On cyanobacterial community diversity and its quantification

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ABSTRACT

In spite of the importance of diversity as a central parameter in ecology, novel methodologies to assess microbial "phylogenetic diversity" have not yet contributed to the quantitative estimations of natural diversity in the ecological sense. Using cyanobacterial communities as examples, we review the various relevant concepts of diversity, the problems attached to using various differentiating criteria - among them species- to estimate it, and argue for the simultaneous use of traditional, molecular and chemotaxonomic approaches, as a promising tool in the quantification of community diversity.

The importance of Diversity for ecological studies

The concept of diversity has a central, ever-growing role in ecological theory. It has long been held, not without controversy, that inherent ecosystem (or community) parameters such as stability, resilience, predictability and productivity are a function of the diversity contained within them. The possible direct dependency of ecosystem stability on diversity, has obvious implications for ecosystem management and for nascent disciplines like conservation biology, as it would offer a scientific basis for conservation efforts. Microbial ecology has lagged behind in this respect, most probably because of a lack of appropriate methodology to assess and quantify microbial diversity. Thanks to the advent of molecular techniques aimed at cultivation-free identification of microbes and the characterization of community structure (reviewed in [1]), microbial ecology has witnessed a bloom of contributions dealing with natural microbial diversity. However, in spite of the high standards that the measurement of functional parameters (i.e., biogeochemical processes) has attained in microbial ecology, there have been few attempts to combine diversity assessments with functional characteristics of microbial communities. We have been trying to go beyond mere qualitative descriptions of microbial diversity in nature, and have tried to obtain quantitative estimates thereof using cyanobacterial communities. Here we discuss some of the problems and possible solutions to this endeavour.

Diversity, which diversity?

The word diversity has an intuitively straightforward but vague meaning. It implies variety, the quality of containing manifold units according to a certain differentiating criterion. It necessitates adjectives or prefixes to become descriptive, and one currently uses precise expressions like phylogenetic diversity or metabolic diversity, and more mystifying ones like biodiversity or microdiversity. In ecology, however, "*Diversity*" (hereafter, capitalized) is a strictly defined parameter applied to an ecosystem or part thereof (a population, for example). Traditionally, "richness" is the parameter used to quantify the number of ecologically meaningful units contained within an ecosystem.

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Diversity takes into account not only the number of discernible units contained within the ecosystem, but also the relative prominence of each unit. This entails both the absolute number of units and the evenness of the distribution of relative prominence; all contributing to increased Diversity. In the 70's and early 80's the measurement of Diversity had been sought in microbial ecology, in spite of the difficulties involved (reviewed in [2]). Short of contributing directly to the measurement of Diversity, molecular techniques have rather redirected research into cataloguing efforts and exemplary demonstrations of bacterial phylogenetic richness. A community´s "phylogenetic diversity" or biodiversity, as used nowadays by microbiologists, reflects something closer to richness than to Diversity, since the quantitation of the relative prominence distributions is not carried out.

Both biodiversity and Diversity have their place in the study of nature, but they are not tantamount. The link between both concepts, in the words of Margalef [3], is that "the functional diversity of ecosystems processes the genetic material from the local stock of available biodiversity". Community or ecosystem Diversity is the parameter that one expects to determine (or be determined by) other ecologically important properties of ecosystems, not (necessarily) biodiversity. This is because Diversity is ultimately a measure of functional diversity, a reflection of the variety of actions and interactions of community members weighed by their proportional importance, and this is what will determine ecosystem responses to the environment. We learn, for example, on the basis of 16S rRNA gene sequence comparisons, that the phylogenetic diversity contained in a single hot spring spans a range comparable to the whole variety of microbial life [4], and that the phylogenetic diversity of the morphologically uniform cyanobacterial inhabitants of a hot spring microbial mat spans a range comparable to that of all known cyanobacteria [5]. If so, then how about the old statement that extreme environments harbour low-diversity communities? It may still hold true, because, in fact, we still do not know much about the relative contributions of each phylogenetic type to the community, nor do we know how phylogenetic diversity relates to functional diversity in those environments. If the majority of those "phylotypes" are rare and most of the populations are made up of only a few dominant types, then the Diversity of the communities is still low.

The study of phylogenetic diversity in natural communities provides priceless insights into the evolution and distribution of bacteria, as well as overwhelming prospects and humbling directions for those of us still attempting to cultivate novel isolates. But the phylogenetic diversity of a community does not necessarily have to be relevant for the function of the community. In fact, for the case of cyanobacteria, relating the phylogenetic diversity assessed directly in natural communities to functional properties has necessitated a return to traditional cultivation and characterization [6, 5]. In spite of the large efforts they require, studies searching for correlations between phylogeny and physiology in a variety of isolates [7-10] should be most welcome in this respect, since they will likely improve our ability to interpret information on phylogenetic diversity in terms of functional diversity.

Species as units to estimate Diversity?

