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Diversity of Fungi, Bacteria, and Actinomycetes on Leaves Decomposing in a Stream[∇]

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Although fungi, bacteria, and specific bacterial taxa, such as the actinomycetes, have been studied extensively in various habitats, few studies have examined them simultaneously, especially on decomposing leaves in streams. In this study, sugar maple and white oak leaves were incubated in a stream in northeastern Ohio for 181 days during which samples were collected at regular intervals. Following DNA extraction, PCR-denaturing gradient gel electrophoresis (DGGE) was performed using fungus-, bacterium-, and actinomycete-specific primers. In addition, fungal and bacterial biomass was estimated. Fungal biomass differed on different days but not between leaves of the two species and was always greater than bacterial biomass. There were significant differences in bacterial biomass through time and between leaf types on some days. Generally, on the basis of DGGE, few differences in community structure were found for different leaf types. However, the ribotype richness of fungi was significantly greater than those of the bacteria and actinomycetes, which were similar to each other. Ribotype richness decreased toward the end of the study for each group except bacteria. Lack of differences between the two leaf types suggests that the microorganisms colonizing the leaf biofilm were primarily generalists that could exploit the resources of the leaves of either species equally well. Thus, we conclude that factors, such as the ecological role of the taxa (generalists versus specialists), stage of decay, and time of exposure, appeared to be more important determinants of microbial community structure than leaf quality.

In many streams, leaf litter is an important source of energy and carbon (15, 36). Microorganisms like bacteria and fungi are among the few organisms that secrete enzymes that can break down large molecules, such as cellulose, chitin, and lignin, into smaller compounds that can be taken up by the biota (57). This “conditioned” leaf litter that has been modified by microbial activities provides nutrition for invertebrates and forms a central part of many stream food webs (4, 25, 61).

To understand the ecology of these microorganisms, their interactions, and the functions they perform, it is important to study them in their natural habitats (60). Yet, most studies of leaf decomposition in streams provide no information on the diversity of bacteria (1, 2), focusing solely on fungi (21, 28). In some studies, the aquatic hyphomycete species are listed (2, 70), but for bacteria, generally total numbers, biomass, or production rates are reported without information on the taxa present (1, 2, 23, 63).

Because microbial leaf decomposition results from the combined actions of fungi and bacteria, it is important to elucidate the roles of each in the process. Different groups of bacteria and fungi have various biochemical and physiological capabilities, and these differences may influence the leaf decomposition process. This ultimately affects the bioavailability and supply rate of organic carbon to other trophic levels and underscores the need to examine microbial community structure and its potential influence on ecosystem processes.

The importance of microbial community structure is further reflected in the unique roles that certain taxa, such as actinomycetes, play. Actinomycetes are a group of gram-positive bacteria (Class *Actinobacteria*) characterized by a high G+C content (12) that perform a wide array of important functions in various habitats (58), including contributing significantly to organic matter processing (59). Actinomycetes, like *Actinokineospora* (48), *Streptomyces*, *Nocardiodetes*, *Pseudonocardia*, *Nocardia*, and *Micromonospora* (73), have been found on decomposing plant litter. However, the diversity of this bacterial group on leaves decomposing in streams has not been examined using molecular methods, which can reveal community structure with higher resolution and a greater degree of completeness than traditional approaches.

Recently, in leaf decomposition studies, molecular methods have been employed to look at microbial diversity to circumvent inadequacies of culture-based methods (5, 43). Denaturing gradient gel electrophoresis (DGGE) has been used extensively for examination of complex microbial community assemblages (6, 20, 30, 46, 52). DGGE analyses are influenced by methodological limitations, as with any PCR-based approach (55), and the prevalent consensus is that major populations are reflected in the molecular fingerprint of an environmental sample (38, 41). Nevertheless, Fromin et al. (16) suggest that possible biases in DNA extraction and amplification become homogenous if identical methods are used to process samples, which are then subjected to DGGE analysis.

Our goal in this study was to assess over time the diversity of fungi, bacteria, and actinomycetes on decaying leaves of sugar maple and white oak in a small, forested stream. The leaves of these two species were selected because of differences in their

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TABLE 1. Primers used in the PCRs

Microorganism and primer ^a	16S or 18S ribosomal DNA target (positions) ^b	Sequence (5'–3') ^c	Reference
Bacteria			
F984GC	968–984	GC.-AACGCGAAGAACCTTAC	45
R1378	1378–1401	CGGTGTGTACAAGGCCCGGGAACG	22
Actinomycetes			
F243	226–243	GGATGAGCCCGCGGCCTA	22
F984GC	968–984	GC.-AACGCGAAGAACCTTAC	45
R1378	1378–1401	CGGTGTGTACAAGGCCCGGGAACG	22
Fungi			
NS1	20–38	GTAGTCATATGCTTGTCTC	71
GCfung	351–368	GC.-ATTCCCCGTTACCCGTTG	29
GC(bacteria)		<u>CGCCCGGGGCGCGCCCGGGGCGGGGCGGGGACACGGGGG</u>	45
GC(fungi)		<u>CGCCCGCCGCGCCCGCGCCCGGCCCGCCCGCCCGCCCG</u>	29

^a F, forward primer; R, reverse primer.

^b Numbering (7) denotes positions in *E. coli* for bacteria and actinomycetes and positions in fungi for *Saccharomyces cerevisiae* (GenBank accession no. J01353).

^c A G+C-rich sequence (GC.-) attached to the 5' end of sequence is indicated. Underlined sequences represent the nucleotide sequences in the GC clamps of the respective primers.

chemical composition and decomposition rates as reported in a study examining the relationship between breakdown rates and chemistry of autumn-shed leaves (47). Specifically, white oak leaves had greater lignin content, C/N ratio, and toughness than did sugar maple leaves, which decayed five times faster than oak leaves. Hence, we hypothesized that these differences would impact microbial community structure. To assess community structure, DGGE (39) was performed on PCR products representing the 18S rRNA genes of fungi and the 16S rRNA genes of bacteria. Nested PCR (37) was used to obtain actinomycete amplification products.

MATERIALS AND METHODS

Sample collection. Senescent leaves of sugar maple (*Acer saccharum*) and white oak (*Quercus alba*) collected from several trees along the west branch of the Mahoning River in northeastern Ohio were incubated in the stream channel from October 2003 to May 2004 in 3-mm-mesh litterbags. At each sampling time (1, 6, 15, 30, 60, 90, 120, 150, and 181 days), triplicate litterbags of each species were collected, brought to the laboratory on ice, and gently rinsed with distilled water; leaf disks (14-mm diameter) were excised for further analysis (described below). The dry weight of the remaining leaf material was measured and corrected for excised leaf disks. For both leaf types, decay constants, k , expressed per day, were calculated by fitting the loss of leaf mass to the exponential decay model: $y_t = y_o \times e^{-kt}$, where y_t represents the mass (dry weight) remaining at time t (in days), and y_o is the initial mass (dry weight).

To assess water conditions, selected physical and chemical variables were measured on each date. An Oakton meter (Singapore) was used to measure temperature and pH, while turbidity and conductivity were determined using a Hach turbidity meter (model 2100P) and a Hach conductivity meter (model 44600). Analysis of nitrate and nitrite (as N) and soluble reactive phosphorus concentrations were determined colorimetrically (Hach models 41100-13 and 41100-16, respectively). Dissolved organic carbon (DOC) concentration was analyzed with a TOC 5000 total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD).

Fungal biomass. Fungal biomass was estimated by extracting ergosterol from the leaves. On each sampling date, 10 leaf disks from each litterbag were blotted dry, weighed, and stored in methanol in the dark at 4°C until extraction. To extract the ergosterol, samples were heated at 65°C for 2 h, saponified with KOH, and centrifuged, and ergosterol in the supernatant was extracted with hexane (66). After evaporation of the hexane, ergosterol was dissolved in high-performance liquid chromatography-grade methanol and filtered through 0.2-µm membrane filters (Gelman Supor-200; Fisher Scientific, PA). The final ergosterol extracts were injected (10-µl injection volume) into a high-performance liquid chromatography C₁₈ column (Prevail analytical column [25 cm × 4.6 mm, 5 µm];

Alltech, Deerfield, IL) and eluted with methanol at 2.0 ml min⁻¹ at 20°C (elution time between 10 and 12.5 min). Ergosterol content was estimated by comparing the peak areas with those of external standards (0.25 to 5 mg/liter, ergosterol [Fluka and Calbiochem]). Using an ergosterol-to-biomass conversion factor of 5.5 mg/g of fungal biomass (35) and assuming that 43% of this biomass is carbon (17), the measured ergosterol contents were converted to milligrams of fungal C/gram of leaf mass (dry weight). The detection limit for ergosterol on the instrument was 0.02 mg/liter.

