Freshwater Biology

Freshwater Biology (2009) **54**, 2616–2627 doi:10.1111/j.1365-2427.2009.02270.x

Genetic and environmental controls of microbial communities on leaf litter in streams

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SUMMARY

1. Despite the importance of microorganisms for leaf litter decomposition in streams, little is known about which factors affect community composition of bacterial and fungal communities. Standard morphological techniques probably underestimate microbial diversity.
2. We used terminal restriction fragment length polymorphisms of the ITS regions for fungi, and the 16S region for bacteria, to compare fungal and bacterial communities on four cross types of cottonwood leaves (*Populus fremontii*, *P. angustifolia*, and their naturally occurring F_1 and backcross hybrids). Decomposing leaves were studied in two Arizona rivers that differ in water chemistry and macroinvertebrates.
3. Hybridising cottonwoods are an ideal model system to test how genetic differences in leaf litter chemistry affect microbial communities because cross types have different decomposition rates and leaf litter chemistry. Leaves were incubated in litter bags for 2 weeks and brought to the laboratory for genetic analysis. Communities were analysed using non-metric multi dimensional scaling (NMDS) and diversity indices.
4. Fungal and bacterial communities differed between the two rivers, even when growing on identical substrates. There were also significant differences in microbial communities among the four cross types, indicating that genetically based differences in leaf litter translate to differences in microbial communities.
5. Diversity increased along the hybridising complex from *P. fremontii* to *P. angustifolia*, with hybrids showing intermediate values. Fungal and bacterial diversity were significantly higher on cross types with higher tannin concentrations and slower decomposition rates.
6. Environmental conditions most strongly structured microbial communities, but within an environment, genetic-based differences in leaf litter quality yielded differences in diversity and community structure.
7. Molecular tools are making it possible to understand patterns of microbial diversity in river ecosystems, paving the way for a better understanding of how differences in microbial species affect ecosystem processes and higher trophic levels.

Keywords: bacteria, decomposition, fungi, microbes, streams

Introduction

Understanding how microbial communities vary spatially and temporally across abiotic and biotic gradients is fundamental for developing a framework for using microorganisms to test general ecological theories and for integrating community and

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ecosystem ecology. In streams, microbial communities are important for leaf litter decomposition, yet they are poorly characterised because of difficulties describing species composition using traditional morphological and culturing techniques. Responses of fungal and bacterial biomass to abiotic (e.g. water chemistry, pH and temperature) and biotic (e.g. leaf type and invertebrates) variables on decomposing leaves are well documented (see Gessner *et al.*, 2007 for literature review), but less is known about how species assemblages change under different environmental conditions. Taxonomic challenges have prevented microbial ecologists from developing distribution maps of microbial taxa similar to those for plants and animals (Leff & Lemke, 1998; Findlay & Sinsabaugh, 2006). There is compelling evidence from laboratory studies that fungal species composition and diversity affect decomposition (Bärlocher & Corkum, 2003; Dang, Chauvet & Gessner, 2005) and energy transfer up food webs (Arsuffi & Suberkropp, 1986; Lecerf *et al.*, 2005), but field studies of microbial communities have been limited by methodological constraints.

Fungal communities on decomposing leaf litter have traditionally been studied by counting conidia, which are limited to aquatic hyphomycetes and require the induction of sporulation (Bärlocher, 1992; Suberkropp, 1992; Gessner, Bärlocher & Chauvet, 2003; Graça, Bärlocher & Gessner, 2005). Bacterial community analyses relied primarily on culturing-based techniques although many taxa have not been successfully cultured. Recent advances in molecular taxonomy offer a new approach for analysing community structure for both fungi and bacteria (e.g. Blackwood *et al.*, 2003; Nikolcheva & Bärlocher, 2004). DNA profiles are generally quicker to generate than morphological profiles, do not require sporulation or culturing, and can detect all life stages of microbes even when populations are small (Nikolcheva & Bärlocher, 2005; Nikolcheva, Bourque & Bärlocher, 2005). Comparisons of morphological and molecular approaches indicate that diversity is underestimated by morphology (Nikolcheva & Bärlocher, 2004; Nikolcheva *et al.*, 2005; Gessner *et al.*, 2007). Polymerase chain reaction based techniques, such as terminal restriction fragment (TRF) length polymorphisms (TRFLP) and de-naturing gradient gel electrophoresis, enable processing large sample sizes (Liu *et al.*, 1997). Although these methods can both underestimate and overestimate taxonomic richness (Liu *et al.*, 1997; Osborn, Moore & Timmis, 2000; Dahllöf, 2002; Forney, Zhou & Brown, 2004) they are generally accepted as high quality techniques for determining fungal and bacterial 'species' richness in freshwaters (Dorigo, Volatier & Humbert, 2005; Nikolcheva & Bärlocher, 2005; Mitchell & Zuccaro, 2006; Fierer *et al.*, 2008).

