Effect of single-species and mixed-species leaf leachate on bacterial communities in biofilms

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Abstract Dissolved organic matter in the form of leaf leachate represents an important carbon and energy source in many lotic ecosystems. In this study, we investigated utilization of mono-specific and mixed-species leaf leachate and impacts on biofilm bacterial community structure. Ceramic tiles were incubated in a Northeast Ohio stream to allow for biofilm development and then exposed in the laboratory to glucose or leachate from: sugar maple (*Acer saccharum*), pin oak (*Quercus palustris*), maple + oak, American beech (*Fagus grandifolia*), witch hazel (*Hamamelis virginiana*), or beech + witch hazel. Bacterial responses to these amendments were compared to un-amended controls based on fluorescent in situ hybridization (FISH) targeting selected taxa and terminal restriction fragment length polymorphism (T-RFLP) of bacterial 16S rRNA genes; also changes in DOC concentrations were quantified. Generally, there were limited differences among communities as a result of leachate amendment, although specific taxa monitored by FISH exhibited differential responses. There was no evidence that mixing of leachate from different leaf species created an effect different than what could be expected based on monospecific experiments. Witch hazel solicited the greatest response, based on T-RFLP data, regardless of whether the community was exposed to witch hazel alone or witch hazel + beech, accounting for 19% of the variability in Jaccard distance ($P < 0.05$) and 27% of the variability in Hellinger distance among profiles. In conclusion, we found that leaf leachate can be readily degraded but only in some cases did differences in leaf leachate among tree species cause an alteration in community structure. Mixing of leachate from different leaf species did have an impact on DOC loss but did not alter community structure. The occurrence of particular compounds, such as those in witch hazel, may alter community structure suggesting that the presence and abundance of specific plant taxa can impact bacterial communities.

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Introduction

Bacteria are an important component of stream biofilms and play a dominant role in nutrient cycling and energy/carbon flow in lotic systems (Mulholland et al., 1984; Meyer, 1994; Battin et al., 2003). In turn, allochthonous organic matter is a central contributor to carbon and energy in many streams, and impacts the metabolism and abundance of bacteria (Fisher & Likens, 1973; Webster et al., 1995). In particular, dissolved organic matter (DOM) is the dominant form of organic carbon in aquatic systems (Wetzel, 1992; Münster, 1993) and can be directly used by bacteria (Hall, 1995).

One major form of DOM entering many streams is leaf leachate (Giller & Malmqvist, 1998). For example, up to 25% of initial leaf mass is lost within 1 day through leaching (Webster & Benfield, 1986). Although there have been several studies focused on the effects of leaf leachate on microbial communities in streams (e.g., Koetsier et al., 1997; Strauss & Lamberti, 2002; Schlief & Mutz, 2007), little is known about the impact of leachate from different leaf species on specific bacterial taxa or how mixtures of leachate from different leaf species are utilized. The latter point is particularly relevant because, in a given watershed, a variety of tree species are intermingled, creating mixtures of leaf leachate from different sources (Koetsier et al., 1997).

Variations in leachate chemistry among leaf species likely contribute to differences in overall utilization of leachate (Wallace et al., 2008) as well as specific bacterial community structural responses. DOM from leaves includes, among other compounds, sugars (that are utilized rapidly) and polyphenols (Sasaki et al., 2007). Labile compounds can stimulate some bacteria while phenolics can have inhibitory or toxic effects (Fierer et al., 2001; Schlief & Mutz, 2007). Leachate from different leaf species differs in chemical composition which subsequently effects lability (Cleveland et al., 2004; Wallace et al., 2008).

In addition to differences among leachates from leaves of different species in bioavailability, responses to leachate vary among bacterial taxa. For example, in a study using maple leachate amendments, *Acinetobacter calcoaceticus* was not affected, the population size of *Burkholderia cepacia* increased, and *Pseudomonas putida* was inhibited by the highest leachate concentrations (McNamara & Leff, 2004a).

In order to examine biofilm bacterial community structure in this study, we used fluorescent in situ hybridization (FISH; Delong et al., 1989) and terminal restriction fragment length polymorphism (T-RFLP; Liu et al., 1997) to detect responses to leaf leachate. FISH has been widely used to describe temporal and spatial changes in structure of aquatic bacterial communities (e. g., Koetsier et al., 1997; Pernthaler et al., 1998; Manz et al., 1999) as well as to examine responses to different environmental conditions (Amann et al., 1990, 1995; Olapade & Leff, 2004), in spite of methodological limitations (Bouvier & Giorgio, 2003). Specifically, in this study, we targeted the *Beta-* and *Gamma-Proteobacteria* along with *A. calcoaceticus* and *B. cepacia* because of their abundance and importance in streams (Leff, 2000; Liu & Leff, 2002; Araya et al., 2003; Olapade & Leff, 2006; Santmire & Leff, 2007). For example, responses to DOM differ between the two species (McNamara & Leff, 2004a, b), perhaps because *A. calcoaceticus* can use few simple sugars (Juni, 1978), whereas *B. cepacia* can utilize a broad array of compounds (Lessie & Gaffney, 1986). Therefore, we hypothesized that responses to different types of leaf leachate would vary among these groups. T-RFLP is a genetic fingerprinting tool that has been widely used in various environments to examine microbial community structure (e.g., Kent et al., 2003; Blackwood et al., 2006; Danovaro et al., 2006; Blackwood & Buyer, 2007). Marsh (1999) suggests that T-RFLP is a sensitive method which has the ability to detect less-abundant taxa within assemblages, although it does have drawbacks inherent to many PCR-based community fingerprinting techniques.

