion of the role of biodiversity in ecosystem services (Díaz et al., 2018) and buffering against tipping points and regime shifts (Nyström et al., 2019). However, detecting non native species, and teasing them out from natives can be an almost impossibly difficult task (Guiasu, 2016). Perhaps because of this difficulty, some have argued that non native species must be incorporated into conservation decisions (Sax, Schlaepfer, & Olden, 2022), though the scientific rationale is difficult to establish and there is a substantial literature indicating that the co-evolved nature of species in a given area is critical to its resilience (Pauchard et al., 2018). High throughput approaches are now providing entirely novel avenues for the study of non native species. First, the use of eDNA can provide unprecedented levels of detectability, both in aquatic and terrestrial systems (Valentin et al., 2020). In addition, an intriguing new analytical tool uses the genetic signature derived from metabarcoding studies to separate bioinformatically, native from non native species (Andersen et al., 2019); this method was employed in several studies in this special issue to provide insights into the impact of non native species and the associated biotic homogenization (Graham et al., 2022; Kennedy et al., 2022).

The modeling approaches developed in the context of community assembly can equally be applied to understanding invasions. Thus, Overcast et al. (Overcast, Ruffley, Rosindell, Harmon, Borges, Emerson, Etienne, Gillespie, Krehenwinkel, Mahler, et al., 2021) highlight the importance of neutral processes in invaded communities. The paper by **Zhang et al. 2022** (2022) used a chronological gradient of smooth cordgrass invasion in salt marshes (Yellow River Estuary, China) with a combination of metabarcoding and GeoChip approaches to show a positive correlation between microbial diversity and the duration age of invasion, and both bacterial and fungal communities showed consistent changes with invasion. Soil microbial metabolic

potential, as indicated by the abundance of microbial functional genes involved in biogeochemical cycling, decreased in response to invasion. As a consequence, declining soil microbial metabolisms as a result of plant invasion facilitated carbon accumulation in invaded salt marshes. Bacteria and fungi exhibited distinct contributions to assembly processes along the invasion gradient: bacterial communities were mainly driven by selection and dispersal limitation, while fungi were dramatically shaped by stochastic processes.

Metabarcoding approaches can clearly identify the effect of anthropogenic habitat modification on species assemblages, including key taxa that are associated with modified environments, as well as the overall homogenizing effects of invasions. For example, **Hampel et al.** (Hampel, Moseley, & Hamdan, 2022) show that the presence of undersea “built habitats” (shipwrecks) causes increased microbial biodiversity and a predictable core microbiome in their surrounding deep-sea sediments (extending up to 300m from the wrecks). Specific archaeal groups showed enrichment around shipwrecks, suggesting metabolic shifts towards chemolithoautotrophy in these proximate sediments. Similarly, Andrés et al. (2023) used eukaryotic environmental DNA (eDNA) to reveal the interplay between environmental factors in the homogenizing effects of shipping, with routed-based models of ship-borne species showing that environmental dissimilarity, shipping, and their interaction reduce biological dissimilarity among commercial port habitats.

As in the previous sections, metabarcoding across gradients provide insights into processes of invasion and in particular, the phenomenon of biotic resistance, or the reduction in invasion success caused by the resident community (Levine, Adler, & Yelenik, 2004). Notably, the paper by Graham et al. (2022) used the geological age gradient of the Hawaiian Islands in which comparable sites of high elevation native forest show increasing diversity of native species over the 5 my timeframe. Results from metabarcoding of entire arthropod communities demonstrate that, where species diversity is lowest (on the youngest island), infiltration of non-native species is highest. Likewise, Kennedy et al. (2022) used DNA metabarcoding and statistical modeling to survey community-wide arthropod richness, the proportion of native and non-native species, and the incursion of non-natives into primary habitats on three archipelagos in the Pacific. Focusing on one island from each of the three archipelagos that differ with respect to age, area and proportion of native habitat, there were three alternative hypotheses defined by fundamental eco-evolutionary processes with associated predictions that were detectable from the high-throughput metabarcoding surveys. The study showed that older age and correspondingly higher taxonomic richness was associated with higher resistance to invasion, and that invasion did not lead to homogenization of arthropod assemblages across the different degraded forests on the three archipelagos.