In streams, molecular tools have revealed large spatial and temporal variation among microbial communities and more subtle variation among substrate types within a habitat. In particular, fungal communities on decomposing leaves vary across sites, seasons and during decomposition (Nikolcheva, Cockshutt & Bärlocher, 2003; Nikolcheva & Bärlocher, 2004; Ferreira, Gulis & Graça, 2006; Gessner *et al.*, 2007). Although fungal and bacterial biomass vary across leaf type, differences in community structure of microbes are small (Nikolcheva *et al.*, 2003; Das, Royer & Leff, 2007). The limited research conducted on bacterial communities in streams indicates that benthic communities differ among sites and are particularly responsive to organic pollution, metal contamination and differences in pH (Williams & Fulthorpe, 2003; Cordova-Kreylos *et al.*, 2006; Fierer *et al.*, 2008) but do not show strong differences by leaf type (Das *et al.*, 2007; Harrop, Marks & Watwood, 2009).

To date, only Schweitzer *et al.* (2008) has shown that microbial communities differ in composition among different genotypes within a single plant species, although several studies have shown that the effects of plant genotypes on ecosystem processes can be important and even rival those found among distantly related plant species (Driebe & Whitham, 2000; Treseder & Vitousek, 2001; Schweitzer *et al.*, 2004; Madritch & Hunter, 2005; LeRoy *et al.*, 2006). Phenotypic differences within plant hybrids and their parental species provide a sensitive model for describing how plant traits affect communities and ecosystems because it is easier to isolate a few traits from the vast array of correlated traits that vary among species (e.g. leaf chemistry, leaf size and cuticle thickness). Hybridising cottonwoods are an ideal model system to test how genetically based differences in leaf litter chemistry affect microbial communities because differences in decomposition rates and leaf litter chemistry have been well documented in both terrestrial (Schweitzer *et al.*, 2004, 2005, 2008) and aquatic ecosystems (Driebe & Whitham, 2000; LeRoy *et al.*, 2006,

2007). Cottonwoods are a dominant species in many western United States and European catchments, and have large effects on the community structure of associated arthropods (Bangert *et al.*, 2006a,b; Whitham *et al.*, 2006; Wimp *et al.*, 2007) and soil bacteria (Schweitzer *et al.*, 2008). Genetic-based traits in cottonwoods also influence ecosystem processes other than decomposition, including nutrient and carbon cycling and root productivity (Fischer *et al.*, 2004, 2007; Schweitzer *et al.*, 2004). As our understanding of how genes affect plant traits is refined, genotypic studies will facilitate elucidate which plant traits are most important for influencing decomposition rates and community structure of decomposers (Whitham *et al.*, 2008; Woolbright *et al.*, 2008). Furthermore, because the majority of stream restoration projects in the United States focus on re-vegetating riparian zones (Bernhardt *et al.*, 2005), understanding if and how genetic variation within plant species affects community and ecosystem processes could be important for designing restoration projects.

Here we use a factorial design to compare bacterial and fungal communities between two streams and among four leaf litter types to address the following three questions: (1) Do microbial communities on decomposing leaf litter differ between two headwater streams that differ in water chemistry, geomorphology and invertebrate assemblages? (2) Are there differences in microbial community structure among litters from genetically distinct cross types? (3) Is microbial diversity positively or negatively correlated with decomposition rate? We used four cross types of cottonwoods grown in a common garden to isolate genetic effects on leaf litter quality: the two parent species *Populus fremontii* S. Watson (Fremont cottonwood), *P. angustifolia* James (narrowleaf cottonwood), and their natural occurring F_1 and backcross hybrids. Backcross hybrids are formed from unidirectional crosses between F_1 hybrids, pure *P. angustifolia* and other backcrosses and have fewer 'Fremont' alleles than F_1 hybrids (Keim *et al.*, 1989). Leaves from *P. angustifolia* and backcross hybrids decompose more slowly than *P. fremontii* and F_1 hybrids both in streams and soils, and generally have higher tannin levels (Driebe & Whitham, 2000; LeRoy *et al.*, 2006; Rehill *et al.*, 2006).

This design was used to test if stream condition or substrate type is more important in structuring microbial communities. We predicted that microbial communities would differ most strongly between the two rivers based on differences in water chemistry, geomorphology and invertebrate assemblages (LeRoy & Marks, 2006). We predicted that community structure and diversity would also differ among the four cross types based on the large community and ecosystem effects described above. We expected higher diversity on slower decomposing cross types (*P. angustifolia* and backcross hybrids) because leaves with more complex chemical constituents may host unique species with the enzymatic capability of breaking down these compounds.

