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Towards meaningful scales in ecosystem microbiome research

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Running title. The problem of scale in ecosystem microbiomes

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Originality-Significance Statement

This is a critical article that articulates on the main issues to be considered in properly addressing the problem of scale in ecosystem microbiome research. We aim at stimulating debate on this challenging and often neglected topic. We advocate that efforts towards defining and using meaningful scales in ecosystem microbiome assessments will promote study reproducibility and advance both conceptual and theoretical developments. This will also contribute to redirect research priorities to enhance our mechanistic understanding of taxa distributional patterns and the ecosystem functions they provide.

Summary

Studies of microbial communities in natural ecosystems have been generally focused on mapping patterns of species and gene distributions. Although highly instrumental in expanding our understanding of microbial diversity and distribution patterns, such census studies often lack a meaningful and explicit definition of scale. Here, we discuss the importance of scale in environmental microbiology assessments and consider how patterning ecology can be redirected towards advancing concept and theory formation in ecosystem microbiome research.

Introduction

Increasing attention is being given to mapping 'global' and/or 'cross-continental' patterns of microbial communities across ecosystems (e.g., Ladau *et al.*, 2013; Bates *et al.*, 2013; Tedersoo *et al.*, 2014; Zhou *et al.*, 2016; Delgado-Baquerizo *et al.*, 2018; Baham *et al.*, 2018). The majority of these studies are founded on the notion that cataloguing large-scale diversity and distributional patterns of microbes will improve our understanding of the importance of microbiomes and specific taxon/gene abundances for predicting regional and global ecosystem processes; e.g. carbon source-sink dynamics and positive or negative microbially mediated effects on global warming (Crowther *et al.*, 2019). These studies have increased knowledge of, for instance, the ubiquitous versus rare distribution of taxa and functions. However, less attention has been given to assessing the reliability or value of the often-arbitrary scales used. We argue that without an explicit consideration of scale and its limitations, extrapolating findings obtained from limited datasets can lead to misleading information, thus hindering our ability to effectively understand and predict the functional capacity of environmental microbiomes. To stimulate debate on this challenging topic, we consider three main categorical issues associated with terminology, concept formation and theory construction.

The terminology problem

The use of ‘global/regional/cross-continental’ and ‘pattern/distribution/biogeography’ terminologies has been broadly used to refer to large-scale surveys of ecosystem microbiomes. It is important to realize that data obtained from these assessments are derived from a limited set of samples collected at particularly small scales (e.g., 1-10 mL of water or <1 g of soil), with results later extrapolated to larger spatial scales using scattered sampling designs without accounting for spatial environmental variation. Although typically not stated explicitly, it is often implied that such datasets are in fact representative of all sites at that scale, including non-sampled sites for which information is unavailable. In defence of this approach, it could be argued that it is not feasible to perform fine-scale sampling for broad-scale studies that range from thousands of kilometres to continental scales. In addition, meaningful or not, correlational outcomes are often robust and sufficiently significant to justify hypothesis development and the further exploration of potential underlying processes and mechanisms governing taxon distribution patterns. In fact, both of these arguments suffer from the same fallacy, which is an assumption that is best illustrated by the logical argument from classical scientific philosophy: “Absence of evidence is not evidence of absence”. That is, since it is impossible to cover at a fine-scale potential site variation across broad spatial scales (i.e., absence of evidence), whatever patterns emerge from a limited (often scattered) collection of samples will hold for all missing samples within that scale (evidence of absence). Thus, as a consequence of this fundamental design problem, outcomes will be biased by non-random datasets, at least to some extent, and there is no explicitly defined/optimal sampling plan. Eliminating these issues – or at least diminishing their importance – requires the use of proper language, the explicit definition of scale and its limitations, and proper accounting for both replication and assessment of within-site variation when developing broad-scale sampling designs.

Concept formation

The debate about patterns and scale in ecology is not new (see Chave, 2013) but these concepts remain poorly developed in environmental microbiology (Ladau and Eloe-Fadrosh, 2019). As revised by Levin (1992) and Chave (2013), the main objective of patterning ecology is to inform theory. Thus, it is critically important that patterns have some degree of repetition and replicability if they are to have any level of predictive power (MacArthur, 1972). Replication and spatially independent representative sampling are therefore essential to infer both small-scale and large-scale patterns, as is the incorporation of nested designs in accordance with scale to account for autocorrelation (Tedersoo, 2017). Moreover, reliable and informed prediction can only be achieved through sufficient longitudinal data and/or by clear elucidation of mechanisms that underlie patterns (Levin, 1992), both of which require a meaningful and explicit definition of scale. Importantly, the relative influences of distinct mechanisms underpinning taxa distributions are known to vary as a function of scale, so-called scale-dependency (Dini-Andreote *et al.*, 2015; Chase *et al.*, 2018). Thus, starting with a clean slate and going from processes to patterns, and not backwards, may represent a more fruitful strategy to advance mechanistic understanding of microbial distributional patterns.

