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SOIL BACTERIAL COMMUNITY STRUCTURE CHANGES FOLLOWING DISTURBANCE OF THE OVERLYING PLANT COMMUNITY

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The effect of plant species identity and diversity on the spatial and temporal differentiation in bacterial community structure in the active root zone was studied at a field site that was treated with herbicide, burned, and then reseeded with native grass species. Treatments were arranged in three blocks. The blocks corresponded to an elevational gradient that was used as a surrogate for moisture differences across the field. Samples were collected before and after devegetation, and eight times thereafter, over the subsequent year. The soil samples were examined for bacterial abundance (direct counts) and bacterial community structure by the genetic fingerprinting techniques randomly amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE) of whole-community DNA extracts. The bacterial abundance was relatively constant in both time and space. Discriminant function analysis and the Mantel test revealed clear temporal trends in community structure throughout the entire 2-year study that coincided with the overall development of the grasses in the field. Spatial differentiation of community structure was clear among the blocks, suggesting differences in community structure based on soil moisture content. Significant differences were also observed between control and treated plots. Spatial and temporal change in the bacterial community was evident, and bacterial and plant succession coincided temporally. Differences in the bacterial communities that developed were also affected by the soil moisture. (Soil Science 2004;Volume 169:55–65)

Key words: Soil microbes, root zone, microbial community, succession, disturbance, genetic fingerprinting.

THE relationship between plants and soil microorganisms in the active root zone is one of the best examples of community dynamics within an ecosystem. Hiltner (1904) first defined the rhizosphere as "... that zone of soil in which the microflora are influenced by plant roots." Although the rhizosphere is often defined operationally as occurring with soil that adheres to plant roots when they are shaken, the rhizosphere effect must be felt to some extent throughout the active root zone. The rhizosphere effect results from the deposition of (i) water-soluble root exudates such as sugars, amino acids, organic acids, hormones, and vitamins that leak from the root; (ii) secretions such as polymeric carbohydrates and enzymes; (iii) lysates released when cells autolyse; (iv) solid materials, including cell walls, sloughed whole cells, and, with time, whole roots; and (v) gases such as ethylene and CO_2 (Whipps, 1990). Rhizodeposition is carbon loss from roots (Lynch and Whipps, 1991). The rhizosphere effect is often manifested as increased bacterial abundance, metabolic activity, and community diversity (Clark, 1949; Katznelson et al., 1948; Krasil'nikov, 1954; Starkey, 1929), although

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some recent articles have noted a decrease in diversity (Marilley and Aragno, 1999). Numbers of microorganisms in the rhizosphere can reach 10^{10}–10^{12} organisms g^{-1} of rhizosphere soil (Foster, 1988). Estimates of bacterial growth in the rhizosphere (Barber and Lynch, 1977; Bowen and Rovira, 1973) show that the soil bacterial community depends largely on rhizodeposition.

The rhizosphere is a zone of commensal and mutualistic interactions between plants and microorganisms. In addition to mutualistic mycorrhizal relationships, a great many free-living microorganisms are observed as a result of the favorable environment associated with the provision by plant roots, for example, of root litter and exudates (Atlas and Bartha, 1998; Franklin et al., 1999; Kowalchuk et al., 2002) that can be used by the microbes as a high quality source of carbon and energy. The relationship between the plants and microorganisms is not always mutually beneficial. There are frequent reports of negative effects, such as herbicidal and pathogenic activities of certain viruses, bacteria, and fungi (Shabana et al., 1997), and high competition between the plants and microorganisms for limited resources, such as nitrogen, (Newman, 1985) or phosphorous (Tilman, 1982). For instance, heterotrophic microorganisms can limit plant growth by controlling the amount of inorganic nitrogen available in the root zone caused by immobilization of N during organic matter decay (Kaye and Hart, 1997).

Early work established bacterial associations with various plant species (Katznelson et al., 1948; Katznelson and Rouatt, 1957; Rovira, 1965), and more recent work is consistent with those findings (Burke et al., 1989; Söderberg et al., 2002). The early effort led to development of the rhizosphere concept (enhanced abundance and activity of bacterial community) that is commonly accepted as a property of the plant root zone. However, even though there are strong associations of some microbes with some types of plants, with the exception of specific plant symbionts such as the rhizobia and the mycorrhizae, it is not known to what extent the vegetation controls the bacterial community structure (i.e., the composition and distribution of the individuals among the species assembled) in the underlying soil.

