

Predicting Ecosystem Metaphenome from Community Metagenome:

A Grand Challenge for Environmental Biology

Neo D. Martinez*

Center for Complex Networks and Systems

School of Informatics, Computing, and Engineering

919 E. 10th Street, Room 302

Bloomington, Indiana 47408

Indiana University, Bloomington, IN USA

Email: neo@PEaCELab.net Phone: +1 510 295 7624

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*to whom correspondence should be sent

ABSTRACT: Elucidating how an organism’s characteristics emerge from its DNA sequence has been one of the great triumphs of biology. This triumph has cumulated in sophisticated computational models that successfully predict how an organism’s detailed phenotype emerges from its specific genotype. Inspired by that effort’s vision and empowered by its methodologies, this Viewpoint describes a grand challenge to predict the biotic characteristics of an ecosystem, its metaphenome, from nucleic acid sequences of all the species in its community, its metagenome. Meeting this challenge would integrate rapidly advancing abilities of environmental nucleic acids (eDNA and eRNA) to identify organisms, their ecological interactions, and their evolutionary relationships with advances in mechanistic models of complex ecosystems. Addressing the challenge aims to help integrate ecology and evolutionary biology into a more unified and successfully predictive science that can better help describe and manage ecosystems and the services they provide to humanity.

INTRODUCTION

“Grand Challenges” have emerged as one of the most compelling tools to motivate, engage, and organize major research programs across the sciences and engineering (Omenn 2006; Kaldewey 2018). This is especially true for biology. Grand challenges in evolution include assembling a great tree of life summarizing the evolution of all life on our planet (Hinchliff *et al.* 2015). Grand challenges in ecology include understanding the relationship between biodiversity and ecosystem function (National Research Council 2001; van der Plas 2019). One of the largest and most successful grand challenges since sequencing the human genome includes molecular and cell biologists’ project to predict individual organisms’ characteristics from their DNA sequence otherwise known as predicting an organism’s phenotype from its genotype (Fig. 1, National Research Council 2010). This challenge was largely met for one species by a whole-cell simulation of a human pathogen (Karr *et al.* 2012) as proposed a decade earlier (Tomita 2001).

Mechanistic models of phenotypes emerging from their genotypes continue to be developed for different organisms using a variety of approaches. Several employ empirically parameterized models of complex networks comprised of modules using mathematical and other algorithms to represent DNA transcription, translation of RNA into proteins, and metabolic processes involving those proteins (Fig. 1, Karr *et al.* 2012; Fang *et al.* 2020). Others model individual molecules and the cell's physical structure created by those molecules to generate much more highly resolved representations of cellular structure such as membranes, organelles, and proteins as well as their interactions (Feig & Sugita 2019). Both approaches include many types of interactions involving thousands of biochemical species, interactions, and parameters operating on multiple scales from molecular dynamics to cell division. The sophistication and coordinated research and engineering applied to modeling whole cells vastly exceeds that dedicated to similarly detailed modelling of whole ecosystems. Both inspired and informed by whole-cell modeling as well as a long history of more modest behavioral, population, community and ecosystem theory and modeling, the grand challenge to predict ecosystem metaphenome from community metagenome seeks to motivate a multiscale mechanistic understanding of how the detailed structure and function of ecosystems emerge from the interactions among organisms coexisting within an environment (Jansson & Hofmockel 2018).

Addressing this challenge helps integrate behavioral, population, community, and ecosystem ecology, several of the largest subdisciplines of ecology, with phylogenetics and population genetics, two of the largest subdisciplines of evolutionary biology. Together, ecology and evolution comprise the lion's share of environmental biology which has yet to achieve the synthetic and predictive successes enjoyed by physics, chemistry, and molecular and cell biology. Such limitations may be surmounted by predicting ecosystem metaphenomes from the metagenomes within the ecosystem's communities starting first with relatively simple experimental ecosystems in the lab and eventually extending to ecosystems more generally. Ecological communities are described by the diversity and identity of

species within a habitat and are identifiable by their genetic “barcodes.” The community metagenome consists of the genomes of all species within the ecosystem’s communities. A key metaphenome is the distribution of organisms among all trophic levels within a habitat and the dynamics of their populations and biomass over time. Cell and molecular biologists’ success at meeting their genotype-to-phenotype challenge points towards the tractability of a similar challenge at the ecosystem level and contributes powerful social (e.g., structured collaborations), scientific (e.g., networks of networks) and technical (e.g., sequencers and software) methodologies for addressing the challenge. What the grand challenge is, how it may be met, and why it is worth pursuing are described further below.