"The description of patterns is the description of variation, and the quantification of variation requires the determination of scales" (Levin, 1992). This raises a critical limitation, which is the extent to which patterns or variation that emerge from spatially limited designs and lack of temporal components preclude many studies from appropriate replication. In other words, are these assessments providing global patterns of biological diversity or intrinsic variation within sample sets collected at sites scattered at a global scale? For example, samples may be collected across a range of environmental variable(s), and these variable(s) may turn out to fit correlational patterns. However, these patterns remain in the absence of evidence of 'how' and 'the extent at which' these variable(s) might physiologically constrain or promote the abundance or activities of specific taxa. Considering pH, which is often found to be the highest correlate to community composition in terrestrial (soil) systems. Although pH can have some direct effect on microbial physiology, it will also have many indirect effects that might be equally or even more important to species distributions. In addition, millimetre-scale gradients in O_2 in soil particles have been shown to drive community divergences that can be analogous to those resulting from selection imposed by large-scale abiotic variables (e.g., pH, salinity, etc.) or anthropogenic disturbances (agricultural practices, land-use conversion, etc.) at a broader scale (Konopka, 2009; Vos *et al.*, 2013). Moreover, it is worth noting that there are fundamental differences when comparing aquatic and terrestrial ecosystems. Most of these differences relate to the mechanisms structuring these systems and how these relate to organismal dispersal limitation, spatial connectivity and environmental filtering. For example, one can argue that microbiomes in surface waters in the Pacific and Atlantic oceans (similar to 'continental scales') may be more similar than those collected in a single area but will differ in the photic and twilight zones (mesopelagic zone, 200-1,000 m). Furthermore, samples for soil microbiome assessments usually undergo homogenization of micro-scale 'habitats' during processing. This is less of a problem in aquatic systems, in which the distribution of habitat gradients varies at a larger scale (ca. 1-10 m) than in terrestrial systems (ca. 0.1-1 mm). Thus, attention should be given to the intrinsic characteristics of divergent systems, the scales at

which biogeochemical gradients are formed and those at which ecological processes operate. There is, therefore, no such thing as a 'gold standard' by which one can define an 'optimum scale' (also called 'characteristic scale', i.e. one that 'maximizes the ratio of deterministic information to stochastic fluctuations' *sensu* Pascual and Levin, 1999). Rather, there is an urgent need for reproducibility with an explicit consideration of scale from which patterns emerge, thus paving the way for conceptual and theoretical developments.

Theory construction

"Theory is used to classify, interpret and predict the world around us. Without it, microbial ecology is merely the accumulation of situation-bound statements that are of limited predictive ability, providing microbiologists with few insights" (Prosser *et al.*, 2009).

An ecological theory is built upon a contemplative amalgamate of information that aims to explain generalized patterns in nature. Most importantly, an ecological theory requires an 'explanatory surplus' (Gillies, 2015), that is, the ability to describe phenomena outside of the immediate realm in which it was formulated. This means that patterning microbiomes across ecosystems require a clear consideration of scale that results in consistency, a level of reproducibility and integration of information, as well as empirical tests and patterning validation assessments. Non-reproducible patterns are likely to result in obscure noise and/or lead to an overall assessment that microbiomes are stochastically assembled; or that patterns emerging from a reductionist approach are applicable at broad scales. This would reinforce assumptions that predicting, monitoring and/or manipulating microbiomes are unrealistic tasks and promote the argument of context-dependency and absence of realistic trackable mechanisms underpinning divergence in community assemblages across divergent systems and scales.

Two of the main objectives of theory are to inform applied ecology and experimental design. Patterns in ecosystem microbiomes can promote theory development by providing cross-system assessments using microbe-centric sampling strategies that also include appropriate measures of the local environmental complexity. The emerging data, together with other macroscale data, can then be used to develop validation experiments or environmental assessments that test hypotheses about potential underlying ecological and evolutionary mechanisms. As a path forward, and as similarly debated in general ecology, focus should be given to study systems at appropriate and pre-defined scales. This will later inform the development of models that bridge different scales (Melbourne and Chesson, 2006; Holt and Chesson, 2016; Chesson, 2010; Chase *et al.*, 2018), and avoid the accumulation of an anecdotal collection of datasets that may confound concept formation and therefore limit, rather than advance, the development of ecological theory.