Bacterial biomass. Ten leaf disks were suspended in 0.1% tetrasodium pyrophosphate (Na₄P₂O₇ · 10H₂O) and sonicated at 40 KHz for 5 min in an Ultrasonic cleaner (model 2210; Branson Ultrasonics Co., Danbury, CT) to dislodge bacteria (32). Aliquots of samples preserved in PO₄-buffered saline (1× phosphate-buffered saline, pH 7.2) and paraformaldehyde (8%) were concentrated onto 0.2-µm-pore-size black polycarbonate filters (Poretics, Livermore, CA). Total bacterial number was determined with epifluorescence microscopy after staining with 15 µg/µl of DAPI (4',6-diamidino-2-phenylindole) for 3 min (50). The length and breadth for each bacterial cell were measured using Metamorph image analysis software version 4.5r4 (Molecular Devices Corporation, Downingtown, PA), and biovolume was calculated. Biovolume was converted to biomass estimates using the conversion factor of Loferer-Kröbber et al. (26), which is $CC = 218 \times V^{0.86}$, where CC is the carbon content and V is bacterial cell volume (µm³).

DNA extraction and PCR. For DNA extraction, leaf disks were frozen (-70°C) and subsequently freeze-dried. Approximately 50 mg of freeze-dried leaf material was ground in liquid nitrogen, and DNA was isolated with the PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions.

For bacteria, the primer pair F984GC (45) and R1378 (22) was used, whereas a nested PCR approach was used for actinomycetes (Table 1) (primers from Sigma-Genosys, St. Louis, MO). For this group, primer F243 (22) that specifically amplifies actinomycete 16S rRNA genes was used with the reverse primer R1378 to amplify a fragment from positions 226 to 1401. The products from primer pair F243-R1378 were then used as a template for a second PCR with primer pair F984GC-R1378, which allowed us to obtain a fragment from positions 968 to 1401 which is suitable for DGGE. For the fungi, the primer pair NS1 (71) and the fungus-specific primer GCfung (29) were used to amplify the 5' end (370 bp) of the 18S rRNA gene. Both F984GC and GCfung had GC clamps, as is needed for optimal DGGE analysis (13).

PCR (54) was performed with a PTC-100 programmable thermal controller (M.J. Research, Inc., Watertown, MA) using Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ) with a 0.8 µM concentration of each primer and 2 µl of the eluted template DNA. Positive and negative controls were run for every PCR.

The reaction conditions for bacterial PCR and the nested PCR for actinomycetes followed the protocol of Heuer et al. (22) as modified by van Dillewijn et al. (67). For actinomycetes, the nested PCR consisted of two rounds of amplification. In the first step, primers F243 and R1378 were used, and in the second

TABLE 2. Physical and chemical variables measured over the course of the study

Day	Temp (°C) ^a	pH ^a	DOC ^b (mg/liter)	NO ₃ + NO ₂ (mg/liter) ^a	SRP ^c (mg/liter) ^a	Conductivity (μS/cm) ^a	Turbidity (NTU) ^d
0	21.4 ± 0.28	7.4 ± 0.00	11.2	0.06 ± 0.01	0.20 ± 0.01	588 ± 10.27	3 ± 0.06
1	12.9 ± 0.00	7.4 ± 0.00	9.3	0.07 ± 0.01	0.17 ± 0.02	625 ± 1.76	5 ± 0.82
6	11.8 ± 0.00	7.4 ± 0.00	11.2	0.06 ± 0.01	0.19 ± 0.06	640 ± 1.00	2 ± 0.04
15	5.6 ± 0.03	7.4 ± 0.01	8.6	0.09 ± 0.01	0.23 ± 0.04	546 ± 1.86	9 ± 3.30
30	3.6 ± 0.00	7.1 ± 0.01	34.8	0.06 ± 0.01	0.13 ± 0.01	534 ± 51.70	12 ± 0.36
60	3.3 ± 0.00	7.1 ± 0.02	5.4	0.29 ± 0.02	0.12 ± 0.01	524 ± 10.68	37 ± 1.85
90	0.7 ± 0.00	8.0 ± 0.12	11.6	0.48 ± 0.02	0.21 ± 0.04	1,777 ± 97.33	5 ± 0.13
120	2.3 ± 0.00	7.3 ± 0.06	14.8	0.31 ± 0.06	0.19 ± 0.04	526 ± 4.33	8 ± 0.37
150	8.8 ± 0.00	7.2 ± 0.01	12.9	0.08 ± 0.01	0.21 ± 0.04	463 ± 2.40	58 ± 0.69
181	15.4 ± 0.03	7.8 ± 0.04	7.8	0.12 ± 0.03	0.04 ± 0.01	607 ± 33.24	4 ± 0.34

^a Mean values ($n = 3$) ± 1 standard error are shown.

^b DOC, dissolved organic carbon (measured on a single sample).

^c SRP, soluble reactive phosphorus.

^d NTU, nephelometric turbidity units.

amplification reaction, 2 μl of the first PCR product was used as a template against the primer pair F984GC and R1378. Reaction conditions were similar to those for the bacterial PCR; the only difference was in annealing temperature, which was 63°C for primers F243 and R1378 in the first PCR and 53°C for primers F984GC and R1378 in the second reaction as in the bacterial PCR. Fungal PCR was performed by the method of Nikolcheva et al. (42) with slight modification of the primer concentration. Amplification products were checked on a 1% agarose gel in 0.5× Tris-borate-EDTA buffer at the end of each PCR.

Denaturing gradient gel electrophoresis. The DCode system (Bio-Rad Laboratories, Hercules, CA) was used to analyze the PCR products (433 bp for bacteria and actinomycetes and 370 bp for fungi). Samples were loaded in 6% (bacteria and actinomycetes) or 8% (wt/vol) (for fungi) polyacrylamide gels in 1× Tris-acetate-EDTA (TAE) or 0.5× TAE, respectively. For bacteria and actinomycetes, the denaturant gradient was 30 to 60%, and electrophoresis was performed for 12 h at 70V. As for fungal DGGE, a gradient of 25 to 55% was used at 50 V for 16 h. Fungal DGGE was performed by the method of Nikolcheva et al. (42), with slight modification, and a modified protocol of Heuer et al. (22) was followed for bacteria and actinomycetes. All replicates were

analyzed for the leaves for each species and sampling day, unless no amplifiable DNA was obtained.

A 100-bp molecular ladder (Promega, Madison, WI) was loaded on either end of each gel. Controls with known concentrations of PCR products from fungal, bacterial, or actinomycete isolates were run between samples. For bacteria and actinomycetes, known concentrations of purified PCR products of *Escherichia coli* and *Streptomyces californicus* were used. For fungi, purified PCR products of isolates obtained from the leaves (11) that were used were *Sirococcus conigenus* isolate H3 (GenBank accession no. DQ310765), *Hypocrea* sp. strain H5 (GenBank accession no. DQ310767), *Anguillospora longissima* isolate H28 (GenBank accession no. DQ310784), and *Tetrachaetum elegans* isolate H30 (GenBank accession no. DQ310786).

After electrophoresis, gels were removed and stained in SYBR gold (1:10,000 dilution), and digital images were taken using a Gel Doc imaging system (Bio-Rad Laboratories, Hercules, CA).

According to Muyzer et al. (40) and van Hannen et al. (68), a band in a denaturing gel represents a distinct "sequence type" or "phylogroup." In our study, each band was considered a discrete ribotype (42). There was a high degree of gel reproducibility based on migration of standards. Therefore, a binary matrix was

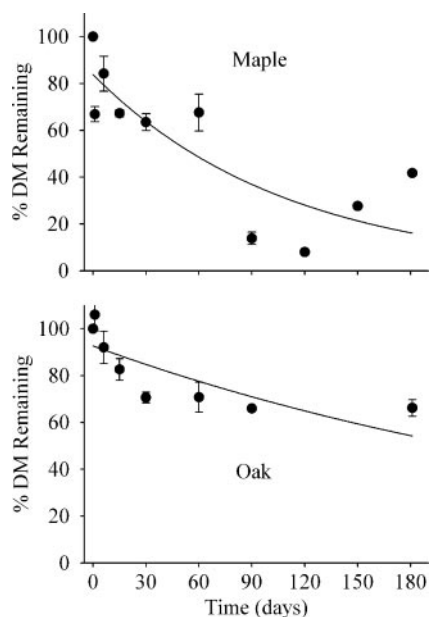


FIG. 1. Decomposition of sugar maple and white oak leaves during the study. The percent remaining dry mass (%DM) of leaves is given. Values are given as means ± 1 standard error (error bars).