The traditional concept of community development involves the movement of the community toward more a stable state (Ajwa et al., 1999; Atlas et al., 1991; Bardgett et al., 2001; Griffiths et al., 1999). In the terminology used to describe succession, the community at climax is relatively stable and is at dynamic equilibrium. It is not clear, however, that the climax concept applies to natural bacterial communities (Atlas and Bartha, 1998) because even small disturbances (e.g., a single rainfall) can disrupt succession (Ahlgren and Ahlgren, 1965), and prevent the community from reaching equilibrium. Furthermore, given the hyperdiversity associated with bacterial communities (Curtis et al., 2002) and the tremendous redundancy of function among many different species of microbes (Garland et al., in press), identical environmental conditions may not force development of bacterial communities that are compositionally identical. The similarity of complex bacterial communities can vary substantially over time, with little alteration in function, even among controlled reactors operated as replicates (Fernández et al., 2000; Mills et al., 2003).

A catastrophic disturbance, such as fire, may decimate the biota at a location, replacing it with a sparsely populated environment in which bacterial communities can undergo secondary succession (DeBano et al., 1998). Soil bacterial communities, because of their rapid turnover, might be expected to go through successional sera more rapidly than communities of other organisms in the repopulating ecosystem. The microorganisms can modify the environment to more favorable conditions for other groups of microbes and for higher organisms (i.e., autogenic succession). After the bacterial pioneers initiate the succession, subsequent sera may interact among different cell domains, especially between plants and microorganisms. Disturbed systems offer a unique opportunity to examine the control that vegetation exerts on the structure of the soil bacterial community. Strong control should be manifested by a major shift in structure of the bacterial community that is correlated to the identity of the plant cover that develops after the disturbance, i.e., different plants should yield different communities. In addition, development of a diverse plant community might be expected to provide the soil microbes with, for example, a broader selection of organic energy sources, resulting in a richer, more diverse bacterial community.

The present research examined the temporal change in bacterial community structure coincident with a major alteration in the plant species inhabiting a field site as a result of a catastrophic disturbance (total devegetation followed by replanting of different assemblages of plant species). We also examined the degree to which soil moisture forced different bacterial communities to develop.

MATERIALS AND METHODS

The study was conducted on a 14-ha field at Blandy Experimental Farm (BEF) in Clarke County, Virginia (78°00 W, 39°00 N) where a meadow comprising native temperate tallgrass species is being established. The average slope of the experimental field is approximately 3.7% from north to south, with an elevation difference of 8 m over the (210 m) distance. Soils at the crest of the hill tend to be moderately droughty whereas the soils at the base are often wet; a swampy area extends into the lower part of the field. Thus, the elevation gradient can be described as representing an analog of a soil moisture gradient, with the soils at the top termed xeric, those in the middle mesic, and those at the base hydric, although use of these terms does not conform to any quantitative standard.

Soils at the site are dominated by the Poplimento silt loam series (Fine, mixed, mesic Ultic Hapludults), with an area of Timberville silt loam (Clayey, mixed, mesic Typic Hapludults) in the southeast corner (in areas H1 and H4, see Fig. 1) (NRCS, 1982). Both of these soils are well drained, but the Poplimento is generally found on side slopes and hillcrests, and the Timberville is more characteristic of narrow to moderately broad drainage ways. Neither of these series is considered a hydric soil; the descriptors used in this paper are only to separate the perceived relative soil moisture regimes of the three portions of the hillslope.

Although the field has long been maintained as a meadow by mowing and occasional prescribed burnings, the grasses in the field have been dominated by exotic species, as is typical of most such areas in Virginia. The revegetation was initiated by an herbicidal treatment (Roundup®) in October 1999. In April 2000, the field was treated with another application of herbicide, followed shortly by a prescribed burning. After the devegetation treatment (herbicide on April 13, 2000, and burning on May 3, 2000), the field was sown with a variety of native grasses: big bluestem (*Andropogon gerardii);* broom sedge (*Andropogon virginicus);* little bluestem (*Schizachyrium scoparium);* side oats (*Bouteloua cultipendula);* switch grass (*Panicum virgatum);* Indian grass (*Sorghastrum nutans*); and forbs (44 species) on May 10, 2000.