Methods

Study sites

Fossil Creek (FC) and Beaver Creek (BC), described in detail in LeRoy & Marks (2006), are spring-fed headwater streams of the Upper Verde River catchment flowing off the southern part of the Colorado Plateau in Arizona, U.S.A. Flow during this experiment was significantly higher in BC (340 L s^{-1}) than in FC (56 L s^{-1}) where water was diverted for hydropower (LeRoy & Marks, 2006). Water temperature, conductivity, pH and concentrations of calcium carbonate are all higher in FC than in BC. FC is a travertine stream, where high concentrations of calcium carbonate precipitate to form large travertine terraces (LeRoy & Marks, 2006; Marks *et al.*, 2006; Carter & Marks, 2007). Decomposition rates of five species of leaf litter and mixtures of leaf litter do not differ significantly between the two rivers but each has a different macroinvertebrate assemblage (LeRoy & Marks, 2006).

Leaf substrates

Leaves were collected from multiple mature trees of each cross type growing in a common garden at the Ogden Nature Center, Ogden, UT, U.S.A. Mesh bags were wrapped around branches to collect leaves before they fell to the ground. Leaves were allowed to air dry and 4 g of each leaf type were sewn into litterbags made of Vexar netting (DuPont, Wilmington, DE, U.S.A.) with 6 mm mesh size. In February 2004, three litterbags of each leaf type were attached to 1 m long steel reinforcement bars and placed in FC and BC. Litterbags were removed from each creek after 2 weeks. This incubation time was used because

it corresponds to high levels of microbial activity and most terrestrial species of fungi that are present during the first few days after leaves are placed in streams are gone (Harrop *et al*., 2009). Leaf punches were collected from each litterbag using a 5 mm diameter cork borer and stored on glycerol at −20 °C.

Microbial community analyses

DNA extraction. Community DNA was extracted from three litterbags of each leaf type at each site using the FastDNA Spin Kit for Soil (Qbiogene, Solon, OH, U.S.A.) and the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, U.S.A.). Approximately 20 leaf punches were vortexed in 500 μL of 10 mM $MgSO_4$ buffer for 10 min at setting 10 on a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY, U.S.A.) then the punches and buffer were added to each kit. Bead tubes were homogenised for 30 s on a Mini-Beadbeater (Biospec Products, Bartlesville, OK, U.S.A.). DNA obtained via the two kits was visualised by agarose gel electrophoresis, and DNA for each litter bag was pooled prior to PCR.

Terminal restriction fragment length polymorphism. Microbial communities were characterised via TRFLP analysis based on 16S rDNA sequences for bacteria and internal transcribed spacer (ITS) ribosomal region sequences for fungi. Bacterial 16S rDNA was amplified from community DNA using primers 8F-FAM (5′-/56-FAM/AGA GTT TGA TCC TGG CTC AG-3′) and 907R1 (5′-CCG TCA ATT CCT TTG AGT TT-3′). The forward primer, 8F-FAM, was labelled with fluoresceine. Each reaction included 1× PCR buffer (Qiagen, Valencia, CA, U.S.A.), 200 μM of each dNTP (Qiagen), 0.5 μM of each primer (Integrated DNA Technologies, Inc., Coralville, IA, U.S.A.), 0.4 mg mL^{-1} of bovine serum albumin, 2.5 U Taq DNA polymerase (Qiagen), 2 μL of community DNA, and molecular grade water added to a final volume of 50 μL. The thermal cycler protocol included initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, and final elongation at 72 °C for 5 min.

Fungal ITS regions were amplified from community DNA using primers ITS1-F-FAM (5′-/56-FAM/CTT GGT CAT TTA GAG GAA GTA A-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) that target partial 18S rDNA sequence, complete ITS 1 sequence, complete 5.8S rDNA sequence, complete ITS 2 sequence and partial 28S rDNA sequence (White *et al*., 1990). The forward primer, ITS1F-FAM, was labelled with fluoresceine. Each 50 μL reaction had the same reagents as described for bacteria except that 0.2 μM of each primer was used. The thermal cycler protocol included initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min; and final elongation at 72 °C for 10 min. Negative controls replaced DNA template with molecular water. Amplicons were visualised by agarose gel electrophoresis with ethidium bromide staining.

Bacterial 16s rDNA amplicons were digested using restriction endonucleases *Hae*III and *Msp*I. Fungal ITS amplicon digestions used endonucleases *Hae*III and *Rsa*I. Each digest included 1× RE buffer (Promega, Madison, WI, U.S.A.), 0.1 mg mL^{-1} bovine serum albumin, 0.25 U μL^{-1} of each endonuclease (Promega), 3 μL of PCR product, and molecular water added to a final volume of 20 μL. Amplicons were digested at 37 °C for 6 h, then visualised by agarose gel electrophoresis.

Digested DNA was precipitated and stored at −20 °C. DNA was resuspended in 10.8 μL of Hi-Di formamide (Applied Biosystems, Foster City, CA, U.S.A.) and 0.2 μL of X-Rhodamine MapMarker 1000 (BioVentures, Inc., Murfreesboro, TN, U.S.A.) and was denatured at 96 °C for 5 min prior to sequence analysis. An Applied Biosystems 3730 DNA sequencer and GeneMapper software (version 4.0; Applied Biosystems) were used for fragment length analysis at Northern Arizona University's Environmental Genetics and Genomics (EnGGen) Laboratory.

