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PRIMARY RESEARCH PAPER

Effects of plant species on stream bacterial communities via leachate from leaf litter

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Abstract Leaf litter provides an important resource to forested stream ecosystems. During leaf fall a significant amount of dissolved organic carbon (DOC) enters streams as leaf leachate. We compared the effects of plant species and leaf leachate bioavailability on the composition of stream bacterial communities and rates of DOC decomposition. We used four common riparian tree species that varied in foliar chemistry, leachate optical properties, and litter decomposition rate. We used laboratory microcosms from two streams and amended with a standard concentration of DOC derived from leaf leachate of the four tree species. After 24 h, we measured rates of DOC biodegradation and determined the composition of the bacterial communities via bar-coded pyrosequencing of the 16S rRNA gene. The composition, diversity, and abundance of the bacterial community differed significantly among plant species from both streams. The phylogenetic distance of the different bacterial communities correlated with species-specific leachate optical properties and rates of DOC biodegradation. Highest rates of DOC decomposition were associated with high tannin and lignin leaf types. Results demonstrate that riparian plant species strongly influences stream bacterial communities via

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their leachate suggesting that alterations to the presence or abundance of riparian plant taxa may influence these communities and associated ecosystem processes.

Keywords Dissolved organic carbon · Leaf litter · Streams · 16S rRNA · Fluorescence spectroscopy

Introduction

The leaching phase of leaf litter decomposition provides a highly labile source of energy to ecosystems in the form of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON; Kuserk et al., 1984). Although the potential significance of leaf leaching to aquatic and terrestrial ecosystems has been recognized for decades (Gosz et al., 1972; McDowell & Fisher, 1976) the impacts of this highly labile material on ecosystem structure and function are not well understood. This is in part due to its inherently ephemeral nature, as it is rapidly removed from solution in both aquatic (Kuserk et al., 1984) and terrestrial (McDowell & Likens, 1988) ecosystems. Although leaf litter leachate in terrestrial systems has received attention (Qualls & Haines, 1992; Magill & Aber, 2000; Park et al., 2002; Cleveland et al., 2007; Leff et al., 2012), fluxes of DOC from soils have previously not been considered important for ecosystem carbon budgets (Qualls & Haines, 1992; Neff & Asner, 2001). The effects of leachate from fresh litter, however, may be especially strong in streams (McDowell & Fisher, 1976; Webster & Benfield, 1986; Meyer et al., 1998) due to the rapid release of leachate into stream water. During autumn, the total DOC pool can comprise 30–42% leaf litter DOC (McDowell & Fisher, 1976; Meyer et al., 1998), impacting secondary production and abundance of heterotrophic bacteria (Meyer et al., 1987) affecting fluxes of C through both the microbial loop as well as aquatic food webs.

Variation among plant species in leachate-derived nutrients and DOC may affect heterotrophic communities. In soils, DOC amendments from leaf litter can promote shifts in bacterial communities compared to water-amended controls (Cleveland et al., 2007); however, differences among plant species are weak (Leff et al., 2012). In contrast, effects of labile leaf leachate in aquatic systems may be particularly strong. For example, leaching can cause up to 30% mass loss of leaves (Webster & Benfield, 1986 and references therein). Leachate from different species of leaf litter can also vary in quality and bioavailability to aquatic microbes, differentially affecting rates of nitrification, for example (Strauss & Lamberti, 2002). Differences in leachate composition and bioavailability among species may exert strong effects on the structure and composition of aquatic bacterial communities, in turn determining rates of DOC decomposition.

Our work was motivated by the general goal to understand how species-level differences in leaf litter inputs structure microbial communities, helping to link the presence of certain plant species with aquatic ecosystem processes. To test the effects of leachate from different leaf species on the composition of aquatic bacterial communities, we amended stream water and sediment from two streams with leaf litter leachate from four common riparian tree species. We predicted that bacterial community composition and diversity would differ among the four plant species and that community differences would be associated with the leachate optical properties (a proxy for molecular composition and leachate quality) of the leachate-derived dissolved organic matter (DOM). Specifically, we hypothesized that leachates with similar chemistry and optical properties, measured via fluorescence and absorbance spectroscopy (Cory et al., 2011), would have more similar bacterial communities. Based on previously published tannin concentrations and decomposition rates of the four species used in this study (LeRoy & Marks, 2006), we hypothesized that the plant species would differ in leachate chemistry, nutrient stoichiometry, optical properties, and rates of DOC biodegradation. Leaf litter with higher concentrations of tannin and lignin leaf types have slower rates of decomposition in both aquatic (Gessner & Chauvet, 1994; LeRoy & Marks, 2006; LeRoy et al. 2007) and terrestrial systems (Schweitzer et al., 2008), influence microbial abundance and community composition throughout decomposition (Wymore et al., 2013, 2016), and produce less bioavailable DOM (Wymore et al., 2015). Thus, we postulated that similar to longer-term decomposition of the tissue, leaf litter high in tannin and lignin would produce more recalcitrant DOC measured as the proportion of aromatic compounds present in 24-h leachate and these recalcitrant

leachates would be associated with lower bacterial abundance (measured as 16S rRNA gene quantities; see Wymore et al., 2013), would have slower rates of DOC decomposition, and would be associated with unique bacterial communities.

