

Spatial and Resource Factors Influencing High Microbial Diversity in Soil

Jizhong Zhou,^{1,2*} Beicheng Xia,^{2†} David S. Treves,² L.-Y. Wu,^{1,2} Terry L. Marsh,²
Robert V. O'Neill,¹ Anthony V. Palumbo,¹ and James M. Tiedje²

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831,¹ and Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824²

Received 11 June 2001/Accepted 9 October 2001

To begin defining the key determinants that drive microbial community structure in soil, we examined 29 soil samples from four geographically distinct locations taken from the surface, vadose zone, and saturated subsurface using a small-subunit rRNA-based cloning approach. While microbial communities in low-carbon, saturated, subsurface soils showed dominance, microbial communities in low-carbon surface soils showed remarkably uniform distributions, and all species were equally abundant. Two diversity indices, the reciprocal of Simpson's index ($1/D$) and the log series index, effectively distinguished between the dominant and uniform diversity patterns. For example, the uniform profiles characteristic of the surface communities had diversity index values that were 2 to 3 orders of magnitude greater than those for the high-dominance, saturated, subsurface communities. In a site richer in organic carbon, microbial communities consistently exhibited the uniform distribution pattern regardless of soil water content and depth. The uniform distribution implies that competition does not shape the structure of these microbial communities. Theoretical studies based on mathematical modeling suggested that spatial isolation could limit competition in surface soils, thereby supporting the high diversity and a uniform community structure. Carbon resource heterogeneity may explain the uniform diversity patterns observed in the high-carbon samples even in the saturated zone. Very high levels of chromium contamination (e.g., >20%) in the high-organic-matter soils did not greatly reduce the diversity. Understanding mechanisms that may control community structure, such as spatial isolation, has important implications for preservation of biodiversity, management of microbial communities for bioremediation, biocontrol of root diseases, and improved soil fertility.

Both theoretical and empirical studies suggest that in plant and animal communities competitive interaction is the key determinant of species abundance and diversity (11). Competition also appears to drive the structure of aqueous laboratory-maintained microbial communities (24), but little is known about the driving forces that determine the structure of more complex natural microbial communities, especially those in soil. Application of nucleic acid-based methods to soil microbial communities has revealed high prokaryote diversity (2–4, 6, 9, 13, 15, 20, 29–31, 38), but the studies have not yet provided a common mechanism that explains the maintenance of diversity in this environment.

To identify possible determinants that control microbial community structure in soil, we characterized microbial communities from 29 soil samples using a small-subunit (SSU) rRNA-based cloning approach. This allowed us to evaluate more than 9,000 restriction fragment length polymorphism (RFLP) profiles and to examine whether diversity profiles were correlated with particular environmental conditions. Our findings show that surface soils from low-carbon sites exhibited a uniform diversity pattern quite distinct from the pattern of saturated subsurface soils, which exhibited the more common

competitive diversity pattern found in most biological communities. In contrast, microbial communities from high-carbon sites displayed the uniform diversity pattern regardless of depth or the water content of the sample. We hypothesized that spatial isolation can explain the community structure differences at the low-carbon sites but that high soil carbon contents can overcome such differences, as we observed at the high-carbon site.

MATERIALS AND METHODS

Site characteristics. The Abbott's Pit site is located near the Atlantic coast on the southern Delmarva Peninsula (37°50.198'N, 75°32.135'W). The soil is medium and fine sand derived from beach and upper shore surface sediments and is classified as a thermic Hapludult. The vegetation is primarily low grasses and sparse shrubs. The water table was 3.75 m below the surface at the time of sampling but may fluctuate annually by up to 1 m. The numbers of CFU, which were determined as described previously (1), were 3.8×10^6 CFU g^{-1} in the surface soil, 1.4×10^4 CFU in the vadose (unsaturated subsurface) soil, and 6.4×10^3 CFU g^{-1} in the saturated subsurface soil. The soil color indicated that oxidizing conditions were present.

The Delaware site is on Dover Air Force Base in an area where the vegetation is grass and cultivated shrubs (near well IR-07D; 39°7.374'N, 75°29.047'W). This site was contaminated with low levels of chloroethenes (17). The soil is primarily coarse sands with some lenses enriched in clay and is classified as a mesic Hapludult. The zones analyzed were all sands. The water table at the time of sampling was 5.5 m below the surface. The number of CFU, was 1.1×10^7 CFU g^{-1} in the surface soil, but the concentration dropped sharply with depth and was $<2.0 \times 10^2$ CFU g^{-1} in samples obtained at depths of more than 6 m. The oxygen contents of water samples obtained at the Dover site are typically 2 to 3 mg/liter (7).

The Kellogg Biological Station surface samples were taken from noncultivated poplar plots in the long-term ecological research study site in southwest Michigan. The soil at this site is fine to coarse loamy sand and is classified as mesic Typic Hapludalf.

* Corresponding author. Mailing address: Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6038. Phone: (865) 576-7544. Fax: (865) 576-8646. E-mail: zhouj@ornl.gov.

† Present address: Institute of Environmental Science, Zhongshan University, Guangzhou, China, 510275.

The Cannelton site is a wetland in northern Michigan on the shore of the St. Mary's River. The soil is sandy and is classified as a frigid Entic Hapaquod. A tannery operated nearby from 1900 to 1958 and contaminated the area with high levels of chromium. The water table was 1.8, 1.1, and 1.4 m below the surface for the J, H, and O sample series, respectively. On the basis of color samples H17-2 and -3 showed some evidence of reducing conditions. The mean viable plate counts on R2A medium for Cannelton sites J, H, and O were 3.5×10^7 and 1.4×10^6 CFU g^{-1} for the surface and saturated subsurface samples, respectively. Chromium concentrations in the Cannelton soils were determined with an inductively coupled plasma-mass spectrometer. Briefly, soils were extracted with nitric acid in pressurized vessels heated in a microwave (10). The extracted liquid was diluted, and metal concentrations were determined by inductively coupled plasma-mass spectrometry with a hexapole collision cell at the ICP-HEX-MS Laboratory at Michigan State University. Calibration standards for analyses were prepared by using distilled, deionized water and stock standards (J. T. Baker Analyzed). Standards and sample dilutions were prepared under identical solution conditions. All chemicals and reagents used were analytical metal grade or better.