To control for PCR bias and sequencer run variation, PCR reactions were run in triplicate for each community DNA sample, the triplicate PCR products were pooled and digested, and digests were run in triplicate on the sequencer. TRF that appeared in all three TRFLP profiles (within 0.5 bp in length) and were at least 0.5% of total fluorescent intensity were included in community analyses.

For each community profile, richness (S) was determined (where S = total number of TRF). Relative abundance (P_i) of each TRF was calculated based on fluorescent intensity (where P_i = the proportion of fluorescent intensity of the total community profile attributable to the ith TRF). The Shannon Diversity

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Index (H) was calculated for each community profile: $H = -\sum Pi \ln(Pi)$, where P_i is the relative abundance of each TRF as described above. Evenness (E) was calculated for each profile: $E = H/H$ max and H max = ln (S), where H is the diversity of TRF as described above and H_{max} is the maximum value of H if all TRF had the same fluorescent intensity.

Statistical analyses

Analysis of variance (ANOVA) was used to test for significant differences in microbial community diversity indices among sites and leaf types using JMP software (release 5.1.2; SAS Institute Inc., Cary, NC, USA). TRFLP community profiles were compared using multi-response permutation procedures (MRPP) and non-metric multidimensional scaling (NMDS) with Sorensen (Bray-Curtis) distance measure using PC-ORD (version 4.41; MjM Software, Gleneden Beach, OR, U.S.A.). In the MRPP test, the statistic A is a measure of effect size, where $A = 1$ indicates that samples within a treatment group are identical but different from other treatments, and an $A = 0$ indicates that samples are heterogeneous between treatments.

Results

Environmental effects

Results indicate that stream identity was more important in structuring microbial communities than leaf type. Differences among streams were evident in three measures of microbial community structure. First, each stream had multiple unique phylotypes (Table 1). The TRFLP analysis generated 176 distinct phylotypes of fungi and bacteria. Of the 80 bacterial phylotypes, 35 were unique to FC, 17 to BC and 28 were found in both streams (Table 1). Fungal phylotype richness was slightly higher with 96 phylotypes, 37 of which were unique to FC, 48 to BC and only 11 found in both streams (Table 1). Most of the fungal phylotypes (89%) were found in only one of the two rivers (Table 1). Overall, only 22% of microbial phylotypes were found in both streams. Second, all measures of phylotype diversity at the leaf pack scale differed between streams (Fig. 1; Table 2). Specifically, mean richness, evenness, and overall diversity of both fungi and bacteria were significantly higher in FC than in BC (Fig. 1; Table 2). Third, the NMDS and

Table 1 The distribution of bacterial and fungal phylotypes found on four genetically distinct cross types of cottonwood leaves incubated in two Arizona rivers

	Bacteria	Fungi
	Phylotypes (#)	
Unique to Fossil Creek	35	37
Unique to Beaver Creek	17	48
Found in both rivers	28	11
Total	80	96
Percent unique to one river	65	89
	Beaver Creek bacteria	Beaver Creek fungi
Unique to *Populus fremontii*	1	3
Unique to F_1 hybrids	2	18
Unique to backcross hybrids	4	6
Unique to *Populus angustifolia*	10	10
Shared by 2 cross types	14	12
Shared by 3 cross types	4	3
Shared by 4 cross types	10	7
Total	45	59
Percent unique to a single cross type	38	63
	Fossil Creek bacteria	Fossil Creek fungi
Unique to *P. freemontii*	5	4
Unique to F_1 hybrids	7	2
Unique to backcross hybrids	9	21
Unique to *P. angustifolia*	2	0
Shared by 2 cross types	14	10
Shared by 3 cross types	10	0
Shared by 4 cross types	16	11
Total	63	48
Percent unique to a single cross type	37	56

MRRP analyses showed significant differences in the community composition of both fungi and bacteria between streams (Fig. 2; Table 3). The NMDS ordination, which plots similar samples closer to each other, illustrates that all samples collected within a river were more similar to each other than they were to any samples collected from the other river, regardless of the leaf type on which they were incubated.

