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MICROBIOLOGY OF AQUATIC SYSTEMS

The Influence of Time and Plant Species on the Composition of the Decomposing Bacterial Community in a Stream Ecosystem

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Abstract Foliar chemistry influences leaf decomposition, but little is known about how litter chemistry affects the assemblage of bacterial communities during decomposition. Here we examined relationships between initial litter chemistry and the composition of the bacterial community in a stream ecosystem. We incubated replicated genotypes of *Populus fremontii* and *P. angustifolia* leaf litter that differ in percent tannin and lignin, then followed changes in bacterial community composition during 28 days of decomposition using 16S rRNA gene-based pyrosequencing. Using a nested experimental design, the majority of variation in bacterial community composition was explained by time (i.e., harvest day) ($R^2 = 0.50$). Plant species, nested within harvest date, explained a significant but smaller proportion of the variation ($R^2 = 0.03$). Significant differences in community composition between leaf species were apparent at day 14, but no significant differences existed among genotypes. Foliar chemistry correlated significantly with community composition at day 14 ($r = 0.46$) indicating that leaf litter with more similar phytochemistry harbor bacterial communities that are alike. Bacteroidetes and β-proteobacteria dominated the bacterial assemblage on decomposing leaves, and Verrucomicrobia and α- and δ-proteobacteria became more abundant over time. After 14 days, bacterial diversity diverged significantly between leaf litter types with fast-decomposing *P. fremontii* hosting greater richness than slowly decomposing *P. angustifolia*; however, differences were no longer present after 28 days in the stream. Leaf litter tannin, lignin, and lignin: N ratios all correlated negatively with diversity. This work shows that the bacterial community on decomposing leaves in streams changes rapidly over time, influenced by leaf species via differences in genotype-level foliar chemistry.

Keywords Streams · Bacteria · 16S rRNA · Pyrosequencing · Leaf litter chemistry · *Populus*

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Introduction

Leaf litter is a major energy source to forested stream ecosystems [1]. Bacteria contribute to leaf litter decomposition and nutrient cycling [2, 3]. Litter chemistry strongly influences bacterial biomass e.g. [4] and the rate at which litter decomposes [5, 6], yet we do not fully understand how variation in litter traits and decomposition rate influence the composition and diversity of the microbial community catalyzing litter decomposition in aquatic environments. This is mainly because the relationship between the diversity of stream bacterial communities and decomposing leaf litter is inconsistent. In some streams, bacterial diversity and composition do not differ with leaf litter type, even when the litter types have significantly different decomposition rates [7–10]. On the other hand,

greater diversity has been found on both fast-decomposing [11] and slow-decomposing and more recalcitrant leaf litter [12–14]. However, much of the previous work describing bacterial communities on decomposing leaf litter in freshwater systems has used low-resolution fingerprinting techniques such as DGGE or T-RFLP analysis [7, 14, 15]. High-throughput and deep sequencing of the bacterial 16S rRNA gene now provide a more refined description of bacterial community composition including the abundance of rare taxa, e.g., [16, 17]. By using a high-resolution and more powerful molecular technique, we can potentially describe how bacterial community composition and diversity respond to gradients of leaf litter chemistry in greater detail and provide more insight into how resource chemistry structures the composition of this important group of decomposers.

In this study, we assessed changes in community composition and diversity of the bacterial community during decomposition using leaf litter of replicated genotypes from two closely related species (*Populus fremontii* and *P. angustifolia*). These species were selected for their large interspecific and intraspecific phytochemical variation including tannin and lignin concentration [6, 18, 19]. For example, *Populus* species can vary 10–30-fold in leaf litter tannin and lignin concentration [18, 20] while genotypes within a species can vary 2–7-fold [6, 18, 20]. In addition, rates of decomposition span the range of decomposition coefficients (k) for multiple plant families [6]. The use of the *Populus* model system is ideal to examine the bacterial community's response to leaf litter chemistry because the response can be analyzed at both the leaf litter species and genotypic scales, e.g., [6, 19, 21]. This allows us to set our results within a community genetics framework, e.g., [19] which examines the effects of genetic variation (e.g., foliar chemistry) within foundation species [22] on community and ecosystem processes. Leaf litter with higher concentrations of tannin and lignin has slower rates of decomposition [6, 18, 23], produces less bioavailable dissolved organic matter [21], and influences microbial abundance and biomass during decomposition [4, 20]. Foliar chemistry may also affect bacterial community composition if different groups of bacteria use different ecological strategies to compete for and access nutrients, e.g., [24–26].

Our objective was to understand how genetic-based differences in resource chemistry, in this case leaf litter, structures bacterial community composition throughout decomposition in stream ecosystems. While fungi are important players in the decomposition process and appear to dominate based on biomass measurements [27], this study focuses on the bacterial community because less is known about how factors such as resource chemistry (e.g., leaf litter chemistry) affect the community composition and structure of this group [28]. We hypothesized that community composition would differ between the two leaf species and among genotypes within each species based on differences in foliar tannin and lignin concentration. We specifically hypothesized that genotypes with more similar foliar chemistry would have more alike bacterial communities. Species-time relationships may also be particularly strong in bacterial communities [29] with distinct ecological groups fulfilling early and later roles during decomposition [24–26]. Thus, we also hypothesized that time in the stream (i.e., harvest day) would explain a significant amount of the variation in community composition. We predicted that diversity would increase on both leaf species with time, but the highest overall diversity would occur on recalcitrant leaves as substrates with more complex chemical compounds require an array of enzymes to access nutrients [30, 31]. These hypotheses use the natural gradient of foliar chemistry provided by the *Populus* model system at both the species and genotypic scale to make strong inferences regarding the role of leaf litter chemistry in structuring bacterial communities. Understanding the factors that control bacterial assembly in detrital-based food webs is needed to develop an ecological framework for how microorganisms vary across time and resource gradients and the role of microbial diversity in ecosystem processes.