Soil coring was done by using steam-cleaned Lexan core liners and a sonic drilling technique at the Virginia and Delaware sites. At the Cannelton site coring was done with a split-spoon sampler driven by hand, and greater depths were reached by hand auguring before sampling. Kellogg Biological Station samples were collected from surface soils only with a standard soil corer. Soil from the center of each core was removed with a sterile spatula, collected in sterile Whirl-pak bags, and either immediately frozen (Abbott's Pit and Dover samples) or dispensed into sterile plastic bottles, transported on ice, and then frozen within 12 to 48 h (Cannelton and Kellogg Biological Station samples).

DNA extraction. DNA was extracted from 5 or 10 g of soil by grinding, freeze-thawing, and using sodium dodecyl sulfate for cell lysis (37). This method effectively lyses various types of bacteria, such as gram-positive bacteria and methanogens. The crude DNA was purified by the minicolumn purification method (37), except that the DNA was eluted twice from the resin column with 50 μ l of hot (80°C) water.

SSU rRNA gene amplification and cloning. The oligonucleotides used to amplify bacterial SSU rRNA genes were the fD1-rP1 primer set described by Weisburg et al. (35) with modifications to the linker sequences (39). All stock preparations used for PCR amplification were made and all procedures were performed with the precautions suggested by Kwok and Higuchi (14). One microliter of purified DNA (1 to 20 ng) was used as the template in a 20- μ l reaction mixture. The conditions used to amplify SSU rRNA genes were those described previously (39).

The concentrations of PCR-amplified SSU rRNA gene products were estimated by comparing the band intensities on agarose gels to the band intensities for known concentrations of standard lambda DNA. The amplified PCR products were directly ligated to the PCR II vector obtained from Invitrogen (San Diego, Calif.). Ligation and transformation were carried out according to the manufacturer's instructions. The ratios of inserts to vectors were 0.5:1 to 1:1. Two microliters of a ligation reaction mixture was transformed by the heat pulse method into 50 μ l of *Escherichia coli* Top 10F⁺ competent cells (Invitrogen). The transformed cells were then incubated in 450 μ l of SOC medium at 37°C for 30 min, after which the entire SOC-cell mixture was plated onto Luria-Bertani agar plates with ampicillin.

SSU rDNA RFLP analysis. All white colonies were picked and screened for SSU ribosomal DNA (rDNA) inserts, which were amplified (20 μ l) with the primers specific for the polylinker of vector pCR II (38). One-third of the remaining 18 μ l of PCR amplified products was digested with 0.1 U of *Msp*I plus 0.1 U of *Rsa*I or with 0.1 U of *Hha*I plus 0.1 U of *Hae*III (Gibco BRL Life Technologies, Gaithersburg, Md.) overnight at 37°C. The resulting RFLP products were separated by gel electrophoresis in 3.5% Metaphor agarose (FMC Bioproducts, Rockland, Maine) in 1 \times Tris-borate-EDTA by using 7 V/cm for 4 h. The gel was stained with 0.5 μ g of ethidium bromide per ml and visualized by UV excitation. RFLP patterns were compared by using the Molecular Analyst program (Bio-Rad, Hercules, Calif.). Clones with unique patterns were considered operational taxonomic units (OTUs), and their dominance patterns were evaluated as abundance profiles.

Diversity indices. The reciprocal of Simpson's index ($1/D$) and the log series index were chosen to characterize the microbial communities in our soil samples because they have good to moderate discriminating ability and are used widely in ecological studies (18). The use of $1/D$ instead of the original formulation of Simpson's index ensures that an increase in the reciprocal index reflects an increase in diversity (18).

RESULTS AND DISCUSSION

We examined OTU diversity patterns for total of 29 soil samples taken from four geographically distinct sites at three soil depths: the surface, the vadose zone, and the saturated subsurface (Table 1). Only surface samples were available from the Kellogg Biological Station. Sample Ab-5 was taken from a capillary fringe zone that is saturated part of the year, and hence the conditions that affect community structure should be most similar to those in the saturated zone. The Abbott's Pit and Delaware sites contained low concentrations of total organic carbon (0.27 to 4.75 mg g^{-1}), whereas the Cannelton site had much higher total organic carbon concentrations (30 to 750 mg g^{-1}) (Table 1). The total organic carbon levels of the Kellogg Biological Station soil (12.8 mg g^{-1}) are on the low side of the range for most temperate region surface soils.

Microbial community structure at low-carbon sites. Overall, the structure of the soil communities from the low-carbon surface soils was quite different from that of the low-carbon saturated subsurface communities (Fig. 1A and C). Whereas the saturated subsurface soil communities showed low OTU diversity and greater dominance by fewer OTUs, the surface soil communities showed high OTU diversity and a complete lack of dominance. Visual inspection of the vadose zone soil communities suggested that they are more variable across sites, but overall the vadose zone samples had profiles intermediate between the profiles of the surface and saturated subsurface samples (Fig. 1B). In addition, the shape of the community structure curve is the same for all saturated sandy samples regardless of the trichloroethylene concentration (which ranged from 0 to 3 ppm), and hence the microbial community structure appeared to be not affected.

To quantitatively measure diversity in the samples, we used the inverse of Simpson's index ($1/D$), which is sensitive to the level of dominance in a community (18). The reciprocal of Simpson's index (Fig. 1) effectively distinguished the low-carbon surface communities from the low-carbon saturated subsurface communities. For example, the uniform profiles characteristic of the surface communities had values that were 2 to 3 orders of magnitude greater than the values for the high-dominance, saturated subsurface communities (Fig. 1A and C).

To provide a second estimate of community diversity, we applied the log series index (α) to our samples. This index is most sensitive to species richness (18) but has been recommended as a reasonable diversity measure even when species abundance does not follow the log series distribution (28). Visual inspection of the profiles suggested that samples with $1/D$ index values below about 50 and α values below 100 show typical dominance profiles (Fig. 2A). One low-carbon vadose zone sample (DB-1) showed a dominance profile, while the other two vadose zone samples did not, although their index values were lower than those of the surface soils (Fig. 2A).

