Advances in MICROBIAL ECOLOGY

Volume 7

Edited by
K. C. Marshall

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PLENUM PRESS · NEW YORK AND LONDON

The Library of Congress cataloged the first volume of this title as follows:

Advances in microbial ecology. v. 1–
New York, Plenum Press c1977–
v. ill. 24 cm.
Key title: Advances in microbial ecology, ISSN 0147-4863
1. Microbial ecology—Collected works.
QR100.A36 576'.15 77-649698

Library of Congress Catalog Card Number 77-649698
ISBN 0-306-41558-9

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

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ADVANCES IN MICROBIAL ECOLOGY

Sponsored by the International Committee on Microbial Ecology (ICOME), a committee of the International Union of Microbiological Societies (IUMS) and the International Union of Biological Sciences (IUBS)

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Preface

Since the appearance of the first volume of *Advances in Microbial Ecology* in 1977 under the editorship of Martin Alexander, the series has achieved wide recognition as a source of in-depth, critical, and sometimes provocative reviews on the ecology of microorganisms in natural and man-made ecosystems. Most reviews published in *Advances* have been prepared by experts at the invitation of the Editorial Board. The Board intends to continue its policy of soliciting reviews, but individuals are encouraged to submit outlines of unsolicited contributions for consideration of their suitability for publication in *Advances*.

Volume 7 of *Advances in Microbial Ecology* covers a range of topics related to the ecology of microorganisms in natural and artificial habitats. R. M. Atlas discusses the measurement and significance of diversity in microbial communities. The nature of deserts and the activity of microorganisms in desert soils are considered by J. Skujiņš. D. B. Nedwell examines both the input and the mineralization of organic carbon in anaerobic aquatic sediments. The role of microcosms in the evaluation of interactions between pollutants and microorganisms is the basis of a major review by P. H. Pritchard and A. W. Bourquin.

The Editor and members of the Editorial Board of *Advances in Microbial Ecology* are appointed by the International Committee on Microbial Ecology (ICOME) for fixed terms. Martin Alexander and Thomas Rosswall have completed their terms as Board members, and we wish to offer them sincere thanks for their efforts in establishing the series. With the publication of this volume, we welcome Ron Atlas and Bo Barker Jørgensen to the Editorial Board.

K. C. Marshall, Editor
R. Atlas
B. B. Jørgensen
J. H. Slater

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Diversity of Microbial Communities

RONALD M. ATLAS

1. Introduction

As used by microbiologists, the term *diversity* has various meanings, often describing qualitative morphological or physiological variances among microorganisms (Starr and Skerman, 1965; Belser, 1979; Hamada and Farrand, 1980; Hanson, 1980; Stanley and Schmidt, 1981; Walker, 1978; Yeh and Ornston, 1980). Microbial populations indeed exhibit great heterogeneity or diversity in their morphological, physiological, and ultimately genetic characteristics. An extensive list of diversifying factors that act to establish differentiating characteristics between microbial species has been discussed by Starr and Schmidt (1981). Some examples of these diversifying features are listed in Table I. These diversifying features have traditionally been employed by bacteriologists as the criteria for differentiating species. Often, the ability to recognize and distinguish species of microorganisms is difficult, but it is essential for assessing diversity.

In its ecological sense, the term *diversity* describes the assemblage of species within a community, and it is in this restricted sense that ecologists synonomously describe ecological and species diversity (Margalef, 1979; Pielou, 1975; Whittaker, 1975). Species diversity is a measure of entropy (disorder or randomness) of the community; an index of diversity measures the degree of uncertainty that an individual picked at random from a multispecies assemblage will belong to a particular species within that community (Legendre and Legendre, 1982). The greater the heterogeneity of the assemblage of populations and individuals within those populations, the greater the diversity of the

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Table I. Diversification Elements of Prokaryotes

Morphological	
Cellular size	Cell envelope: gram staining and correlated properties, membrane diversity (lipopolysaccharide, protein, lipid), wall (and peptidoglycan) diversity
Cellular shape	
Cellular flexibility vs. cellular rigidity	
Morphogenesis and life cycles	
Endospores, cysts, conidiospores, and sporangiospores	Flagella and other locomotor devices
	Prosthecae and other cellular appendages
Cell division, binary fission, budding, fragmentation	Noncellular appendages, including pili and similar structures
Filaments, trichomes, multicellularity	Holdfasts and other adhesive devices
Cytoplasmic inclusions and vacuoles	Sheaths, capsules, zoogleae, other extra-wall structures (including encrustation by iron and manganese oxides)
Mesosomes, thylakoids, and chromatophores	
Associative relationships	
Colonies, clones, multicellularity	Symbiotic associations including endosymbiotic relationships
Cooperation and competition	Host cell–parasite interactions
Physiological	
Relationship to oxygen	Relationship to light (photosynthesis, other phototransducers, other effects of visible light)
Nutrition, mechanisms of energy conversion, metabolic potentialities—catabolic capabilities, growth factor requirements, dinitrogen fixation and other nitrogen metabolism	Relationship to radiation: UV, X-rays, and other ionizing radiation
	Temperature relationships
Pigments	Hydrogen ion concentrations
Secondary metabolites	Barophily
Luminescence	Halophily
	Osmotic relationships
Genetic and epigenic	
Nucleoids, including genome size, multinuclearity, peculiar karyology	Ribosomal RNA sequencing
	Isoenzymes
Plasmids, episomes, temperate phages, other nonnucleate elements containing nucleic acid	

community. Diversity is equivalent to a measure of variance for the species parameter of the community; it is a measure of the species composition of an ecosystem in terms of the number and relative abundances of the species.