Methods

Study System

We collected naturally abscised leaf litter in 2008 from a common garden (i.e., trees planted in the same environment—*see below*) in Ogden, Utah from replicated genotypes of *P. fremontii* (four replicated genotypes) and *P. angustifolia* (six replicated genotypes). Trees were genotyped using 35 specific *P. fremontii* markers [32, 33]. Leaf litter from the common garden provides a model system for studying how species and genetic level differences in litter quality and chemistry affect bacterial communities. Leaves were air dried in the laboratory prior to analyses. We characterized phytochemicals prior to stream incubation. We measured soluble and bound condensed tannin concentration, lignin concentration, and C/N ratios for each genotype. Condensed tannins were measured with the acid butanol assay [34] with purified *P. angustifolia* condensed tannins as standards. Lignin concentration was estimated gravimetrically using an Ankom 2000 fiber analyzer (Ankom Technology, Macedon, NY), and percent C and N was determined via combustion using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies, Valencia, CA). These chemistry data (and subsequent PCA analysis—*see below*) have been previously published [20]; however, they are used in a new context here. In particular, we use these data to address unique hypotheses regarding the community composition and diversity of bacteria on decomposing leaves. There were significant differences

in phytochemistry between *Populus* species (Table 1); [20]. *P. angustifolia* had significantly higher concentrations of soluble ($P<0.01$) and bound condensed tannin ($P<0.01$), lignin ($P<0.001$), and lignin/N ratios ($P<0.01$). There were no significant differences in C/N ratios between the two species ($P=0.22$).

Field Study

To permit analyses at the species and genotype level, we mixed litter from three replicate clones of each *Populus* genotype that were randomly grown in a common garden. Because replicated clones were grown in a common garden, significant differences in phytochemistry and other traits among genotypes and species can be assumed to be genetically based and not due to differences in the environment (see [6, 19] for further description). Two grams of this mixture was placed into 20 × 20 cm 6.4-mm mesh Vexar® bags (Trical netting, aquatic Eco-Systems, Apopka, Florida). Using a fully randomized design, bags were secured to rebar and placed in the headwaters of Oak Creek, Arizona (November, 2010), a high-elevation first-order stream. Oak Creek is a perennial headwater stream flowing off the southern edge of the Mogollon Rim south of Flagstaff, Arizona. Oak Creek has an annual average flow of 368 L s^{-1} and is underlain by Paleozoic sandstone and tertiary igneous formations causing the stream to have slightly higher alkalinity. During the experiment, Oak Creek had a mean water temperature of 10.5 ± 0.1 °C and a mean pH of 8.3 ± 0.04. Mean dissolved oxygen (% saturation) was 95.2 ± 0.6 and mean specific conductance was 290 ± 1.6 μS cm^{-1}. Ambient nutrient concentrations for Oak Creek range from 0.12–0.28 mg L^{-1} for ammonium (NH_4^+) and nitrate (NO_3^-), respectively, and 0.17 mg L^{-1} for phosphate (PO_4^{-3}) [35]. Three to four replicate litter bags per genotype were harvested on days 6, 14, and 28. Bags were stored on ice, transferred to a −20 °C freezer, and processed within 24 h.

Sample Processing and DNA Extraction

In the laboratory, leaves were removed from bags and placed on a sterile wax surface. Twenty-five leaf cores (~0.5 g) were randomly taken from all leaves in a bag. Cores were spatially distributed across the leaf surface to obtain a full representation of the bacterial community. Cores were placed in 15 % glycerol and stored at −80 °C until DNA extraction (<3 months).

Bacterial cells were lysed chemically and mechanically. Chemical lysis was performed by adding 600 μl RLT buffer to each leaf core sample (Qiagen Inc., Valencia, CA), and mechanical lysis was performed using a Barocycler NEP 2320 (Pressure Bioscience Inc. Easton, MA) at room temperature with 15 cycles of 10 s at 35,000 psi followed by 10 s at atmospheric pressure. After lysis, genomic DNA was isolated and purified using the AllPrep DNA/RNA Kit (Qiagen Inc., Valencia, CA).

Table 1 Phytochemical characteristics of the two *Populus* leaf types and genotypes

	C/N⁺	% SCT⁺	% BCT⁺	% Lignin⁺
P. fremontii	58.60a	0.11a	0.17a	9.58a
17	58.82	<0.02	0.19	9.52
31	71.10	0.22	0.17	9.25
KH8	66.34	0.23	0.13	9.45
KSCR-1	38.14	<0.02	0.20	10.11
P. angustifolia	49.23a	1.94b	2.91b	23.04b
1008	58.28	2.83	2.97	22.38
HE-10	45.94	0.41	1.35	17.46
T-15	39.50	1.02	2.95	23.34
1007	56.18	3.04	3.70	25.07
RM-2	54.70	3.18	3.45	22.33
WC-5	40.80	1.18	3.03	27.69

%SCT soluble condensed tannin (SCT), %BCT bound condensed tannin (BCT). Data are species means and individual genotype values. Different letters represent statistically significant differences between species (α = 0.05). Data originally presented in [20]