Methods

Leaf litter and stream sediment collection

We collected naturally abscised leaf litter from four common riparian species along Oak Creek, Arizona during the autumn 2011: Arizona alder (*Alnus oblongifolia* Torr.), Fremont cottonwood (*Populus fremontii* S. Wats.), Gambel oak (*Quercus gambelii* Nutt.), and Arizona sycamore (*Platanus wrightii* S. Wats.). Leaf litter from these four species differs in tannin, N and P concentration and in rates of decomposition (see Table 2; LeRoy & Marks, 2006). Leaf litter was collected from 5 individual trees per species (except Fremont cottonwood; $n = 4$) for a total of 19 trees. We obtained litter by covering multiple branches per tree with bridal veil netting. Leaf litter never came in contact with the ground and was air-dried in the lab. Leaf litter from all individual trees within a species was then mixed together.

We collected stream water and stream sediments from Oak Creek, Arizona (35°0′N, 111°44′W) and Wet Beaver Creek, Arizona (34°40′N, 111°44′W). Both streams are perennial headwater streams and flow off the southern edge of the Colorado Plateau in north-central Arizona. Oak Creek and Wet Beaver Creek have average annual flows of 368 and 340 l s^{-1}, respectively. Both streams are characterized by a similar geology of Palaeozoic sandstone and tertiary igneous formations that contribute to high alkalinity (LeRoy & Marks, 2006). Oak Creek and Wet Beaver Creek are comparable in salinity, pH, specific conductivity, alkalinity, and total dissolved solids. Oak Creek often has higher levels of ammonium, nitrate, and phosphate (LeRoy & Marks, 2006 for specific water chemistry values). The riparian areas of both streams include the four species described above. Other riparian taxa found within these watersheds include: narrowleaf cottonwood (*Populus angustifolia* James), box elder (*Acer negundo* L.), velvet ash (*Fraxinus velutina* Torr.), coyote willow (*Salix exigua* Nutt.), and Gooding's willow (*Salix gooddingii* Ball) (LeRoy & Marks, 2006). Water and sediments were collected from both streams on the same day, which was the day when the laboratory portion of the experiment began.

DOC concentration and optical properties of leaf species

We conducted a 24-h leaching to extract the most soluble portion of the leaf litter from each species by leaching 1 g of whole leaf litter in 400 ml laboratory-grade de-ionized water (Milli-Q). Leaching was conducted in acid-washed glass beakers at room temperature. Each species was replicated four times. Leachate was syringe-filtered through pre-combusted 0.47 µm glass microfiber filers (Whatman GF/F) into amber glass vials, stored at 4°C and protected from UV light. We determined total DOC and total dissolved nitrogen (TDN) in leachate solutions using a Shimadzu TOCV analyzer with total nitrogen mode and analysis of DOC as non-purgeable organic carbon. We determined the nutrient content of leachate using a robotic analyzer (Westco Smartchem), measuring ammonium (phenate method), soluble reactive phosphorus (SRP; molybdate blue), and nitrate + nitrite (Cd–Cu reduction). DON was calculated as the difference between TDN and inorganic nitrogen ($NO_3 + NH_4$).

We assessed differences in leachate DOM composition and optical properties with fluorescence spectroscopy using a Horiba JY scanning fluorescent spectrophotometer and UV absorbance using a Shimadzu photo diode array detector with HPLC (200–700 nm in 1-nm intervals). Raw excitation and emission matrices (EEMs) were collected at excitation wavelengths of 240–450 nm in 5-nm intervals and emission wavelengths of 300–600 nm in 2-nm intervals. EEMs were corrected for blanks (Milli-Q water), Raman scans (excitation = 350 nm, emission = 365–450 nm in 0.5-nm intervals of Milli-Q water), and inner filter using protocols outlined in Murphy et al. (2010). We then determined specifically the optical metrics of fluorescence index (FI), T280, and specific ultraviolet absorbance at 254 nm ($SUVA_{254}$; Cory et al., 2011). FI and $SUVA_{254}$ both provide measurements of aromaticity based on established correlations with ^{13}C nuclear magnetic resonance data (McKnight et al., 2001; Weishaar et al., 2003). FI correlates negatively with aromaticity

(McKnight et al., 2001) and positively with bioavailability (Johnson et al., 2011) while $SUVA_{254}$ is positively correlated with aromaticity (Weishaar et al., 2003) and negatively with bioavailability (Balcarczyk et al., 2009; Wickland et al., 2012). The T280 peak is associated with tryptophan- and tyrosine-like fluorescence (Coble 1996; Leenheer & Croué, 2003; Stedmon et al., 2003) and correlates positively with DOC uptake rates and bioavailability (Baker & Inverarity, 2004; Wickland et al., 2007; Fellman et al., 2009). As quality control for experimental data, FI values were checked against 'end-member' fulvic acid standards (International Humic Substances Society) following methods outlined in Cory et al. (2010) with an associated dilution series. $SUVA_{254}$ was calculated by dividing the UV absorbance at 254 nm measured in inverse meters (m^{-1}) by DOC concentration (mg l^{-1}) and is reported in units of l mg C^{-1} m^{-1}.