The structure of the vadose zone communities in the low-carbon soil is difficult to interpret since one of the three samples showed a dominance pattern while the other two did not. Several unsuccessful attempts were made to obtain larger clone libraries from the Abbott's Pit vadose zone samples, and this limited our ability to clearly resolve the community struc-

TABLE 1. Site characteristics and OTUs for 29 soil samples from four geographically distinct sites

Site	Sample ^a	Depth (m)	Zone	Total organic C concn (mg g ⁻¹)	Total no. of OTUs	No. of unique OTUs
Abbott's Pit, Virginia	Ab-2	0.05	Surface	4.08	695	665
	Ab-4	1.57	Vadose	1.83	52	40
	Ab-5	3.25	Capillary fringe		81	36
	Ab-10	4.04	Saturated	0.27	252	85
Dover Air Force Base, Delaware	DH-0	0.07	Surface	4.75	906	891
	DH-1	1.8	Vadose	0.33	196	172
	DH-3	8.0 ^b	Saturated	0.58	178	65
	D1-1	0.05	Surface	4.51	396	355
	D1-4	6.0 ^c	Saturated	0.84	220	89
	D1-8	7.5 ^c	Saturated	0.51	218	65
	DB-0	0.16	Surface	4.42	920	886
	DB-1	1.8	Vadose	1.17	176	44
	DB-4	8.0 ^b	Saturated	0.51	223	42
	DB-5	8.1 ^b	Saturated	0.51	161	74
Kellogg Biological Station, Michigan	W-1	0.04	Surface	12.8 ^b	599	551
	W-2	0.15	Surface	12.8 ^b	697	685
	WF-1	0.04	Surface	12.8 ^b	209	192
	WF-2	0.15	Surface	12.8 ^b	321	312
Cannelton, Michigan	H17-1	0.15 ^d	Surface	340	127	119
	H17-2	0.46 ^d	Vadose	300	165	155
	H17-3	1.1 ^e	Saturated	250	91	72
	J19-1	0.15 ^f	Surface	190	271	258
	J19-2	0.46 ^f	Vadose	420	272	224
	J19-3	1.1 ^f	Vadose	750	378	303
	O22-1	0.15 ^f	Surface	380	166	153
	O22-2	0.46 ^f	Vadose	300	218	210
	O22-3	1.1 ^e	Vadose	50	310	295
	O22-4	1.4 ^e	Saturated	30	181	179
	M24-1	0.15 ^g	Surface	38	400	392

^a Most samples were 5-g samples; the only exceptions were the subsurface samples from Abbott's Pit and Dover Air Force Base, which were 10-g samples.

^b Sample with intermediate level of chloroethenes (>2 mg/liter).

^c Sample with high level of chloroethenes (>3 mg/liter).

^d Sample with high chromium level (>200,000 mg/kg).

^e Sample with low chromium level (700 to 10,000 mg/kg).

^f Sample with intermediate chromium level (13,000 to 32,000 mg/kg).

^g Control sample with approximately 90 mg of chromium per kg.

^h Average value for the site.

ture of this zone. One explanation may be that the very low stable carbon content in this zone reduced survival, thereby reducing community richness, but more study of this zone is needed to resolve whether it has a characteristic community structure.

Microbial community structure at high-carbon sites. In contrast to the communities at the low-carbon sites, the microbial communities at the high-carbon sites showed a uniform diversity pattern, regardless of depth (Fig. 1D to F). Not surprisingly, the $1/D$ values for these samples were similar to the low-carbon surface sample values and did not decrease with increasing depth of the sample (Fig. 1). Also, a comparison of the reciprocal of Simpson's index versus α showed that no high-carbon samples approached index values reflecting dominance profiles (Fig. 2B).

Besides having high total organic carbon contents, the Cannelton samples differed markedly in their chromium(III) contents, which was the dominant form of chromium at this site. This provided an opportunity to examine the effects of this contaminant on microbial community structure and diversity. The two samples with low chromium levels (M24-1 and O22-4) showed greater diversity than the samples with higher chro-

mium levels based on the reciprocal of Simpson's index (Fig. 3). Beyond this, however, there was no clear relationship between microbial community diversity and chromium content. It is noteworthy that soils with very high chromium concentrations (20 to 30% Cr) and with severely stunted vegetation still had a reasonably high microbial diversity index.

PCR artifacts. Since PCR bias and errors are well-known issues associated with PCR-based rDNA methods (12, 16, 23, 26, 27, 32, 33, 36), it is important to consider to what extent they influenced the observations in this study. Most important in this evaluation was the fact that there were major differences in the diversity patterns for the surface and saturated subsurface samples analyzed under identical experimental conditions, including the same sufficient amount of template DNA (~5 ng). Hence, the difference in patterns should be attributable to differences in rDNA diversity in the templates. To further evaluate this possibility, we estimated the number of chimeras by selecting, at random, 30 to 40 clones from each of the Abbott's Pit and Dover libraries and sequenced both the 5' and 3' ends. We then constructed independent phylogenetic trees and compared the branching orders to identify discrepancies which were indicative of different sources for the 3' and 5'