Bacterial 16S rRNA Gene-Based Pyrosequencing Analysis

We generated barcoded V3–V6 amplicons using broad-coverage fusion PCR primers. Amplification of the 16S rRNA genes in each DNA sample was performed in a 96-well format using broad-range fusion forward primer 341 F (5′-CCTACGGGDGGCWGCA-3′) and fusion reverse primer 807R (5′-CTGACGACRRCCRTGCA-3′) [36]. Amplicons were pooled and sequenced on the Genome Sequencer FLX (Roche Applied Sciences, Branford, USA). We identified chimeric sequences *de novo* using U-Search's cluster utility (U-Search version 5.0.144) and U-Chime at the 99 % threshold [37, 38]. Next, non-chimeric sequences were demultiplexed and quality-checked [37]. Each pyrosequence was classified with a 99 % bootstrap confidence level at each taxonomic level using the Ribosomal Database Project Naïve Bayesian Classifier (RDP Release 10, Update 28) [38] to generate an abundance-based data matrix. Phylotypes were identified at the 97 % sequence similarity level. Additional methodological details can be found in the supplementary file. After removing samples with insufficient number of sequences (<1600 sequences per sample), pyrosequencing produced 277,020 individual sequences with an average of 2789 sequences per sample and a mean sequence length of 344.2 bases. The number of sequences per sample ranged from 1670 to 5005. Since one of the objectives of this study was to estimate biodiversity during leaf decomposition, we did not subsample our data to an equivalent sequencing depth. Subsampling to an equivalent depth removes valuable data underestimating biodiversity. Importantly, there were no significant differences in the

number of sequences per sample between species or within a species across harvest days.

Statistical Analyses

We used non-metric multidimensional scaling (NMDS) ordination to visualize and compare the structure of bacterial communities between *Populus* species and genotypes across and within harvests using a Bray-Curtis distance matrix. Unlike other ordination techniques, NMDS can be used with any distance measure and it uses rank-order correlation which minimizes the horseshoe/detrending-effect associated with other ordination techniques (e.g., PCoA) [39]. We used a nested PERMANOVA design (*permutations* = *1000*) to partition the variation in community composition by the factors incubation times (i.e., harvest dates) and leaf litter species. We nested leaf species within harvest date. PERMANOVA is appropriate in this context as ecological community data is often not normally distributed making tests like MANOVA less effective in partitioning variation among groups [40]. We also used goodness of fit tests at each harvest date to determine if the bacterial community was significantly different between the leaf species and among genotypes independent of time. We specifically tested the influence of phytochemistry using Mantel tests [41] (*permutations* = *1000*) by correlating Bray-Curtis-based dissimilarity matrices of community composition at each harvest date with a Euclidean-based dissimilarity matrix of phytochemistry based on a principle component analysis (PCA) (*data originally presented in* [20]). The use of a Mantel test specifically allows us to ask the question: do leaf types with more similar chemistry harbor more similar bacterial communities? We used PCA to summarize phytochemical variation among genotypes and loaded percent soluble condensed tannins, percent bound condensed tannins, percent lignin, and C/N ratios into the model. These analyses were performed in R using the *vegan* package version 2.15.3 [42].

Richness (number of unique genera) and evenness and Shannon's diversity (H) were compared using a one-tailed Student's *t* test ($\alpha = 0.05$). Diversity metrics at each harvest were correlated with C/N ratios, soluble and bound condensed tannin (%), lignin (%) and lignin/N ratios, and the first axis of the phytochemistry PCA. These analyses were performed in SPSS for Windows [43]. We also tested genera fidelity between the leaf species using indicator species analysis. We identified those bacterial genera that had significant indicator values greater than 0.5 for each leaf type at each harvest date. These analyses were performed in R using the *vegan* package version 2.15.3 [42].

Results

Community Composition

As predicted, time in the stream, expressed as harvest date, explained a large percentage of the variation in the composition of the bacterial community ($R^2 = 0.50$, $P < 0.001$). Also, as hypothesized, the relationship between the composition of the bacterial community and leaf species was significant but the amount of variation partitioned to leaf species was considerably smaller than the factor of time (Fig. 1a; $R^2 = 0.03$, $P < 0.001$). Within a harvest date, the bacterial community only differed significantly between the leaf species at day 14 (Fig. 1b; $R^2 = 0.20$, $P < 0.001$). There were no significant