Experimental set-up

To test for differences among plant species and leaf leachate composition on stream bacterial communities, we amended Oak Creek and Wet Beaver Creek microcosms with 50 ml of a standardized concentration of the 24-h leachate solution (8 mg C l^{-1}) of each of the riparian species. We created stream microcosms by combining 5 g of stream sediments and 250 ml of stream water in pint-sized (473 ml) Mason jars. Both streams had a background DOC concentration of 1.6 mg C l^{-1}. Our amendment raised DOC concentration to 2.7 mg C l^{-1}, an increase of 69%. We then covered microcosms loosely with aluminum foil and placed them on a shaker table at 100 rpm at 25°C. Four replicates of each leaf type × stream combination were created. Three control microcosms were also created for each stream and received no DOC amendments for a total of 38 experimental units.

Dissolved organic carbon biodegradation

From each of the six control jars, samples were removed to determine ambient DOC concentration for both stream treatments prior to leachate additions. At the end of the experiment, ($t = 24$ h) samples were collected from all 38 experimental units to determine the percentage of DOC consumed during the incubation period.

Sample processing and DNA extraction

To assess changes in the bacterial community we collected water samples and associated bacterial samples from Mason jars at the beginning of the experiment ($t = 0$ h) and at the end of the incubation ($t = 24$ h). We selected this first 24 h period based on other studies that demonstrate a rapid response of bacterial communities to DOC amendments within this time frame including changes in community composition (Young et al., 2005; Docherty et al., 2006; Eilers et al., 2010; Wymore et al., 2015). Prior to sampling, microcosms were covered and hand-shaken to create a slurry. To assess the initial bacterial community, we removed samples from each of the six control jars ($n = 3$ for each stream environment). After 24 h of incubation, we created another slurry and extracted community samples from each of the steam × leachate experimental units. Samples were placed in 15% glycerol and stored at −80° until DNA extraction.

To extract bacterial DNA, cells were lysed chemically and mechanically while minimizing exogenous DNA contamination from reagents. Chemical lysis was performed by adding 600 µl RLT buffer to each sample (Qiagen, Inc., Valencia, CA, USA). Next, mechanical lysis was performed using a Barocycler NEP 2320 (Pressure Bioscience, Inc., Easton, MA, USA) at room temperature with 15 cycles of 10 s at 35,000 psi followed by 10 s at atmospheric pressure. After lysis, genomic DNA isolation and purification were performed following the manufacturer's instructions using the AllPrep Kit (Qiagen, Inc.).

Bacterial load quantification and 16S rRNA gene-based pyrosequencing analysis

We quantified bacterial load, measured as the quantity of bacterial 16S rRNA gene copy using a broad-coverage qPCR assay (Liu et al., 2012; Wymore et al., 2013). We also generated bar-coded V3–V6 amplicons using broad-coverage fusion PCR primers, which were pooled and sequenced on the Genome Sequencer FLX (Roche Applied Sciences, Branford, USA). Generated pyrosequences were chimera-checked (Edgar et al., 2011), de-multiplexed, and quality-checked (Caporaso et al., 2010). We performed taxonomic classification using the Ribosomal Database Project Naïve Bayesian Classifier (RDP Release

10, Update 28) (Cole et al., 2009). Phylotypes were identified at the 97% sequence similarity level. Pyrosequencing produced 75,739 individual sequences with an average of 1,993 sequences per sample. Generated sequence data has been deposited in NCBI's Sequence Read Archive (accession number SRP114706). Additional pyrosequencing details and rarefaction curves can be found in Supplementary File 1 and Supplementary Files 2 and 3, respectively.

Statistical analysis

We used three methods to assess differences in the composition of the bacterial community among leaf litter types. First, we used the unweighted UniFrac algorithm to visualize differences in community composition between plant species using principle coordinate analysis (PCoA). UniFrac is a phylogenetic-based method used to qualitatively compare β diversity between communities (Lozupone & Knight, 2005; Lozupone et al., 2007, 2010). We then used analysis of similarity (ANOSIM) to determine statistically if community composition between stream environments and among leachate types was significantly different. Partial Mantel tests were used to test for significant relationships between the phylogenetic distance of the bacterial communities and metrics of leachate chemistry and optical properties. Values of leachate chemistry and optics were transformed to dissimilarity matrices using Euclidean distance while phylogenetic distance was obtained via the unweighted UniFrac algorithm. Tests were performed using Pearson's product-moment correlation coefficient (r) with 999 permutations. Analyses were performed in Qiime version 1.7.0 using the default settings (Caporaso et al., 2010) and samples were rarefied at 237 sequences per sample. Due to Qiime's stringent requirements for primer selection, we also repeated the analyses using a relaxed primer selection to determine if patterns and results changed with the addition of more reads with samples rarefied at 920 sequences per sample (please see results under "Community composition" section). Here, we present the Qiime results using the default settings to facilitate comparison with other studies using the same analytical technique (e.g., Leff et al., 2012). We used independent t-tests to assess differences in leachate optical properties, leachate chemistry, mass loss, leachate nutrient stoichiometry, DOC biodegradation, and 16S rRNA gene quantities and diversity metrics among leachates within a stream environment. Diversity metrics including number of unique OTUs, evenness and Shannon Diversity Index were calculated using Qiime following the calculations originally outlined in Shannon (1948) and Spellerberg & Fedor (2003). 16S rRNA gene quantity data was log transformed prior to analysis using the natural logarithm. If the assumption of homogeneity of variance was violated (e.g., mass loss data), we used the Welch–Satterthwaite method to compare means. t-tests and ANOVAs were performed in SPSS for Windows (2011).