In this study, we assessed the utilization of leaf leachate from different tree species (singly and in combination) by aquatic bacterial biofilm communities and evaluated the impacts on community structure. Biofilms were selected as the focus because of their widespread occurrence and importance in biogeochemistry of aquatic systems, particularly streams. Responses to monospecific or mixed-species leaf leachate were examined, along with responses to glucose. We hypothesized that bacterial taxa would vary in their responses to leachate of different types because of chemical differences among leaf species which would also be reflected in differences in utilization of leachate among leaves of different species. We also predicted higher bacterial abundance

on leachate from mixed-species leaves compared with monospecific leachate due to an increased array of potential biochemical niches in the presence of a more diverse suite of chemical resources.

Materials and methods

Experimental design

Unglazed, autoclaved ceramic tiles (5.12 cm × 5.12 cm) were incubated to allow for biofilm development in the West Branch of Mahoning River, a stream located in Northeastern Ohio (USA), as in Olapade & Leff (2006). Sets of tiles were positioned in a riffle on 29 November 2007 and 23 February 2008 and retrieved after about 5 weeks for use in two experiments described below. Stream temperature, pH, conductivity (Symphony SP90M5), water velocity (Marsh-McBirney model 201/201D), and turbidity (Hach model 2100P) were measured at the site of deployment in triplicate on each deployment date. Also, triplicate water samples were collected and concentrations of dissolved organic carbon (DOC) (Shimadzu Scientific Instruments TOC5000), nitrate/nitrite, and soluble reactive phosphate (SRP) (LachAT Quikchem 800 FLA$^+$) were measured.

After incubation in the stream, tiles were retrieved and brought to the laboratory where they were placed in 2 l beakers with 600 ml of sterile artificial stream water (ASW) containing the following (per liter): 12 mg NaHCO$_3$, 7.5 mg CaSO$_4$·2H$_2$O, 7.5 mg MgSO$_4$, 0.5 mg KCL, 10 mg CaCO$_3$ (pH 6.4) (APHA, 1998). Beakers were subjected to various DOM amendments. Leaf leachate was extracted from senescent sugar maple (*Acer saccharum*), pin oak (*Quercus palustris*), American beech (*Fagus grandifolia*), and witch hazel (*Hamamelis virginiana*), collected from trees at the study site, as described in McNamara & Leff (2004b). In the first experiment, treatments included: controls with no added leachate, maple leachate, oak leachate, and maple + oak leachate. In the second experiment, treatments included: controls with no added leachate, beech leachate, witch hazel leachate, beech + witch hazel leachate, and glucose. All treatments, including controls, were performed in triplicate, and there were seven tiles per beaker. The concentrations of DOC from leaf leachate and glucose used were 15 mg/l. For mixed-species leachate, the concentrations of DOC from the two species were the same and totaled 15 mg/l.

A portion of leachate from each leaf species was used for chemical analysis. DOC and DON concentrations were measured using a TOC5000 (Shimadzu Scientific Instruments). Total sugar concentrations were determined based on phenol–sulfuric assay (Dubois et al., 1956), and were expressed as glucose equivalents. Concentrations of total phenol compounds were determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965); tannic acid was used as the standard.

Beakers of tiles were incubated for 11 days (at room temperature in the dark) with stirring at 20 rpm using Phipps and Bird paddle stirrers (Phipps and Bird, Richmond, Virginia) as in Olapade & Leff (2006). After incubation, three randomly selected tiles were scraped with a sterile toothbrush and scrapings were preserved in 1× phosphate-buffered saline and 8% paraformaldehyde, then sonicated (Branson model 2210 ultrasonic bath, Trenton, NJ) for 5 min in 0.1% tetrasodium pyrophosphate; samples were stored at 4°C. The remaining tiles were frozen at −80°C for subsequent DNA extraction. pH and DOC concentrations of water in the beakers were measured following the same methods (above) for water samples collected from the stream.