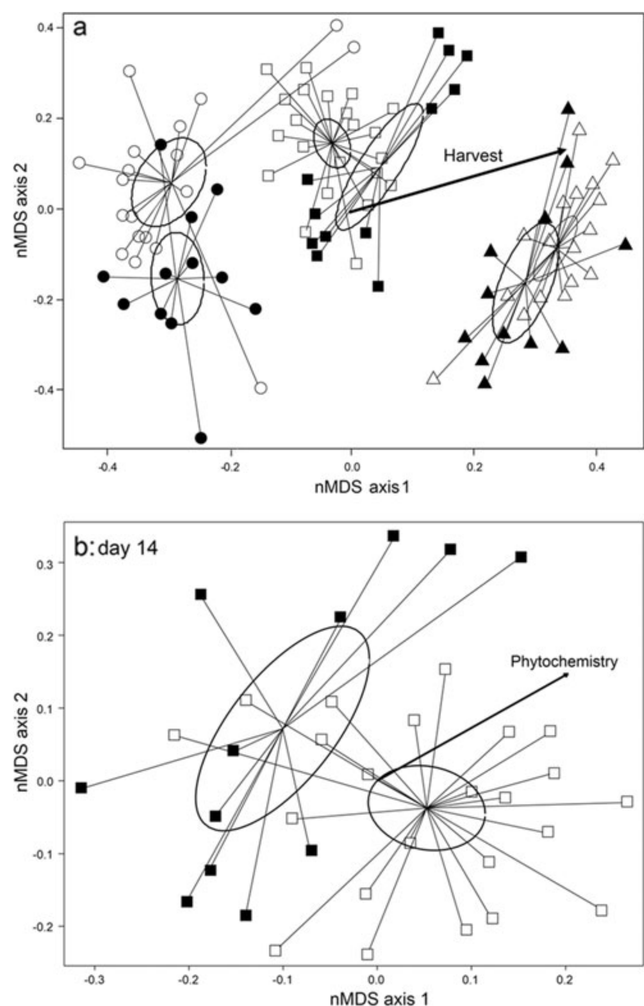

Fig. 1 Non-metric multidimensional scaling (NMDS) comparing bacterial communities on two types of *Populus* leaf litter through 28 days of decomposition in a stream. **a** NMDS constructed using harvest day and leaf litter species as factors. **b** NMDS of leaf litter species at day 14 (i.e., time excluded from analysis). *Closed shapes*: *P. fremontii*; *open shapes*: *P. angustifolia*. *Circles*: 6 days, *squares*: 14 days, *triangles*: 28 days. *Spheres* reflect 95 % confidence intervals of the data from each group, and *lines* connect each sample to its associated centroid [42].

differences among genotypes within plant species including day 14 (e.g., Fig. 1c; $R^2=0.46$, $P=0.10$). Bacterial community composition measured as Bray-Curtis distance correlated significantly with the first axis of the phytochemistry PCA at day 14 (Mantel test, $r=0.46$, $P=0.0001$) and marginally at days 6 and 28 ($r=0.09$, $P=0.10$; $r=0.08$, $P=0.06$, respectively), indicating that leaf litter with similar measurements of phytochemistry harbor more alike bacterial communities.

In agreement with our hypothesis that bacterial communities would diverge over time among leaf types, we found that the bacterial community changed significantly throughout decomposition (Table 2, Fig. 1a). Proteobacteria, specifically β-proteobacteria, together with Bacteroidetes, dominated both leaf types at all harvest dates but showed variable responses over time depending on leaf type (Table 2). Alpha and δ-proteobacteria and the Verrucomicrobia increased on both litter types throughout decomposition. No genera were unique to either leaf species at day 6. By the second harvest, at day 14, both leaf species had 2 unique species and by day 28, *P. fremontii* and *P. angustifolia* had 14 and 13 unique species, respectively (Table 3).

Bacterial Diversity and Phytochemistry

In contrast to our expectations, labile *P. fremontii* litter hosted higher levels of bacterial diversity; however, significant differences between plant species were only observed at day 14 ($P<0.01$) (Table 4). The effect of phytochemistry was also strongest at harvest day 14 (Table 5) with no significant relationships found at day 6 or day 28. Also, in contrast to our hypothesis that higher diversity would occur on more recalcitrant leaves, richness correlated negatively and significantly with percent bound condensed tannin, percent lignin, and lignin/N ratios. Evenness and Shannon diversity also correlated negatively and significantly with percent soluble condensed tannin, percent bound condensed tannin, percent lignin, and lignin/N ratios. C/N ratios did not correlate significantly with any diversity metric during decomposition.

The first two axes of the PCA explained 96.8 % of the variation in foliar chemistry (Table 6). Soluble and bound condensed tannins and lignin correlated significantly with the first PCA axes. In a pattern similar to the univariate linear regressions, the first axis of the PCA correlated significantly and negatively with metrics of biodiversity (Fig. 2). *P. fremontii* clones had relatively low concentrations of tannins and lignin and higher bacterial diversity than *P. angustifolia* clones which had higher concentrations of recalcitrant compounds.

Discussion

The Development of Bacterial Assemblages Through Time

Bacterial assemblages in freshwater ecosystems, including streams, are determined by a mix of stochastic and sorting processes [16, 44–47]. Our results show that time in the stream is a major factor in controlling bacterial assemblages, similar to patterns found in other freshwater systems [7, 17, 29, 46, 47]. Litter species is less important, although there were differences in composition and diversity after 2 weeks of decomposition. One explanation for these patterns is that early in decomposition, communities are dominated by taxa that can colonize new substrates quickly regardless of litter species, e.g., [7]. Communities 2 weeks later are sorted by litter chemistry. And later in decomposition, bacterial communities converge as both litter species are mostly comprised of recalcitrant compounds. The relative strength of stochastic and species-sorting processes likely change during different phases of decomposition [16, 44–46]. For example, the initial development of stream biofilms can be a random assemblage from the larger community of potential colonizers [44, 45]

Table 2 Community composition presented as relative abundance (%) of the dominant bacterial phyla and five classes within the Proteobacteria phylum on *Populus* leaf litter during 28 days of decomposition