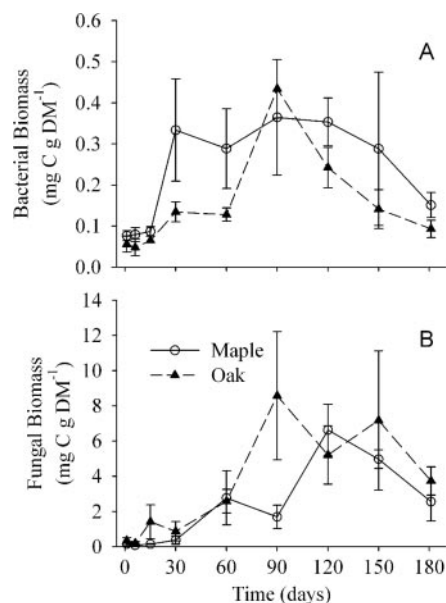


FIG. 2. Bacterial biomass (A) and fungal biomass (B) on sugar maple and white oak leaves. Both bacterial and fungal biomass are expressed in milligrams of C/gram of leaf dry mass (DM). Values are given as means ± 1 standard error (error bars).

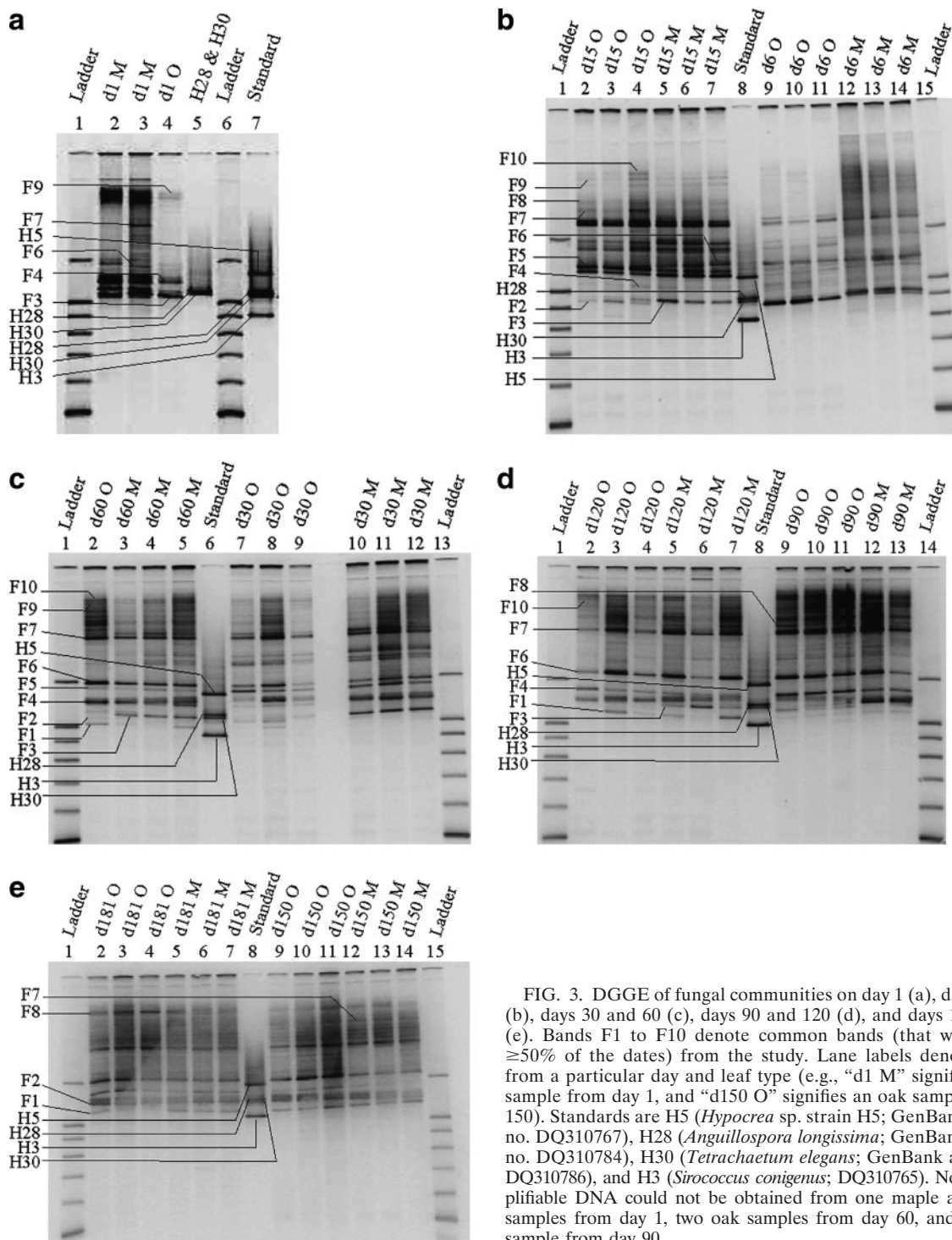


FIG. 3. DGGE of fungal communities on day 1 (a), days 6 and 15 (b), days 30 and 60 (c), days 90 and 120 (d), and days 150 and 181 (e). Bands F1 to F10 denote common bands (that were present $\geq 50\%$ of the dates) from the study. Lane labels denote samples from a particular day and leaf type (e.g., “d1 M” signifies a maple sample from day 1, and “d150 O” signifies an oak sample from day 150). Standards are H5 (*Hypocrea* sp. strain H5; GenBank accession no. DQ310767), H28 (*Anguillospora longissima*; GenBank accession no. DQ310784), H30 (*Tetrachaetum elegans*; GenBank accession no. DQ310786), and H3 (*Sirococcus conigenus*; DQ310765). Note that amplifiable DNA could not be obtained from one maple and two oak samples from day 1, two oak samples from day 60, and one maple sample from day 90.

constructed for each microbial community based on the presence and absence of bands. The matrix was used to calculate Jaccard’s coefficient (a similarity measure) with MVSP version 3.1a (Kovach Computing Services, Pentraeth, Wales, United Kingdom). The data were subjected to clustering based on the unweighted pair group method using arithmetic averages (UPGMA) to identify samples that generated patterns similar to each other (6, 24). Two-way analysis of variance (ANOVA) (with time of exposure and leaf type as treatments) was used to evaluate temporal changes in ribotype richness and microbial biomass.

Correlations between microbial biomass and environmental variables were assessed using Pearson correlations.

RESULTS

Physical and chemical measurements. The measured physicochemical variables showed typical seasonal variations dur-

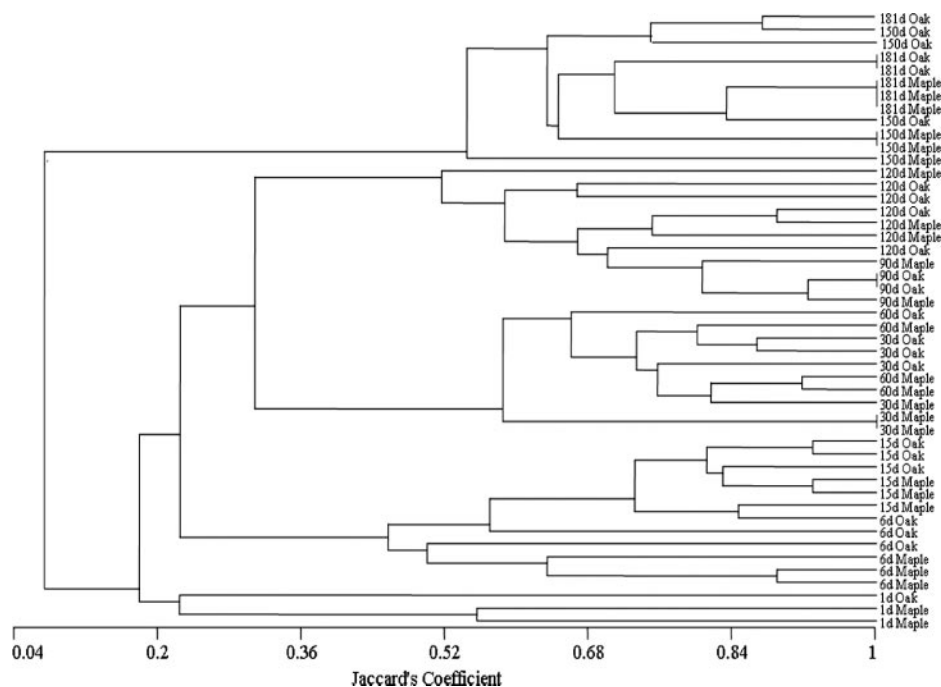


FIG. 4. UPGMA clustering of fungal DGGE bands according to Jaccard's coefficient. Short vertical lines at the end of branches signify that the patterns in those samples are identical. The sampling date (day 1 [1d] to day 181 [181d]) and leaf type are given at the end of branches.

ing the course of the study (Table 2). The water temperature was highest (21.7°C) at the beginning of the study, decreasing to the lowest values (0.7°C) on day 90. Overall, pH remained stable during the study. Conductivity usually did not fluctuate greatly except for a maximum on day 90, which may be attributable to runoff from road salt upstream of the study site. This is supported by high values of Na⁺ and Cl⁻ ion concentrations on day 90 (11). Turbidity ranged from approximately 2 to 60 nephelometric turbidity units, and the higher values were recorded when substantial precipitation preceded the sampling date. Soluble reactive phosphorus varied between 0.03 and 0.28 mg/liter, and nitrate and nitrite (as N) varied between 0.04 and 0.5 mg/liter with a peak on day 90. DOC concentrations were as low as 5.4 mg/liter and as high as 34.8 mg/liter on day 30.