Bacterial enumeration

Total bacterial number was determined by staining cells, concentrated on 0.2 μm-pore-size black polycarbonate filters (Poretics, Livemore, CA), with 15 μg/ml DAPI for 3 min (Porter & Feig, 1980); bacteria in 10 fields per slide were enumerated. The abundance of cells belonging to *Beta-Proteobacteria*, *Gamma-Proteobacteria*, *A. calcoaceticus*, and *B. cepacia* was enumerated using FISH as described previously (e.g., Olapade & Leff, 2005; Rubin & Leff, 2007). Details of methods, probes, and hybridization conditions can be found in Olapade & Leff (2005). Briefly, samples were concentrated on 0.2 μm white polycarbonate filters (Poretics) or Anodiscs (Whatman) depending on the probe and washed with 0.1% Nonidet P-40 (Sigma). Filters were placed in Petri plates and treated with 40 μl Texas Red-labeled probe (Sigma Genosys, Cambridge, UK) at 5 ng/μl final concentration and incubated in the dark at the appropriate temperature for 4 h; probe was diluted in hybridization buffer

[6 × standard saline citrate (SSC), 0.02 M Trizma base (pH7.0), 0.1% sodium dodecyl sulfate (SDS), and 0.01% polyadenylic acid]. Then, cells were washed twice with 400 μl washing buffer (0.9 M NaCl or 0.009 M NaCl depending on the probe) with a 10 min incubation time in between. After a second 10 min incubation, filters were rinsed twice with 400 μl water, and hybridized cells of 50 fields per filter were enumerated.

DNA extraction and terminal restriction fragment length polymorphism (T-RFLP)

For DNA extraction, biofilms were scraped from tiles using a razor blade; approximately 150 mg wet weight was recovered per beaker. DNA was isolated from the biofilms using the PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's instructions. Bacterial 16S rRNA genes were amplified with Eub338F-0-I-Fam mixed with Eub338F-II–III-Fam (5′-ACT CCT ACG GGA GGC WGC-3′ and 5′-ACA CCT ACG GGT GGC WGC-3′, Fam labeled) and 1392R (5′-ACG GGC GGT GTG TAC A-3′) (Blackwood et al., 2005). Primers were from Integrated DNA Tech (Coralville, IA). PCR was performed with a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad, Hercules, CA) using Hot Start GoTaq (Promega, Madison, WI) using 0.1 μM concentration for primers and 2 μl of template. The amplification program started with initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation for 30 s at 94°C, primer annealing for 30 s at 57°C and extension for 90 s at 72°C. The program ended with a final extension for 7 min at 72°C. PCR was performed thrice on each sample and amplicons were pooled. Negative controls were run for every PCR.

After verifying fragment sizes with electrophoresis, PCR products were digested using HaeIII restriction enzyme (New England Biolabs, Ipswich, MA) overnight at 37°C; then, samples were purified using the Qiaquick Nucleotide Removal Kit (Qiagen, Valencia,CA). T-RFLP profiles were generated at the Ohio State Plant Microbe Genomics Facility on an Applied Biosytems 3730 DNA Analyzer (Foster, CA). Peaks with heights comprising >0.5% of cumulative peak height between 50 and 600 bp were retained for analysis.

Statistical analysis

Results of the two experiments were analyzed separately. Statistical tests were considered significant at the level $α = 0.05$. T tests were performed to examine differences in relative sugar and phenol concentrations between leachates used in the two experiments.

Two-way ANOVA was used to examine the influence of leaf leachate on bacteria numbers (stained by DAPI or FISH), pH, and DOC concentration (i.e., we tested for effects of species 1 treatment, species 2 treatment, and an interaction between species 1 and 2 treatments). A significant interaction effect in this ANOVA supports the hypothesis that mixing of leaf leachate of different species impacts the response variable in question. Final pH and DOC values for each treatment were also compared to initial values by t tests.

For T-RFLP profiles, statistical significance of differences among treatments within each experiment was determined by redundancy analysis according to a two-way ANOVA design (Legendre & Anderson, 1999; Blackwood & Buyer, 2007). Analyses were conducted using one ecological distance metric that includes information contained in peak height (Hellinger distance) and one that includes presence–absence information (Jaccard distance). Distance-based redundancy analysis was performed using the software Canoco (MicroComputer Power, Ithaca, NY), with the significance assessed by 999 random permutations (Legendre & Anderson, 1999). The similarity of the glucose-amended community to control was assessed in a separate principal components analysis (PCA) ordination after Hellinger-transformation (Legendre & Gallagher, 2001).

Results

Physical and chemical parameters

Physical and chemical parameters on the two dates on which tiles were deployed are presented in Table 1. There were typical seasonal changes in several parameters (such as DOC, pH, and temperature) between the two dates.