There are several assumptions inherent in assessing species diversity. The first is that populations occupying a particular habitat initiate interrelationships that result in the establishment of an organized community. There is ample evidence that biological populations, including microbial populations,

establish interpopulation relationships that lead to the formation of a defined and stable community structure. Microbiologists have long recognized the "normal microflora" associated with various ecosystems, e.g., the normal microbiota of man. The occurrence of a normal microbiota must result from the establishment of community structure. Population (species) interactions that lead to the establishment of a defined community have been assumed to be based on various physiological interactions. The functional roles of specific populations (niches) within the communities of certain ecosystems have been defined, e.g., for the rumen ecosystem (Hungate, 1975); the bases for interspecific population relationships within such communities have been defined as well, leading to a relatively complete understanding of community structure and ecosystem function. Work using chemostats has elucidated some of the interactions between microbial populations that lead to the establishment of stable community structure in aquatic ecosystems; the nature of these interactions has been summarized by Slater (1978, 1980). In chemostat studies, it is often found that stability occurs when several interacting populations cooperate to best exploit the available resources. In some cases, two species constitute a stable community structure, whereas in other experiments, additional member populations are needed before community stability is achieved.

Whereas interrelationships among populations are clearly dynamic, the assumption that the populations within the community reach points of stability is an underlying principle of diversity calculations. In fact, perhaps the premise of greatest importance in considering ecological diversity and community structure is that species diversity is a community parameter that relates to the degree of stability of that community, i.e., that stable and resilient biological communities must contain a certain level of diversity; it is this hypothesis that is often used to justify the estimation of community diversity (Pielou, 1975; Peet, 1974). Communities with too much or too little diversity would be subject to continuous or catastrophic change.

2. Measurement of Species Diversity

Before considering how diversity measurements have been applied to microbial communities, we must examine the meaning of diversity and how it is measured. Ecologists have developed several indices of species diversity. Several extensive discussions of ecological diversity indices have been published (Dennis and Patil, 1977; Hurlbert, 1971; Legendre and Legendre, 1982; Margalef, 1968; Peet, 1974; Pielou, 1966a,b, 1969, 1975, 1977; Whittaker, 1975; Woodwell and Smith, 1969). As discussed in these reviews, the meaning, interpretation, and proper use of particular indices are often the subject of controversy among ecologists. Hurlbert (1971), for example, argues that species diversity, as defined by a variety of indices, has no biological meaning; com-

munities having different species compositions are not intrinsically arrangeable in a linear order on a diversity scale. Pielou (1975, 1977) points out the problems with the methods of calculating species diversity that make interpretation difficult. Peet (1974) also considers the use and misuse of diversity indices. It is not the intent of this review to settle these long-standing debates, but rather to consider how the concepts embodied in the measures of ecological diversity can be extended to microbial communities. The expression of ecological diversity as a species-diversity index is an outgrowth of information theory. Essentially, any diversity index must measure the heterogeneity of information stored within the component populations of the community; the species-diversity indices that have been developed aim to describe the way in which information is apportioned within the community.

2.1. Species Richness

In its simplest form, the species-diversity index simply represents a count of the number of different species found occurring together. Communities with many different species have high diversity and those in which few species are found have low diversity. The number of species (n) can be used as a measure of the biological richness (species richness) of a community (Patrick, 1949): diversity (D) = n. Margalef (1951) proposed a standardization of the number of species (n) by the number of individuals (N): $D = (n - 1)/\ln N$. Similarly, Odum *et al.* (1960) proposed using $n/\log N$ and Menhinick (1964) suggested using $n/\sqrt{N}$ as measures of species richness.

To overcome the problem of estimating the numbers of species when samples are not the same size, Sanders (1968) developed the method of species rarefaction. This method consists of calculating the number of species that the samples would contain if they were the same size. Sanders's original formula was corrected by Hurlbert (1971) so that one obtains the expectancy of a number of species (n') in a standardized sample of N' specimens from a nonstandard sample containing n species, N specimens, and N_i specimens in species i according to the formula

$$E(S) = E(n') = \sum \left[1 - \frac{\left| \begin{matrix} N - N_i \\ N' \end{matrix} \right|}{\left| \begin{matrix} N \\ N' \end{matrix} \right|} \right] \quad \text{where} \quad \left| \begin{matrix} N \\ N' \end{matrix} \right| = N!/N'!(N - N')!$$

The proper use of rarefaction has been considered by Tipper (1979) and Simberloff (1972). To facilitate the calculation of $E(S)$, Simberloff (1978) has developed a computer program for computing the expected number of species.