Leaf decomposition and microbial biomass. The decay constant for sugar maple was 0.009/day, which was three times that of white oak (0.003/day). Overall, mass loss in maple was significantly greater than in oak (Fig. 1, ANOVA, $P < 0.05$). The coefficient of determination values for the exponential decay model were 0.69 and 0.61 for maple and oak, respectively.

On maple leaves, bacterial biomass quickly peaked in the first part of the study but then decreased gradually, while on oak, it peaked later and subsequently decreased rapidly (Fig. 2A). During the first 15 days of the study, bacterial biomass on sugar maple leaves was significantly higher than on oak leaves (ANOVA, with Tukey's post-hoc comparisons, $P < 0.05$), but as the study progressed, large variation among replicate leaf packs was observed and there were no statistical differences in biomass between maple and oak leaves. Bacterial biomass was significantly negatively correlated with temperature ($r = -0.535$; $P < 0.01$) and positively correlated with nitrate and nitrite (as N) concentrations ($r = 0.537$; $P < 0.05$).

For fungal biomass on maple leaves, there was a steady increase initially; biomass reached a maximum on day 120 after which it declined (Fig. 2B). On oak, the peak was earlier than on maple. Yet, there were no significant differences in fungal biomass between the leaves of the two species ($P = 0.121$), while there were significant differences among dates ($P < 0.05$). Fungal biomass was significantly correlated with nitrate and nitrite (as N) concentration ($r = 0.290$; $P < 0.05$). In addition, bacterial and fungal biomass were significantly correlated with each other ($r = 0.329$; $P < 0.05$), and on most dates, fungal biomass was 10 times higher than bacterial biomass.

DGGE. For fungi, 33 ribotypes were found throughout the study of which 10 ribotypes (labeled F1 to F10 in Fig. 3) occurred frequently; bands were considered common if they were found on $\geq 50\%$ of the dates. While some bands were more common on oak than maple (F1, F2, and F8), the patterns of occurrence of the rest of these bands (F3 to F7) were similar for the leaf types. Bands F2 and F3 had the same migration distances as the standards H30 and H28, respectively. UPGMA of the DGGE gels was used to examine temporal changes and differences in the leaves of the two species. In the fungal UPGMA dendrogram, maple and oak samples did not cluster separately; rather, samples clustered on the basis of sampling date (Fig. 4). There were two primary clusters: a small group encompassing samples from days 150 and 181 and a larger cluster representing the other dates. Typically, patterns were similar between samples collected on successive sampling dates.

Bacterial DGGE revealed 30 ribotypes of which 5 were common bands (labeled B1 to B5 in Fig. 5). Of the five bands, four (B2, B3, B4, and B5) were found on almost all dates, while band B1 was interspersed throughout the study. Ribotype B4

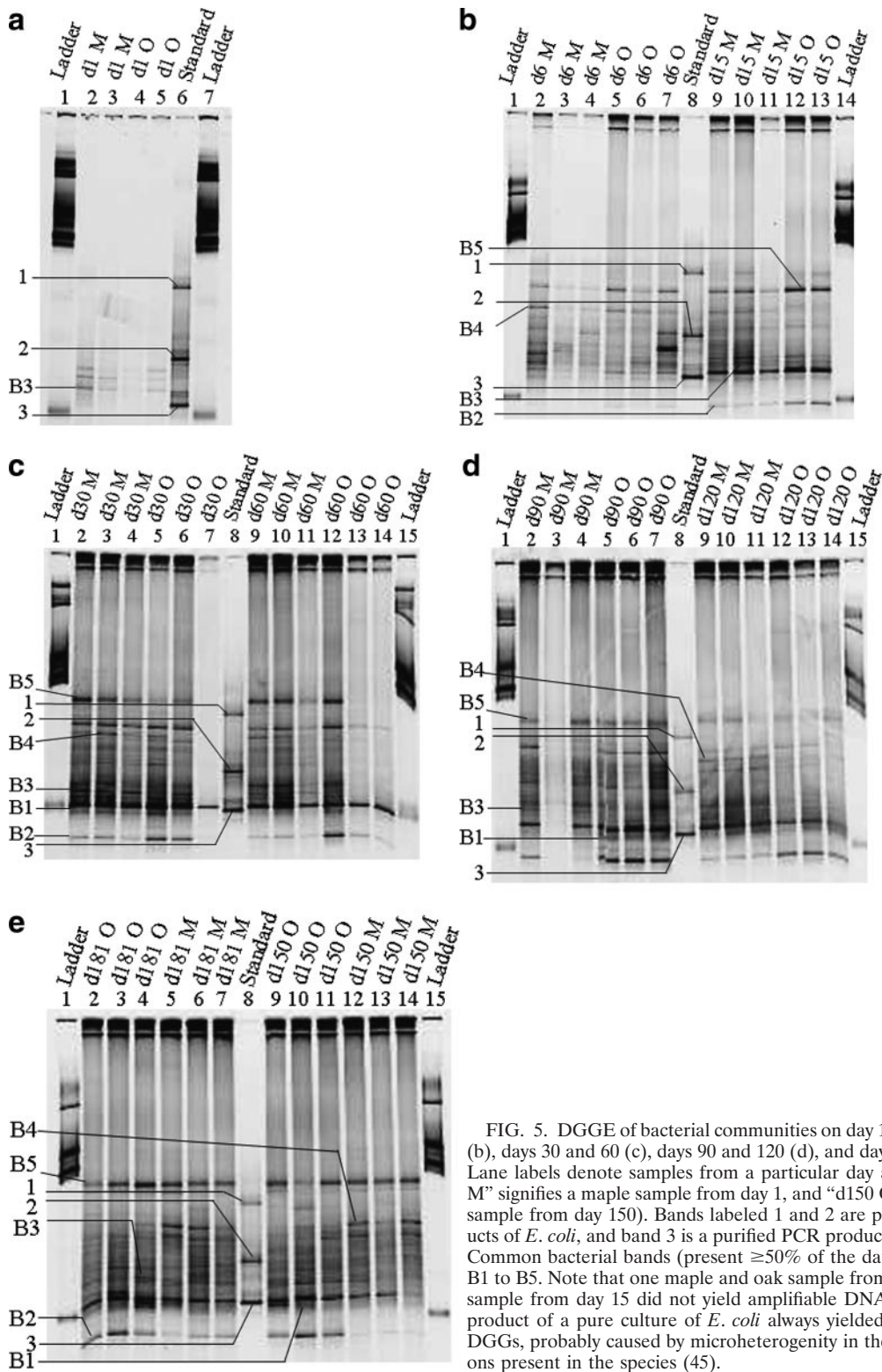


FIG. 5. DGGE of bacterial communities on day 1 (a), days 6 and 15 (b), days 30 and 60 (c), days 90 and 120 (d), and days 150 and 181 (e). Lane labels denote samples from a particular day and leaf type (“d1 M” signifies a maple sample from day 1, and “d150 O” signifies an oak sample from day 150). Bands labeled 1 and 2 are purified PCR products of *E. coli*, and band 3 is a purified PCR product of *S. californicus*. Common bacterial bands (present $\geq 50\%$ of the dates) are labeled as B1 to B5. Note that one maple and oak sample from day 1 and an oak sample from day 15 did not yield amplifiable DNA. A purified PCR product of a pure culture of *E. coli* always yielded two bands in the DGGEs, probably caused by microheterogeneity in the 16S rRNA operons present in the species (45).

occurred more frequently on maple leaves than on oak leaves, and B1 colonized oak to a greater extent, while the rest occurred equally on maple and oak leaves. The UPGMA tree for the bacterial community did not reveal any marked differenti-

ation on the basis of leaf type (Fig. 6). Samples collected on the same or successive dates generally clustered together but not as cleanly as for the fungi.