Results

General overview

Overall, the addition of leaf litter leachate caused a significant shift from the initial ambient community (Fig. 1) in both stream environments, and the resultant bacterial community composition was unique to the leaf species (Fig. 2). Metrics of leachate optical properties correlated significantly with community composition (Table 1). In particular, samples with similar FIs and $SUVA_{254}$ values had more similar bacterial communities (Table 1). DOC biodegradation rate was also strongly correlated with bacterial community composition (Table 1). Although no obvious pattern between leachate nitrogen and DOC biodegradation emerged, the leachate with the lowest DOC:DON ratio (i.e., sycamore; Table 2) produced communities distinctly separated in PCoA ordination space that were associated with the highest rates of DOC biodegradation (Figs. 1, 4).

Leachate chemistry and optical properties

The four riparian species produced 24-h leaf litter leachates with significantly different chemistries (Table 2). Cottonwood leachate had the highest concentrations of both DOC ($P < 0.05$) and DON ($P < 0.05$). Oak had significantly higher concentrations of SRP ($P < 0.05$) even though there was considerable variation among replicates. As hypothesized, 24-h leachates differed significantly in optical properties among the four species (Table 3). Sycamore leachate had the highest FI ($P < 0.001$) and

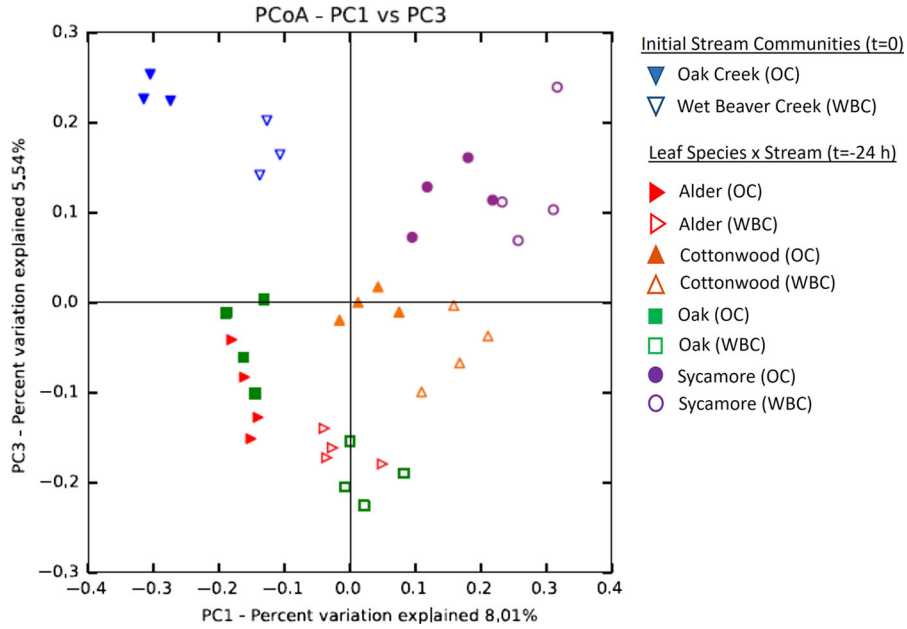

Fig. 1 Unweighted UniFrac principle coordinates analysis (PCoA) of stream bacterial communities before and after leaf leachate amendment. Upside down blue triangles represent initial stream communities ($t = 0$ h) prior to leachate amendment. Red, orange, green, and purple shapes represent leaf leachate treatments after 24 h of incubation. The identity of the stream environment is maintained via the solid and open shapes