Recommendations

This article has aimed to stimulate debate on how scale can be reliable and meaningfully defined and taken into practice in ecosystem microbiome research. The arguments and recommendations are not intended to discredit or demerit previous research efforts. We also do not claim to have now closed this debate nor do we have the ultimate solutions. However, we provide some initial recommendations that we believe will benefit future research. In brief, to promote conceptual and theory formation, studies should consider the following recommendations: (1) Explicitly define scale and the extent to which it is representative and relevant to potentially underlying mechanisms. This should lead to a level of reproducibility that allows for the proper elucidation and validation of underlying mechanisms and processes that govern community divergences. (2) Access variability within local sites and make use of new methods to assist sampling design (e.g., response-surface methodology, see Albert *et al.*, 2010). This is critical for the development of experimental plans that enhance sample/site representativeness. Information on local variability can also be used to feed model predictions and indicate the level of uncertainty when attempting to extrapolate patterns. (3) Account for inaccurate extrapolations by indicating levels of uncertainty in model predictions and data visualization, as well as acknowledging the existence of sites for which information is unavailable. Together, these will enhance the clarity of data presentation and promote discussion on challenges and limitations associated with sampling design. (4) When similar ecosystems are surveyed across large-scale gradients, studies should consider using replicable spatial designs with consistent sample sizes that potentially control for autocorrelation of variables. Particularly in the case of autocorrelation and structured data, models using k-fold cross-validation on geographically-partitioned datasets have been successfully used in species distribution modelling and has led to much more generalizable model fits (e.g. Roberts *et al.*, 2017). (5) Since correlational outcomes can be meaningfully applied to generate and test hypotheses empirically, value should be given to studies that develop prospective experimental designs aimed at falsifying putative mechanisms. This represents a challenging yet elegant way towards advancing this fast-moving field of science. In conclusion, despite the various ways in which scale and spatiotemporal variability can subvert our interpretation of microbial taxa and gene distributional patterns (also see Armitage and Jones, 2019), we hope new studies in 'global/regional/cross-continental' microbiome assessments will take these constructive suggestions into account. Collectively, these will help to orient new experimental designs and enhance precision in data analysis, interpretation and communication. Thus, paving the way for a more realistic appreciation of the advances and impacts ecosystem microbiome surveys truly provide.

Conflict if interests

The authors declare no conflict of interest.

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The terminology problem

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"The description of patterns is the description of variation, and the quantification of variation requires the determination of scales" (Levin, 1992). This raises a critical limitation, which is the extent to which patterns or variation that emerge from spatially limited designs and lack of temporal components preclude many studies from appropriate replication. In other words, are these assessments providing global patterns of biological diversity or intrinsic variation within sample sets collected at sites scattered at a global scale? For example, samples may be collected across a range of environmental variable(s), and these variable(s) may turn out to fit correlational patterns. However, these patterns remain in the absence of evidence of 'how' and 'the extent at which' these variable(s) might physiologically constrain or promote the abundance or activities of specific taxa. Considering pH, which is often found to be the highest correlate to community composition in terrestrial (soil) systems. Although pH can have some direct effect on microbial physiology, it will also have many indirect effects that might be equally or even more important to species distributions. In addition, millimetre-scale gradients in O_2 in soil particles have been shown to drive community divergences that can be analogous to those resulting from selection imposed by large-scale abiotic variables (e.g., pH, salinity, etc.) or anthropogenic disturbances (agricultural practices, land-use conversion, etc.) at a broader scale (Konopka, 2009; Vos *et al.*, 2013). Moreover, it is worth noting that there are fundamental differences when comparing aquatic and terrestrial ecosystems. Most of these differences relate to the mechanisms structuring these systems and how these relate to organismal dispersal limitation, spatial connectivity and environmental filtering. For example, one can argue that microbiomes in surface waters in the Pacific and Atlantic oceans (similar to 'continental scales') may be more similar than those collected in a single area but will differ in the photic and twilight zones (mesopelagic zone, 200-1,000 m). Furthermore, samples for soil microbiome assessments usually undergo homogenization of micro-scale 'habitats' during processing. This is less of a problem in aquatic systems, in which the distribution of habitat gradients varies at a larger scale (ca. 1-10 m) than in terrestrial systems (ca. 0.1-1 mm). Thus, attention should be given to the intrinsic characteristics of divergent systems, the scales at

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Theory construction

"Theory is used to classify, interpret and predict the world around us. Without it, microbial ecology is merely the accumulation of situation-bound statements that are of limited predictive ability, providing microbiologists with few insights" (Prosser *et al.*, 2009).