As observed for the fungal and bacterial communities, the

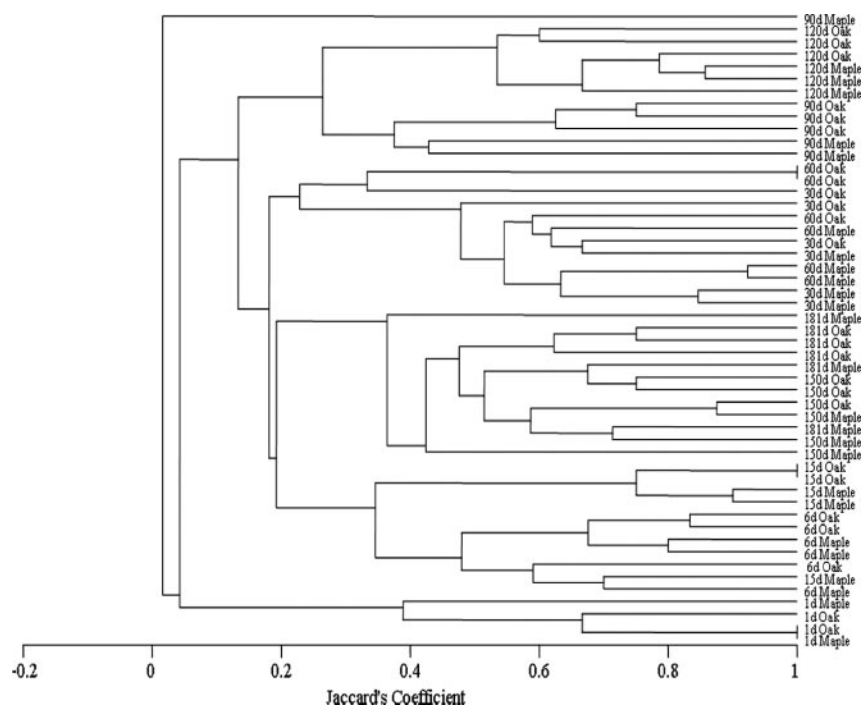


FIG. 6. UPGMA clustering of bacterial DGGE bands according to Jaccard's coefficient. Short vertical lines at the end of branches signify that the patterns in those samples are identical. The sampling date (day 1 [1d] to day 181 [181d]) and leaf type are given at the end of branches.

actinomycete DGGE also did not show any leaf-specific differences (Fig. 7). Eighteen ribotypes were detected of which seven (labeled A1 to A7 in Fig. 7) were common. Two of these bands (A2 and A4) were more common on oak than on maple, and two (A6 and A7) were found more frequently on maple than on oak. The patterns of occurrence of ribotypes A1, A3, and A5 were similar on leaves of both species. Band A1 had the same migration distance as *S. californicus*. Out of 18 bands, 10 had similar electrophoretic mobilities as ribotypes observed in the bacterial DGGE. UPGMA of the actinomycete community revealed two primary clusters as in the fungi: one, representing the communities on days 150 and 181 and the other consisting of samples from the other dates (Fig. 8). Overall, maple samples from a particular day grouped together as did oak samples. However, no major clusters consisting only of maple or oak samples were observed for the actinomycete community.

Overall, there were statistically significant differences in ribotype richness across all three microbial groups on different days ($P < 0.05$) but not between leaf species (Fig. 9). The numbers of ribotypes of fungi were significantly higher than those of bacteria and actinomycetes ($P < 0.05$). In general, bacterial and actinomycete ribotype abundances were not significantly different from each other. By the end of the study, these values were similar for all three groups of microorganisms.

DISCUSSION

The decay rates of maple and oak leaves we determined are comparable to those reported from other studies which also found oak leaves to be processed more slowly than maple

leaves (47, 53). The exponential decay model explained 69 and 61% of the variability for maple and oak leaves, respectively, in our study. Other factors, like scouring and fragmentation caused by stream flow, especially after precipitation (on days 30, 60, and 150), were likely responsible for the variation that could not be accounted for by the model.

Although the initial patterns of decay were comparable between the two leaf types, maple leaves decomposed more consistently during the study than did oak leaves. White oak leaves have a higher lignin content and greater toughness than sugar maple leaves that should require greater microbial action to breakdown (47). Yet, in spite of these differences in decomposition and known differences in leaf chemistry (47), few differences in the microbial community were detected between the leaf species. For example, fungal biomass did not differ between the leaf types, although peak fungal biomass estimates were within the range of values reported in the literature for leaves in streams (2, 14, 70).

Overall, bacterial biomass values obtained in our study were comparable to those in other studies (2, 14, 70). In a study by Baldy et al. (1), the greatest bacterial biomass was observed on leaves of the London plane tree (*Platanus hybrida*), the species with the slowest breakdown rate in their study. The low rates of fungal colonization on the lignin-rich London plane leaves most likely allowed for the greater than expected bacterial biomass in their study. However, on the basis of our results, we suggest that the increased availability of colonizable physical substrate (due to faster leaf decay and fragmentation) in sugar maple leaves in the initial phase of the study allowed for greater bacterial biomass on sugar maple leaves compared to oak leaves, as fungal biomass on the leaves of the two species was not different. In

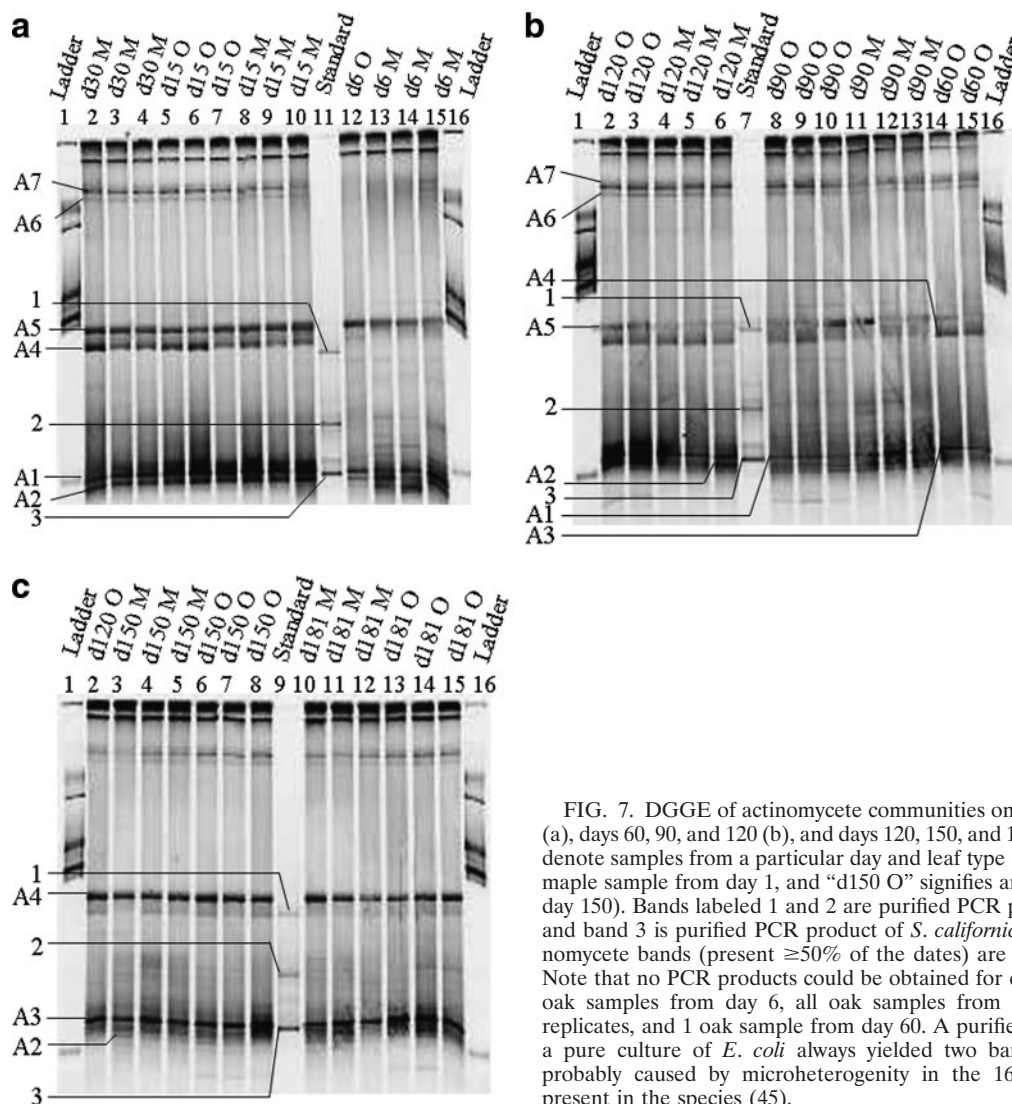


FIG. 7. DGGE of actinomycete communities on days 6, 15, and 30 (a), days 60, 90, and 120 (b), and days 120, 150, and 181 (c). Lane labels denote samples from a particular day and leaf type (“d1 M” signifies a maple sample from day 1, and “d150 O” signifies an oak sample from day 150). Bands labeled 1 and 2 are purified PCR products of *E. coli*, and band 3 is purified PCR product of *S. californicus*. Common actinomycete bands (present $\geq 50\%$ of the dates) are labeled A1 to A7. Note that no PCR products could be obtained for day 1 samples, two oak samples from day 6, all oak samples from day 30, all maple replicates, and 1 oak sample from day 60. A purified PCR product of a pure culture of *E. coli* always yielded two bands in the DGGEs probably caused by microheterogeneity in the 16S rRNA operons present in the species (45).