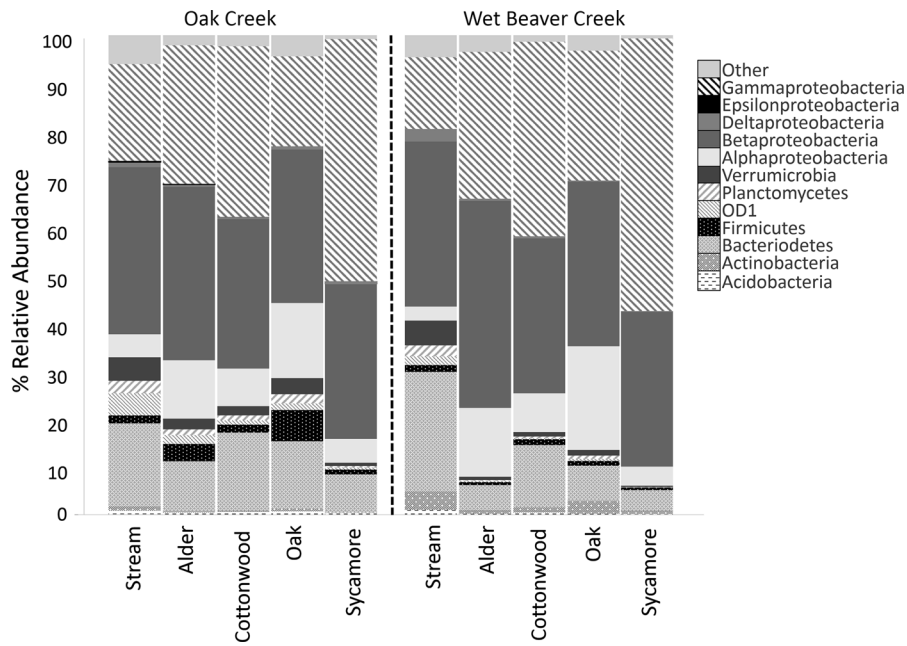

Fig. 2 Relative abundance of dominant bacterial phyla and classes within the Proteobacteria phylum in stream water and sediment microcosms with and without the addition of leaf litter leachate. Stream: (no leachate added, 0 h), alder (24 h), cottonwood (24 h), oak (24 h), sycamore (24 h)

$SUVA_{254}$ values ($P < 0.05$), whereas cottonwood had the highest T280 values ($P < 0.005$).

Community composition

As predicted, communities differed among plant species treatments (ANOSIM global $R = 0.70$, $P < 0.001$; Fig. 1). Significant differences also existed between the initial stream communities prior to leachate exposure (ANOSIM global $R = 0.40$, $P < 0.001$). These results were robust to different levels of primer stringency (Supplemental File 4). Additionally, all pairwise comparisons of leaf species treatments were significant (ANOSIM: global R 0.30–0.91, all P values < 0.014). Prior to leachate amendment, Bacteriodetes and Proteobacteria were

Table 1 Partial Mantel tests of dissimilarity between bacterial phylogenetic distance and leachate optical properties and DOC biodegradation

Distance matrix 2	Mantel *r* coefficient	*P* values
Fluorescence index	0.24	0.001*
$SUVA_{254}$	0.40	0.001*
T280	−0.06	0.88
DOC biodegradation	0.34	0.001*

For all tests the first distance matrix is phylogenetic distance calculated using the unweighted UniFrac algorithm. Asterisks represent significant *P* values ($\alpha < 0.05$)

$SUVA_{254}$ specific ultraviolet absorbance at 254 nm, *T280* fluorescence peak at excitation wavelength 280 nm

the two most dominant phyla. The Proteobacteria phylum was comprised primarily of members of the β- and γ-Proteobacteria class. In both streams, the Bacteriodetes, OD1, and Verrucomicrobia all decreased in both relative and absolute abundance with the addition of all leachate types with some of the largest decreases associated with sycamore leachate (Fig. 2; Table 4). In contrast, the Proteobacteria phylum experienced some of the largest increases in abundance with the addition of leachate (Fig. 2; Table 4). Changes in the absolute abundance of the γ-proteobacteria class were also substantial including increases of 232 and 327% with the addition of sycamore leachate in Oak Creek and Wet Beaver Creek samples, respectively.

Bacterial abundance and diversity

As expected, the addition of leaf litter leachate to the microcosms increased bacterial gene abundance with significant differences among plant species ($P < 0.05$; Fig. 3). In contrast to our predictions, it was not leachate from labile and low tannin and lignin litter types that supported the greatest 16S rRNA gene quantities. Rather it was leachate from the leaves with higher tannin and lignin (sycamore) that was associated with the highest 16S rRNA gene quantities, a pattern observed in both streams.

Biodiversity of the initial stream community did not differ between the two environments (Table 5). However, leaf litter leachate did have a significant impact on community biodiversity (Table 5). Leachate from alder resulted in the greatest richness followed by oak, cottonwood, and lastly sycamore, a pattern that held in

Table 2 Mean leachate chemistry and nutrient stoichiometry values from 24-h leachates and initial leaf litter chemistry values

