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ACCELERATED PAPER

FUNCTIONAL DIVERSITY OF MICROBIAL COMMUNITIES: A QUANTITATIVE APPROACH

JOHN C. ZAK,[1]* MICHAEL R. WILLIG,[1] DARYL L. MOORHEAD[1] and HOWARD G. WILDMAN[2]

[1]Ecology Program, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, U.S.A. and [2]Ecology Group, Natural Products Discovery Department, Glaxo Group Research Limited, Greenford, Middlesex UB6 0HE, U.K.

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Summary—Evaluating the biodiversity of microbial communities remains an elusive task because of taxonomic and methodological difficulties. An alternative approach is to examine components of biodiversity for which there exists a reasonable chance of detecting patterns that are biologically meaningful. One such alternative is functional diversity. We propose a procedure based on the Biolog identification system to quickly, effectively, and inexpensively assess aspects of the functional diversity of microbial communities. The numbers and types of substrates utilized by bacterial communities, as well as the levels of activities on various substrates and patterns of temporal development, constitute an information-rich data set from which to assess functional diversity. Data from six plant communities (black grama grassland, *Sporobolus* grassland, creosotebush bajada, herbaceous bajada, mesquite-playa fringe, and playa grassland) located along an elevational and moisture gradient at the Jornada Long-Term Ecological Research site in the northern Chihuahuan Desert, are analyzed to illustrate the procedure and its relevance to biodiversity. Our analyses demonstrate that the Biolog system can detect considerable variation in the ability of microbial communities to metabolize different carbon compounds. Variation in substrate use was compartmentalized differently along the moisture gradient. Differences in functional diversity were dependent upon the class of carbon sources (guild-specific results). A multifaceted approach to biodiversity that comprises both functional and taxonomic perspectives represents fertile ground for future research endeavors.

INTRODUCTION

Although current emphases in ecology, systematics, and conservation biology include issues of biodiversity, its very definition remains controversial (Lubchenco *et al.*, 1991; Sayer and Whitmore, 1991). Solbrig (1991) provided an incisive treatment of underlying conceptual issues, and defined biodiversity as comprising three interrelated elements: genetic, functional and taxonomic diversity (Fig. 1). Most research on biodiversity focuses on taxonomic richness or diversity, although increasing attention is being directed to genetic diversity, especially as it relates to rare or endangered species. However, few investigations have examined *functional* diversity, and its theoretical foundations are not well understood. Indeed, we understand little about the degree to which genetic diversity is translated into taxonomic diversity, and even less about the manner in which genetic and taxonomic diversity affects functional diversity or ecosystem properties.

*Author for correspondence.

Historically, studies of biodiversity and its relation to ecosystem structure and function have focused primarily on macro-organisms (see Ricklefs and Schluter, 1993), with little consideration of microorganisms, even though the latter perform key ecological roles (Parkinson and Coleman, 1991). Decomposition is dominated by microbial activities and is as fundamental as primary production to the long-term functioning of ecosystems. In addition, microorganisms are primarily responsible for the degradation and detoxification of many environmental contaminants (e.g. Lamar and Dietrich, 1990; Aelion and Bradley, 1991). For these reasons, changes in the composition or activity of microbial communities might have immediate or lasting effects on ecosystem functioning (Perry *et al.*, 1989).

Our limited understanding of microbial biodiversity results from methodological limitations and a basic lack of taxonomic information (Klopatek *et al.*, 1993). The Microbial Diversity 21 Action Statement, issued by the International Unions of Biologists and Microbiologists (Colwell and Hawksworth, 1991), directed research toward microorganisms of primary