Phylum Class	*P. fremontii* Day 6, Day 14, Day 28			*P. angustifolia* Day 6, Day 14, Day 28		
Bacteroidetes	36.7 (5.6)	19.8 (2.2)	17.3 (0.7)	19.3 (2.7)	21.5 (1.4)	21.4 (0.9)
Proteobacteria	62.1 (5.6)	74.7 (1.8)	73.7 (2.1)	79.3 (2.6)	73.8 (1.3)	68.7 (1.5)
α-Proteobacteria	8.9 (1.1)	10.3 (1.4)	14.2 (1.9)	12.3 (1.3)	7.7 (0.4)	21.2 (2.5)
β-Proteobacteria	88.7 (1.3)	80.0 (2.7)	74.7 (2.7)	84.7 (1.3)	86.2 (0.5)	69.2 (2.7)
δ-Proteobacteria	0. (0.0)	2.5 (0.2)	4.9 (0.6)	0.5 (0.1)	1.5 (0.1)	3.3 (0.2)
ε-Proteobacteria	0.0 (0.0)	0.1 (0.1)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)
γ-Proteobacteria	2.0 (0.3)	7.3 (1.5)	6.2 (0.4)	2.6 (0.4)	4.6 (0.3)	6.2 (0.4)
Verrucomicrobia	0.5 (0.1)	2.5 (0.5)	5.3 (1.4)	0.5 (0.1)	0.9 (0.1)	6.6 (0.7)
Other	0.7 (0.0)	3.0 (0.3)	3.6 (0.6)	0.9 (0.1)	3.8 (0.4)	3.3 (0.3)

The relative abundances of the five classes are expressed as a percentage of the Proteobacteria phylum. Values are means (±1 SE)

Table 3 Indicator species analysis

Species (day)	*Genus*	Indicator value	*P* value
P. fremontii (6)	N/A	N/A	N/A
P. fremontii (14)	*Rheinheimera*	0.56	0.001
	Naxibacter	0.57	0.001
P. fremontii (28)	*Methylopila*	0.50	0.001
	Blastopirellula	0.50	0.001
	Rhodopseudomonas	0.51	0.001
	Chondromyces	0.53	0.001
	Haliea	0.53	0.001
	Gp4	0.56	0.001
	Aspromonas	0.57	0.001
	Filimonas	0.57 5757	0.001
	Derxia	0.57	0.001
	Phaselicystis	0.62	0.001
	GpXIII	0.64	0.001
	Aquimonas	0.64	0.001
	Simiduia	0.65	0.001
	Limnobacter	0.68	0.001
P. angustifolia (6)	N/A	N/A	N/A
P. angustifolia (14)	*Solirubrobacter*	0.57	0.001
	Conexibacter	0.65	0.001
P. angustifolia (28)	*Reichenbachiella*	0.50	0.001
	Sporocytophaga	0.51	0.001
	Rhizobacter	0.51	0.001
	Lysobacter	0.54	0.001
	Coraliomargarita	0.55	0.001
	Marixanthomonas	0.57	0.001
	Denitratisoma	0.59	0.001
	Azospira	0.61	0.001
	Actinoplanes	0.63	0.001
	Sterolibacterium	0.65	0.001
	Microvirga	0.65	0.001
	Fulvivirga	0.70	0.001
	Fabibacter	0.72	0.001

Only genera with a significant indicator value equal or greater than 0.5 are included. N/A means that there were no bacterial genera that met the criteria for that leaf species and that harvest date

with species-sorting mechanisms operating later during decomposition [16]. The pronounced changes in community composition through time regardless of litter species indicates that bacterial assemblages do not form randomly.

Temporal shifts indicate that bacteria have different ecological strategies. The copiotrophic–oligotrophic framework of community composition describes ecological strategies for bacteria [24, 25] in which copiotrophs are r-selected taxa that are fast growing and preferentially consume labile C pools, whereas oligotrophs decompose recalcitrant and low quality nutrients and grow more slowly [24, 48]; see also [49].

Table 4 Measurements of bacterial diversity on *Populus* leaf litter during 28 days of decomposition

Metric		Day 6	Day 14	Day 28
Richness	*P. fremontii*	91.0 (3.9)a	185 (8.4)a	232 (9.0)a
	P. angustifolia	88.2 (3.9)a	150 (4.0)b	201 (7.2)a
Evenness	*P. fremontii*	0.57 (0.01)a	0.69 (0.01)a	0.74 (0.01)a
	P. angustifolia	0.60 (0.01)a	0.67 (0.01)b	0.74 (0.01)a
Shannon	*P. fremontii*	2.6 (0.08)a	3.6 (0.05)a	4.0 (0.06)a
	P. angustifolia	2.7 (0.06)a	3.4 (0.03)b	3.9 (0.05)a

Biodiversity metrics include species richness, species evenness, and Shannon's diversity. Different letters reflect significant differences at $\alpha = 0.05$

Bacteroidetes and β-proteobacteria, which were more abundant during early decomposition especially on fast decomposing and labile *P. fremontii*, also increased in relative abundance in terrestrial [24, 49] and aquatic systems [50, 51] when C was readily available, suggesting a copiotrophic strategy for these groups. Bacteria exhibiting oligotrophic strategies in this study include the α- and δ-proteobacteria and the Verrucomicrobia which is consistent with other field studies. For example, leaf litter with higher concentrations of phenolics and tannins (due to growth under elevated CO_2 concentrations) also had greater abundance of α-proteobacteria [13]. Culture-based experiments also support the oligotrophic categorization for these groups as lower-nutrient media and longer-incubation times are often required for the growth of, for instance, Verrucomicrobia [52].