THE CHALLENGE

To scale up our understanding of organismal behavior and interactions to the structure and function of ecosystems, it is eminently clear that knowing which organisms and interactions occur in an ecosystem is a useful place to start. Documenting these ecosystem traits has been a priority since well before Darwin’s voyages. More recently, rapidly emerging technologies based on environmental nucleic acids (eNA) including eDNA and eRNA have greatly increased our ability to describe these traits at a vastly higher degree of resolution with extraordinarily lower effort and cost (Beng & Corlett 2020; Deiner *et al.* 2021). These technologies illuminate the community metagenome constituted by the genomes within ecosystems which provides a uniquely powerful description of the current state and dynamic potential of the ecosystem (Jansson & Hofmockel 2018; Deiner *et al.* 2021). Sequences of eDNA within metagenomes may identify organisms at virtually any level of taxonomic resolution from the species level and above down through the population level (Luck *et al.* 2003) to the individual organism itself. Such sequences also provide phylogenetic information about organisms’ evolutionary history. Thus, metagenomes elucidate who organisms are, where they come from, their evolutionary potential, and help leverage existing data on well-known organisms (e.g., metabolic capabilities) that often accurately describe less familiar close relatives (Davies 2021). The location of metagenomes such

as within the tissue, gut, or pollen sacks of an organism, combined with the organism's known biology such whether it's an animal, plant, parasite, pollinator, etc., can illuminate interactions such as predation, parasitism, herbivory, symbiosis, and mutualism (Kennedy *et al.* 2020). Innovative methods, such as separating differently sized DNA molecules, can distinguish endosymbionts and parasites from prey (Kreherwinkel *et al.* 2017). The number of eDNA copies can even elucidate species' biomass, population size, life-stage structure, eco-evolutionary dynamics (Hao *et al.* 2020), organismal interactions (e.g., feeding, pollination, and growth, Deiner *et al.* 2021; Yates *et al.* 2021) and rates of ecosystem processes (Kennedy *et al.* 2020).

Combined with the vast literature on the physiology, natural history, and ecology of identified organisms, the metagenome may unlock most of the empirical information essential to predicting ecosystem behavior including virtually all co-existing organisms' identities and their most important and cryptic interactions. Correlations with species composition and habitat may allow inferences such as the size, location, and hydrologic regime of a lake or the soil type and fertility of a grassland. As such, the metagenome may elucidate the biotic and abiotic structure of the ecosystem needed to be modeled and ecosystem dynamics to be simulated. These dynamics form much of the ecosystem's metaphenome to be predicted from, and test, models that formalize theories of how ecosystem metaphenomes emerge from community metagenomes (Table 1).

HOW THE CHALLENGE MAY BE ADDRESSED

After deriving the observed and inferred biotic potential and abiotic context of an ecosystem from its metagenome, the next step of the challenge is to describe the mechanisms by which its characteristics persist and change through time. This has been achieved more generically by leveraging the large degree of conformity in organismal behavior and their interactions (Evans *et al.* 2013; Martinez 2020). For example, organisms' metabolic, growth, and maximum consumption rates typically increase

as a three quarters power law of their body size (Brown *et al.* 2004). Additionally, feeding interactions from herbivory through carnivory and parasitism to decomposition that determine carbon and population dynamics are highly conserved both within evolutionary lineages (Edger *et al.* 2015; Davies 2021) and among ecosystems (Williams & Martinez 2008). Mechanisms responsible for this consistency include the trophic hierarchy whereby organic energy necessarily flows from autotrophs through heterotrophs at higher trophic levels and ultimately to decomposers. That constraint plus a species-level mechanism that constrains generalists to consume organisms adjacent in this hierarchy enables the trophic “niche model” to successfully predict food-web characteristics (e.g., means and variances of species’ trophic levels, generality, vulnerability, connectedness, etc.) within a wide range of terrestrial and aquatic ecosystems (Williams & Martinez 2000, 2008; Dunne *et al.* 2004) including Cambrian ecosystems over a half billion years old (Dunne *et al.* 2008). Yet more constraints emerge from the relatively consistent body size ratios between consumers and resources such as predators and their prey (Brose *et al.* 2019) and parasites and their hosts (Hechinger 2013). Allometric trophic network (ATN) models integrate these constraints with the identities and abundance of species as inputs into relatively comprehensive models of the structure and dynamics of complex ecosystems (Table 1). ATN model’s output richly describes ecosystems’ metaphenomes including how the abundance of multiple species change over time when subjected to environmental change, biodiversity loss, and extraction of ecosystem services (Table 1).