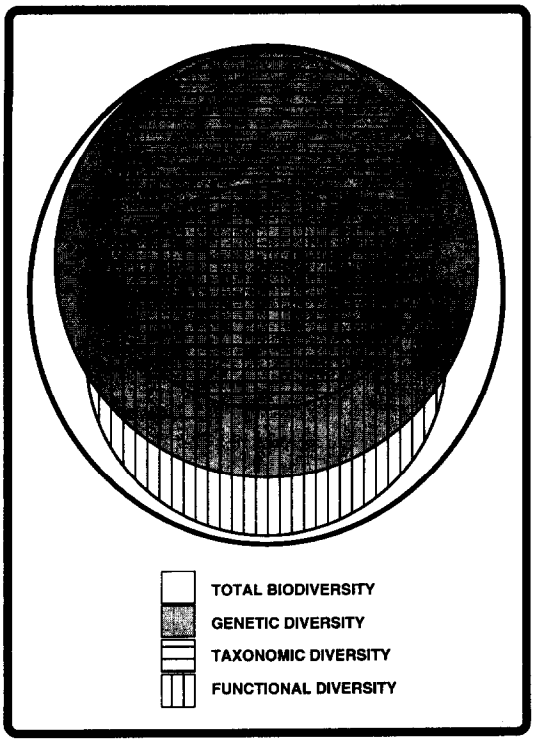

Fig. 1. A conceptual model relating genetic, taxonomic and functional diversity to total biodiversity. Biodiversity has a genetic foundation because diversity arises as a consequence of evolutionary differentiation (e.g. speciation). Functional diversity results as a consequence of genetic variability within a taxon, environmental effects on gene expression, and ecological interactions among taxa. A comprehensive understanding of biodiversity, especially as it applies to ecosystem properties, requires consideration of genetic, taxonomic and functional components.

importance to ecosystem functioning because taxonomic assessments on a large scale are clearly impractical. Moreover, a recent statement by the U.S. Soil Ecology Society (Executive Summary; May 1993) noted a general lack of information relating taxonomic diversity to aspects of ecosystem function (e.g. decomposition and nutrient cycling). For these reasons, functional rather than taxonomic diversity may provide greater insight to microbial roles in ecosystems.

Using the Biolog microplate identification system, Garland and Mills (1991) were able to obtain an assessment of functional differences in microbial communities from a variety of soil and water samples based on sole-source carbon utilization patterns of bacterial communities. With this approach, they were able to characterize differences among habitats and between samples within the same habitat based on patterns of substrate usage. Furthermore, they showed that separation of samples along axes of principle components was related directly to differences in the use of a particular carbon source.

Our objective is to expand upon the procedure of Garland and Mills (1991) by integrating the procedure into current discussions of biodiversity and by illustrating the wealth of information that can be obtained on functional diversity of bacteria by using the Biolog system. Unlike alternative approaches, the Biolog method is easy to use and produces data that are rich in information about functional biodiversity of bacteria. Moreover, these data are particularly amenable to multivariate analyses and other statistical procedures that are commonly used to examine taxonomic diversity in macro-organisms.

In the context of our approach, microbial functional diversity is defined operationally as the numbers, types, activities, and rates at which a suite of substrates are utilized by the bacterial community. Although the exact numbers and taxonomic identities of the bacterial species responsible for the Biolog reactions remain uncertain, patterns of functional diversity within and among communities provide insight to soil microbial communities where little understanding currently exists.

MATERIALS AND METHODS

Sample collection

The sampling approach that is employed when examining aspects of microbial biodiversity is determined by the scale and focus of the question. For example, meso-scale investigations that address differences in functional biodiversity among communities within a landscape require a different sampling strategy (see Ludwig and Reynolds, 1988) than do questions that examine the partitioning of biodiversity within a community. Because we were interested in patterns of functional biodiversity among bacterial communities, soil samples were bulked to obtain a composite assessment of that plant community. In particular, soil samples were collected during early December 1993 from six plant communities (black grama grassland, *Sporobolus* grassland, creosotebush bajada, herbaceous bajada, mesquite-playa fringe and playa grassland) along the Jornada Long-Term Ecological Research (LTER) site control transect (see Van Cleve and Martin, 1991) in the northern Chihuahuan Desert near Las Cruces, New Mexico (Lat. 32°31′17″ Long. 106°47′50″). In each of the six plant communities, 10 soil samples were collected to a depth of 15 cm using a soil corer (2 cm dia); five samples were obtained from under plants and five from between plants.