Three blocks were assigned to the field by topographic position corresponding to the assumed soil moisture gradient. A control plot was assigned to each block; the controls were neither treated with herbicide nor burned and thus represent the community of exotic plants. Six species of two grass types ranging from tall, turf-forming species (big bluestem) to small-statured bunch grasses (e.g., side-oats grama) were chosen and sown in different combinations, including monocultures of *A. gerardii* and *B. cultipendula.*

Samples were taken 10 times over a 2-year period. An initial sampling was conducted immediately before the devegetation procedure (April 13 and May 4) and again immediately after re-

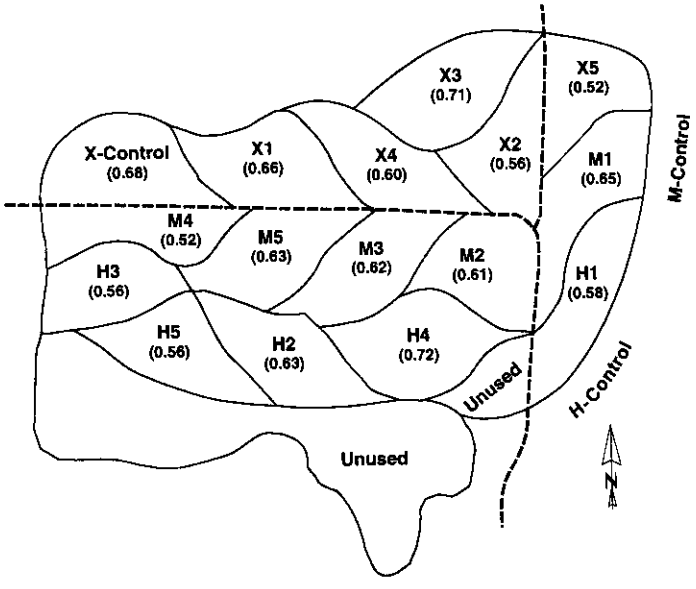

Fig. 1. Map of experimental site showing the layout of experimental blocks. The dotted line indicates an unpaved road through the field. The highest (driest, xeric) points in the field are at the northernmost points, and the lowest (wettest, hydric) areas are at the southernmost parts. Numbers in parentheses in each plot represent the area of the plot in ha.

planting (May 18). Additional samples were collected periodically during the growing season and once in winter for the first year (June 1, June 22, and July 20). The second-year sampling schedule was similar to that of the first year (May 3, June 13 and July 18) except that the midwinter (non-growing season, January 11, 2001) sample was not taken. At each sampling time, soil was collected from three randomly chosen locations within each plot. Approximately 100 g of soil was taken with a small shovel from the 5- to 10-cm depth interval, i.e., the active root zone, placed in polyethylene Ziploc® sampling bags, and stored at −20 °C until analysis (usually within 1 week). Obvious plant root debris was removed manually, but no specific discrimination was made between rhizosphere and bulk soil. The samples were analyzed individually as within-treatment replicates.

Epifluorescent-microscopic direct counting with acridine orange staining was used to measure bacterial abundance (Bottomley, 1994). Molecular analysis of total community DNA was conducted on material extracted from the samples using the UltraClean™ soil DNA isolation kit (#12800, MoBio Laboratories, Solana Beach, CA). Subsamples (0.25 g) of the soil were extracted by "bead beating" to break cell walls, followed by treatment with a PCR inhibitor removal solution included in the kit. This process produced a final volume of 50 μL of extract. The concentration of the extracted DNA was measured using PicoGreen® dsDNA Quantitation Reagent (Molecular Probes, Eugene, OR). The total amount of DNA was approximately 1 μg in each 50 μL of 10 m*M* Tris buffer.