Species	% Mass loss	Leachate chemistry					Initial leaf litter chemistry			
		DOC (g C g^{-1} LL)	DON (mg N g^{-1} LL)	SRP (μg P g^{-1} LL)	DOC:DON	DOC:SRP	% Lignin	% CT†	% N†	% P†
Alder	12.3^{a} (1.07)	0.033^{a} (0.003)	0.33^{a} (0.08)	13.6^{a} (8.2)	132.2^{ab} (33.0)	$20{,}900^{a}$ (10,555)	8.02^{a} (0.60)	0.61^{a} (0.07)	1.31^{c} (0.03)	0.05^{a} (0.01)
Cottonwood	12.9^{a} (0.91)	0.058^{b} (0.004)	0.49^{b} (0.03)	96.4^{b} (6.4)	142.6^{ac} (16.5)	1567^{ac} (63)	10.41^{b} (0.80)	0.06^{a} (0.05)	0.42^{a} (0.00)	0.04^{a} (0.01)
Oak	6.80^{b} (1.31)	0.031^{a} (0.006)	0.38^{ac} (0.05)	683.1^{c} (69.1)	92.6^{ab} (12.0)	127^{ab} (36)	18.33^{c} (0.84)	2.13^{b} (0.18)	0.85^{b} (0.01)	0.25^{c} (0.02)
Sycamore	2.50^{c} (0.47)	0.011^{c} (0.001)	0.29^{ac} (0.02)	44.3^{ab} (32.2)	55.0^{d} (10.3)	2088^{ac} (1085)	45.48^{d} (1.52)	4.72^{c} (0.12)	0.60^{a} (0.01)	0.13^{b} (0.01)

% Mass loss is loss due to 24 leaching. Leachate C, N, and P data presented as mass per gram leaf litter (LL). Stoichiometric ratios are presented as molar ratios. Values are means with ± 1SE in parentheses. Different superscript letters represent statistically significant differences among leaf litter leachate types

DOC dissolved organic carbon, *DON* dissolved organic nitrogen, *SRP* soluble reactive phosphorous, *CT* condensed tannin

† Data and statistical analyses originally presented in LeRoy & Marks (2006)

Table 3 Optical properties of 24-h leaf litter leachates

Species	Fluorescence index	$SUVA_{254}$	T280
Alder	1.30 (0.03)[a]	1.4 (0.02)[a]	0.14 (0.01)[a]
Cottonwood	1.34 (0.02)[a]	0.5 (0.001)[b]	0.27 (0.03)[b]
Oak	2.07 (0.04)[b]	1.9 (0.01)[c]	0.16 (0.02)[a]
Sycamore	2.35 (0.03)[c]	4.8 (0.15)[c]	0.25 (0.01)[b]

Values are means ± 1SE. Different superscript letters represent statistically significant differences ($\alpha = 0.05$)

both stream environments. Community evenness and Shannon diversity were similar among plant types, except for sycamore leachate, which resulted in the lowest community evenness and Shannon diversity.

DOC biodegradation

The biodegradation of leachate DOC varied significantly among the four species ($P < 0.001$; Fig. 4). Contrary to our predictions it was DOC from high tannin and lignin litter (sycamore) that experienced the highest biodegradation. Within both stream systems approximately 80% of sycamore DOC was consumed. The remaining three plant species had a mean DOC consumption of 45% (range 39–57%).

Discussion

Plant species exerted strong effects on the composition, diversity, and abundance of stream bacterial communities associated with the leaching of DOM. Patterns were consistent between the two stream environments. This plant species effect can be partitioned into two possible, but not mutually exclusive, mechanisms. First, different bacterial communities could have entered the stream microcosms with the leachate itself having originating from the surface of the leaf litter. Plant species harbor distinct phyllosphere bacterial communities (Redford et al., 2010) and these taxa may be specifically adapted to the associated leachate. However, most terrestrial microbes are not known to persist in aquatic environments (Harrop et al., 2009). Second, the diverse bacterial taxa in the stream sediments may have responded differently to the leachate and community-wide differences could be a result of differences in leachate composition. Both pathways would explain the strong correlation between species-specific leachate optical properties and community composition. This experiment was designed to measure the total "leaf effect" and cannot differentiate between the two mechanisms. This study demonstrates a strong effect of plant species on stream bacterial communities closely associated with leachate optical properties. With the possibility that the inoculum (i.e., original phyllosphere community) may have contributed directly to the community, there is a good likelihood that in situ interactions exist between the two communities (i.e., sediment community and phyllosphere inoculum). Differences among plant species and their associated inocula may be highly relevant as they represent different ecological starting points which may have ultimately sent these communities along different trajectories. These mechanistic hypotheses are both testable and offer interesting lines of future research to understand how microbial communities move across ecosystem boundaries.

Bacterial communities may be more sensitive to the DOC from leaves compared to the leaf tissue itself as other studies have found a weaker response of bacterial communities to variation in leaf type later in the decomposition process (Marks et al., 2009; Wymore et al., 2016). Regardless of the mechanistic pathways discussed above, the differentiation of the bacterial communities in this study is strongly associated with species-specific leachate (i.e., plant) optical properties. For example, communities metabolizing sycamore-derived leachate, which is both highly aromatic (i.e., high $SUVA_{254}$) and highly labile (i.e., DOC biodegradation), experienced especially large changes in the Proteobacteria phyla, specifically the γ-Proteobacteria class. Other studies examining the effect of soluble and labile C additions have also measured large increases in γ-Proteobacteria (Cleveland et al., 2007). All leachate types promoted increases in the α-proteobacteria, with larger increases in alder and oak leachate. These changes within the bacterial community associated with our general plant species effect, suggest that certain aquatic bacteria preferentially decompose certain types of leaf litter DOC.