Augmenting this ATN approach (Martinez 2020) with the greatly increased resolution and precision of network interactions derived from metagenomes may predict metaphenomes as modular, differential-equation-based, whole-cell modeling efforts have predicted phenotypes (Karr *et al.* 2012). One ATN module may determine network structure from empirical observations assisted by the probabilistic niche model to suggest mistaken interactions (Williams *et al.* 2010). Another module parameterizes the equations with metabolic and consumption rates again either specifically from

empirical observations or from those allometrically derived from the metabolic theory of ecology. A third module runs the simulations with a 1-day time step in contrast to the 1-second time step of whole-cell models. Other approaches may mimic physical models of whole cells that simulate the spatial structure and dynamics of each molecule within a cell by doing the same for each organism within an ecosystem (Katz *et al.* 2011). More plausible may be hybrid approaches where small organisms including microbes are simulated using differential equations (Weitz *et al.* 2015; Jansson & Hofmockel 2018) while large organisms are simulated with individual-based models (DeAngelis 2018). Rapid advances in automated observation (Dell *et al.* 2014), large scale computing, and eNA-based analyses will make current challenges much less prohibitive in the near future when modeling whole environmental systems may eventually focus on socio-ecological sustainability (Martinez *et al.* 2012; Davies *et al.* 2016).

WHY ADDRESS THE CHALLENGE? POSSIBILITY AND UTILITY

Systems biologists have already shown how efforts connecting genotype to phenotype can far surpass those that ecologists employ in terms of empirical and mechanistic richness, computational sophistication, and predictive success. Much of conceptual and technical heavy lifting has already been achieved or at least initiated in the modeling of both whole cells and whole ecosystems. The challenge to predict ecosystem metaphenome from community metagenome aims to focus environmental biologists as the genotype-to-phenotype challenge has focused molecular and cell biologists.

At least since Darwin famously concluded his Origin of Species describing a “tangled bank” of species “so different from each other, and dependent on each other in so complex a manner, [that] have all been produced by laws acting around us,” scientists have sought to discover how the “integrity of eco-systems” (Sir David Attenborough’s interpretation of “tangle bank,” Thorpe 2000) emerges from the complex interactions among diverse organisms in nature. While many biologists focused on how

molecules such as DNA determine the structure and function of organisms, environmental biologists focus more on how organisms determine the structure (e.g., diversity) and function (e.g., stability) of ecosystems. Both sets of scientists focus on highly complex interdependent biological systems with many different types of interactions operating on vastly different scales. However, cell and molecular biologists have more ambitiously and successfully achieved whole system understanding than have environmental biologists. The grand challenge to predict ecosystem metaphenomes from community metagenomes aims to collaboratively leverage the talents and achievements of the former group for the benefit of the latter. While retaining a focus on nucleic acids, the shift in scale from cell to ecosystem shifts genome analyses from identifying genes, their interactions and their products, and their products' interactions to a more straightforward identification of organisms and their interactions (Fig. 1). Instead of interactions among genes and biochemical species (Fang *et al.* 2020), environmental biologists focus on interactions within and among taxonomic species. Despite this shift, much of the conceptual, mathematical, and computational methods are remarkably similar (Fig. 1). Both endeavors also benefit from simultaneously achieving other key challenges while pursuing a grander one. They include "integration of heterogeneous databases, identification of the limits of our knowledge, predicting complex, multi-network phenotypes, and suggesting future experiments that may lead to new knowledge" (Carrera & Covert 2015). Perhaps most importantly, both pursue more comprehensive and synthetically predictive understanding of the biological systems they study (Fig. 1). Interdisciplinary collaborations that leverage these similarities may advance environment biology to where it more effectively helps solve some of the most difficult environmental problems on the planet.

