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# Spatial and Resource Factors Influencing High Microbial Diversity in Soil

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**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 $3.8 \times 10^6$ CFU $g^{-1}$ in the surface soil, $1.4 \times 10^4$ $g^{-1}$ CFU in the vadose (unsaturated subsurface) soil, and $6.4 \times 10^3$ 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 $1.1 \times 10^7$ 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.

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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 $3.5 \times 10^7$ and $1.4 \times 10^6$ 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 μl 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-μl 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 μl of *Escherichia coli* Top 10F′ competent cells (Invitrogen). The transformed cells were then incubated in 450 μl 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 μl) with the primers specific for the polylinker of vector pCR II (38). One-third of the remaining 18 μl 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× Tris-borate-EDTA by using 7 V/cm for 4 h. The gel was stained with 0.5 μg 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 ($\alpha$) 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 $\alpha$ 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[h] | 599 | 551 |
| | W-2 | 0.15 | Surface | 12.8[h] | 697 | 685 |
| | WF-1 | 0.04 | Surface | 12.8[h] | 209 | 192 |
| | WF-2 | 0.15 | Surface | 12.8[h] | 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 chromium 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′

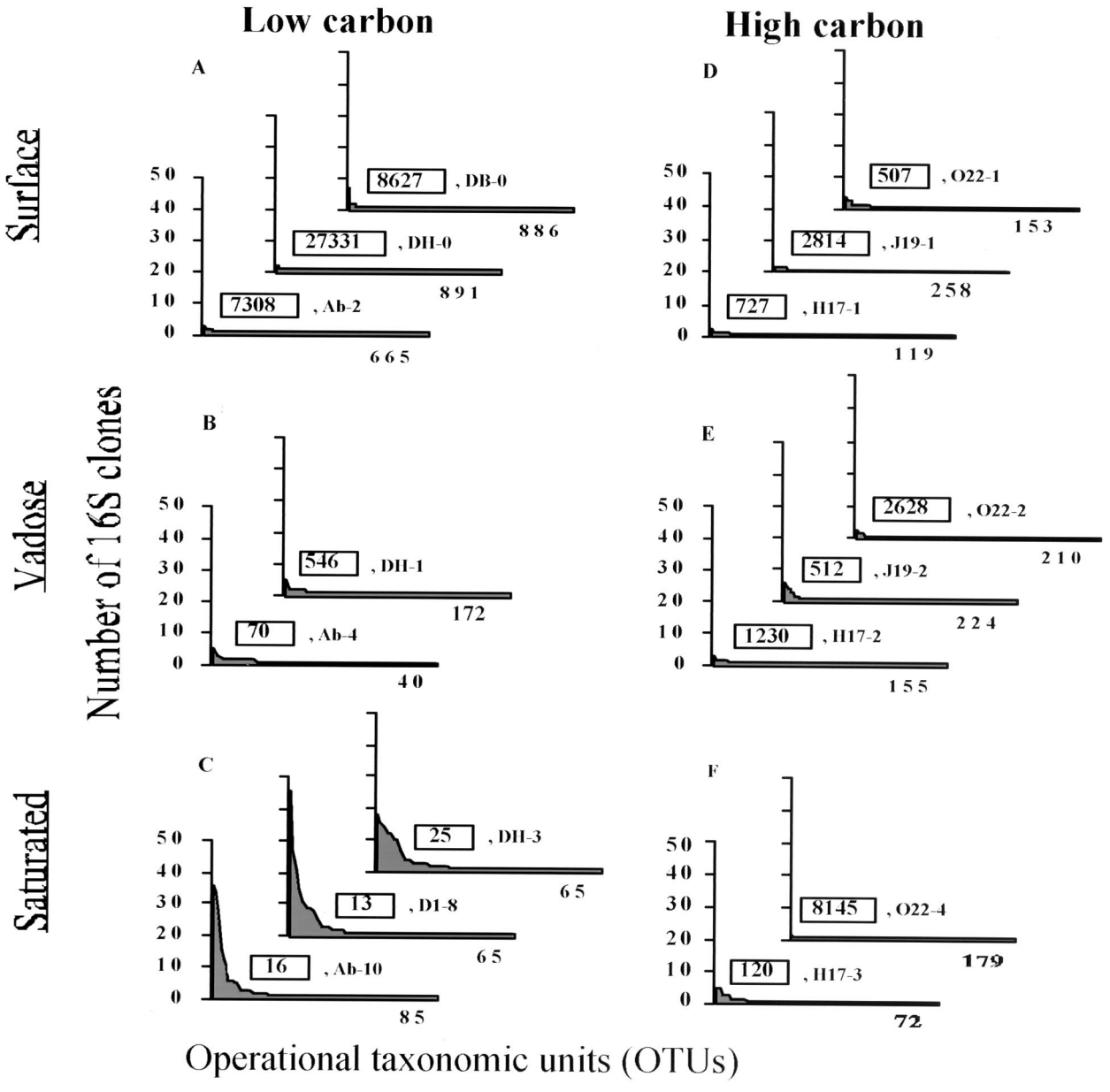


FIG. 1. Representative microbial community diversity patterns based on OTU abundance in surface, vadose, and saturated soils from low- and high-carbon sites. The values in boxes are community diversity values based on the reciprocal of Simpson's index.