General chemical characteristics of the initial leaf leachate are presented in Table 2. There were similar

Table 1 Environmental variables measured at the site used for biofilm development

Date	pH	Temperature (°C)	Conductivity (us/cm)	FV (M/S)	DOC (mg/l)	Nitrate/Nitrite (mg N/l)	SRP (mg P/l)	Turbidity (NTU)
29Nov2007	8.4 (0.07)	4.3 (0.09)	175.0 (25.00)	0.39 (0.02)	16.7 (0.37)	0.89 (0)	15.1 (0.72)	5.91 (0.19)
23Feb2008	6.4 (0.11)	2.0 (0.13)	269.5 (0.97)	0.27 (0.01)	8.8 (0.11)	0.88 (0.02)	ND	5 (0)

$N = 3$; Numbers in parentheses are standard errors

FV flow velocity, *NTU* nephelometric turbidity units, *ND* not determined, *SRP* soluble reactive phosphate

Table 2 Chemical parameters of leaf leachate

	mg DON/ mg DOC	Relative sugar concentration[a]	Relative phenolic concentration[b]
Maple	0.02	0.94 (0.10)	0.56 (0.00)
Oak	0.03	1.17 (0.11)	0.16 (0.13)
Beech	0.03	0.87 (0.11)	0.31 (0.31)
Witch hazel	0.01	0.90 (0.11)	0.34 (0.02)

$N = 3$ for sugar and phenol analysis. $N = 1$ for DON measurement. Numbers in parentheses are standard errors

[a] Glucose equivalents/mg DOC

[b] Tannic acid equivalents/mg DOC

relative sugar concentrations (total sugar concentration/DOC concentration) between maple leachate and oak leachate ($P > 0.05$, *t* test), but relative phenol concentration (total phenol concentration/DOC concentration) of maple leachate was significantly higher than that of oak leachate ($P < 0.05$, *t* test). In contrast, relative sugar and phenol concentrations were not different between beech and witch hazel leachate ($P > 0.05$, *t* test).

pH and DOC concentrations in the experimental units were measured before and after the experiments. The initial pH of the ASW was 6.4 and DOC concentrations were 15 mg/l in DOM-treated units. pH and DOC concentrations at the end of both experiments are shown in Table 3. Final pH was unaffected by treatment ($P > 0.05$), but increased relatively to the initial pH of the ASW ($P < 0.05$ for

all treatments). DOC concentrations declined over time from the initial concentrations added as expected due to heterotrophic activity ($P < 0.05$ for all treatments); DOC concentration increased in controls because of the biofilm present on the tiles at the time of inoculation.

In the first experiment, final DOC concentration was significantly higher (relative to other treatments and controls) in units amended with maple leachate ($F = 27.44$, $P < 0.05$), but was not affected by oak leachate ($F = 2.90$, $P > 0.05$), or the interaction between maple and oak leachate ($F = 3.90$, $P > 0.05$). In the second experiment, final DOC concentrations were significantly higher in both beech ($F = 21.30$, $P < 0.05$) and witch hazel leachate amendments relative to controls ($F = 6.50$, $P < 0.05$; Table 3). The interaction between beech and witch hazel leachate was also significant ($F = 34.34$, $P < 0.001$). For the glucose-amended beakers, final pH was similar to that of the controls ($P > 0.05$, *t* test), while final DOC concentration was significantly higher than the controls ($P = 0.05$, *t* test).

Bacterial abundance

In the first experiment, total bacterial number on the tiles (based on DAPI staining) was not significantly affected by the treatments ($P > 0.05$; Fig. 1a). In the second experiment, total bacterial numbers were significantly higher in witch hazel leachate-treated

Table 3 pH and DOC concentrations of water in the beakers measured 11 days after the DOM amendment

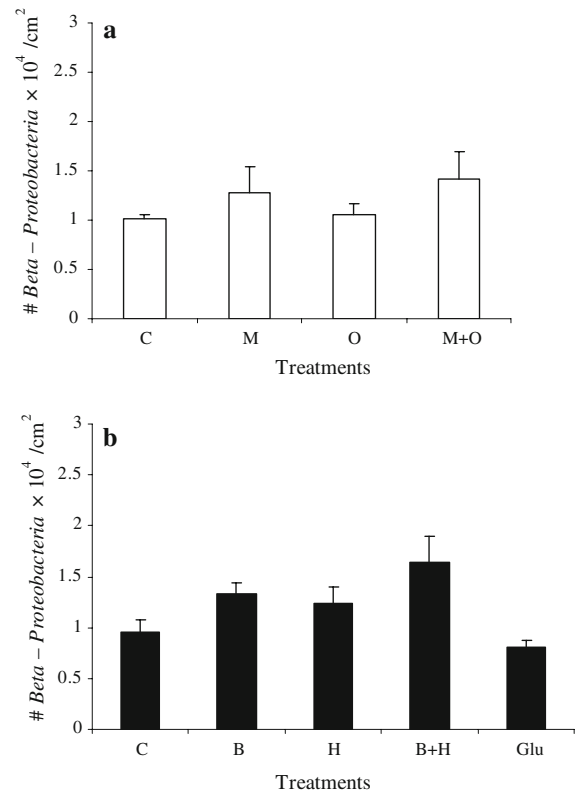|	Experiment 1				Experiment 2				
	Control	Maple	Oak	Maple + Oak	Control	Beech	Witch hazel	Beech + witch hazel	Glucose
pH	6.8 (0.03)	6.7 (0.06)	6.7 (0.01)	6.7 (0.01)	6.6 (0.05)	6.7 (0.05)	6.8 (0.04)	6.7 (0.03)	6.6 (0.03)
DOC (mg/l)	3.3 (0.39)	6.7 (0.85)	3.4 (0.1)	5.0 (0.13)	2.0 (0.15)	4.2 (0.32)	3.8 (0.22)	3.5 (0.04)	4.5 (0.89)