The bacterial community was fingerprinted using two approaches. The total community DNA from sampling times 3, 6, and 8 was examined using the randomly amplified polymorphic DNA (RAPD) procedure (Franklin et al., 1999; Wikstrom et al., 1999). Bacterial DNA from all samples collected was then fingerprinted by DGGE separation of the 16S-rDNA (Heuer and Smalla, 1996; Muyzer and Smalla, 1998; Nakatsu et al., 2000). For RAPD, 36 arbitrary short primers were chosen from the Operon (Alameda, CA) catalog and tested for use in this study. Final selection of primers was based on both the total number of polymorphisms and the contribution of new polymorphisms to the accumulated set. Ten primers were chosen: A7, GAAACGGGTG; A8, GTGACGTAGG; A12, TCGGCGATAG; A19, CAAACGTCGG; A20, GTTGCGATCC; C4, CCGCATCTAC; D18, GAGAGCCAAC; F3, CCTGATCACC; S14, AAAGGGGTCC; and T7, GGCAGGCTGT. The PCR amplifications were performed using the protocol of Franklin et al. (1999) with slight modification. A 5-μL portion of one-tenth diluted template DNA (5 to 10 ng) was added to 20 μL of a reaction mixture containing 1.5 m*M* $MgCl_2$, 0.2 μ*M* primer, 100 μ*M* dNTP mixture, 20 μg BSA (bovine serum albumin, New England Biolabs, Beverly, MD), PCR buffer, and 1 unit of AmpliTaq® DNA polymerase (Applied Biosystems, Foster City, CA). The amplification protocol consisted of an initial denaturation of 1 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C, with final elongation for 10 min at 72 °C. Amplified products were separated by electrophoresis in 1.5% agarose gels and visualized with ethidium bromide staining under UV light.

16S rDNA was obtained for DGGE by the amplification of 2 μL of template DNA (20 to 40 ng) with a universal primer set for soil bacteria P63f (5′-CAGGCCTAACACATGCAAGTC-3′) and P518r (5′-ATTACCGCGGCTGCTGG-3′) (El Fantroussi et al., 1999). A GC clamp of 40 bases (Sheffield et al., 1989) was added to the 5′ terminus of the P63f primer. The PCR reaction mixture contained 3.75 m*M* $MgCl_2$, 0.2 μ*M* primers, 200 μ*M* dNTP mixture, 40 μg BSA, PCR buffer, and 2 units of AmpliTaq® DNA polymerase. Amplification was performed as follows: 5 min at 94 °C, and 30 cycles of 1 min at 92 °C, 1 min at 64 °C, and 2 min at 72 °C, with a final elongation of 10 min at 72 °C. Amplified 16S rDNA was separated on a polyacrylamide gel with a denaturing gradient of 33 to 47% of ureaformamide, following protocols established by the equipment manufacturer (CBS Scientific Company, Del Mar, CA). The electrophoretic separation was run for 4 h at 175 V, and products were visualized using SYBR Green I staining (Molecular Probes, Eugene, OR) under UV light.

Analysis of variance (ANOVA) with SAS v. 8.2 (SAS Institute Inc., 1989) was used to compare the effects of moisture gradient and seasonal change on abundance. Collected band patterns of RAPD and DGGE were stored as binary data and analyzed by discriminant function analysis (DFA), which provides explicit hypothesis testing, using SPSS v. 11 (SPSS Inc., Chicago, IL). Classification results and discriminant function (DF) plots using the first two functions were used to examine hypotheses related to the multivariate (DGGE) data. In addition to grouping efficiency as a hypothesis testing procedure, DFA plots contain centroids and their associated standard errors

of the mean for visual determination of significant differences among group means. Similarity matrices representing every treatment combination were computed using the Jaccard coefficient to prevent formation of groups based solely on negative data (i.e., to preclude reliance on absence of bands rather than presence) (Lamboy, 1994). For example, the treatment involving the time progress of community structure included a matrix that had dimensions of 10 × 56 DGGE bands. Other analyses required a matrix of the number of treatments times the total number of bands obtained for the treatments of interest. A Mantel test was performed with constructed similarity matrices using Mantel-Struct v. 1.0 (Mark P. Miller, Northern Arizona University) to reveal temporal trends in community structure. The Mantel test is used to estimate the correlation of two matrices (usually dissimilarity or distance matrices, although it is not limited solely to those types of data). The standardized Mantel coefficient (r, a correlation coefficient) is used for practical analysis (Legendre and Fortin, 1989; Legendre and Legendre, 1998; Sokal and Rohlf, 1995).