Sample preparation

Standard dilutions were performed on each bulked sample with 100 μl aliquots of the final dilution (10^{-4}) added to each of the 96 wells on a Biolog microplate (Biolog Inc., 3938 Trust Way, Hayward, CA 94545, U.S.A., 800-284-4949). To ensure homogenous dispersion of the soil particles in the initial dilution, we recommend a 1 min blending at high speed with the soil material dispersed in 0.2% water

agar. Although the Biolog instructions indicate that 150 μl should be added to each well, we had found that with this amount of inoculant, the sensor of the manual plate reader would touch the liquid surface in a well. Because a series of readings over time is desirable, a lesser amount of inoculum was used to avoid exchange of material between wells during operation of the plate reader. With an automated plate reader, 150 μl of inoculum can be added without danger of cross contamination.

The presence of soluble organic material or colloidal particles in the final dilution can interfere with measurements of microbial activity. As a consequence, final dilutions should be chosen to minimize background color in the inoculant. Also, the dilution that is chosen for inoculating the wells must be constant for all sites within a study to eliminate confounding effects related to differences in initial microbial densities (Garland and Mills, 1991).

Microbial activities

The Biolog system detects the utilization of specific carbon sources by bacteria from a set of 95 different carbon compounds on each of either Gram (−) or Gram (+) microplates (Biolog, 1993). The microplates are designed to provide standardized biochemical tests for identifying a broad range of either Gram (+) or Gram (−) bacteria. Differences in the particular suite of carbon sources on the microplates relate to characteristics useful in distinguishing among species. Nonetheless, the Biolog microplates are not selective for either Gram (−) or Gram (+) taxa, and by combining the two types of microplates in the analysis, a large number (128) of carbon compounds (Table 1) can be used to evaluate the functional diversity of microbial communities.

The degree to which a particular substrate is utilized can be quantified by measuring the intensity of color change caused by incorporation of tetra-

Table 1. Carbon substrates included in the Gram (−) and Gram (+) Biolog microplates categorized by substrate guilds

Carbohydrates	*Carboxylic acids*	*Amino acids*
adonitol	acetic acid	D,L-carnitine
α-D-galactoside	α-hydroxybutyric acid	D-alanine
α-D-glucose	α-ketobutyric acid	D-serine
α-D-lactose	α-ketoglutaric acid	γ-aminobutyric acid
α-methyl-D-glucoside	α-ketovaleric acid	glycyl-L-aspartic acid
α-methyl-D-mannoside	β-hydroxybutyric acid	glycyl-L-glutamic acid
arbutin	*cis*-aconitic acid	hydroxy-L-proline
β-methyl-D-galactoside	citric acid	L-alanine
β-methyl-D-glucoside	D,L-lactic acid	L-alanyl-glycine
cellobiose	D-galactonic acid lactone	L-asparagine
D-arabitol	D-galacturonic acid	L-aspartic acid
D-fructose	D-gluconic acid	L-glutamic acid
D-galactose	D-glucosaminic acid	L-histidine
D-mannitol	D-glucuronic acid	L-leucine
D-mannose	D-malic acid	L-ornithine
D-melezitose	D-saccharic acid	L-phenylalanine
D-meliobiose	formic acid	L-proline
D-raffinose	γ-hydroxy-butyric acid	L-pyroglutamic acid
D-ribose	itaconic acid	L-serine
D-psicose	L-lactic acid	L-threonine
D-sorbitol	L-malic acid	
D-tagatose	malonic acid	*Miscellaneous*
D-trehalose	*N*-acetyl-L-glutamic acid	2-deoxyadenosine
D-xylose	*p*-hydroxy-phenylacetic acid	2,3-butanediol
gentiobiose	propionic acid	adenosine
i-erythritol	pyruvic acid	adenosine-5-monophosphate
L-arabinose	quinic acid	amygdalin
L-fucose	sebacic acid	bromosuccinic acid
L-rhamnose	succinic acid	D,L-α-glycerolphosphate
lactulose		D-lactic acid methyl ester
m-inositol	*Polymers*	fructose-6-phosphate
maltose	α-cyclodextrin	glucose-1-phosphate
maltotriose	β-cyclodextrin	glucose-6-phosphate
mannan	dextrin	glycerol
3-methyl glucose	glycogen	inosine
methyl pyruvate	inulin	salicin
mono-methyl succinate	tween 40	thymidine
N-acetyl-D-galactosamine	tween 80	thymidine-5-monophosphate
N-acetyl-D-glucosamine		uridine
N-acetyl-D-mannosamine	*Amines/amides*	uridine-5-monophosphate
palatinose	2-amino-ethanol	urocanic acid
sedoheptulosan	alaninamide	
stachyose	glucuronamide	
sucrose	lactamide	
turanose	phenyl-ethylamine	
xylitol	putrescine	
	succinamic acid	