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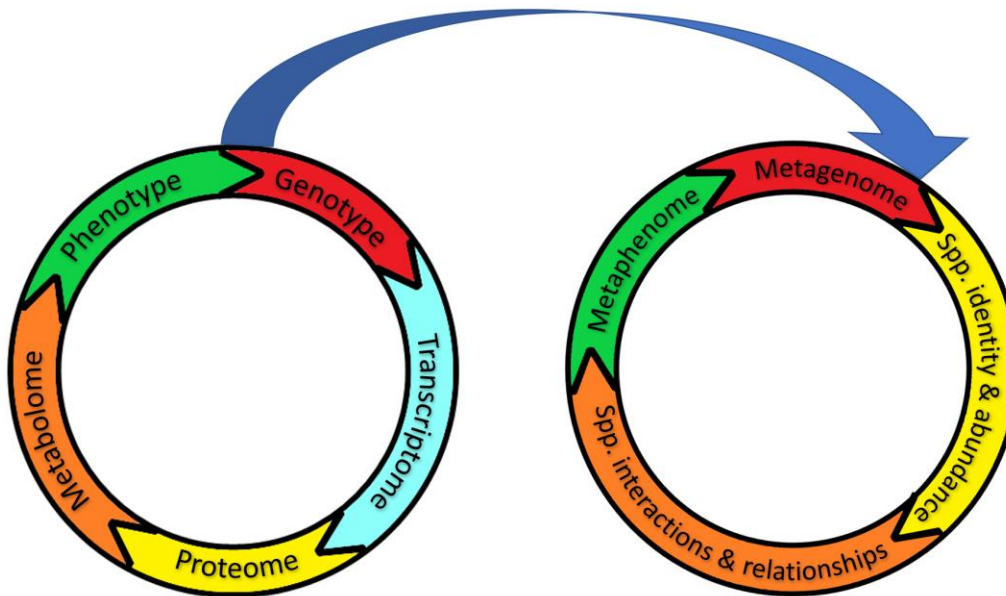
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282 Figure 1. Simplified depiction (after Bathe & Farshidfar 2014) of how an organism's phenotype emerges
283 from its genotype (left) and its role in an analogous depiction (right) of how an ecosystem's biotic
284 characteristics, its metaphenome, emerge from the genomes of its constituent species, its metagenome.
285 The ability to identify species and quantify their abundance stems from (blue arrow) the ability of
286 genotypes to identify the phenotype (e.g., body type and size, taxonomy, etc.) of organisms. Similar
287 colors between left and right depictions indicate analogous steps of emergence. While proteomes are
288 biologically derived from genotypes via the transcriptome, the identity and abundance of species may
289 be bioinformatically derived from an ecosystem's metagenome. This enables the identification of
290 species' ecological interactions that function similarly to the metabolome in generating essential
291 characteristics of organisms and ecosystems. For example, a key similarity is that both phenotypes and
292 metaphenomes (green) emerge from networks of interactions (orange) among functionally similar
293 molecules (left) and organisms (right) grouped into functionally distinct species (yellow) that interact at
294 concentration- (left) and density- (right) dependent rates modeled by Michaelis-Menten functions (left)
295 also called "functional responses" by ecologists (right, Fang *et al.* 2020; Martinez 2020).

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Step	Challenge	Focus	Allometric trophic network theory
1	Metagenotyping	Habitats, organisms, guts, tissues	30 yr observing L. Constance biota
2	Identify and quantify	Taxa, diversity, phylogenies, populations, growth rates, interactions	Field and lab observations & experiments
3	Assemble and parameterize consumer-resource networks	Feeding, infection, pollination, seed dispersal, nutrient transport, habitat provision, decomposition, preferences, adaptive responses (fear, cooperation, interference, defense, etc.)	25 Nodes: detritus, decomposers, bacteria, phytoplankton, zooplankton, fishes. Empirically observed & allometrically estimated parameters.
4	Code and conduct simulations	Uncertainty, variability, stability, species abundance & distribution. Effects of species loss & invasion, warming & eutrophication, extraction	Seasonal dynamics of a complex food web with 1-day time steps initialized with spring conditions (Boit <i>et al.</i> 2012)
5	Test against observations	Self-consistency, controlled & uncontrolled experiments	Explains 83% & 88% seasonal biomass & production variability of 20 auto- & heterotrophic spp.+ detritus
6	Repeat 1-5 as necessary	Test novel predictions, explore new hypotheses	Effects of age-structure, fishing, environmental noise & mutualism

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299 Table 1. Grand challenge steps for generally predicting ecosystem metaphenomes from community
300 metagenomes compared to specifically predicting the metaphenome of Lake Constance (Boit *et al.*
301 2012) with Allometric Trophic Network (ATN) theory (Martinez 2020). ATN predictions of ecosystem
302 metaphenomes involve simulations of empirical and niche-model networks of consumer-resource
303 interactions that have been broadly corroborated empirically (Martinez 2020): Vucic-Pestic *et al.* (2011)
304 predicted the recently observed decreases in trophic transfer efficiency (Barneche *et al.* 2021) caused by
305 global warming. Others (Jonsson *et al.* 2018; Curtsdotter *et al.* 2019) predicted observed effects of
306 species loss and invasion (Romanuk *et al.* 2009, 2017; Smith-Ramesh *et al.* 2017). ATN theory (Martinez
307 2020) has been extended to include detritus (Boit *et al.* 2012), evolutionary and spatial ecology (Allhoff
308 *et al.* 2015), ontogenetic niche shifts (Kuparinen *et al.* 2016; Bland *et al.* 2019), environmental variability
309 (Kuparinen *et al.* 2018), and mutualism within pollination networks (Hale *et al.* 2020).