RESULTS

The 10 RAPD primers used generated 88 genetic polymorphisms from the three samplings (3, 6, and 8) to which the approach was applied. A much larger proportion of rare polymorphisms was obtained with RAPD than with DGGE, and a larger total number of bands was obtained with RAPD (88 vs 56). Simultaneous comparison of the two genetic fingerprinting techniques with the Mantel test for samples on which both approaches were employed (sampling 3, 6, and 8) showed no correlation between them ($r = 0.003$, $P = 0.128$). The two genetic fingerprinting techniques examined the community genome in different ways (RAPD examines the frequency of arbitrary sequences in the genome, whereas DGGE examines the sequence information of the most dominant ribotypes). For that limited sample set, however, DFA of temporal trends yielded 98.1% (DGGE) and 100% (RAPD) matching of original group membership, suggesting that although they work differently, the two genetic fingerprinting techniques were equally effective in detecting differences in the bacterial communities.

The field was burned about 20 days after the second herbicide treatment. Although the burning lasted the entire day, there was a portion of field that was burned less intensively than rest of the field, specifically, an area with dense perennial vegetation (included in M4 and H4) near the road in the lower southeast part of the field. As shown in Fig. 2, the overall abundance of microorganisms obtained with direct counts was not only not decreased by the burning, in contrast to what has frequently been observed elsewhere using culture methods (Ahlgren and Ahlgren, 1960; Borchers and Perry, 1990; Stendell et al., 1999), there was actually an apparent increase in bacterial abundance after the burning. These data suggest that the intensity of the burning was not sufficient to cause a major decrease in bacterial abundance, and that conjecture is supported by the lack of a significant change in bacterial community structure (Fig. 3), although it is possible that intact DNA in killed cells could confound that observation. Samples obtained 2 weeks after the fire showed a drastic change in bacterial community structure.

Microbial communities within the three blocks established on the presumed moisture regime gradient were separated, as shown by the discriminant function analysis (Fig. 4) of the DGGE data. The overall matching of the samples to the groups to which they were assigned in the experimental design was 58.9% for all groups. The large number of samples allowed a territory map to be generated as a part of the DFA (overlaid on the DFA plot, Fig. 4) by the statistical software; that map indicated separation of the samples into three distinct groups. However, bacterial abundance among the locations on the hillslope did not show segregation similar to that of the community structure. The only exception was

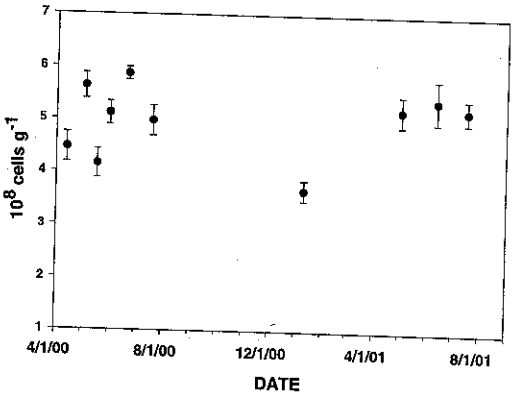

Fig. 2. Average bacterial abundance at each sampling during the 2 years of study. Error bars are 1 standard error of the mean.

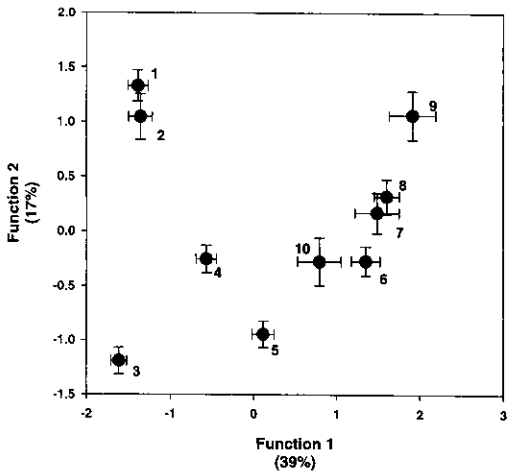

Fig. 3. Plot of first and second discriminant functions of DGGE data according to the group membership of sampling time from sampling 1 to 10. Points indicates centroids with 1 standard error. Number in parentheses indicates the amount of variance explained by that function.