zolium dye into a respiring bacterial community. Thus, each Biolog microplate yields a specific pattern of activities representing the functional attributes of the inoculated bacterial community with respect to a suite of substrates (Bochner, 1989). The number and categories of utilized substrates, as well as activities, constitute a data set from which we assessed functional diversity. Color development in each well reflects species activity and density, as well as the ability of the bacterial community to respond to particular substrates. Inoculated microplates from the Jornada were incubated at 25°C and examined every 12 h for 72 h. Although the peak absorbance of the tetrazolium dye occurs at 590 nm, we used absorbance at 405 nm because our plate reader was equipped with only that filter. In that our measures of activity are consequently conservative, we can be confident of site differences detected by our technique.

Although the duration of the observation period for assessing the Biolog microplates may differ among studies, the appropriate observation period is determined by the rate at which color develops, the time at which most of the substrates are used, a color change in the water well (control), or by fungi growing in a well from the original inoculum. For the Jornada study, color development occurred in several control wells at 72 h, apparently from bacteria using dissolved organic material contained in the inoculum. Furthermore, fungal growth was apparent in several wells at this time.

RESULTS

Functional biodiversity

Functional diversity can be examined from a variety of perspectives, paralleling concepts analogous to those of taxonomic diversity. The simplest aspect is substrate richness, the number of different substrates that are used by the microbial community. This index also has a temporal dimension that develops during incubation (Fig. 2). Substrate diversity (H), which encompasses both substrate richness and substrate evenness, may be quantified according to information theory (Magurran, 1988):

$$H = -\Sigma\, p_i (\ln p_i)$$

where p_i is the ratio of the activity on a particular substrate to the sum of activities on all substrates. Similarly, substrate evenness (E) measures the equitability of activities across all utilized substrates and is given by:

$$E = H/H_{max} = H/\log S$$

where H is substrate diversity and S is substrate richness. Analyses of functional richness, diversity, or evenness are univariate in nature (Sokal and Rohlf, 1981) and address if the functional aspects of biodiversity differ among sites or among habitats within a site.