An ecological theory is built upon a contemplative amalgamate of information that aims to explain generalized patterns in nature. Most importantly, an ecological theory requires an 'explanatory surplus' (Gillies, 2015), that is, the ability to describe phenomena outside of the immediate realm in which it was formulated. This means that patterning microbiomes across ecosystems require a clear consideration of scale that results in consistency, a level of reproducibility and integration of information, as well as empirical tests and patterning validation assessments. Non-reproducible patterns are likely to result in obscure noise and/or lead to an overall assessment that microbiomes are stochastically assembled; or that patterns emerging from a reductionist approach are applicable at broad scales. This would reinforce assumptions that predicting, monitoring and/or manipulating microbiomes are unrealistic tasks and promote the argument of context-dependency and absence of realistic trackable mechanisms underpinning divergence in community assemblages across divergent systems and scales.

Two of the main objectives of theory are to inform applied ecology and experimental design. Patterns in ecosystem microbiomes can promote theory development by providing cross-system assessments using microbe-centric sampling strategies that also include appropriate measures of the local environmental complexity. The emerging data, together with other macroscale data, can then be used to develop validation experiments or environmental assessments that test hypotheses about potential underlying ecological and evolutionary mechanisms. As a path forward, and as similarly debated in general ecology, focus should be given to study systems at appropriate and pre-defined scales. This will later inform the development of models that bridge different scales (Melbourne and Chesson, 2006; Holt and Chesson, 2016; Chesson, 2010; Chase *et al.*, 2018), and avoid the accumulation of an anecdotal collection of datasets that may confound concept formation and therefore limit, rather than advance, the development of ecological theory.

Recommendations ~~moving forward~~

This article has aimed to stimulate debate on how scale can be reliable and meaningfully defined and taken into practice in ecosystem microbiome research. The arguments and recommendations are not intended to discredit or demerit previous research efforts. We also do not claim to have now closed this debate nor do we have the ultimate solutions. However, we provide some initial recommendations that we believe will benefit future research. In brief, to promote conceptual and theory formation, studies should consider the following recommendations: (~~i~~1) E~~e~~xplicitly define scale and the extent to which it is representative and relevant to potentially underlying mechanisms. This should ~~then~~ lead to ~~(*ii*)~~ a level of reproducibility that ~~(*iii*)~~ allows for the proper elucidation and validation of underlying mechanisms and processes that govern community divergences. (2) Access variability within local sites and make use of new ~~We suggest~~

~~that accessing variability within local sites and introducing new~~ methods to assist sampling design (~~for instance~~e.g., response-surface methodology, see Albert *et al.*, 2010). This is critical for ~~can lead~~ the development of experimental plans ~~that enhances~~that enhance sample/site representativeness. Information on local variability can also be used to feed model predictions and indicate the level of uncertainty when attempting to extrapolate patterns. (3) ~~Accounting~~ Account for inaccurate extrapolations by indicating levels of uncertainty in model predictions and data visualization, as well as acknowledging the existence of sites for which information is unavailable. Together, these~~,~~ ~~can~~ will enhance the clarity of data presentation ~~. This will improve~~and promote discussion ~~of~~ on challenges and limitations associated with sampling design~~, thus avoiding overarching assumptions and statements and the noise these may echo~~. (4) ~~Moreover, w~~When similar ecosystems are surveyed across large-scale gradients, ~~the~~ studies should consider ~~use~~ using ~~of~~ replicable spatial designs with consistent sample sizes that potentially control for autocorrelation of variables~~can be beneficial~~. ~~It is worth noting, p~~Particularly in the case of autocorrelation and structured data, ~~that~~ models using k-fold cross-validation on geographically-partitioned datasets have been successfully used in species distribution modelling and has led to much more generalizable model fits (e.g. Roberts *et al.*, 2017). (5) Since correlational outcomes can be meaningfully applied to generate and test hypotheses empirically, value should be given to studies that develop prospective experimental designs aimed at falsifying putative mechanisms. This represents a challenging yet elegant way towards advancing this fast-moving field of science.~~I~~ In conclusion, despite the various ways in which scale and spatiotemporal variability can subvert our interpretation of microbial taxa and gene distributional patterns (also see Armitage and Jones, 2019), we hope new studies in 'global/regional/cross-continental' microbiome assessments will take ~~into account~~ these constructive suggestions into account. Collectively, these will help to orient new experimental designs and enhance precision in data analysis, interpretation and communication. Thus, paving the way for a more realistic appreciation of the advances and impacts ecosystem microbiome surveys truly provide.~~.~~

~~Finally, since correlational outcomes can be meaningfully used to generate and test hypotheses empirically, switching gears towards developing prospective experimental designs for falsifying putative mechanisms represents another elegant way towards advancing this fast-moving field of science.~~

Conflict if interests

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