The observation of greater diversity after 14 days of decomposition on more labile litter differed from our prediction and prior research [12–14]. Specifically, we show that fast-decomposing and labile litter supports greater bacterial diversity after 2 weeks of decomposition. This pattern is opposite than a similar *Populus*-based study that found greater diversity on slow-decomposing leaf litter [14]. The fast rates of decomposition associated with *P. fremontii* [6] may be driven by this

Table 5 R^2 values from linear regressions between initial leaf litter chemistry and bacterial diversity at the day 14 harvest for *Populus fremontii* and *P. angustifolia* genotypes

	Community Richness	*Community Evenness*	*Shannon Diversity*
C:N	0.03	0.02	0.01
% SCT	0.40	0.40	0.40
% BCT	0.54	0.55	0.61
% Lignin	0.56	0.56	0.67
Lignin: N	0.54	0.74	0.70

Bold font indicates statistical significance ($\alpha = 0.05$) and the directionality of the significant regressions is negative ($N = 10$ for each regression)

%SCT soluble condensed tannin, *% BCT* bound condensed tannin

Table 6 Results of a principle component analysis based on initial phytochemistry of the four *Populus fremontii* and six *P. angustifolia* genotypes

	Principle component	
Result	I	II
% variation explained	70.5	26.3
Cumulative % total variance explained	70.5	96.8
Correlations of original variables with PC1		
% soluble condensed tannins	**0.86**	
% bound condensed tannins	**0.99**	
% lignin	**0.97**	
C: N	−0.39	

Bold indicated statistical significance ($P<0.01$). Data originally presented in [20]

greater diversity [53]. These differences may have been observed because pyrosequencing detects more rare species than DNA fingerprinting techniques employed in previous studies [16]. Once labile litter types like *P. fremontii* lose their high proportion of soluble dissolved organic carbon [21] and other labile substrates [54–56], what might remain is a highly complex assortment of polymeric C [57]. With time, labile litter types may ultimately require a suite of enzymes produced by more diverse microbial consortia [31]. Moore et al. [31] suggest that ontogenetic shifts occur during decomposition which changes resource chemistry. Changes in leaf litter chemistry during decomposition may ultimately be responsible for the observed changes in the bacterial community. Although results presented here demonstrate the effects of initial chemistry, future studies would benefit from progressive sampling of leaf litter chemistry which could connect more directly temporal changes in resource chemistry to simultaneous changes in the decomposer community. Such studies would place changes in the bacterial community into a classic ecological succession framework. The effect of plant species may be larger for fungal communities if fungi are more responsive to litter quality and chemistry. However, results are mixed [7, 9, 11, 14, 58, 59] necessitating further study.

Effect of Populus Foliar Chemistry on Bacterial Assemblages

Day 14 patterns of diversity and community composition are predictable by genotype level foliar chemistry. To our knowledge, this is one of the first studies to show the relationship between lignin, lignin/N ratios, and bacterial diversity in stream ecosystems. Lignin/N ratios predict a high amount of the variation observed in ecosystem processes such as decomposition [60], but here, we demonstrate that the ratio can also drive patterns of bacterial diversity. C/N ratios also correlate with rates of leaf litter decomposition

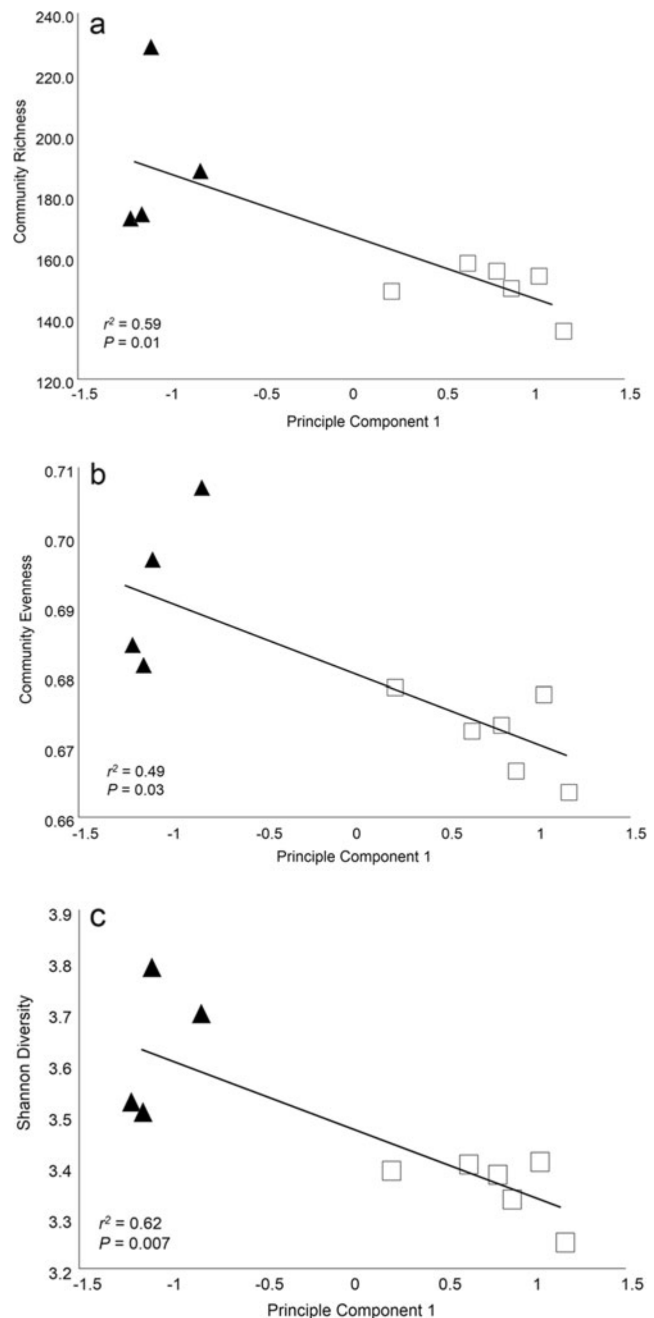

Fig. 2 Linear regression analysis of a composite phytochemical gradient of percent soluble condensed tannins, percent bound condensed tannins, percent lignin, and C/N ratio presented as the first axis scores of a principle component analysis and metrics of bacterial diversity. **a** Community richness; **b** Community evenness; **c** Shannon diversity. *Closed triangles*: *Populus fremontii* genotypes. *Open squares*: *P. angustifolia* genotypes.