2.2. Dominance and Evenness

Whereas $E(n')$ indicates the expected distribution of species within the community, the concept of species richness alone, as measured by a simple species-richness index, does not account for the evenness or equitability with which species (bits of information) are distributed within the community. The way in which information is distributed within the community is an important component of the heterogeneity of the assemblage of biological populations. Communities can be dominated by individual populations even if the species richness of the community is high.

To assess the degree of dominance, a species-diversity index was developed by Simpson (1949). The Simpson index is expressed as the function $\Sigma p_i^2 = \lambda$, where λ represents the probability that any two individuals picked independently at random from the community will belong to the same species. The function λ is a measure of the expected commonness of an event; the probability that two randomly chosen specimens belong to the same species is a measure of concentration as proposed by Simpson. The Simpson index is the opposite of diversity: the greater the homogeneity of a community, the greater the chance two randomly picked individuals will be of the same species; i.e., the lower the heterogeneity (diversity) of the community, the higher the value for λ. When a community is dominated by a single species, λ is high and approaches unity. Conversely, when there are numerous species that are relatively evenly represented, λ is low and is the probability of selecting two different species at random.

Another procedure for measuring diversity, based on a geometrical approach, was proposed by McIntosh (1967). The McIntosh index measures uniformity (U) within the community; it is expressed as $U = (\Sigma N_i^2)^{1/2}$, where N is the total number of individuals in the collection (N_i is the total number of specimens in a species). The larger the number of species, the smaller the value of U; the maximum value of U occurs when the sample contains but one species. The diversity (D) of the community is inversely related to U, and a diversity index based on McIntosh's U can be expressed as $D' = N - U$. The McIntosh diversity measures species diversity on the basis of the evenness of the apportionment of populations within the community.

2.3. General Measures of Species Diversity—The Shannon Index

In contrast to the McIntosh uniformity index and the Simpson dominance index, a general information index measures both the species richness and equitability components of community diversity. The Shannon diversity index, known with slight mathematical variations as the Shannon–Weaver and Shannon–Wiener indices, is probably the most widely used index for measuring species diversity (Shannon, 1948; Shannon and Weaver, 1949). The Shannon

index is expressed as $H' = -\Sigma p_i \log p_i$, where p_i is the proportion of the community belonging to the ith species. The calculation of H' was simplified by Lloyd *et al.* (1968), who developed the following log base 10 formula: $H' = C/N(n \log_{10} N - \Sigma n_i \log_{10} n_i)$, where C is 3.3219, N is the total number of individuals, and n_i is the total number of individuals in the ith species.

The Shannon index has the following properties: (1) for a given number of species (S), H' has its greatest value when $p_i = 1/S$ for all values of i; i.e., H' is at its maximum when there is a completely even distribution of species within the community; (2) H' is at its minimum value (0) when a community is composed of only one species; (3) the diversity, H', increases with species richness such that H' is greater for a completely even community with $S + 1$ species than for a completely even community with only S species; and (4) the diversity of the community measured with the Shannon index can be partitioned into different fractions; i.e., the individual diversity indices for component groups, such as taxonomic families or genera, can be added to determine the total diversity of a biological community. As stated by Legendre and Legendre (1982), a probabilistic interpretation of the Shannon index lends itself to a measure of uncertainty regarding the identity of a randomly chosen specimen from the community; this uncertainty is smaller when the community in the sample is dominated by a few species, in which case H' is low; the value of H' also diminishes when the number of species gets lower, which also diminishes the uncertainty associated with the identification of a randomly chosen specimen.

There are certain limitations to the use of the Shannon index (Pielou, 1969, 1975, 1977). The Shannon index is appropriately used only when examining communities that are sufficiently large so that removing samples in a census does not cause any perceptible change within the community. In cases where the community is small and fully censused to determine diversity, it is necessary to use another measure of diversity; in such cases, Margalef (1958) has proposed the use of Brillouin's index (Brillouin, 1962). Brillouin's index, expressed as $H = (1/N) \log (N!/\Pi n_i!)$, where N is the number of individuals in the whole collection and n_i is the number in the ith species, has also been proposed for use when one performs sampling without knowing whether the sample is representative and the sample is therefore best treated as a collection. Peet (1974) suggests that contrary to the Shannon index, Brillouin's measure of uncertainty is not a good measure of diversity and its use as a community descriptor is rarely necessary. A problem with both the Shannon index and the Brillouin index is that both species richness and evenness play a role in determining the value of the index. Quite different communities, as a consequence, can have the same index. It is sometimes necessary to assess the species richness and evenness components individually in order to understand the factors controlling the structure of a community and the reasons a community has attained a certain level of species diversity. Further, when addressing the ques-