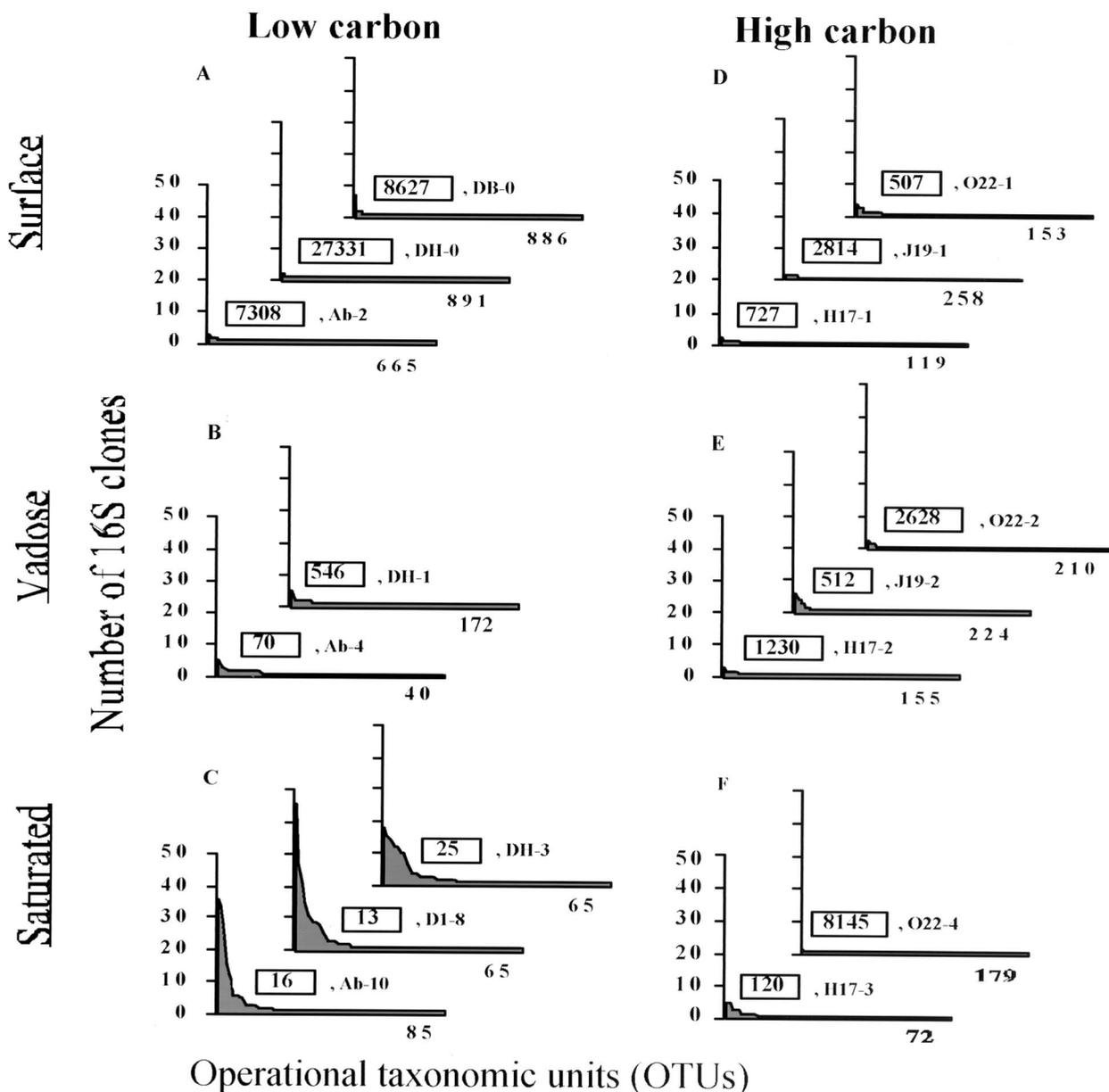


FIG. 1. Representative microbial community diversity patterns based on OTU abundance in surface, vadose, and saturated soils from low- and high-carbon sites. The values in boxes are community diversity values based on the reciprocal of Simpson's index.

sequences. The proportions of chimeric sequences were 5.2 and 7.4% for Abbott's Pit saturated subsurface and surface soil samples respectively, and 5.5 and 10.5% for Dover saturated subsurface and surface soil samples, respectively. These results suggest that the chimeric structure, at least for dissimilar genotypes, was not a severe problem in our results. Probably the second most significant potential artifact that could increase apparent diversity is the cloning of heteroduplexes. We found that the proportions of heteroduplexes were 3.6 and 4.5% for Abbott's Pit and Dover samples, respectively. Other potential errors that could artificially increase diversity, such as *rnn* heterogeneity (8) in the same organism and PCR fidelity, have been shown to occur less frequently (22, 23). In summary, while we believe that the libraries used contained some arti-

cial OTUs, we think that the extents of the errors should not exceed 10 to 20% and that they should be similar in libraries yielding the two types of profiles. Since the differences in the indices for the dominance and uniform profiles are much larger (i.e., 2 to 3 orders of magnitude), we believe that the distinction between the two patterns is real and reflects a difference in underlying community structure. Indeed, the median value for the reciprocal of Simpson's index for the low-carbon surface samples was 5,490, compared to a value of just 15 for the low-carbon saturated samples.

How different are different OTUs? We also used the 5' and 3' end sequence information to evaluate the differences between OTUs that varied by only one or two bands when they were cut with four tetrameric restriction enzymes. This should