addition, variability of bacterial biomass on sugar maple leaves suggests that as it decays, it can provide a more dynamic and variable environment than oak leaves.

We observed greater fungal biomass than bacterial biomass throughout our study, as has been reported by others (2, 14, 21, 70). Greater fungal biomass likely occurs because fungi are more efficient than bacteria (except for a few tunneling bacteria [10, 49, 69]) in exploiting available resources through invasion and enzymatic hydrolysis of leaf material and lysed hyphae (9, 56). Bacteria can benefit from the compounds released by this process and take advantage of the increased surface area provided by the macerated plant tissue (through fungal action) and fungal hyphae for colonization (21). This hypothesis is supported by the correlation between fungal and bacterial biomass we observed. Biomass estimations reported here for fungi and bacteria are based on different methods, following the approach of earlier studies (1, 2), suggesting that direct comparisons of the two groups be made with caution. For bacteria, conversion of biovolume to biomass via allometric or linear conversion factors (51) was utilized, but this is not feasible for

fungi. This is primarily because estimation of fungal biomass from biovolume based on hyphal length measurements suffers from inherent biases and uncertainties (see reference 18 for a detailed review on fungal biomass estimation methods). Therefore, ergosterol is used as an indicator molecule for fungal biomass (18).

The effects of environmental variables on microbial communities are routinely evaluated to explain changes in biomass and community structure (44, 70). Among physical variables, the role of temperature in controlling bacterial biomass on decaying litter is not consistent across studies, with some reporting significant temperature effects (70) and others reporting no effect of temperature (14). We found higher bacterial biomass on the colder sampling dates and attribute this to either the low-temperature optima of the litter-associated bacteria or an increase in useable area for bacteria at that stage of leaf decay. Microbial biomass was related positively to dissolved inorganic nitrogen concentrations in our study, which supports the idea that nutrient concentrations in the water column can be a limiting factor for microbial activity (34, 64).

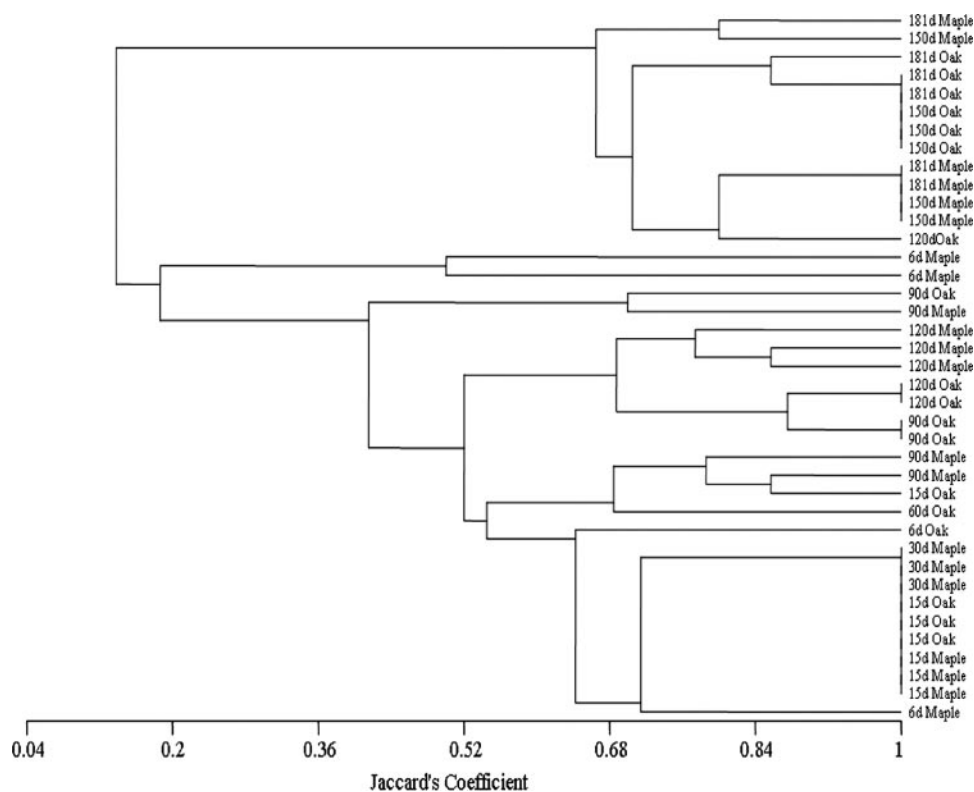


FIG. 8. UPGMA clustering of actinomycete DGGE bands according to Jaccard's coefficient. Short vertical lines at the ends of branches signify that the patterns in those samples are identical. The sampling date (day 6 [6d] to day 181 [181d]) and leaf type are given at the ends of branches.

However, the stronger correlation with bacterial biomass suggests that bacteria, which mostly exist in a biofilm (unlike the invasive fungal mycelia), may have depended more on the dissolved nitrogen, while fungi could perhaps utilize N from both leaves and the overlying water column (62).

UPGMA analyses of DGGE profiles revealed that the microbial community fingerprints were similar between the two leaf species throughout the study. For the fungal assemblage, the patterns from the latter stages of decomposition differed from that of the rest of the study. This is attributable to the disappearance of some of the common bands that resulted in a community with diminished ribotype richness. Temporal changes in the actinomycete assemblage were also explained by a loss of some of the common ribotypes during the later part of the study. Thus, the fungal and actinomycete assemblages were characterized by successional changes where an initial establishment of ribotypes was ultimately followed by the loss of some common types from the leaf assemblage. However, for bacteria, although the common ribotypes persisted, there was both a loss and reappearance of a few bands by the end of the study that were present earlier. The niches left unutilized by the loss of the common fungal and actinomycete ribotypes may have allowed those bacterial ribotypes to colonize the leaf biofilm again. Overall, the ribotypes that disappeared were probably unable to persist as the leaf resources became limiting. Although we do not know what proportion of bands from the bacterial DGGE represent actinomycetes, it is possible that this group makes a significant contribution to the overall bacterial community (based on previous research as well as simi-

larities in the electrophoretic mobilities of bands on the bacterial and actinomycete DGGE gels), as we observed some actinomycete ribotypes that had similar migration distances to bands found in the bacterial DGGE. The role of actinomycetes in streams is largely unexplored, and we suggest that future research address the contribution of this bacterial taxon to decay of leaf litter in streams. Specifically, actinomycetes like *Streptomyces*, *Micromonospora*, *Actinomadura*, and *Pseudonocardia* play a significant role in the degradation of lignocellulose (reviewed by McCarthy [31]). Tamura et al. (65) found *Actinokineospora* spp. in plant litter in soil, while Wohl and McArthur (73) documented the presence of a suite of actinomycete genera from submersed macrophytes. This is not surprising, considering the importance of this bacterial group in the transformation of organic matter (58, 59).