$N = 3$ for each treatment and control; Numbers in parentheses are standard errors
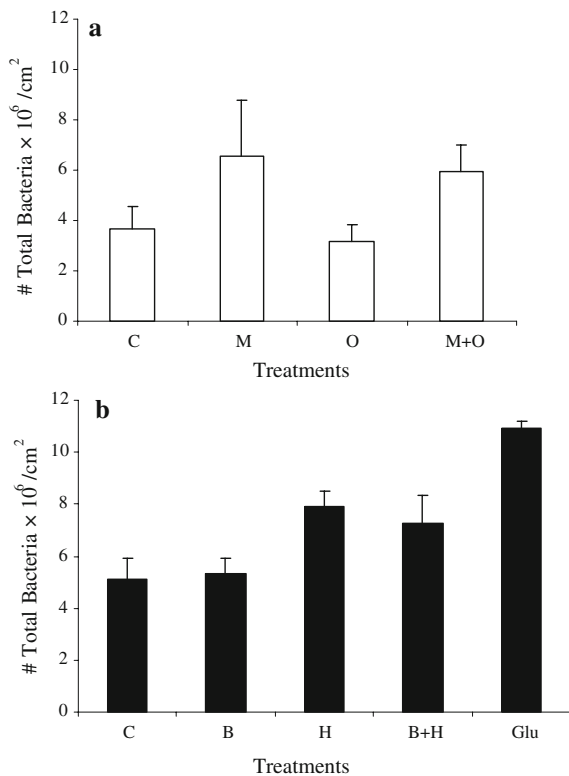
Fig. 1 Mean (+SE) number of total bacteria (determined by DAPI staining on tiles) from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment); *M* maple leachate, *O* oak leachate, *M* + *O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate; *B* + *H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

Fig. 2 Mean (+SE) abundance of *Beta-Proteobacteria* (determined by FISH) on tiles from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment), *M* maple leachate, *O* oak leachate, *M* + *O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate, *B* + *H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

beakers ($F = 9.18$, $P < 0.05$), but were unaffected by beech leachate amendment ($F = 0.08$, $P > 0.05$). The interaction between beech and witch hazel treatments was not significant ($F = 0.31$, $P > 0.05$; Fig. 1b). During this experiment, there were significantly higher abundances on the glucose-treated tiles than control tiles ($P < 0.05$, *t* test).

Like total bacterial numbers, there were no significant differences for *Beta-Proteobacteria* abundance among treatments in the first experiment ($P > 0.05$; Fig. 2a). However, in the second experiment, the abundance of *Beta-Proteobacteria* was significantly higher on tiles incubated in beech leachate compared to tiles not exposed to beech leachate ($F = 5.48$, $P < 0.05$) but was not significantly affected by witch hazel leachate ($F = 3.17$, $P > 0.05$) or the interaction of beech and witch hazel ($F = 0.01$, $P > 0.05$; Fig. 2b). The number of *Beta-Proteobacteria* after

glucose treatment was not significantly different than the control ($P > 0.05$, *t* test).

The number of *Gamma-Proteobacteria* in the biofilms differed among different leaf leachate treatments. In the first experiment, abundance of *Gamma-Proteobacteria* was significantly higher in the maple leachate amendment than in beakers without maple leachate ($F = 6.81$, $P < 0.05$). In contrast, there was no significant effect of oak leachate treatment ($F = 0.68$, $P > 0.05$); also there was no significant interaction between maple and oak leachate ($F = 1.16$, $P > 0.05$; Fig. 3a).