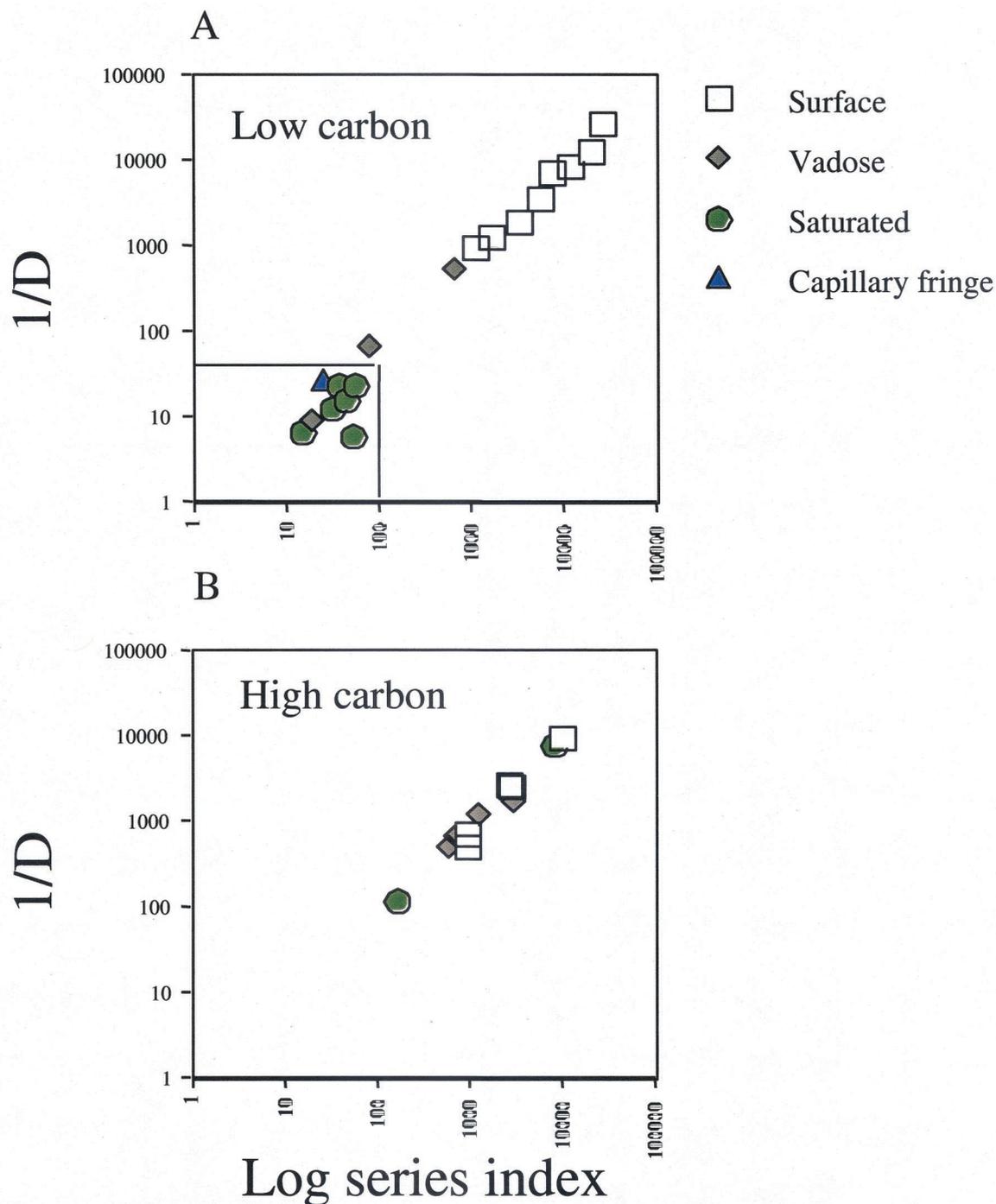


FIG. 2. Plots of the reciprocal of Simpson's index ($1/D$) versus the log series index for the microbial communities at different soil depths from low-carbon (A) and high-carbon (B) sites. The values in the box are index values that indicate dominance in community structure.

be the minimum level of phylotype difference detected by this method. OTUs that differed by one or two bands had SSU rRNA gene sequence similarities of $97.0\% \pm 2.4\%$ at the 5' end and $97.6\% \pm 2.1\%$ at the 3' end. Differences in SSU rDNA sequences of 2.5% or more correspond to $<70\%$ DNA-DNA hybridization (25), a key criterion indicating different bacterial species (34). This interpretation is supported by in silico digestion of SSU rDNA database sequences (20). Hence, in this study a unique OTU suggested a different species for

nonartificial clones. A more detailed phylogenetic analysis of clones from Abbott's Pit and Dover surface soils also supported this interpretation (J. Zhou, H. Huang, B. Xia, D. S. Treves, L. J. Hauser, R. J. Mural, A. V. Palumbo, and J. M. Tiedje, unpublished data).

Mechanisms producing the noncompetitive diversity pattern in low-carbon soils. We propose that the dominance structure seen in the low-carbon saturated subsurface samples is a common result of competitive interactions. Some species are

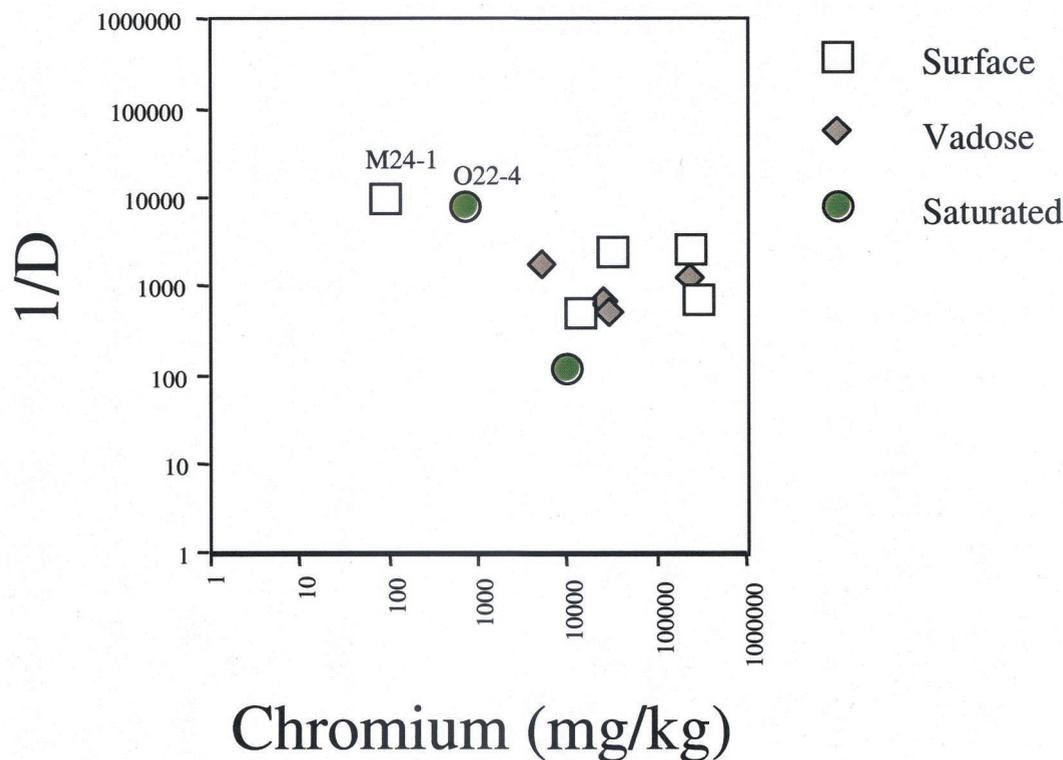


FIG. 3. Comparison of microbial community diversity (based on Simpson's index) and chromium levels at the high-carbon Cannelton site.

eliminated by competitive exclusion, and a few species, better adapted to the conditions, dominate. The lack of dominance observed in the surface soils is highly unusual in ecological communities. This extreme pattern implies that the microbial community must be experiencing an almost complete lack of competition. Many genotypes are maintained, and yet no genotype appears to be superior.