Shifts in the structure and composition of the bacterial community reveal that leaf litter leachate promotes the formation of unique communities

Table 4 Absolute abundance of bacterial phyla and classes within the Proteobacteria phylum in stream water and sediment microcosms with and without the addition of leaf litter leachate

Phyla/class	Oak Creek					Wet Beaver Creek				
	STR	ALD	CWD	OAK	SYC	STR	ALD	CWD	OAK	SYC
Acidobacteria	17.0 (2.5)	6.30 (2.7)	9.30 (2.4)	12.0 (2.7)	8.80 (3.4)	20.3 (3.9)	4.30 (0.9)	8.50 (1.9)	3.30 (1.3)	1.80 (0.3)
Actinobacteria	18.0 (3.8)	4.50 (1.8)	6.30 (1.4)	8.50 (1.3)	3.30 (1.1)	74.3 (5.2)	18.3 (2.5)	23.0 (2.5)	43.0 (7.4)	13.3 (2.0)
Bacteriodetes	359 (9.5)	173 (31)	302 (41)	251 (22)	204 (32)	490 (53)	128 (19)	264 (42)	115 (10)	86.0 (22)
Firmicutes	36.0 (7.8)	60.3 (14)	32.0 (6.7)	116.5 (7.6)	24.8 (11)	26.7 (7.3)	16.5 (1.2)	23.5 (5.2)	16.3 (0.5)	10.5 (4.9)
OD1	96.0 (17)	31.8 (8.5)	15.3 (1.8)	25.0 (1.8)	11.0 (3.0)	35.7 (7.7)	8.50 (2.9)	3.80 (1.0)	6.50 (1.9)	1.80 (0.5)
Planctomycetes	54.0 (10)	19.3 (8.5)	19.5 (1.2)	33.3 (8.3)	7.80 (2.6)	45.0 (2.6)	2.50 (1.2)	8.50 (2.6)	11.5 (5.3)	1.50 (0.6)
Verrucomicrobia	101.0 (9.3)	36.8 (15)	34.8 (13)	60.5 (8.2)	16.8 (5.5)	99.3 (4.3)	18.3 (1.4)	18.8 (5.8)	18.3 (5.8)	3.50 (1.2)
Proteobacteria	1270 (37)	1297 (83)	1386 (95)	1195 (56)	2256 (287)	1079 (145)	2189 (201)	1651 (155)	1305 (101)	1857 (160)
α-Proteobacteria	89.3 (8.7)	195 (7.8)	140 (13)	260 (21)	122 (16)	50.7 (5.5)	347 (48)	160 (19)	331 (20)	78.0 (19)
β-Proteobacteria	654 (39)	578 (38)	555 (32)	536 (28)	804 (111)	599 (125)	1043 (122)	639 (92)	523 (45)	628 (83)
δ-Proteobacteria	20.0 (2.1)	8.50 (2.5)	6.30 (1.1)	10.8 (2.9)	6.30 (2.3)	46.0 (32)	10.3 (1.3)	4.80 (0.9)	3.80 (1.4)	1.80 (0.8)
ε-Proteobacteria	5.00 (1.7)	0.80 (0.8)	1.30 (0.5)	0.50 (0.3)	3.50 (1.0)	0.00 (0.0)	0.80 (0.8)	5.00 (1.8)	0.30 (0.3)	1.80 (0.9)
γ-Proteobacteria	378 (21)	463 (31)	635 (58)	314 (18)	1255 (220)	260 (18)	741 (56)	803 (77)	414 (33)	1110 (173)

Values are means (± 1SE) and calculated as the number of 16S rRNA gene copies * relative abundance

STR stream/initial community (no leachate added, 0 h), *ALD* alder (24 h), *CWD* cottonwood (24 h), *OAK* oak (24 h), *SYC* sycamore (24 h)