In the second experiment, the abundance of *Gamma-Proteobacteria* was significantly higher in beakers amended with witch hazel leachate compared to those without witch hazel ($F = 12.54$, $P < 0.01$). No significant effect was found with beech leachate amendment ($F = 3.07$, $P > 0.05$); also there was not
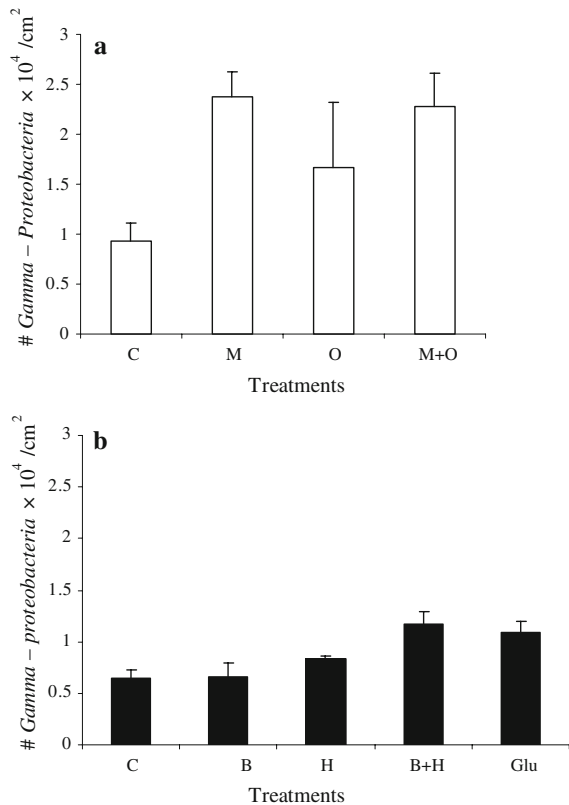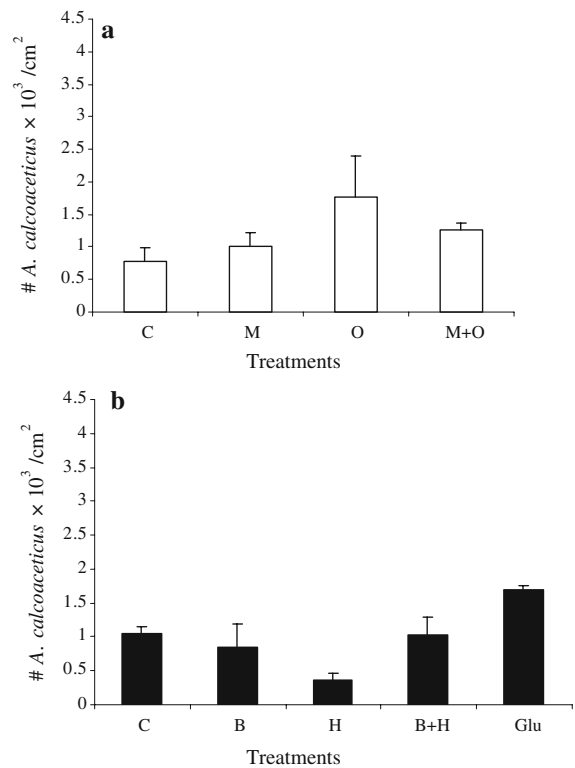
Fig. 3 Mean (+SE) abundance of *Gamma-proteobacteria* (determined by FISH) on tiles from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment), *M* maple leachate, *O* oak leachate, *M + O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate, *B + H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

Fig. 4 Mean (+SE) abundance of *A. calcoaceticus* (determined by FISH) on tiles from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment), *M* maple leachate, *O* oak leachate, *M + O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate, *B + H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

a significant interaction between beech and witch hazel leachate treatments ($F = 2.54$, $P > 0.05$; Fig. 3b). The numbers of *Gamma-Proteobacteria* after glucose amendment were significantly higher than the controls ($P < 0.05$, *t* test).

No significant differences were observed among different leaf leachate treatments, in either experiment, for *A. calcoaceticus* ($P > 0.05$; Fig. 4a and b). *A. calcoaceticus* numbers were significantly higher on tiles treated with glucose than the controls ($P < 0.01$, *t* test; Fig. 4b).

When *B. cepacia* was examined, numbers were significantly lower in response to oak leachate amendment ($F = 11.72$, $P < 0.01$) but not influenced by maple leachate amendment ($F = 2.72$, $P > 0.05$); the interaction between maple and oak leachate treatments was not significant ($F = 2.36$, $P > 0.05$; Fig. 5a). In the second experiment, there were no

significant effects of beech leachate ($F = 0.13$, $P > 0.05$), witch hazel, ($F = 0.00$, $P > 0.05$), or interaction between beech and witch hazel leachate ($F = 0.43$, $P > 0.05$; Fig. 5b). Likewise, differences in *B. cepacia* numbers on tiles treated with glucose were not statistically significant different than the controls ($P > 0.05$, *t* test).