Since we hypothesize that the dominant diversity pattern observed in the low-carbon saturated subsurface soils is shaped by competitive interactions within the communities, we refer to such a diversity pattern as a competitive diversity pattern. This pattern has also been observed in the microbial communities in aquatic environments, such as sea vent biofilms (19), and is very common in plant and animal communities. In contrast, we refer to the uniform diversity pattern observed in the surface soils from the low-carbon sites and from all of the high-carbon sites as a noncompetitive diversity pattern. This pattern has also been observed in other surface soil samples, such as arctic tundra soil (38), Hawaiian soils (21), and Wisconsin agricultural soil (3).

The following four mechanisms may produce a noncompetitive diversity pattern: (i) superabundant resources (if more than enough resources are available, there is no competition and communities evolve toward high diversity), (ii) resource heterogeneity (if resources are available in many different forms, populations can avoid competition by specialization), (iii) spatial isolation (if the habitat is subdivided into many separate pockets of resources, populations can avoid competition by physical isolation), and (iv) nonequilibrium conditions (more populations can be maintained under fluctuating environmental conditions).

We examined the first three of these potential mechanisms with a simple model that includes the resource uptake by, transfer between, and loss by the microbes (M_i) adhering to a single soil particle (i) and the nutrient resource (R_i) available in the adjacent soil water (Fig. 4). A simple representation of each particle system is given by:

$$dM_i/dt = a_i M_i [R_i / (R_i + K)] - b_i M_i^2 \text{ and}$$

$$dR_i/dt = I - a_i M_i [R_i / (R_i + K)] + b_i M_i^2 - cR_i + dR_i - eR_i$$

where a_i and b_i are uptake and loss rates, respectively; K is the

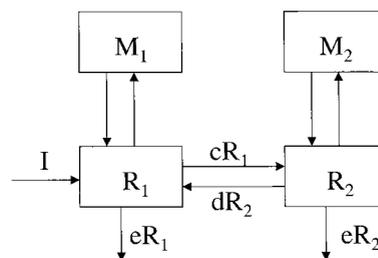


FIG. 4. Conceptual model of two soil particles showing the relationships between microbial biomass (M) and nutrient (R) exchanges. Although a small sample of soil would contain a large number of similar particle systems, this minimal representation suffices for our purposes. cR_1 and dR_2 , nutrient fluxes between soil particles through water film, which also permits migration of microorganisms between particles (albeit slow); I , nutrient input from rainfall; eR_1 and eR_2 nutrient fluxes that become unavailable for microbial uptake, either because of gravitational loss or because of immobilization in compounds that the organisms cannot metabolize.

Michaelis-Menton constant for resource uptake; I is the nutrient input from rainfall; cR_i and dR_i are nutrient fluxes between soil particles through water film; and eR_i is nutrient fluxes that become unavailable for microbial uptake.

Our model indicates that the superabundance mechanism does not explain why the sizes of all of the populations in the surface samples were remarkably similar. Superabundance corresponds to a situation in which R_i is much larger than the limiting concentration, K . Thus, $R_i \gg K$, and the ratio $R_i/(R_i + K)$ approaches 1.0. At equilibrium, $M_i = a_i/b_i$, each population grows to its own potential level, resulting in a variety of population densities. This differs from observations of uniform low population density and is consistent with the simple observation that nutrient resources are not superabundant in the soil environment.

A major difference between the surface and the saturated subsurface is water content, suggesting that free water that interconnects soil particles may be a key factor in determining diversity patterns. Genotypes with higher growth rates could produce larger populations since the limiting resource remains connected through diffusion. The bacteria reduce resources (R_i) by utilization, but diffusion from neighboring sites replaces this loss and bacteria (M_i) continue to grow. The populations that extract nutrients most efficiently dominate. With a continuous water film, to achieve the noncompetitive pattern, all uptake and loss coefficients (i.e., a_i and b_i) must be equal (data not shown). However, if we make a more realistic assumption and change the relative value of the uptake rate (a_i), the population levels change in direct proportion (Fig. 5A). Hence, the populations cannot remain the same, and the noncompetitive pattern cannot be achieved with unrestricted solute diffusion.

Spatial isolation can thus explain the diversity patterns in the low-carbon surface soils. Water-filled pores exist at the surface only immediately following rainfall; thereafter water paths become more tortuous and water activity decreases, reducing both microbe and solute movement. Soil particles become effectively isolated (i.e., $I = c = d = 0.0$), and a potentially available nutrient gradually becomes unavailable (i.e., $eR_i > 0$). When the soil particles are isolated, the equilibrium value of $M_i = a_i/b_i [R_i/(R_i + K)]$ and R_i approaches 0.0, and the populations remain small and nearly equal even if the uptake and loss dynamics are quite different (Fig. 5B), as seen in the noncompetitive pattern. Thus, spatial isolation can explain why we see a large number of genotypes but the size of each genotype remains small.

Mechanisms producing the noncompetitive diversity pattern in high-carbon soils. Since the saturated subsurface samples from the high-carbon Cannelton site show a noncompetitive diversity profile, it is difficult to invoke the spatial isolation hypothesis as the sole explanation for the observations. Instead, it appears that the high carbon content and accompanying resource heterogeneity are a force driving microbial community structure at this site. The soils at this site are marshy, organic soils with very high carbon contents (up to 20% organic C). Thus, the Cannelton soil environment differs dramatically in terms of resource diversity and amount from the low-carbon subsurface soils, which contain less than 0.1% organic carbon.