Genetic effects

Differences in microbial communities across leaf cross types were more subtle and depended upon the type of microbe (fungi versus bacteria) and the stream in which leaves were incubated. The strongest and most consistent differences among cross types were in diversity and evenness (Fig. 3). As predicted,

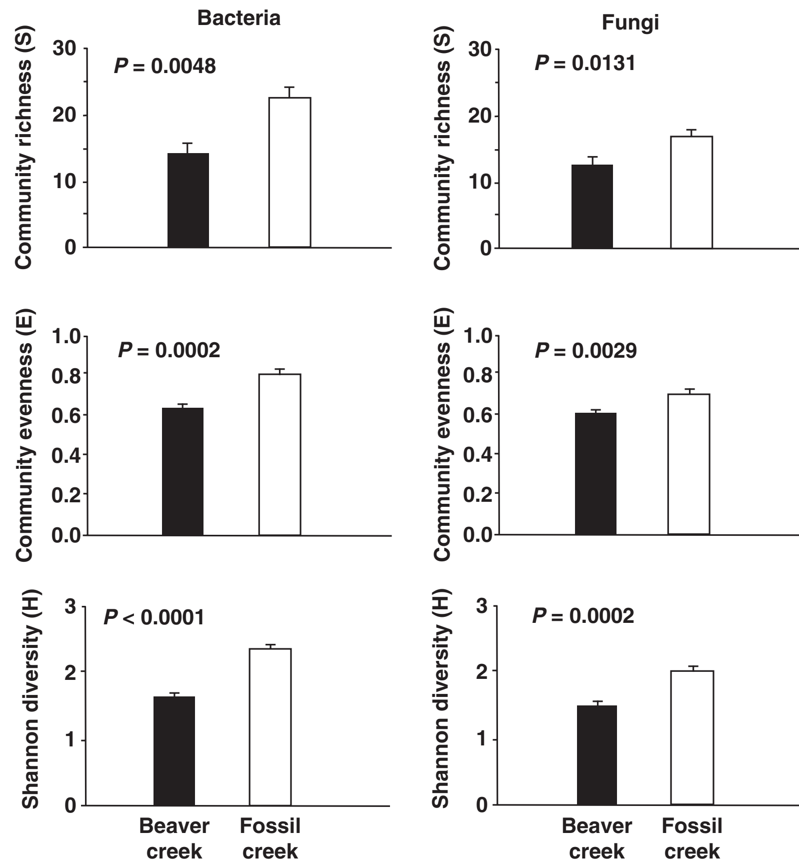

Fig. 1 Average richness, evenness and total diversity of bacterial and fungal communities on leaf packs in Beaver Creek (BC) show that phylotype diversity is significantly higher in Fossil Creek than BC. Corresponding statistical analyses are shown in Table 2.

Table 2 Two-way ANOVA results for richness, evenness and diversity of fungal and bacterial communities growing on leaves incubated in Fossil Creek and Beaver Creek

		Richness		Evenness		Diversity	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Fungi							
Stream	1	7.78	0.0131*	12.33	0.0029*	24.12	0.0002*
Leaf type	3	0.66	0.5899	5.81	0.0070*	3.93	0.0281*
Stream*leaf	3	3.83	0.0305*	0.77	0.5258	2.84	0.0708
Bacteria							
Stream	1	10.95	0.0048*	22.76	0.0002*	42.42	<0.0001*
Leaf type	3	2.25	0.1243	1.28	0.3186	7.25	0.0031*
Stream*leaf	3	1.36	0.2926	0.83	0.4966	2.38	0.1103

Stream (Fossil Creek and Beaver Creek) and leaf cross type are the two independent variables.
*Significant *P* values.

diversity of both bacteria and fungi increased along the hybridising complex from *P. fremontii* to *P. angustifolia* cottonwoods, with hybrids showing intermediate values (Fig. 3; Table 2). This pattern was consistent across rivers. Diversity was higher on backcross hybrids and pure *P. angustifolia* which both have high tannin concentrations and slower decomposition rates than either *P. fremontii* or F_1 hybrids (Driebe & Whitham, 2000; LeRoy *et al.*, 2006, 2007). Leaf litter quality affected diversity and evenness but not species richness suggesting that substrate quality primarily affects the relative abundance of microbial taxa rather than the numbers of species that colonise different leaves. The only significant interaction term (stream * leaf type) was for fungal species richness, indicating that fungi responded more

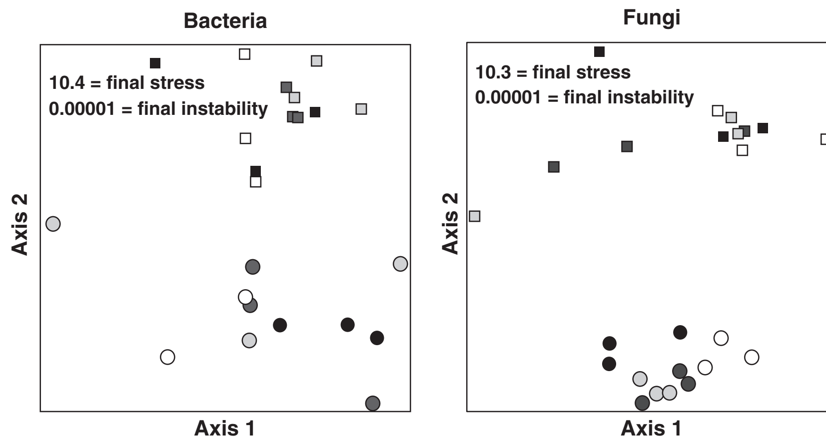

Fig. 2 Non-metric multidimensional scaling ordination comparing bacterial and fungal communities among four leaf cross types incubated in two rivers. Beaver Creek is represented by squares, and Fossil Creek by circles. Light grey, *Populus fremontii*; dark grey, first generation hybrids; black, backcross hybrids; white, *P. angustifolia*. Multi-response permutation procedures results for this ordination are presented in Table 3.