[61]. In this and other stream-based studies, C/N ratios did not correlate significantly with either decomposition [6] or microbial abundance [20]. Although these *Populus* genotypes provide a range in C/N ratios, bacterial cells may extract dissolved N from the water column [62, 63] alleviating constrains of leaf litter N. In streams with lower

ambient dissolved inorganic nitrogen concentrations, we might expect a stronger effect of leaf nitrogen content on decomposer communities. Our use of high-throughput sequencing over multiple harvest-dates provides much greater detail into the relationship between genetically based differences in leaf litter chemistry and bacterial community composition and diversity.

Interspecific and intraspecific variation in *Populus* foliar chemistry influences multiple community and ecosystem processes. The dominant source of variation (species, cross types, genotypes) however, differs depending on the community or process, e.g., [6, 21, 23, 64]. However, in contrast to much of the *Populus* community genetic works, e.g., [19, 65], aquatic bacterial communities do not appear as sensitive to genetic-level differences in leaf litter chemistry and appear to respond more strongly to species -level differences (*P. fremontii* vs. *P. angustifolia*). Bacterial gene copy abundance [20] and the bacterial response to leaf litter dissolved organic carbon [21] also differ between these species and their associated hybrid cross types but with no significant genotype effect, despite pronounced differences in phytochemistry.

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Reference

1. Fisher SG, Likens GE (1973) Energy flow in Bear Brook. New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol Monogr 43:421–439
2. Hieber M, Gessner MO (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83:1026–1038
3. Suberkropp K, Klug MJ (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707–719
4. Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817
5. Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, Van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Díaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065–1107
6. LeRoy CJ, Whitham TG, Wooley SC, Marks JC (2007) Within species variation in foliar chemistry influences leaf-litter decomposition in a Utah River. J N Am Benthol Soc 26:426–438
7. Das M, Royer TV, Leff LG (2007) Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Appl Environ Microbiol 73:756–767
8. Fernandes I, Duarte S, Cassio F, Pascoal C (2013) Effects of riparian plant diversity loss on aquatic microbial decomposers become more pronounced with time. Microb Ecol 66:763–772
9. Harrop BL, Marks JC, Watwood ME (2009) Early bacterial and fungal colonization of leaf litter in Fossil Creek, Arizona. J N Am Benthol Soc 28:383–396
10. Kominoski JS, Marczak LB, Richardson JS (2011) Riparian forest composition affects stream litter decomposition despite similar microbial and invertebrate communities. Ecology 92:151–159
11. Kominoski JS, Hoellin TJ, Kelly JJ, Pringle CM (2009) Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? Oikos 118:457–463
12. Dilly O, Bloem J, Vos A, Munch JC (2004) Bacterial diversity in agricultural soils during litter decomposition. Appl Environ Microbiol 70:468–474
13. Kelly JJ, Bansal A, Winkelman J, Janus LR, Hell S, Wencel M, Belt P, Kuehn KA, Rier ST, Tuchman NC (2010) Alteration of microbial communities colonizing leaf litter in a temperate woodland stream by growth of trees under condition of elevated atmospheric CO_2. Appl Environ Microbiol 76:4950–4959
14. Marks JC, Haden GA, Harrop BL, Reese EG, Keams JL, Watwood ME, Whitham TG (2009) Genetic and environmental controls of microbial communities on leaf litter in streams. Freshw Biol 54: 2616–2627
15. Duarte S, Pascoal C, Alves A, Correia A, Cassio F (2010) Assessing the dynamic of microbial communities during leaf litter decomposition in a low-order stream by microscopic and molecular techniques. Microbiol Res 165:351–362
16. Besemer K, Peter H, Logue JB, Langenheder S, Lindström ES, Tranvik LJ, Battin TJ (2012) Unraveling assembly of stream biofilm communities. ISME J 6:1459–1468
17. Portillo MC, Anderson SP, Fierer N (2012) Temporal variability in the diversity and composition of stream bacterioplankton communities. Environ Microbiol 14:2417–2428
18. Driebe EM, Whitham TG (2000) Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia 123:99–107
19. Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks JC, Hart SC, Wimp GM, Wooley SC (2006) A framework for community and ecosystem genetics: from genes to ecosystems. Nat Rev Genet 7:510–523
20. Wymore AS, Compson ZG, Liu CM, Price LB, Whitham TG, Keim P, Marks JC (2013) Contrasting rRNA gene abundance patterns for aquatic fungi and bacteria in response to leaf litter chemistry. Freshw Sci 32:663–672
21. Wymore AS, Compson ZG, McDowell WH, Potter JD, Hungate BA, Whitham TG, Marks JC (2015) Leaf litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. Freshwater Sci 34:doi:10.1086/682000.
22. Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM et al (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486
23. Schweitzer JA, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG (2004) Genetically based trait in a dominant tree affects ecosystem processes. Ecol Lett 7:127–134
24. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364
25. Fierer N, Nemergut D, Knight R, Craine JM (2010) Changes through time: integrating microorganisms into the study of succession. Res Microbiol 161:635–642

26. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76:151–174
27. Findlay S, Tank J, Dye S, Valett HM, Mulholland PJ, McDowell WH, Johnson SL, Hamilton SK, Edmonds J, Dodds WK, Bowden WB (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb Ecol 43:55–66
28. Heino J, Tolkkinen M, Pirttilla AM, Aisala H, Mykra H (2014) Microbial diversity and community-environment relationships in boreal streams. J Biogeogr 41:2234–2244
29. Shade A, Caporaso JG, Handelsman J, Knight R, Fierer N (2013) A meta-analysis of changes in bacterial and archaeal communities with time. ISME J. doi:10.1038/ismej.2013.54
30. McIntosh ACS, Macdonald SE, Quideau SA (2013) Linkages between the forest floor microbial community and resource heterogeneity within mature lodgepole pine forests. Soil Biol Biochem 63: 61–72
31. Moore JC, Berlow EL, Coleman DC, de Ruiter PC, Dong Q, Hastings A, Johnson NC, McCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ, Wall DH (2004) Detritus, trophic dynamics, and biodiversity. Ecol Lett 7:584–600
32. Keim P, Paige N, Whitham TG, Lark KG (1989) Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. Genetics 123:557–565
33. Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. Evolution 55:1325–1335
34. Porter LJ, Hrstich LN, Chan BC (1986) The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin. Pythochemistry 25:223–230
35. LeRoy CJ, Marks JC (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshw Biol 51:605–617
36. Liu CM, Hungate BA, Tobian AAR, Serwadda D, Ravel J, Lester R, Kigozi G, Aziz M, Galiwango RM, Nalugoda F, Contente-Cuomo TL, Wawer MJ, Keim P, Gray RH, Price LB (2013) Male circumcision significantly reduces prevalence and load of genital anaerobic bacterial. mBio 4:doi: 10.1128/mBio.00076-13
37. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods. doi:10.1038/nmeth.f.303
38. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulum-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37(Supplement 1):D141–D145
39. Gotelli NJ, Ellison AM (2004) A primer of ecological statistics. Sinauer Associates, Inc, Sunderland, MA. USA
40. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46
41. Mantel NA (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220
42. R Development Core Team (2009) R: a language and environment for statistical computing. doi:10.1159/000115429
43. SPSS. IBM Corporation (2011) IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY.
44. Besemer K, Singer GA, Limberger R, Chlup A-K, Hochedlinger G, Hödl I-A, Baranyi C, Battin TJ (2007) Biophysical controls on community succession in stream biofilms. Appl Environ Microbiol 73:4966–4974
45. Jackson CR, Churchill PF, Roden EE (2001) Successional changes in bacterial assemblage structure during epilithic biofilm development. Ecology 82:555–566
46. Langenheder S, Székely AJ (2011) Species sorting and neutral processes are both important during the initial assembly of bacterial communities. ISME J 5:1086–1094
47. Lyautey E, Jackson CR, Cayrou J, Rols J-L, Garabétian F (2005) Bacterial community succession in natural river biofilm assemblages. Microb Ecol 50:589–601
48. Rui JP, Peng JJ, Lu YH (2009) Succession of bacteria populations during plant residue decomposition in rice field soil. Appl Environ Microbiol 75:4879–4886
49. Padmanabhan P, Padmanabhan S, DeRito C, Gray A, Gannon D, Snape JR, Tsai CS, Park W, Jeon C, Madsen EL (2003) Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. Appl Environ Microbiol 69:1614–1622
50. Fazi S, Amalfitano S, Pernthaler J, Puddu A (2005) Bacterial communities associated with benthic organic matter in headwater stream microhabitats. Environ Microbiol 7: 1633–1640
51. Simon M, Grossart H-P, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat Microb Ecol 28:175–211
52. Stevenson B, Eichorst S, Wertz J, Schmidt T, Breznak J (2004) New strategies for cultivation and detection of previously uncultured microbes. Appl Environ Microbiol 70: 4748–4755
53. Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S (2010) Diversity meets decomposition. Trends Ecol Evol 25:372–380
54. Rinkes ZL, DeForest JL, Grandy AS, Moorhead DL, Weintraub MN (2014) Interactions between leaf litter quality, particle size, and microbial community composition during the earliest stage of decay. Biogeochemistry 117:153–168
55. Snajdr J, Cajthaml T, Valaskova V, Merhautova V, Petrankova M, Spetz P, Leppanen K, Baldrian P (2011) Transformation of *Quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS Microbiol Ecol 75:291–303
56. van Hees PAW, Jones DL, Finlay R, Godbold DL, Lundström US (2005) The carbon we don't see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biol Biochem 37:1–13
57. Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. For Ecol Manag 133:13–22
58. McGuire KL, Fierer N, Bateman C, Treseder KK, Turner BL (2012) Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. Environ Microbiol 63:804–812
59. Nikolcheva LG, Cockshutt AM, Barlocher F (2003) Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. Appl Environ Microbiol 69:2548–2554
60. Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621–626
61. Enriquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia (Berlin) 94:457–471
62. Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw Biol 48:123–134

63. Pastor A, Compson ZG, Dijkstra P, Rivera JL, Marti E, Sabater F, Hungate BA, Marks JC (2014) Stream carbon and nitrogen supplements during leaf litter decomposition; contrasting patterns for two foundation species. Oecologia 176:1111–1121
64. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. Oecologia 170:695–707
65. Schweitzer JA, Madritch MD, Bailey JK, LeRoy CJ, Fisher DG, Rehill BJ, Hagerman AE, Wooley SC, Hart SC, Whitham TG (2008) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in *Populus* model system. Ecosystems 11:1005–1020