It is thought that fluctuations in environmental conditions,

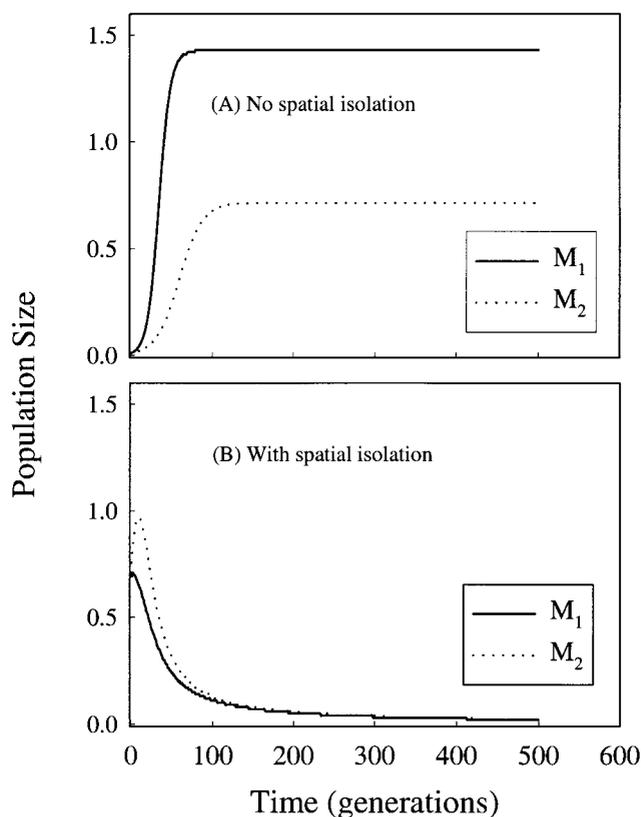


FIG. 5. Effects of spatial isolation on population dynamics. (A) No spatial isolation with unequal uptake and loss coefficients. When $I = 5$, $a_1 = 0.2$, $a_2 = b_1 = b_2 = d = e = 0.1$, $c = 0.2$, $K = 10$, and $R_1 = R_2 = 25$, M_1 approaches 1.428 while M_2 remains 0.714 at equilibrium. Thus, two populations cannot be equally abundant, and hence noncompetitive patterns cannot be achieved. (B) Spatial isolation. The results are similar to those described above, except that when $I = c = d = 0.0$, $M_1 = 0.129$ and $M_2 = 0.112$ after 100 time intervals; that is, initially, M_1 has a slightly faster growth rate. However, $M_1 = M_2 = 0.021$ after 500 time intervals. The initial growth advantage of M_1 is temporary since it uses up its isolated supply of nutrients more rapidly. Thus, the sizes of the isolated populations become nearly identical, as seen in the noncompetitive pattern, even if the populations differ in terms of their growth dynamics.

which can also lead to more evenness in populations, are not a prominent factor in soils, especially below the surface. Even temperature, which does vary annually at the surface, does not vary at the depths of the deeper samples obtained in this study. While environmental variation cannot be ruled out as a factor influencing soil microbial diversity, it does not seem to be as significant in soil as the other two factors identified in this study.

Implications of the causes of high soil microbial diversity. There are several important implications of the noncompetitive profile and the spatial isolation hypothesis. First, spatial isolation helps explain the incredibly high microbial diversity seen in soil on a small scale (e.g., 1 to 5 g). The high diversity seen in this study is consistent with the high diversity found in DNA reannealing studies which revealed at least 5×10^3 genotypes g of soil⁻¹ (30). Second, spatial isolation would enhance the probability of successful colonization by any alien or introduced strain but make it difficult for that strain or any

indigenous strain to achieve dominance. This has important practical implications since it would be difficult for any invader to achieve dominance unless spatial isolation can be eliminated. This might be important in bioremediation of hazardous waste sites, in sequestration of C and N, in biocontrol of root diseases, and in enhancing N₂ fixation by some forest or crop plants. Third, spatial isolation would favor development of microbial genotypes endemic to an area (5), which would further enhance microbial diversity on a global scale.

While spatial isolation may explain the diversity patterns that we observed at the low-carbon sites, our data from the Cannelton site suggests that large amounts of heterogeneous organic carbon also play a significant role in structuring microbial communities. If this is true, then an invading species can also become established in this environment easily; however, whereas we predict that high degrees of spatial isolation could be destroyed by the addition of water, this treatment would have little effect on a community with such rich and diverse carbon resources. The absence of a reduction in diversity in the soils with extremely high chromium contents is surprising and suggests that this carbon-rich environment is able to counteract the expected decrease in microbial diversity.

While our study supports the conclusion that very high microbial diversity is maintained in soil and the conclusion that there is an apparent lack of competition in low-carbon surface soil communities and high-carbon soil communities, further testing of the spatial isolation and resource heterogeneity hypotheses is needed. Understanding the forces that shape soil microbial communities should help in practical management of soil communities and in the wise use and maintenance of microbial diversity.

ACKNOWLEDGMENTS

We thank Alison Murray for suggestions on diversity indices and Tsutomu Hattori for comments, especially on soil structure and modeling.

This research was funded by the Natural and Accelerated Bioremediation Research program, Biological and Environmental Research, U.S. Department of Energy. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the Department of Energy under contract DE-AC05-96OR22464.