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 *rrn* 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 artificial 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

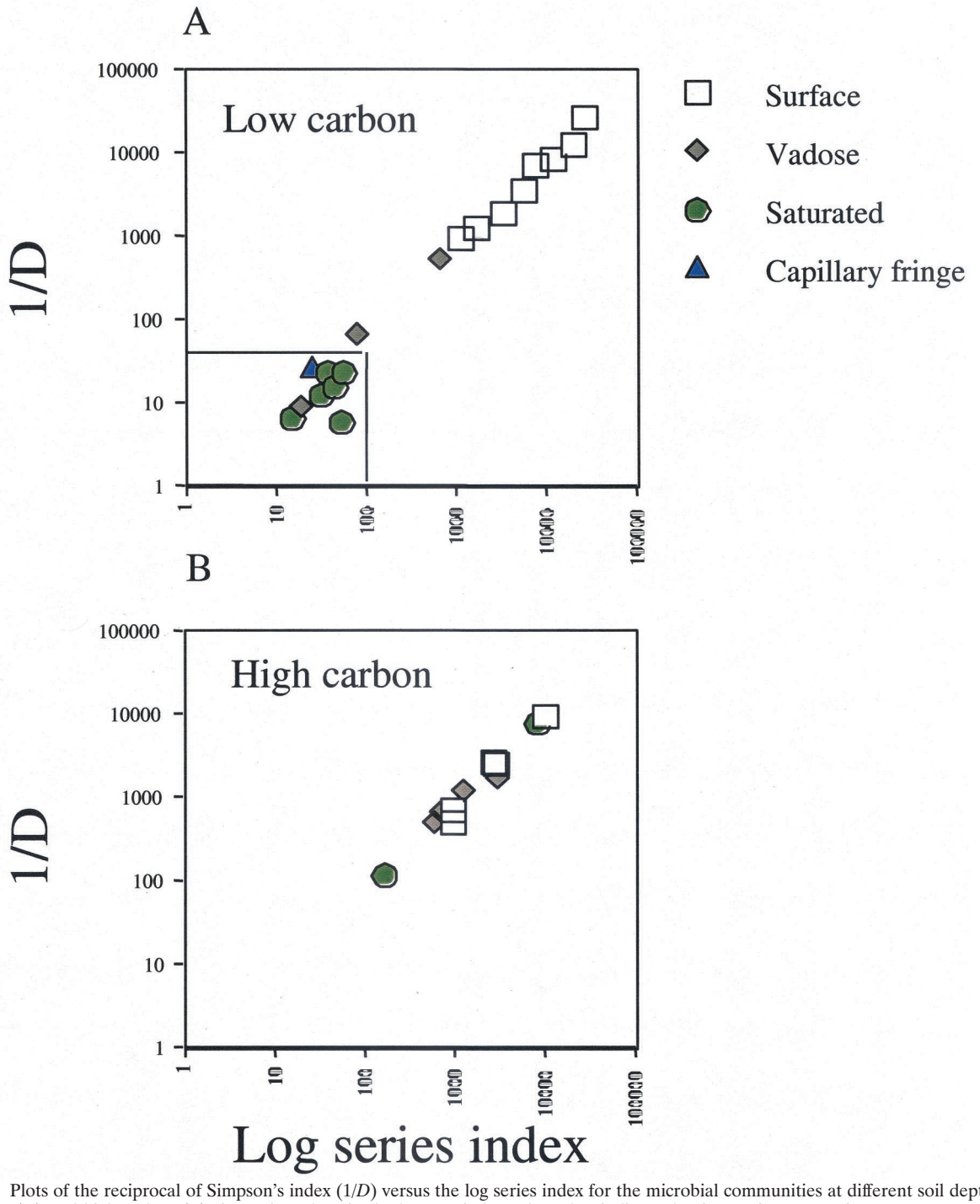


FIG. 2. Plots of the reciprocal of Simpson's index (1/*D*) versus the log series index for the microbial communities at different soil depths from low-carbon (A) and high-carbon (B) sites. The values in the box are index values that indicate dominance in community structure.

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% ± 2.4% at the 5′ end and 97.6% ± 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 nonartifactual 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