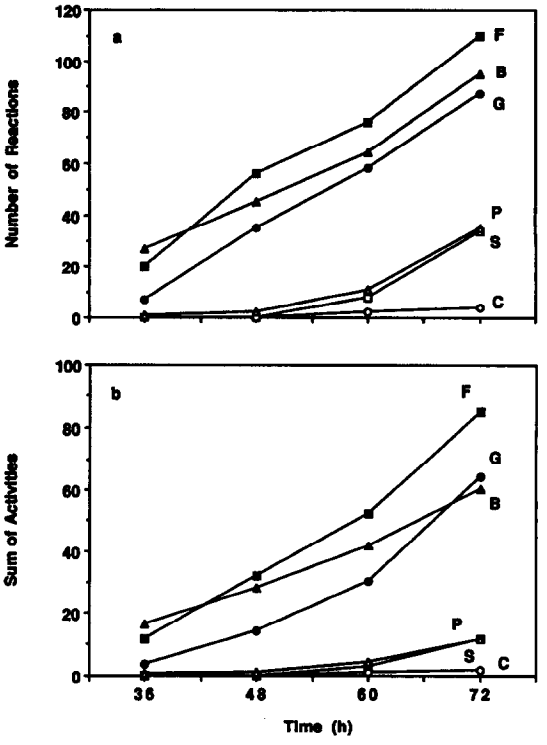

Fig. 2. Substrate utilization patterns for six communities along the Jornada Long-Term Ecological Research transect, northern Chihuahuan Desert, U.S.A. using number of utilized substrates for each community (a) and summation of absorbance values (activity levels) for each community (b). The abbreviations correspond to plant communities: F = mesquite-playa fringe (■), B = herbaceous bajada (▲), G = black grama grassland (●), C = creosotebush bajada (○), P = playa grassland (△), S = *Sporobolus* grassland (□).

Differences in the number of utilized substrates among the six communities demonstrate a spatial dimension of functional diversity at the Jornada (Fig. 2). Both functional richness and total activity were higher for the mesquite-playa fringe, herbaceous bajada and black grama grassland than for the playa grassland, *Sporobolus* grassland and creosotebush bajada. Substrate diversity also was higher for the mesquite-playa fringe, the herbaceous bajada and the black grama grassland (Fig. 3). Evenness remained uniformly high throughout the incubation for the black grama grassland, mesquite-playa fringe and herbaceous bajada; was similarly high for the *Sporobolus* grassland and creosotebush bajada (when sufficient activity was present to evaluate evenness); and generally was lower at the playa grassland location (Fig. 3).

Compositional similarity

Measures of substrate richness, evenness and diversity do not provide information about the types of substrates that are utilized by the bacterial community. Two sites could exhibit identical substrate richness, evenness or diversity but still catabolize totally

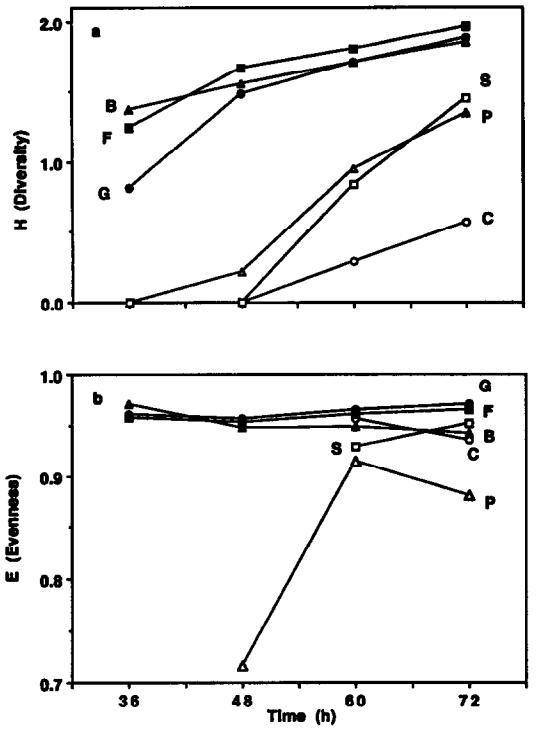

Fig. 3. Patterns of diversity (a) and evenness (b) of substrate utilization for six communities along the Jornada Long-Term Ecological Research transect, northern Chihuahuan Desert, U.S.A. Site codes are the same as in Fig. 2.

different substrates. Alternative approaches incorporate information about the types of substrates that are used as either the presence or absence of specific catabolic abilities or the activity levels exhibited for particular substrates.