REFERENCES

- Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas. 1989. Vertical and horizontal variation in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep southeast coastal plain subsurface sediments. *Appl. Environ. Microbiol.* **55**:1058–1065.
- Bintrim, S. B., T. J. Donohue, J. Handelsman, G. P. Roberts, and R. M. Goodman. 1997. Molecular phylogeny of archaea from soil. *Proc. Natl. Acad. Sci. USA* **94**:277–282.
- Borneman, J., P. W. Skroch, K. M. O'Sullivan, J. A. Palus, N. G. Rumjanek, J. L. Jansen, J. Nienhuis, and E. W. Triplett. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl. Environ. Microbiol.* **62**:1935–1943.
- Borneman, J., and E. W. Triplett. 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl. Environ. Microbiol.* **63**:2647–2653.
- Cho, J.-C., and J. M. Tiedje. 2000. Biogeography and degree of endemism of fluorescent *Pseudomonas* strains from soil. *Appl. Environ. Microbiol.* **66**:5448–5456.
- Dunbar, J., S. Takala, S. M. Barns, J. A. Davis, and C. R. Kuske. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Appl. Environ. Microbiol.* **65**:1662–1669.
- Ellis, D. E., E. J. Lutz, J. M. Odom, R. J. Buchanan, C. L. Bartlett, M. D. Lee, M. R. Harkness, and K. A. Deweed. 2000. Bioaugmentation for accelerated in situ anaerobic bioremediation. *Environ. Sci. Technol.* **34**:2254–2260.
- Farrelly, V., F. A. Rainey, and E. Stackebrandt. 1995. Effect of genome size and *rrn* gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. *Appl. Environ. Microbiol.* **61**:2798–2801.
- Felske, A., A. Wolterink, R. V. Lis, and A. D. L. Akkermans. 1998. Phylogeny of the main bacterial 16S rRNA sequences in Drentse A grassland soils (The Netherlands). *Appl. Environ. Microbiol.* **64**:871–879.
- Hewitt, A. D., and C. M. Reynolds. 1990. Dissolution of metals from soils and sediments with a microwave-nitric digestion technique. *Atomic Spectrosc.* **11**:187–192.
- Huston, M. A. 1994. Biological diversity: the coexistence of species on changing landscapes. Cambridge University Press, New York, N.Y.
- Kopczynski, E. D., M. M. Bateson, and D. M. Ward. 1994. Recognition of chimeric small-subunit ribosomal DNAs composed of genes from uncultivated microorganisms. *Appl. Environ. Microbiol.* **60**:746–748.
- Kuske, C. R., S. M. Barns, and J. D. Busch. 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. *Appl. Environ. Microbiol.* **63**:3614–3621.
- Kwok, S., and R. Higuchi. 1989. Avoiding positives with PCR. *Nature* **339**:237–238.
- Liesack, W., and E. Stackebrandt. 1992. Occurrence of novel groups of the domain *Bacteria* as revealed by analysis of genetic material isolated from an Australian terrestrial environment. *J. Bacteriol.* **174**:5072–5078.
- Liesack, W., H. Weyland, and E. Stackebrandt. 1991. Potential risks of gene amplification by PCR as determined by SSU rDNA analysis of a mixed culture of strict barophilic bacteria. *Microb. Ecol.* **21**:191–198.
- Lollar, B. S., G. F. Slater, B. Sleep, M. Witt, G. M. Klecka, M. Harkness, and J. Spivack. 2001. Stable carbon isotope evidence for intrinsic bioremediation of tetrachloroethene and trichloroethene at area 6, Dover Air Force Base Environ. Sci. Technol. **35**:261–269.
- Magurran, E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.
- Moyer, C. L., F. C. Dobbs, and D. M. Karl. 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl. Environ. Microbiol.* **60**:871–879.
- Moyer, C. L., J. M. Tiedje, F. C. Dobbs, and D. M. Karl. 1996. A computer-simulated restriction fragment length polymorphism analysis of bacterial small-subunit rRNA genes: efficacy of selected tetrameric restriction enzymes for studies of microbial diversity in nature. *Appl. Environ. Microbiol.* **62**:2501–2507.
- Nusslein, K., and J. M. Tiedje. 1998. Characterization of the dominant and rare members of a young Hawaiian soil bacterial community with small-subunit ribosomal DNA amplified from DNA fractionated on the basis of its guanine and cytosine composition. *Appl. Environ. Microbiol.* **64**:1283–1289.
- Pettersson, B., K.-E. Johansson, and M. Uhlen. 1994. Sequence analysis of 16S rRNA from mycoplasmas by direct solid-phase DNA sequencing. *Appl. Environ. Microbiol.* **60**:2456–2461.
- Qiu, X., L. Wu, H. Huang, P. E. McDonal, A. V. Palumbo, J. M. Tiedje, and J.-Z. Zhou. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.* **67**:880–887.
- Rashit, E., and M. Bazin. 1987. Environmental fluctuations, productivity, and species diversity—an experimental study. *Microb. Ecol.* **14**:101–112.
- Stackebrandt, E., W. Liesack, and B. M. Goebel. 1993. Bacterial diversity in a soil sample from a subtropical Australian environment as determined by SSU rDNA analysis. *FASEB J.* **7**:232–236.
- Suzuki, M., M. S. Rappe, and S. J. Giovannoni. 1998. Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Appl. Environ. Microbiol.* **64**:4522–4529.
- Suzuki, M. T., and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol.* **62**:625–630.
- Taylor, L. R. 1978. Bates, Williams, Hutchinson—a variety of diversities, p. 1–18. *In* L. A. Mound and N. Warloff (ed.), Diversity of insect faunas: 9th Symposium of the Royal Entomological Society. Blackwell, Oxford, United Kingdom.
- Tiedje, J. M., J.-Z. Zhou, K. Nusslein, C. L. Moyer, and R. R. Fulthorpe. 1997. Extent and patterns of soil microbial diversity, p. 35–41. *In* M. T. Martins et al. (ed.), Progress in microbial ecology. Brazilian Society for Microbiology, São Paulo, Brazil.
- Torsvik, V., J. Goksoyr, and F. L. Daee. 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* **56**:782–787.
- Ueda, T., Y. Suga, and T. Matsuguchi. 1995. Molecular phylogenetic analysis of a soil microbial community in a soil field. *Eur. J. Soil. Sci.* **46**:415–421.
- Wang, G. C., and Y. Wang. 1996. The frequency of chimeric molecules as a consequence of PCR co-amplification of 16S rRNA genes from different bacterial species. *Microbiology* **142**:1107–1114.
- Wang, G. C., and Y. Wang. 1997. Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16S rRNA genes from mixed bacterial genomes. *Appl. Environ. Microbiol.* **63**:4645–4650.

34. Wayne, L. G., D. J. Brenner, R. R. Colewell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Truper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463–464.
35. Weisburg, W. W., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**:697–703.
36. Wintzingerode, F. V., U. B. Gobel, and E. Stackebrandt. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* **21**:213–229.
37. Zhou, J.-Z., M. Bruns, and J. M. Tiedje. 1996. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol.* **62**:316–322.
38. Zhou, J.-Z., M. E. Davey, J. B. Figueras, E. Rivkina, D. Gilichinsky, and J. M. Tiedje. 1997. Phylogenetic diversity of a bacterial community from Siberian tundra soil DNA. *Microbiology* **143**:3913–3919.
39. Zhou, J.-Z., M. R. Fries, J. C. Chee-Sanford, and J. M. Tiedje. 1995. Phylogenetic analyses of a new group of denitrifiers capable of anaerobic growth on toluene and description of *Azoarcus toluolyticus* sp. nov. *Int. J. Syst. Bacteriol.* **45**:500–506.