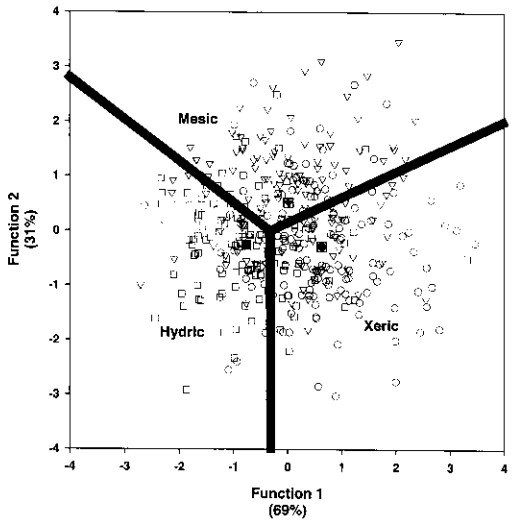

Fig. 4. DFA plot of samples analyzed according to the group membership in the presumed moisture-regime groups. The overlapped territorial map (heavy lines represent territory boundaries) clarifies the data separation. The dark points indicate group centroids with 1 standard error bars indicated, and ●, ▼, and ■ represent the xeric, mesic, and hydric blocks, respectively.

found right after the burning, when Ryan's Q test (Sokal and Rohlf, 1995) revealed a significant difference ($P = 0.0424$) between the xeric- and hydric-block samples.

In the first year after reseeding the field was dominated by weeds, with only sparse patches of the sown species appearing. Larger, more discrete patches appeared in the second growing season, but the field was still unrecognizable by the sown vegetation. Nevertheless, differences between the treated plots were distinguished from the controls by DFA, with 73.6% group membership for samples from the Treated plots and 60.9% membership for samples from the Control plots.

The 10 sampling times were analyzed using both DFA and the Mantel test to examine overall trends of bacterial community structure change. The overall matching of the samples to the appropriate group (DFA, times 1–10) was 61.6%, a high value considering there were 10 different groups with 414 samples to be assigned to them. As shown in Fig. 3, the sampling times were clustered into two distinct groups, with the communities recovered in the first two samplings being decidedly different from all other samplings. In addition, there was also a strong directionality in the shift of bacterial community structure in the multivariate space, suggesting a successional change away from the initial condition. Only at time 10 did the progression not follow the directional pattern. Additional samplings (part of an ongoing study) will reveal if that single sampling (time 10) represents a new direction or simply an anomaly in the successional pattern. The pairwise Mantel test indicated that the overall bacterial community structural change was significant ($r = 0.093$, $P = 0.001$); only 4 of the 45 pairwise comparisons (data not presented) had meaningful correlation between them, and in each case the r value was between 0.31 and 0.38. This result corroborates the DFA results that the bacterial community structure never returned to its original state.

The data were also analyzed for seasonal change and annual patterns. Group membership of non-growing season (samplings 1–3 and 7) and growing season samples (all others) by DFA showed agreement of 83.3% and 77.8%, respectively. In addition, the distinction between the first and second year communities was very clear, with 87.3% and 76.9% agreement for the samples from the first and second years, respectively, an observation consistent with the observed difference in vegetation in those years.

DISCUSSION

The difference in the resident plant species has long been thought to be the driving force in

the structure of the bacterial community within the root zone (rhizosphere effect) (Clark, 1949; Katznelson et al., 1948; Krasil'nikov, 1954; Starkey, 1929). Although grasses have diffuse root systems that permeate the soil and, in some cases (as in salt marshes), dominate the soil volume, some investigators expect the effects of plants on soil bacterial community to be different between the rhizosphere and the bulk soil (Kowalchuk et al., 2002). In the present study, no effort was made to separate the root-attached soil from the remainder; differentiation between rhizosphere and bulk soil was unobtainable. Given the original definition of rhizosphere, the zone where microorganisms are influenced by plant roots (Hiltner, 1904), and given the density of the root mat encountered, we expected all of the soil collected to be influenced by the plants in this case. Some reduction in differentiation might have been derived from mixing the root-associated soil with the bulk soil in the samples, but we consider that to be a minor effect.