Table 3 Multi response permutation procedure (MRPP) results comparing the differences between bacterial, fungal and total microbial community profiles on decomposing leaves

Independent variable	Bacteria		Fungi		Total Microbes	
	A	*P*	*A*	*P*	*A*	*P*
Stream	0.13	<0.0001*	0.18	<0.0001*	0.18	<0.0001*
Leaf type	−0.03	0.77	0.02	0.19	−0.004	0.5
Leaf type (BC)	0.05	0.65	0.01	0.42	−0.03	0.67
Leaf type (FC)	−0.02	0.61	0.43	<0.01*	0.17	0.006*

Leaf type included four hybridising cross types of cottonwoods that were incubated in two streams, Beaver Creek (BC) and Fossil Creek (FC). MRRP analysis was performed separately on bacteria, fungi and the two groups combined. Separate ordinations were also performed for each river. The A statistic is a measure of effect size and ranges from 0 to 1. Non-metric multi dimensional scaling ordinations are shown in Figs 3 & 4.
*Significant *P* values.

strongly to leaf type in FC than in BC (Table 2). The lack of significant interaction terms for other parameters indicates similar responses in evenness and diversity to leaf type in both streams.

Differences in community structure were most pronounced for fungal communities in FC (Fig. 3; Table 3). The percentage of unique phylotypes found on each cross type ranged from 38% to 63% and was generally lower than the percentage of unique phylotypes found within rivers (Table 1). Fungal phylotypes tended to associate more tightly with cross types than bacterial phylotypes (Table 1). The NMDS and MRRP analyses revealed that fungal communities differed among cross types in FC but not in BC, indicating an interaction between leaf quality and environment showing that plant genes play stronger roles in some streams than in others (Fig. 4; Table 3). Fungal communities colonising *P. fremontii* and F_1 hybrids were similar to each other but differed from both backcross hybrids and *P. angustifolia* cottonwoods which differed from each other (Fig. 4; Table 3). This pattern was driven primarily by one phylotype which accounted for 45% of fungal biomass in *P. fremontii* and 40% in F_1 hybrids. This dominant phylotype was not present in either backcross hybrids or *P. angustifolia*. Backcross hybrids had distinct fungal communities from all other leaf types with 21 of the 48 fungal phylotypes found in FC occurring only on backcross hybrids. In contrast to fungi, bacterial communities were similar among the four leaf cross types in both streams (Fig. 3; Table 2).

Discussion

Our results show that both environmental conditions and plant genes drive community structure of microbial communities colonising decomposing leaf litter. As predicted, microbial communities differed more strongly between streams than among cross types within streams. Within a stream, however, plant genes played an important role in determining the diversity of both bacteria and fungi, and in FC had a strong

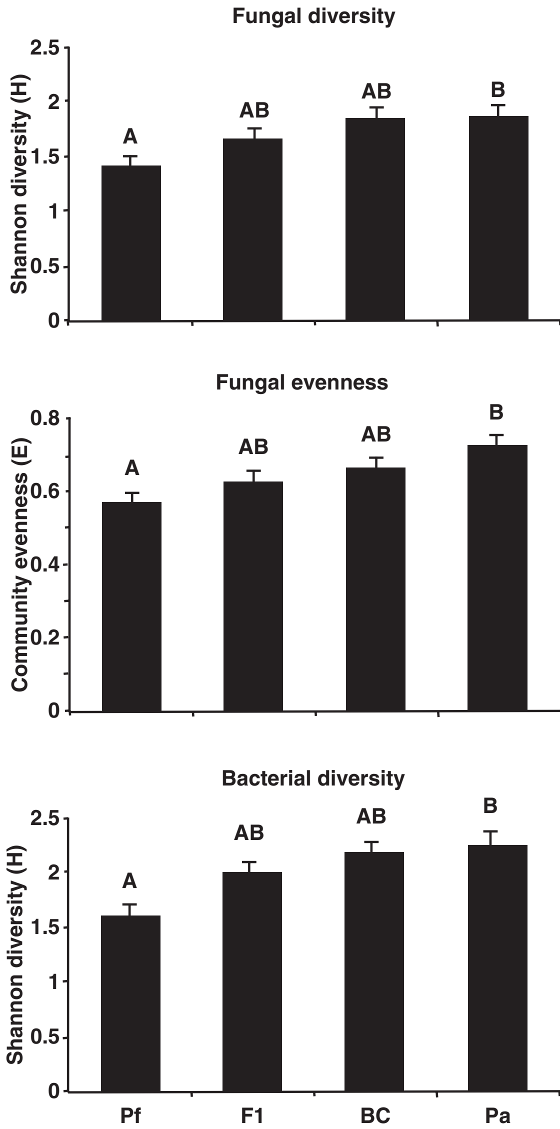

Fig. 3 Comparison of diversity indices for fungi and bacteria colonising four leaf cross types within a hybridising complex of cottonwood trees. Leaves were incubated in Beaver and Fossil Creeks for two weeks. ANOVA results are presented in Table 2. Pf, *Populus fremontii*; F1, first generation hybrids; BC, backcross hybrids; Pa, *Populus angustifolia*.