In general, the total abundance of bacteria, as well as abundance of specific groups, before the beginning of the experiment was not substantially different from the average abundances across treatments after the experiment, implying that experimental incubation in the laboratory did not have a large effect independent of the treatments imposed (data not shown). One exception was *A. calcoaceticus*, which was approximately four times more abundant on tiles before the experiment (483 cells/cm^2) than afterward (average across treatments was 121 cells/cm^2).
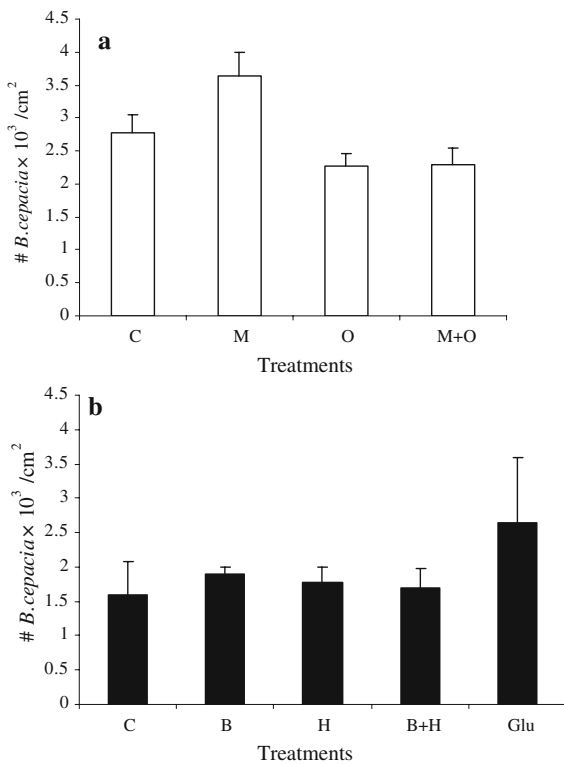
Fig. 5 Mean (+SE) abundance of *B. cepacia* (determined by FISH) on tiles from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment), *M* maple leachate, *O* oak leachate, *M + O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate, *B + H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

Bacterial community composition

T-RFLP was used to examine differences in bacterial community composition among treatments (see Supplementary Material for additional details). In the first experiment, there was no significant effect of maple, oak, or maple + oak interaction ($P > 0.05$, Fig. 6a). However, in the second experiment (Fig. 6b), T-RFLP profiles were significantly affected by DOM amendment; the beech, witch hazel, beech + witch hazel interaction together accounted for 35% of total variability in Jaccard distance between profiles ($P < 0.05$). When beech, witch hazel, and interaction effects were tested individually, only the witch hazel treatment was significant, accounting for 19% of variability in Jaccard distance ($P < 0.05$), and 27% of variability in Hellinger distance between profiles ($P = 0.067$). This is apparent in the PCA plot in Fig. 6b, where control and beech-amended communities are grouped in the upper
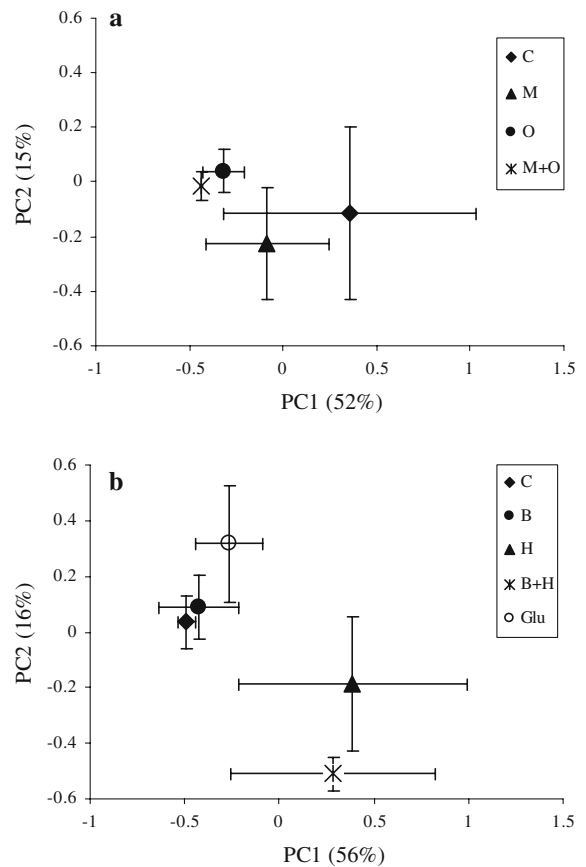
Fig. 6 Principal components plot from bacterial T-RFLP profiles in different DOM treatments from the first experiment (**a**) and the second experiment (**b**). *C* control (no amendment), *M* maple leachate, *O* oak leachate, *M + O* maple + oak leachate, *B* beech leachate, *H* witch hazel leachate, *B + H* beech + witch hazel leachate, *Glu* glucose. $N = 3$ for each treatment

left, separate from witch hazel and beech + witch hazel-amended communities which are grouped in the lower right. Figure 6b also shows that glucose amendment had no affect on bacterial community composition, since this treatment is grouped with the control and beech-amended communities. There was an effect of removing tiles from the stream and incubating in the lab, in that, initial community profiles were separated from post-experiment communities by Hellinger PCA ordination of both experiments (data not shown).

Discussion

DOM is the dominant component of the detrital pool in freshwater systems (e.g., Meyer, 1994; Farjalla

et al., 2006) and is readily used by bacteria. There is ample evidence from a variety of studies that bacteria are limited by the availability of labile organic molecules; however, responses to DOM amendment are not always consistent with expectations (e.g., McArthur & Marzolf, 1986; McArthur et al., 1994; Koetsier et al., 1997; Eiler et al., 2003). Our findings suggest that the unpredictable responses may be at least partially attributable to variability in bacterial community composition as responses varied among bacterial taxa.