Analyses (SPSS, 1990) based upon the presence or absence of substrate-specific catabolic activity requires indices of similarity (e.g. simple matching coefficient or Jaccard's index) to be calculated between all possible pairs of sites (see Willig and Mares, 1989; Hall and Willig, 1994). The resultant similarity matrix is transformed to a dissimilarity matrix, and may be analyzed by a variety of multivariate techniques (e.g. factor analysis, multidimensional scaling, cluster analysis) to assess the relationship among sites in terms of catabolic potential. In an analogous fashion, levels of activity for each substrate can be incorporated directly into multivariate analyses.

Cluster analysis, based on the presence or absence of utilized substrates, revealed consistent relationship among the six communities regardless of the duration of incubation. The creosotebush bajada, *Sporobolus* grassland and playa grassland were indistinguishable from each other. On the other hand, the black grama grassland, mesquite-playa fringe and herbaceous bajada were dissimilar from each other, as well as from the other three communities (Fig. 4). Ordination (principle components analysis) of the six communities based on activity levels also demonstrated a consistent relationship among communities throughout the incubation, even though the numbers of catabolized substrates and levels of activity in the six communities changed substantially during incubation (Fig. 2). The principle components analysis produced a pattern of inter-site relationships similar to that of cluster analysis (Fig. 4). We present the results of cluster analysis and principle components after 60 h incubation because this was the time when maximal substrate richness and the highest activity levels were observed before contamination of Biolog plates. A detailed discussion of the relationships between soil characteristics, vegetation types and microbial functional biodiversity will be presented in a subsequent paper.

Guild structure

Each substrate on the Biolog plate can be characterized according to its chemical nature, thus providing another scheme upon which to base analyses. Garland and Mills (1991) originally assigned the Biolog substrates from Gram (−) microplates into 11 categories; we have condensed their classification into 6 guilds: (1) carbohydrates, mainly simple sugars, (2) carboxylic acids, (3) amino acids, (4) amines and amides, (5) polymers, and (6) miscellaneous, including phosphorylated and aromatic compounds (Table 1). Each of our guilds contains no fewer than five substrates and quantitative measures

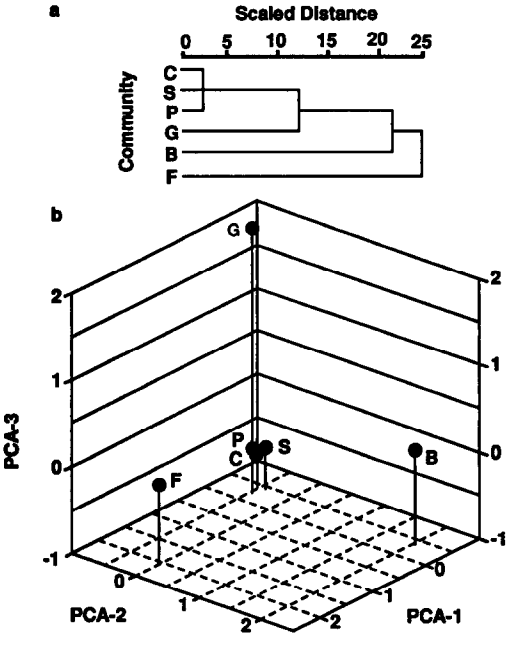

Fig. 4. Compositional relationship among soils collected from the Jornada Long-Term Ecological Research transect, northern Chihuahuan Desert, U.S.A., based on overall substrate utilization (60 h incubation) using (a) cluster analysis (presence or absence of activity) and (b) principle components analysis (levels of activity). Site codes are the same as in Fig. 2.

of functional biodiversity can be calculated for particular guilds in the same manner as for all substrates.

As with total substrate richness and activity, the number of reactions and level of activities within each guild can be evaluated. Using the herbaceous bajada as an example, guild-specific patterns differed in the number of reactions and the total activity over time (Fig. 5). Catabolism of carbohydrate and carboxylic acid substrates was responsible for much of the overall activity pattern observed at all sites, probably because 61% of the substrates on Biolog plates fall within these guilds.