Several earlier studies of burning indicated clearly a significant decrease in bacterial biomass and a drastic change in bacterial community structure (Bååth et al., 1995) after the burn event. Thus, it was surprising that there was no drastic decrease in bacterial abundance during the earliest part of the study. Indeed, the bacterial abundance increased between the first and second sampling (Fig. 2), which was collected 1 day after the field was burned. A partial explanation for the observation might be that the increase occurred because of the combined warming of the soil and the input of a large amount of organic matter because of plant death caused by the herbicide treatment. Because the enumerations were done by direct counts, the immediate effect of the fire may have been different than expected, based on cultural counts. Cells may have been killed by the heat, but not destroyed, and, therefore, uncountable with acridine orange staining. Furthermore, the depth at which the samples were collected (5–10 cm) could have provided protection for the cells from the heat of the fire. Another possibility is that the increase might be part of a general spring warming of the soil.

The patterns of change in bacterial community structure and abundance are somewhat different (Fig. 2 and 3). The change in abundance is a rapidly manifested result of the burning, whereas structural changes are not necessarily expected to be from the same cause since the latter is more likely influenced by selective enhancement or suppression caused by the large input of organic matter. The inconsistency between DFA and the Mantel test for the effect of the fire on community structure might be explained by differences in how the raw data were handled. DFA works directly on the binary data representing the band patterns, whereas the Mantel test is based on the similarity index calculated from the binary data. Discrepancies might arise because of this difference; however, in the present situation the two statistical analyses were, overall, relatively well matched to each other.

Bacterial community recovery after disturbance can be very rapid. In a study by Ahlgren and Ahlgren (1965), plating data indicated a sharp increase in abundance after the burning of a grassy field in each of 2 successive years. The first rainfall was about 1 month after the burning and a drastic increase in abundance (by plating methods) was observed 1 week after that rainfall. In the second year, it rained within 48 h after the burning, and a highly significant increase in bacterial abundance was observed in that period. In neither case was any time course sampling carried out, so there was no determination of the actual time taken for bacterial community recovery. In a study by Choromanska and DeLuca (2002), drastically reduced bacterial biomass C was observed immediately after the fire, but the numbers exceeded the original state in samples collected 14 days after the fire; no community structure data were obtained, and no comment about rainfall or moisture was provided. In the present study, the first three samples were collected about 20 days apart, and this may have been too infrequently to detect rapid changes in bacterial abundance. Other types of microbial communities are also known to respond quickly to disturbance. For example, in a study of impoundment sediments, Bell et al. (1990) noted that the anaerobic community returned to a normal level of abundance and function within about 14 days after the bed was scoured and covered with 15 cm of fresh, aerobic sediment. The commonality of the 2-week time frame in the present and all of the aforementioned studies suggests this may be a reasonable recovery time for heterotrophic microbes in many productive habitats.

To provide a more complete understanding of the bacterial community for the analysis, we combined two different genetic fingerprinting techniques. Several other studies have applied a similar strategy, analyzing samples with two different genetic fingerprinting techniques, and have come up with relatively consistent results with respect to matching the results of the meth-

ods to each other within the same samples. For example, studies with individual genome samples have shown that RAPD provides results that are very consistent with amplified fragment length polymorphism (AFLP) (Powell et al., 1996; Renganayaki et al., 2001). In the present study, the general trend in the results of the DFA was roughly consistent between RAPD and DGGE. 100% of the RAPD and 98.1% of the DGGE profiles matched up with their sample membership for sampling times 3, 6, and 8. However, the acceptance of the null hypothesis in the Mantel test between two genetic fingerprinting techniques indicates that while the two methods discriminated the samples similarly, the basis of that discrimination differed for the two methods. This is a sensible result because DGGE analysis discriminates among communities based on differences in the phylogenetic makeup (i.e., ribotypes based on 16S rDNA sequences), whereas RAPD differentiates the community based on frequency of sequences distributed throughout the total DNA. Both techniques are useful tools for bacterial community structure analysis (Franklin et al., 1999). Indeed, Franklin et al.(2001) reached similar conclusions about analyzing community structure with techniques that use 16S-rDNA compared with other primers after applying AFLP, TRFLP, and community level physiological profiling (Garland and Mills, 1991) to the same sample set, although the methods did not always generate the same patterns because of the different community structure properties examined by each.