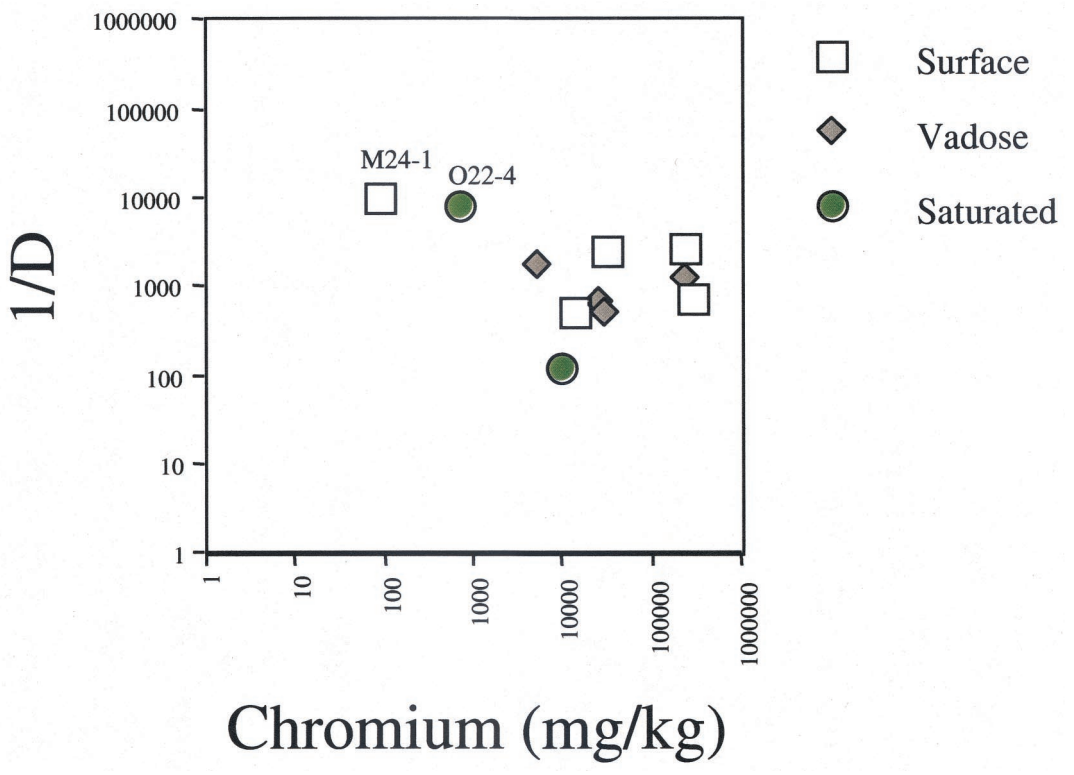


FIG. 3. Comparison of microbial community diversity (based on Simpson's index) and chromium levels at the high-carbon Cannelton site.

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

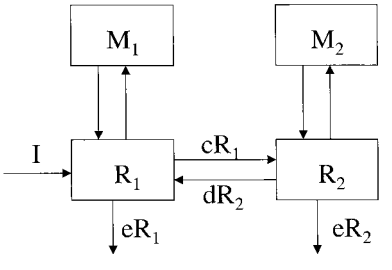


FIG. 4. Conceptual model of two soil particles showing the relationships between microbial biomass (M) and nutrient (R) exchanges. Although a small sample of soil would contain a large number of similar particle systems, this minimal representation suffices for our purposes. $cR_1$ and $dR_2$, nutrient 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 10 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, 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.

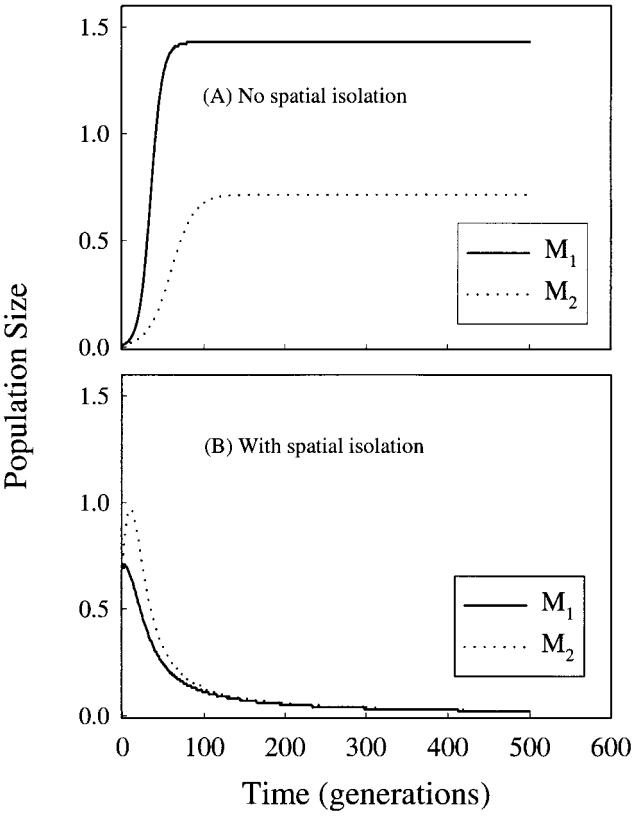


FIG. 5. Effects of spatial isolation on population dynamics. (A) No spatial isolation with unequal uptake and loss coefficients. When $I = 5$, $a_1 = 0.2$, $a_2 = b_1 = b_2 = d = e = 0.1$, $c = 0.2$, $K = 10$, and $R_1 = R_2 = 25$, $M_1$ approaches 1.428 while $M_2$ remains 0.714 at equilibrium. Thus, 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.

**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 $5 \times 10^3$ 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 $N_2$ 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.

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