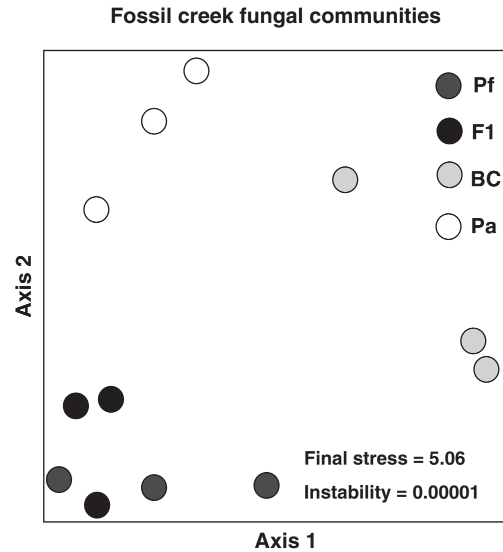

Fig. 4 Non-metric multidimensional scaling ordination comparing fungal communities on four leaf cross types at Fossil Creek. Multi-response permutation procedure values for this ordination are listed in Table 3. Pf, *Populus fremontii*; F1, first generation hybrids; BC, backcross hybrids; Pa, *Populus angustifolia*.

effect on fungal species composition. Diversity of both bacteria and fungi in each stream increased, as predicted, along the genetic gradient from *P. fremontii* to *P. angustifolia* with hybrids showing intermediate values.

Environmental effects

Because this study was designed to discern patterns in microbial communities, it is impossible to attribute a mechanism to the differences between streams or leaf types. These results however are consistent with a study of 23 rivers in Hubbard Brook, New Hampshire, which showed that differences in water chemistry, particularly pH, accounted for most of the variation in bacterial community composition (Fierer *et al*., 2008). Water chemistry may also explain the differences between Beaver and FC. Most studies that have compared the relative importance of stream physicochemistry versus leaf litter quality on microbial community structure have also observed stronger effects of environmental conditions. For example, in a temperate woodland in Nova Scotia, Canada, fungal community structure depended on the number of days leaves were in the stream as well as the season in which they were incubated, but did not vary across different leaf types (Nikolcheva *et al*., 2003; Nikolcheva & Bärlocher, 2005). Similarly, in a stream in Ohio, U.S.A., bacteria and fungal species responded significantly to stage of decay and time of exposure whereas differences among leaf types were relatively small (Das *et al*., 2007). Along the stream gradient within FC, site differences in temperature, flow and water chemistry more strongly structured microbial assemblages than differences in leaf quality (Harrop *et al*., 2009). Microbial patterns also parallel responses of

macroinvertebrates in BC and FC, where differences in macroinvertebrate communities colonising leaves among streams were much larger than differences among leaf type within a stream (LeRoy & Marks, 2006).

Leaf quality/plant genetic effects

Community differences among cross types in FC were more pronounced than those documented using similar molecular techniques among phylogenetically different leaf species, including red maple (*Acer rubrum* L.), linden (*Tilia cordata* Mill.), alder [*Alnus glutinosa* (L.) Gaertn.], beech (*Fagus sylvatica* L.), and oak (*Quercus rubra* L.) (Nikolcheva *et al.*, 2003; Nikolcheva & Bärlocher, 2005). Although our design does not allow attribution of one trait to the differences that we observed, other research has shown that tannin concentration is the best predictor of decomposition among cottonwood leaf types (Driebe & Whitham, 2000; Schweitzer *et al.*, 2004; LeRoy *et al.*, 2007). It is difficult to explain why cross types had more pronounced differences in microbial communities in FC, but it could be that the higher species richness on leaf packs in FC may contribute to the strength of ecological interactions among microbes.