The bacterial community structure within the soil changed substantially during the 2 years after the disturbance and revegetation (Fig. 3). Changes associated with the initial disturbance events were evident followed by a clear succession as the plant community developed after seeding. The successional pattern displayed in discriminant function space was, with the single exception of the last sampling time (time 10), monotonic as the vegetation developed. Given the trajectory over the 2 years, and given the observations associated with the slow rise to dominance of the native grasses planted, we conclude that the bacterial communities and the plant communities were developing in parallel.

The deviation of the last sampling time from the monotonic trajectory might be a simple anomaly, and the successional direction may resume in the future, leaving sampling 10 as an outlier. Alternatively, sample 10 might represent the beginning of a new stage of bacterial community structure shift. In that case, the bacterial community structure might move along a new trajectory in a direction in discriminant function space different from that of the first 2 years. Samples taken in the future will distinguish which of these options is correct.

Although a study by Blume et al. (2002) reported that soil bacterial biomass was relatively constant despite seasonal change, most reports indicate that bacterial abundance undergoes a seasonal trend (Smalla et al., 2001), particularly between summer and winter (Corre et al., 2002; Grayston et al., 2001). A seasonal pattern in the present study was clear, although only a single winter sampling was taken. The annual temperature cycle is very closely related to bacterial abundance and activity (Biederbeck and Campbell, 1971; Dormaar et al., 1984; Kaiser and Heinemeyer, 1993) caused by the direct effects on bacterial growth and the indirect effects of factors such as increased root growth and turnover in the summer months (Lynch and Panting, 1982).

The shifts in bacterial community structure with time were evident before establishment of the sown grasses as the dominant vegetation, a result that is consistent with observations elsewhere. In salt marsh sediments, for example, changes in bacterial community activity (dissolved organic-carbon mineralization rates) were correlated with changes in the relative contribution of *Spartina patens* and *Juncus roemerianus* roots to the total below-ground biomass (Aiosa, 1996). These below-ground responses preceded changes that were documented in above-ground plant community structure in experimental plots that were exposed to an altered hydro period (Tolley and Christian, 1999). Aiosa (1996) speculated that the altered bacterial community activity measured reflected changes in bacterial community composition and that the bacterial community could provide an early indication of plant community structure changes.

The established blocks used in the experimental design were based on the observed topography of the field. Topography can have a major influence on soil moisture, microclimate (Sobieraj et al., 2002), soil type (Pennock et al., 1987), and vegetation (Burke et al., 1989). The obvious wet area at the bottom of the hill slope and the strong influence of soil moisture on many soil factors (Merz and Plate, 1997) led us to consider a gradient in soil moisture content to be a potentially important controlling factor for bacterial abundance and community structure (Corre et al., 2002; Schimel et al., 1999; Van Ges-

tel et al., 1992) at the various locations in this field. Bacterial abundance in this field was virtually identical among blocks representing the elevation gradient (data not shown), and, by inference, the soil moisture gradient, but genetic fingerprinting techniques revealed substantial differences in the bacterial community structure. We measured the *in situ* soil moisture only once during the experimental period, and no gradient was observed at that time. However, Kim and Barros (2002) showed that a strong connection between topography and soil moisture content was observed only during and immediately after rainfall and that the duration of the effect was heavily dependent on soil texture. Additional data are needed to determine if what we are inferring as a moisture gradient effect is just that and not some other factor that varies along the elevation gradient.

We have been able to show that changes in the plant community are associated with changes in the structure of the soil bacterial community and that succession in that community proceeds in concert with visually observable changes in the dominant plants. Bacterial abundance increased after the herbicide treatment in the spring, then decreased in the 3-week period after the field was burned. Numbers of microbes then increased as the vegetation developed and followed the expected seasonal patterns thereafter. The bacterial community structure differed significantly along an assumed moisture gradient based on an elevation gradient across the experimental field. The close association of bacterial community structure with plant cover was clearly, but only generally, demonstrated by the successional patterns. Differences in the bacterial community structure associated with differences in plant identity and plant diversity were not evident in the early stages of revegetation.

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