Assignment of individuals to species has been traditionally the differentiating criterion with which to dissect communities into non-overlapping classes, with catalogues of species yielding more or less directly estimates of richness and with distributions of biomass (or numbers of individuals) into species allowing estimation of Diversity. This approach relies

on the assumption that the quanta of functional diversity (the ecological roles, the "guilds") are neatly and unequivocally distributed among species, so that one can use the latter as a token for the former. The use of the species approach is obviously impracticable in microbial ecology, as the concept of species in bacteriology is arbitrary and often removed from ecological reality. Cyanobacteria are a special case in this respect, since a botanical taxonomy based on morphological traits of uncultured material predates the bacteriological taxonomic treatment based on cultivated material [11]. In principle, it is possible to use the botanical species concept to obtain catalogues of natural cyanobacterial assemblages with which to quantify species richness and Diversity. In fact, cyanobacterial populations treated in this way have always been a part of the diversity estimates in studies of phytoplankton ecology. But, is such an approach licit? Do botanical species correspond to evolutionary clusters so tightly that we can expect phenotypic homogeneity within them and significant differences among them? One finds several blatant disagreements between species (or even genera) and phylogenetic reconstructions based on molecular data, esspecially for morphologically simple cyanobacteria (*Synechococcus, Cyanothece*) [10, 12, 13], but also good correspondence in the case of morphologically more defined species or groups [8] [14] [15]. Thus, there may exist some degree of correlation, but it has to be regarded more like a trend than a direct correspondence. On the other hand, we can infer that wherever cyanobacteria occur, photosynthesis is taking place. We can also expect that whenever heterocystous species occur dinitrogen fixation will take place. These correspondences are, however, a meagre harvest, and, additionally, not reciprocal. Can we then expect to find unequivocal correspondence between species, at least those that are phylogenetically valid, and their functional role in the communities? Culture studies have shown that functional diversity exists within single morphotypes. The so-called ecotypes with respect to single phenotypic traits has been repeatedly demonstrated within botanical (roughly morphotypic) species: for temperature optima in *Synechococcus lividus* from hot-springs [16], for salinity tolerance within *Microcoleus chthonoplastes* strains [17], for anoxygenic photosynthetic capacity in *Oscillatoria amphigranulata* strains [18], for light-adaptation in *Plectonema notatum* [19] and *Prochlorococcus marinus* [6]. At least in some of these examples [6, 17], the species studied corresponded to well-defined phylogenetic units. In addition, phenotypes are really multidimensional entities, so even in the case where we find phenotypic homogeneity with respect to one trait, will expectations for similar homogeneity in other traits be warranted?

In summary, one can expect that the study of morphotypic diversity (traditional botanical species) to bear some information on the Diversity of the community to be studied, but also that this information will carry a certain, variable amount of noise. It is reasonable to expect that the simpler the morphologies involved and the smaller the richness of the community, the more unreliable the estimate will be. Perhaps the situation improves if the definition of bacterial (and cyanobacterial) species changes into a more ecologically sound concept [20], but this is not likely to happen any time soon, as it would require large combined efforts of the characterization of cultures and natural populations.

Alternatives

The use of species is by no means an irreplaceable goal. In fact the quantification of Diversity does not depend on the ability to discern species [21]. Because a direct measure of functional diversity is impossible, as yet and in the foreseeable future, it will be necessary to adopt alternative token units (with their corresponding assumptions) to assess

microbial community Diversity. For the case of cyanobacteria, or any other microbial group, this may include any unit that provides: i) specificity for the group, so that no foreign elements are included, ii) universality within the group so that no cyanobacteria are missed, iii) a large degree of variability so that classes can be made according to it, and iv) ease of identification and quantification so that each class can be easily recognized and its relative contribution to the community can be quantified. Molecular units like genetic sequence variability, either for specifically cyanobacterial genes or for universal genes amplified using cyanobacteria-specific primers, such as 16S rRNA [22], phycocyanin [7] or RNA polymerase[23], are appropriate units. Phenotypic or chemotaxonomic markers such as fatty acid profiles, carotenoids, or mycosporine like amino-acids [24] may offer additional information, if these can be measured in natural communities with sufficient group specificity. Arguments similar to those used in section 2 above for the case of morphotypic diversity, can be used to validate the use of molecular or chemotaxonomic markers in Diversity estimation. These three approaches present inherent and common problems, which will not be discussed here, but they all carry information on the functional diversity of natural communities. Each approach is likely to mirror the community at different levels of resolution (which may not even be the same among all cyanobacterial groups); each will provide a more or less blurred image of the same underlying Diversity. Therefore it is not to be expected that absolute values for Diversity measured with different token parameters will be equal in absolute terms. However, like in a phantom portrait, we argue that the combined use of several sources of information on a single subject, however imprecise, will allow a meaningful picture of Diversity to emerge.

In our recent work using cyanobacterial mats from hypersaline environments as model communities, we have carried out such multiple determinations of Diversity and richness [25]. In these studies, we indeed detected the presence of statistically significant correlations (but also the lack of absolute correspondence) in the different Diversity and richness estimates. This facilitates making sound correlations between functional parameters of the communities and the Diversity they contain. For example, we could demonstrate that in these hypersaline communities, the functional rates of primary productivity and respiration, are not a function of diversity as had been predicted for plankton communities [3], but rather strongly controlled by physicochemical parameters, namely salinity and its effect on gas exchange rates [26]. In fact, analyses of the increase of cumulative richness with the increase in number of samples considered, has shown that using morphotypes as token units for richness, results in lower estimates than the use of 16S rRNA gene sequences, and that the presence of gradients and boundaries in ecological parameters results in increased richness [27]. We are currently working on the possible correlations between Diversity and functional stability against environmental change in our communities. We feel confident that a robust quantification of microbial Diversity will enable us, and others in the near future, to establish its importance in driving ecosystem properties.

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