## Recurring Themes

Besides the insights made in each of the major theme areas above, there were several recurring themes that emerged from multiple studies:

Importance of museum & associated reference collections . Museums play a key role in metabarcoding approaches. First, while many insights can be gained from molecular sequences alone, the availability of a reference collection, ie, molecular barcodes for identified specimens, adds unprecedented dimensionality to the data. The availability of a reference collection allows us to infer the functional traits and morphological attributes of every taxon in a sample, whether it is native or not at a given site, its overall distribution and trophic relationships. Moreover, it is critical that the identity of the specimen has been thoroughly confirmed, as misidentification can lead to flawed interpretations. Thus, rather than diminishing any role of natural history museums in such approaches, the vast data that have been generated through molecular profiling approaches have increasingly highlighted the fundamental importance of barcodes from reliably identified species and populations (Valdivia-Carrillo, Rocha-Olivares, Reyes-Bonilla, Dominguez-Contreras, & Munguia-Vega, 2021). The importance of a reference collection is highlighted by Lue et al. (2022) which describes the importance of a vetted and curated reference library for biological control studies. Likewise, Lu et al. (2022) emphasize the limitations of inference without a reference database, and introduce a fungal rRNA operon database (FRODO) with 1116 linked to taxonomically identified species.

A second role of museum specimens in these approaches is that they can provide historic samples of past

environments. For example, metabarcoding of pollen loads from museum bee specimens has provided key insights into environmental change over decadal scales, both in the availability of plants, and changes in interaction networks (Bell et al., 2022; Gous, Swanevelder, Eardley, & Willows-Munro, 2019). This work adds to the increasing body of research that shows how metabarcoding of museum specimens can provide information on changes in interactions through time, including diet and microbiome (Heindler et al., 2018) and parasite-host interactions (Greiman et al., 2018).

Insights from clustering at different levels : Early metabarcoding studies used clustering approaches and generally grouped ASVs into Operational Taxonomic Units (OTUs), assumed to correspond to species. The purpose of this step was to remove the known noise in the data, while also grouping taxa into species. However, new denoising approaches have presented the opportunity of analyzing ASVs and hence gaining insights into population-level patterns (Noguerales et al., 2023). The most important aspect of the ability to look at different levels of genetic clustering is that the comparison can be tremendously informative into the processes that govern species assembly.

Incorporation of Deep Learning / Artificial Intelligence approaches applied to image analysis to study arthropod biodiversity (Emerson et al., 2022). Supervised learning was used to make predictions of sediment sample proximity to shipwrecks based on frequency of microbial taxa (Hampel et al., 2022).

## Future Outlook

The collection of papers in this Special Issue highlights the critical insights that can be gained using high-throughput approaches, in particular in relation to biodiversity dynamics. We now have a tool for understanding how overall species composition changes across (1) spatial gradients, whether habitat, elevation, precipitation, nutrient, or anthropogenically-associated modifications. Moreover, we can also examine changes through (2) time, whether using museum specimens, ancient sediments, or sub-fossils to show how diet, host-associations, parasitism, and other interactions have changed; and geological or ecological chronosequences that provide insights into how entire communities change over extended time periods. The set of papers includes a mixture of studies, with about half focusing on macro-organisms, the other half on microorganisms. The critical point here is that we have a tool that allows comparison of processes across scales. Thus, concepts developed for understanding biodiversity in macro-organisms can be tested in real time using microorganisms, and dynamics that have been learned from microbial systems can provide insights into factors shaping communities of macro-organisms and their interaction with entire ecosystems. As the approaches become more robust, it will be easier to realize the potential of high-throughput analyses to answer some of the most intractable questions in biodiversity science.

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