With the exception of the polymer guild, principle components and cluster analyses indicated that the positional relationship among community types was similar to that obtained from analyses based on overall substrate utilization (Fig. 4). Cluster analysis of the polymer guild indicated that the black grama grassland and mesquite-playa fringe communities were closely aligned and dissimilar from the other communities; the herbaceous bajada was most closely related to the *Sporobolus* grassland; and the creosotebush bajada was associated with the playa grassland (Fig. 6). In the principle components analysis of the polymer guild, two axes accounted for most of the variation in activity levels among the sites. Four sites were closely related (creosotebush bajada, herbaceous bajada, playa grassland and *Sporobolus* grassland), with the black grama grassland and mesquite-playa fringe sites each being dissimilar from all other sites (Fig. 6).

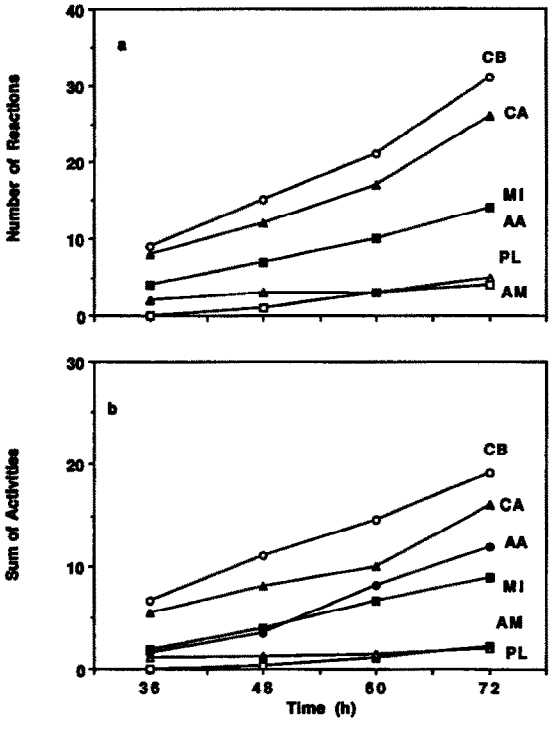

Fig. 5. Substrate utilization patterns for a herbaceous bajada community, Jornada Long-Term Ecological Research transect, northern Chihuahuan Desert, U.S.A., based on number of utilized substrates within each guild (a) and summation of absorbance values (activity levels) for each guild (b). Abbreviations correspond to guilds: CB = carbohydrates (○), CA = carboxylic acids (▲), MI = miscellaneous (■), AA = amino acids (●), PL = polymers (△), AM = amines and amides (□). Note, in panel a, the amino acid results are identical to the results for the miscellaneous guild.

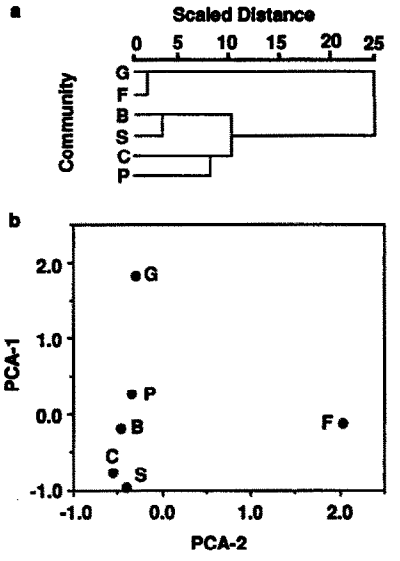

Fig. 6. Compositional relationship among soils collected from the Jornada Long-Term Ecological Research transect, northern Chihuahuan Desert, U.S.A., based on polymer guild substrate utilization (60 h incubation) using (a) cluster analysis (presence or absence of activity) and (b) principle components analysis (levels of activity). Site codes are the same as in Fig. 2.