Although perhaps not all bacteria use leaf leachate effectively as a carbon source, the community was able to effectively degrade the DOM added based on the observed declines in DOC concentration. At the same time, however, the effects of differences in phenolics were reflected in utilization of DOM because loss of DOC from oak (which had lower phenolic concentrations)-treated units was greater than that of maple. Beyond this, we also found that mixing of leachate from different species could increase degradation. Specifically, mixtures of beech and witch hazel were utilized more than leachate from either leaf species alone demonstrating that mixing of leachate may increase its utilization. Glucose amendment generally elicited a positive response consistent with earlier investigations (Gurung & Urabe, 1999; Skoog et al., 2002; Olapade & Leff, 2005, 2006). Collectively, this study reinforces the bacterial preference of carbohydrates because of their extreme lability and common occurrence in streams (Chappelle, 1993).

Our results support the prediction that responses to leaf leachate would differ among the various bacterial groups examined. All groups of bacteria examined, except one, responded to one or more leaf leachate treatments. Specifically, the abundance of *Gamma-proteobacteria* was elevated when exposed to maple and witch hazel leachate, whereas *Beta-proteobacteria* were stimulated by beech leachate. At the species level, *B. cepacia* abundance was suppressed by oak leachate, whereas *A. calcoaceticus* did not respond to any leachate amendments. These results are consistent with those of Olapade & Leff (2006) and McNamara & Leff (2004a). Olapade & Leff (2006) found that the number of *Gamma-proteobacteria* was not affected by leaf leachate exposure but *Beta-proteobacteria*, *A. calcoaceticus* and *B. cepacia* numbers were highest on tiles, in some seasons, after leachate amendments.

McNamara & Leff (2004a) reported no differences in the number of *A. calcoaceticus* after leachate treatment, while *B. cepacia* responded positively.

Community structure, based on T-RFLP fingerprinting, responded to only one of the leachate types examined, witch hazel. Witch hazel plants contain secondary compounds, including those widely known for their medicinal qualities, such as polyphenols, that have clear antioxidant properties (i.e., as free radical scavengers) (Tourino et al., 2008). Therefore, differences between the leachate chemistry of witch hazel and other leaf types likely exist but were not detected by our broad chemical measurements. We hypothesize that the general bacterial community responses observed reflect the effects of high concentrations of particular classes of compounds, such as proanthocyanidins, which have significant impacts on cell function (Cos et al., 2004).

The absence of significant effects of leaf leachate on community structure, other than for witch hazel, is surprising, given that each of the other leaf leachate types significantly affected abundance of at least one taxon detected through FISH, and that the phylogenetic groups differed in their responses to leaf leachates. One potential explanation is that the groups chosen for examination by FISH were purposefully selected because they were likely to respond to different treatments based on prior studies (McNamara & Leff, 2004a, b; Olapade & Leff, 2005, 2006), whereas the T-RFLP analysis included many other groups that did not differentially respond to leaf leachates (other than witch hazel), as well as DNA from inactive, dormant or senescent cells. Cells detected by group-specific FISH represented less than 1% of those detected by DAPI staining, reinforcing this possibility.

We found no evidence supporting our hypothesis that bacterial responses would differ between monospecific and mixed-species leachate, in spite of the observed differences in DOC use in mixed versus monospecific leachate. This was unexpected given that the growth of microorganisms in a mixture of substrates is greater than when only one substrate is available (Egli, 1995). Although the specific chemical composition of the leaf leachate used is unknown and not fully definable, it seems likely that mixing leachates from different species enhances substrate heterogeneity. Perhaps the response of a community to increasing diversity of compounds saturates at a

level of compound diversity much lower than is present in leaf leachate or measures of overall bacterial production would have revealed an affect of mixing leachates. DOM in this study was derived strictly from leaves, but there is also the potential for metabolites produced by microbial communities on different leaf types to have an effect on biofilm communities. In addition, it is possible that community responses are regulated by colonization of bacteria from adjacent leaf types, resulting in a succession which was not captured in this laboratory experiment.

Potentially there are many abiotic and biotic factors that limit biofilm bacteria in freshwater systems. DOM, in particular, has received much attention. Although findings demonstrating DOM limitation are mixed (Sun et al., 1997; McNamara & Leff, 2004a; Docherty et al., 2006; Olapade & Leff, 2006), perhaps because the nature and composition of the DOM pool is not fully accounted for, there is evidence that increasing availability of labile DOM can alter bacterial community structure (Cottrell & Kirchman, 2000) and function (Wehr et al., 1999; Strauss & Lamberti, 2002). Our findings demonstrate that leaf leachate can be readily degraded but that the effects of differences in leaf leachate were very group-specific. Mixing of leaf species had no impact on community structure but, in one of the two experiments, enhanced overall DOC utilization. However, the occurrence of particular compounds, such as those in witch hazel, may alter general community structure suggesting that the presence and abundance of specific plant taxa can play an important role in structuring bacterial communities.

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