The fungal community on plant litter decomposing in aquatic ecosystems is largely dominated by aquatic hyphomycetes (72), but other fungal groups like Ascomycota (8, 19), Oomycota, Zygomycota (3), and Chytridiomycota have also been reported [43]). Using taxon-specific primers, Nikolcheva and Bärlocher (43) demonstrated the presence of these major fungal taxa and found ascomycetes to be the main group of fungi colonizing decaying leaves in streams. This group includes both aquatic hyphomycetes and ascomycetes that reproduce via ascospores (instead of conidia like the hyphomycetes). In our study stream, the majority of fungal isolates cultured from the decaying leaves were ascomycetes including only two hyphomycetes (11). Using DGGE, Nikolcheva et al. (42) found *Articulospora tetracladia* to be the dominant ribotype on decay-

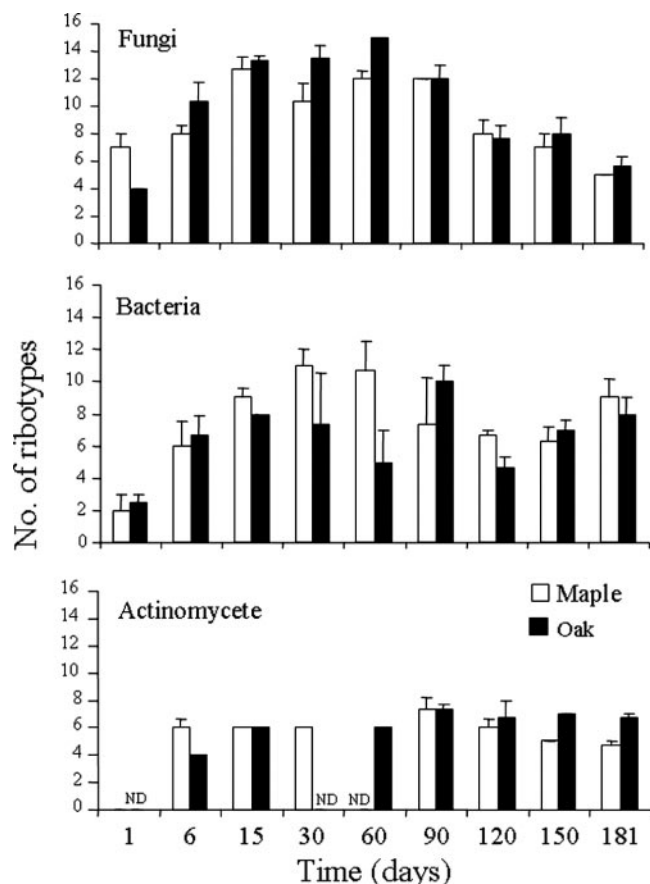


FIG. 9. Number of ribotypes based on DGGE bands from fungal, bacterial, and actinomycete communities on sugar maple and white oak leaves. ND, no data.

ing leaves on the basis of migration distance in DGGE. Similarly, we observed bands that had electrophoretic mobilities similar to those of two aquatic hyphomycete isolates, *Tetrachaetium elegans* isolate H30 (GenBank accession no. DQ310786) and *Anguillospora longissima* isolate H28 (GenBank accession no. DQ310784). This suggests that these ribotypes in our study may also belong to the same broad taxonomic group.

In both aquatic and terrestrial environments, bacteria from the *Cytophaga-Flavobacterium-Bacteroidetes* group have been implicated in the degradation of lignocellulosic litter due to their ability to degrade complex biopolymers (27). This group was found to be dominant in salt-marsh sediments (27) and was one of the major bacterial groups detected in blades of *Spartina alterniflora* mostly during later stage of decay (8). In our study system, we have isolated members for the *Cytophaga-Flavobacterium-Bacteroidetes* group from decomposing leaves (11), and previous studies at this site have identified members of the *Proteobacteria* from biofilms on senescent maple leaves (33). Suberkropp and Klug (63) found mostly gram-negative bacteria (*Flexibacter*, *Flavobacterium*, *Cytophaga*, *Achromobacter*, and *Pseudomonas* species) on decaying leaves in a woodland stream in Michigan; it is unknown why actinomycetes were not recovered to a greater extent in that study.

In contrast to our hypothesis, leaf types of differing quality did not support substantially different microbial communities.

This suggests that the microbes present on the decomposing leaves were generalists capable of exploiting a range of resources and able to perform well on either leaf substrate. Moreover, the results suggest that leaf chemistry is less important than the stage of decomposition and time of exposure in determining microbial community structure. Interestingly, in spite of the large differences in the decomposition of the two leaf species, similar microbial community structure was detected on both leaf types. This suggests that differences in decomposition are attributable to differences in physical fragmentation (due to differences in the toughness of the leaves of the two species) or differences in the activity of the same suite of microorganisms (production of enzymes, secondary production, etc.), rather than differences in the biotic community (macroinvertebrate communities on the leaves were also not different from each other [data not shown]). We believe that future studies on microbial colonization of decomposing litter in aquatic environments will benefit from an examination of the ecological nature (generalists versus specialists) of the microorganisms and state of leaf decay in addition to characterization of the structural and chemical attributes of the leaf substrates.

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REFERENCES

- Baldy, V., E. Chauvet, and M. O. Gessner. 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. *Oikos* 74:93–102.
- Baldy, V., E. Chauvet, J. Y. Charcosset, and M. O. Gessner. 2002. Microbial dynamics associated with leaves decomposing in the mainstem and floodplain of a large river. *Aquat. Microbiol. Ecol.* 28:25–36.
- Bärlocher, F., and B. Kendrick. 1974. Dynamics of fungal populations on leaves in a stream. *J. Ecol.* 62:761–791.
- Bärlocher, F. 1985. The role of fungi in the nutrition of stream invertebrates. *Bot. J. Linn. Soc.* 91:83–94.
- Blackwood, C. B., T. Marsh, S.-H. Kim, and E. A. Paul. 2003. Terminal restriction fragment length polymorphism data analysis for quantitative comparison of microbial communities. *Appl. Environ. Microbiol.* 69:926–932.
- Boon, N., W. De Windt, W. Verstraete, and E. M. Top. 2002. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol. Ecol.* 39:101–112.
- Brosius, J., T. J. Dull, D. D. Sleeter, and H. F. Noller. 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J. Mol. Biol.* 148:107–127.
- Buchan, A., S. Y. Newell, M. Butler, E. J. Biers, J. T. Hollibaugh, and M. A. Moran. 2003. Dynamics of bacterial and fungal communities on decaying salt marsh grass. *Appl. Environ. Microbiol.* 69:6676–6687.
- Chamier, A. C. 1985. Cell-wall degrading enzymes of aquatic hyphomycetes: a review. *Bot. J. Linn. Soc.* 91:67–81.
- Daniel, G. F., T. Nilsson, and A. P. Singh. 1987. Degradation of lignocelluloses by unique tunnel-forming bacteria. *Can. J. Microbiol.* 33:943–948.
- Das, M. 2006. Microbial community structure and interactions in leaf litter in a stream. Ph.D. dissertation. Kent State University, Kent, Ohio.
- Embley, T. M., and E. Stackebrandt. 1994. The molecular phylogeny and systematics of the actinomycetes. *Annu. Rev. Microbiol.* 48:257–289.
- Ferris, M. J., G. Muyzer, and D. M. Ward. 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl. Environ. Microbiol.* 62:340–346.
- Findlay, S., J. Tank, S. Dye, H. M. Valett, P. J. Mulholland, W. H. McDowell, S. L. Johnson, S. K. Hamilton, J. Edmonds, W. K. Dodds, and W. B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detrital pools of headwater streams. *Microb. Ecol.* 43:55–66.
- Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecol. Monogr.* 43:421–439.