DISCUSSION

Even though issues concerning biodiversity and its conservation should apply to all taxa, quantifying and understanding patterns of biodiversity for microorganisms are limited by methodological and taxonomic difficulties. Estimates of microbial diversity at the taxonomic level are labor-intensive, consume large amounts of time, or use expensive equipment. Consequently, such studies are clearly impractical for either extensive inter-site comparisons or for detailed intra-site analyses. To avoid these shortcomings and to broaden our understanding of microbial diversity, we have illustrated a rapid and simple approach to assess initially, functional biodiversity of soil microorganisms using the Biolog system. Unlike traditional culture-based procedures, which generally are selective in the component of the microbial community that is isolated, the Biolog method integrates the activities of a broad range of bacteria into assessments of functional diversity.

Some might argue that the Biolog approach is as selective as traditional isolation procedures because it does not ascertain the proportion of the total bacterial community responsible for the carbon

utilization patterns. Furthermore, recent DNA analyses of soil indicate that the total bacterial community in soils can be considerably greater than that obtained from traditional culture-based methods (Torsvik *et al.*, 1990). The reasons for the discrepancy between culture-based and direct estimates have focused on the physiology of bacteria relative to growth conditions provided by the culture medium (e.g. Xu *et al.*, 1982). Future research using standard isolation methods, as well as DNA or 16 s rRNA analyses, might be useful for comparing the bacteria that develop in each Biolog well to the community that is present in the sample. Like any culture-based procedure, the Biolog method is selective, it only detects activities from bacteria capable of growing on the Biolog medium. Nonetheless, no other taxonomic or functional biases are known to influence observed bacterial activities. Differences in functional characteristics of the bacterial communities among sites consequently represent minimum estimates.

The Jornada study illustrates the wealth of information and insight that can be obtained by using the Biolog method over time. Additionally, patterns of overall substrate use can be viewed separately from the utilization of particular types of substrates (guilds), thus providing information at different levels of functional resolution. Guild-specific analyses facilitate detection of differences among sites that could be obscured in overall analyses of substrate use (compare Figs 4 and 6). Furthermore, guild-specific differences among sites may be related to the ability of the microbial community to degrade specific categories of organic matter that reflect either differences in current vegetation types (e.g. herbaceous vs shrub) or history of exposure to categories of carbon compounds in evolutionary or ecological time.

Guild-specific analyses are especially important to the interpretation of the results of this methodology because the number of substrates per guild differs (e.g. 7 polymers vs 46 carbohydrates). Substrate-rich guilds may make a disproportionate contribution to composite views of functional diversity derived from some types of analyses, although alternative analytical approaches could be used to control for these biases. For example, a weighted multidimensional scaling approach would be appropriate because differences among sites can be determined separately for each guild, and each guild then could be assigned equal weight in composite analyses. Also, the degree to which patterns of activity in different guilds converge or diverge could be quantified by examining weighting space (see SPSS, 1990).

We realize that specific functional abilities of microbial communities in natural systems may be contributed by the presence of rare species (i.e. those with either low densities or those that are spatially hyperdispersed). Depending upon either the dilution or the number of samples, their contribution to functional diversity could be underestimated. The occurrence of 62 duplicate substrates from the Gram (+) and Gram (−) plates permit a preliminary assessment of activities contributed by rare taxa. The number of duplicate substrates for which only one of two wells is positive provides an estimate of activities of rare taxa in a given microbial community.

We acknowledge that the Biolog system has limitations, including sensitivity to inoculum densities, a selection of carbon sources biased toward simple carbohydrates, and the inability to determine fungal activity. Furthermore, the potential implication of consortia on the development of the Biolog responses is unknown at this time. Nonetheless, this methodology produces a rich data set (numerous substrates that are hierarchically arranged in guilds and can be monitored at different times during incubation) that is ideal for detecting site-specific differences in the functional diversity of microorganisms. If progress is to be made in understanding patterns of microbial diversity, we need to broaden our understanding of biodiversity and focus initially on approaches that have a reasonable chance of deducing patterns that are biologically relevant. A multifaceted approach that includes both functional and taxonomic perspectives represents fertile ground for future research.

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