Microbial diversity

The observation that both fungal and bacterial diversity increased as predicted along the genetic gradient from *P. fremontii* to *P. angustifolia* shows that diversity in these rivers is negatively correlated with decomposition rate and positively with tannin concentration. Fungal diversity is likely to be driven by many factors including resource quality, productivity and resource diversity. The negative correlation between diversity and decomposition rate, an index of productivity in this detrital based system, is consistent with grassland studies showing that diversity is negatively correlated with productivity particularly in systems with high nutrients (Wisheu *et al.*, 2000; Tilman *et al.*, 2001). The dominance of one phylotype on the *P. fremontii* and F_1 hybrids leaf litter is consistent with the general observation that high productivity and resource availability can result in competitive exclusion, thereby reducing diversity. We postulate that the higher diversity on *P. angustifolia* is because of its more complex suite of chemical compounds. Although fungal species richness can cause modest increases in decomposition under controlled laboratory conditions (Bärlocher & Corkum, 2003) in the cottonwood system, *P. angustifolia* consistently decomposes more slowly than *P. fremontii* despite higher microbial diversity. A negative correlation between decomposition and fungal diversity was also observed in FC when comparing across leaf types and habitats (Harrop *et al.*, 2009). It is possible that under field conditions, where microbial diversity is much higher than in lab experiments (Bärlocher & Corkum, 2003), increases in decomposition caused by higher diversity may be small relative to the differences in decomposition caused by leaf quality. Potential stimulation of decomposition caused by the increase in diversity on *P. angustifolia* is not sufficient to offset the slower decomposition rates caused by high concentrations of complex carbon molecules such as tannins. Together these field and laboratory studies suggest complex interactions among leaf quality, decomposition and microbial diversity which warrant further study.

The lack of differences in species richness among cross types is consistent with other microbial studies that show that within a river most fungal and bacterial species maintain populations on all leaf types (Nikolcheva *et al.*, 2003; Nikolcheva & Bärlocher, 2005; Das *et al.*, 2007). This result is also consistent with how terrestrial arthropods respond to differences among cross types, showing strong differences in community structure but not necessarily in species richness (Wimp *et al.*, 2005; Bangert *et al.*, 2006a,b). In both rivers, there were many unique phylotypes of bacteria and fungi found on individual leaf packs suggesting a high degree of microhabitat diversity. This could be because the short duration of the study did not allow sufficient time for these phylotypes to colonise more of the leaf packs. Microbial diversity on leaves tends to peak between 2 and 3 weeks (Nikolcheva *et al.*, 2003; Das *et al.*, 2007). Future studies that follow microbes through the entire decomposition process will provide a more refined test of whether these unique phylotypes associate with different leaf types.

Aquatic versus terrestrial microbes

Aquatic fungi and bacteria showed stronger and somewhat different patterns than ectomycorrhizal fungi and soil bacteria associated with the same cross types growing in the common garden where our

leaves were gathered. For example, diversity of ectomycorrhizal fungi was opposite to what we observed and was higher on root tips of Freemont leaves relative to hybrids and *P. angustifolia* (Z. Kovacs, unpubl. data). Community composition of ectomycorrhizal fungi, also measured using PCR-based methods, did not differ among cross types (Z. Kovacs, unpubl. data). Soil bacterial communities living under different trees in the common garden did not differ among cross types, although there were significant and heritable differences among individual genotypes within the *P. angustifolia* cross type (Schweitzer *et al.*, 2008). Together, these studies indicate that plant genes influence many different types of microbial communities, but the genetic effects vary according to the type of microbial association. Microbes associated with leaf litter decomposition are probably responding to differences in foliar chemistry (Driebe & Whitham, 2000; Rehill *et al.*, 2006; LeRoy *et al.*, 2007), whereas microbes associated with roots correlate with differences in root productivity. Free living bacteria in the soil may be responding to multiple factors including both leaf chemistry and soil factors such as soil water content and nutrient availability all of which differ among cross types (Schweitzer *et al.*, 2004; Fischer *et al.*, 2007).

Molecular tools are allowing ecologists to understand how microorganisms are distributed. Our results suggest a hierarchical structure, where environmental conditions of a stream most strongly affect microbes, but within some streams leaf litter quality differences between or within plant species can have a significant effect on diversity and species composition. The close proximity of these two streams suggests that microbial species are not limited by dispersal but rather the environment is selecting, as Baas-Becking (1934) suggested decades ago. The environment by leaf type interaction indicates that the role of leaf quality and plant genes varies across ecosystems and indicates that selection pressures on microbes also differ across ecosystems. Understanding how plant genes affect microbial communities is important for understanding the underlying factors that structure microbial communities. Further integration of laboratory and field studies that manipulate microbial species will facilitate elucidate the conditions under which microbial species composition affects ecosystem processes and the degree to which changes in microbial communities affect higher trophic levels. The interaction between plant genes and microbial communities that we observed may prove to be important in riparian restoration projects where managers may want to ensure that multiple genotypes of plant species are replanted to maintain microbial diversity.

Acknowledgments

We thank the Ogden Nature Center in Utah for providing land and other resources to maintain the common garden facility. Michele James provided valuable editorial assistance. This work was funded by NSF grants DEB 0130487 and FIBR 0425908. Brenda Harrop was supported on an IGERT fellowship while contributing to this paper.

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(Manuscript accepted 10 June 2009)