16. Fromin, N., J. Hamelin, S. Tarnawski, D. Roesti, K. Jourdain-Miserez, B. Forestier, S. Teyssier-Cuvelle, F. Gillet, M. Aragno, and P. Rossi. 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environ. Microbiol.* **4**:634–643.
17. Gessner, M. O., and E. Chauvet. 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Appl. Environ. Microbiol.* **59**:502–507.
18. Gessner, M. O., and S. Y. Newell. 2002. Biomass, growth rate, and production of filamentous fungi in plant litter, p. 390–408. In C. J. Hurst, R. L. Crawford, G. Knudsen, M. McInerney, and L. D. Stetzenbach (ed.), *Manual of environmental microbiology*, 2nd ed. American Society for Microbiology, Washington, DC.
19. Goh, T. K., and K. D. Hyde. 1996. Biodiversity of freshwater fungi. *J. Ind. Microbiol.* **17**:328–345.
20. Griffiths, R. I., A. S. Whiteley, A. G. O'Donnell, and M. J. Bailey. 2003. Influence of depth and sampling time on bacterial community structure. *FEMS Microbiol. Ecol.* **43**:35–43.
21. Gulis, V., and K. Suberkropp. 2003. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microb. Ecol.* **45**:11–19.
22. Heuer, H., M. Krsek, P. Baker, K. Smalla, and E. M. H. Wellington. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* **63**:3233–3241.
23. Hieber, M., and M. O. Gessner. 2002. Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. *Ecology* **83**:1026–1038.
24. Ihekwe, A. M., S. K. Papiernik, J. Gan, S. R. Yates, C.-H. Yang, and D. E. Crowley. 2001. Impact of fumigants on soil microbial communities. *Appl. Environ. Microbiol.* **67**:3245–3257.
25. Kaushik, N. K., and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. *Arch. Hydrobiol.* **68**:465–515.
26. Loferer-Krösbacher, M., J. Kilma, and R. Psenner. 1998. Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis. *Appl. Environ. Microbiol.* **64**:688–694.
27. Lydell, C., L. Dowell, M. Sikaroodi, P. Gillevet, and D. Emerson. 2004. A population survey of the members of the phylum *Bacteroidetes* isolated from salt marsh sediments along the east coast of the United States. *Microb. Ecol.* **48**:263–273.
28. Maamri, A., F. Bärlocher, E. Pattee, and H. Chergui. 2001. Fungal and bacterial colonization of *Salix pedicellata* leaves decaying in permanent and intermittent streams in Eastern Morocco. *Int. Rev. Hydrobiol.* **86**:337–348.
29. May, L. A., B. Smiley, and M. G. Schmidt. 2001. Comparative denaturing gradient gel electrophoresis of fungal communities associated with whole plant corn silage. *Can. J. Microbiol.* **47**:829–841.
30. McCaig, A. E., L. A. Glover, and J. I. Prosser. 2001. Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. *Appl. Environ. Microbiol.* **67**:4554–4559.
31. McCarthy, A. J. 1987. Lignocellulose-degrading actinomycetes. *FEMS Microbiol. Rev.* **46**:145–163.
32. McNamara, C. J., M. J. Lemke, and L. G. Leff. 2002. Culturable and non-culturable fractions of bacterial populations in sediments of a South Carolina stream. *Hydrobiologia* **482**:151–159.
33. McNamara, C. J., and L. G. Leff. 2004. Bacterial community composition in biofilms on leaves in a northeastern Ohio stream. *J. N. Am. Benthol. Soc.* **23**:677–685.
34. Meyer, J. L., and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biol.* **13**:177–183.
35. Mille-Lindblom, C., and L. J. Tranvik. 2003. Antagonism between bacteria and fungi on decomposing aquatic plant litter. *Microb. Ecol.* **45**:173–182.
36. Minshall, G. W. 1967. Role of allochthonous detritus in the trophic structure of an alpine springbrook community. *Ecology* **48**:139–149.
37. Mullis, K. B., and F. A. Faloon. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* **155**:335–350.
38. Muyzer, G., E. C. de Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **59**:695–700.
39. Muyzer, G., and E. C. de Waal. 1994. Determination of the genetic diversity of microbial communities using DGGE analysis of PCR-amplified 16S rRNA. *NATO ASI Ser. G* **35**:207–214.
40. Muyzer, G., A. Teske, C. O. Wirsen, and H. W. Jannasch. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch. Microbiol.* **164**:165–172.
41. Muyzer, G., and K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Leeuwenhoek* **73**:127–141.
42. Nikolcheva, L. G., A. M. Cockshutt, and F. Bärlocher. 2003. Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Appl. Environ. Microbiol.* **69**:2548–2554.
43. Nikolcheva, L. G., and F. Bärlocher. 2004. Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycol. Prog.* **3**:41–49.
44. Nikolcheva, L. G., and F. Bärlocher. 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environ. Microbiol.* **7**:270–280.
45. Nübel, U., B. Engelen, A. Felske, J. Snaidr, A. Weisuber, R. I. Amman, W. Ludwig, and W. Backhaus. 1996. Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *J. Bacteriol.* **178**:5636–5643.
46. Nübel, U., F. Garcia-Pichel, M. Kühl, and G. Muyzer. 1999. Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. *Appl. Environ. Microbiol.* **65**:422–430.
47. Ostrofsky, M. L. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *J. N. Am. Benthol.* **16**:750–759.
48. Otaguro, M., M. Hayakawa, T. Yamazaki, and Y. Iimura. 2001. An integrated method for the enrichment and selective isolation of *Actinokineospora* spp. in soil and plant litter. *J. Appl. Microbiol.* **91**:118–130.
49. Porter, D., S. Y. Newell, and W. L. Lingle. 1989. Tunneling bacteria in decaying leaves of a seagrass. *Aquat. Bot.* **35**:395–401.
50. Porter, K. G., and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**:943–948.
51. Posch, T., M. Loferer-Krösbacher, G. Gao, A. Alfreider, J. Pernthaler, and R. Psenner. 2001. Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors. *Aquat. Microb. Ecol.* **25**:55–63.
52. Reche, I., E. Pulido-Villena, R. Moraes-Baquero, and E. O. Casamayor. 2005. Does ecosystem size determine aquatic bacterial richness? *Ecology* **86**:1715–1722.
53. Rowe, J. M., S. K. Meegan, E. S. Engstrom, S. A. Perry, and W. B. Perry. 1996. Comparison of leaf processing rates under different temperature regimes in three headwater streams. *Freshwater Biol.* **36**:277–288.
54. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* **239**:487–491.
55. Schäfer, H., and G. Muyzer. 2001. Denaturing gradient gel electrophoresis in marine microbial ecology. *Methods Microbiol.* **30**:425–468.
56. Shearer, C. A. 1992. The role of woody debris, p. 77–98. In F. Bärlocher (ed.), *The ecology of aquatic hyphomycetes*. Springer-Verlag, Berlin, Germany.
57. Sinsabaugh, R. L., and A. E. Linkins. 1990. Enzymatic and chemical analysis of particulate organic matter from a boreal river. *Freshwater Biol.* **23**:301–309.
58. Smalla, K., N. Cresswell, L. C. Mendoca-Hagler, A. Wolters, and J. D. van Elsas. 1993. Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. *J. Appl. Bacteriol.* **74**:78–85.
59. Srinivasan, M. C., R. S. Laxman, and M. V. Deshpande. 1991. Physiology and nutrition aspects of actinomycetes—an overview. *World J. Microbiol. Biotechnol.* **7**:171–184.
60. Sterflinger, K., W. E. Krumbein, and A. Schwirtz. 1998. A protocol for PCR *in situ* hybridization of hyphomycetes. *Int. Microbiol.* **1**:217–220.
61. Suberkropp, K. 1992. Interactions with invertebrates, p. 118–134. In F. Bärlocher (ed.), *The ecology of aquatic hyphomycetes*. Springer-Verlag, Berlin, Germany.
62. Suberkropp, K. 1998. Effects of dissolved nutrients on aquatic hyphomycetes growing on leaf litter. *Mycol. Res.* **102**:998–1002.
63. Suberkropp, K., and M. J. Klug. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* **57**:707–719.
64. Suberkropp, K., and E. Chauvet. 1995. Regulation of leaf breakdown in streams: influences of water chemistry. *Ecology* **76**:1433–1445.
65. Tamura, T., M. Hayakawa, H. Nonomura, A. Yokota, and K. Hatano. 1995. Four new species of the genus *Actinokineospora*: *Actinokineospora inagensis* sp. nov., *Actinokineospora globicatena* sp. nov., *Actinokineospora terrae* sp. nov., and *Actinokineospora diospyrosa* sp. nov. *Int. J. Syst. Bacteriol.* **45**:371–378.
66. Tank, J. L., and J. R. Webster. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* **79**:2168–2179.
67. van Dillewijn, P., P. J. Villadas, and N. Toro. 2002. Effect of a *Sinorhizobium meliloti* strain with a modified *pudA* gene on the rhizosphere microbial community of alfalfa. *Appl. Environ. Microbiol.* **68**:4201–4208.
68. van Hannen, E. J., G. Zwart, M. P. van Agterveld, J. Gons, J. Ebert, and H. J. Laanbroek. 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. *Appl. Environ. Microbiol.* **65**:795–801.

69. **Vicuña, R.** 1988. Bacterial degradation of lignin. *Enzyme Microbiol. Technol.* **10**:646–656.
70. **Weyers, H. S., and K. Suberkropp.** 1996. Fungal and bacterial production during leaf breakdown of yellow poplar leaves in two streams. *J. N. Am. Benthol. Soc.* **15**:408–420.
71. **White, T. J., T. Bruns, S. Lee, and J. W. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), *PCR protocols: a guide to methods and applications*. Academic Press, Inc., New York, NY.
72. **Willoughby, L. G.** 1974. Decomposition of litter in freshwater, p. 659–681. *In* C. H. Dickinson and J. F. Pugh (ed.), *Biology of plant litter decomposition*. Academic Press, London, United Kingdom.
73. **Wohl, D., and J. V. McArthur.** 1998. Actinomycete-flora associated with submersed freshwater macrophytes. *FEMS Microbiol. Ecol.* **26**:135–140.