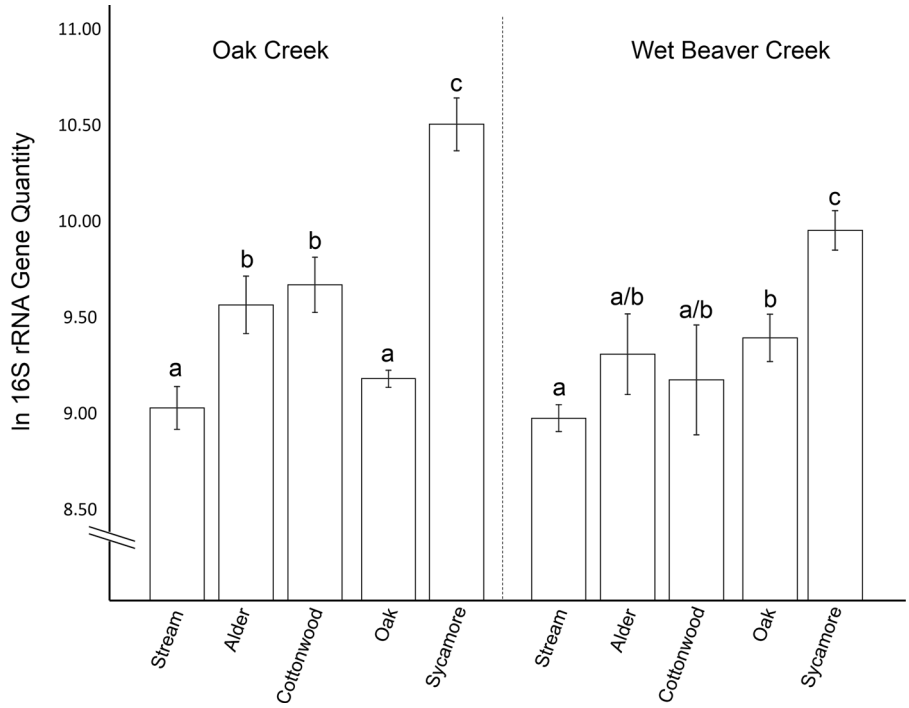

Fig. 3 Bacterial 16S ribosomal ribonucleic acid (rRNA) gene abundances (measured as gene copy number) in water and sediment microcosms from two Arizona streams amended with leaf litter leachate from four common riparian species over a 24-h period. Values are means (± 1SE). Letters represent statistically significant differences among leachates within a stream environment

distinct from ambient stream water and sediments. Members of the Bacteriodetes phyla are common in ambient stream and lake water samples (Young et al., 2005; Docherty et al., 2006; Wilhelm et al., 2013) as are members of the β-proteobacteria class (Young et al., 2005; Docherty et al., 2006; Van Horn et al., 2011; Besemer et al., 2012). Both groups dominated ambient and non-amended stream water samples with the Bacteriodetes experiencing especially large decreases in both absolute and relative abundance with the addition of all leachate types. Collectively, our experimental results highlight how variation among riparian tree species, whether it be due to species-level differences in the phyllosphere community that enter upon leaf submersion, or induced changes within the sediment community by the leachate itself, can potentially drive both community and ecosystem-level processes in stream environments.

One of the surprising results from this study was the large increase in bacterial abundance in response to sycamore leachate and the corresponding high percentage of DOC biodegradation. Sycamore leaf litter tissue is considered highly recalcitrant due to its high concentration of lignin and tannin, and its slow rate of decomposition in streams (LeRoy & Marks, 2006). This suggests that although some leaf litter species have recalcitrant tissue, a proportion of the leaf biomass can be highly soluble and bioavailable. A similar pattern in the decomposition of leachate from high lignin witch hazel leachate (*Hamamelis virginiana*) has also been observed (Wu et al., 2009). We attribute these sycamore patterns to the leachate's low DOC:DON ratios and high T280 values (presence of labile amino acids). A low DOC:DON ratio may indicate high "quality" leachate, much along the same lines that low C:N leaf tissue is considered a high quality resource in streams (Webster & Benfield, 1986; Kominoski et al., 2009). The bioavailability of sycamore leachate compared to its tissue argues that decomposition should be viewed in distinct phases and that the quality and bioavailability of leaf litter as a resource can change over the course of the full decomposition process. We also cannot discount a

Table 5 Bacterial community diversity values between steam types, among leaf litter leachate types, and among leaf litter leachate types within a stream environment

Streams	Leachate types	# of Unique OTUs	Evenness	Shannon
Oak Creek		176 (6.2)[a]	0.96 (0.01)[a]	7.1 (0.1)[a]
Wet Beaver Creek		161 (5.8)[a]	0.94 (0.01)[a]	6.9 (0.1)[a]
	Alder	188 (2.9)[a]	0.98 (0.00)[a]	7.4 (0.0)[a]
	Cottonwood	171 (3.5)[b]	0.96 (0.00)[b]	7.1 (0.1)[b]
	Oak	183 (4.2)[a]	0.97 (0.00)[a]	7.3 (0.1)[a]
	Sycamore	131 (4.4)[c]	0.88 (0.01)[c]	6.2 (0.1)[c]
Oak Creek	Alder	194 (3.2)[a]	0.99 (0.00)[a]	7.5 (0.0)[a]
Oak Creek	Cottonwood	179 (1.4)[b]	0.97 (0.00)[a]	7.2 (0.0)[b]
Oak Creek	Oak	194 (2.7)[a]	0.98 (0.00)[a]	7.5 (0.0)[ab]
Oak Creek	Sycamore	136 (3.6)[c]	0.89 (0.01)[b]	6.3 (0.1)[c]
Wet Beaver Creek	Alder	183 (2.5)[a]	0.97 (0.00)[a]	7.3 (0.0)[a]
Wet Beaver Creek	Cottonwood	162 (2.8)[b]	0.94 (0.00)[b]	6.9 (0.0)[b]
Wet Beaver Creek	Oak	173 (1.9)[ab]	0.97 (0.00)[ab]	7.2 (0.0)[ab]
Wet Beaver Creek	Sycamore	127 (7.8)[c]	0.88 (0.01)[c]	6.1 (0.2)[c]

Values are means ± 1SE in parentheses. Different superscript letters represent significant differences among pairwise comparisons between dashed lines

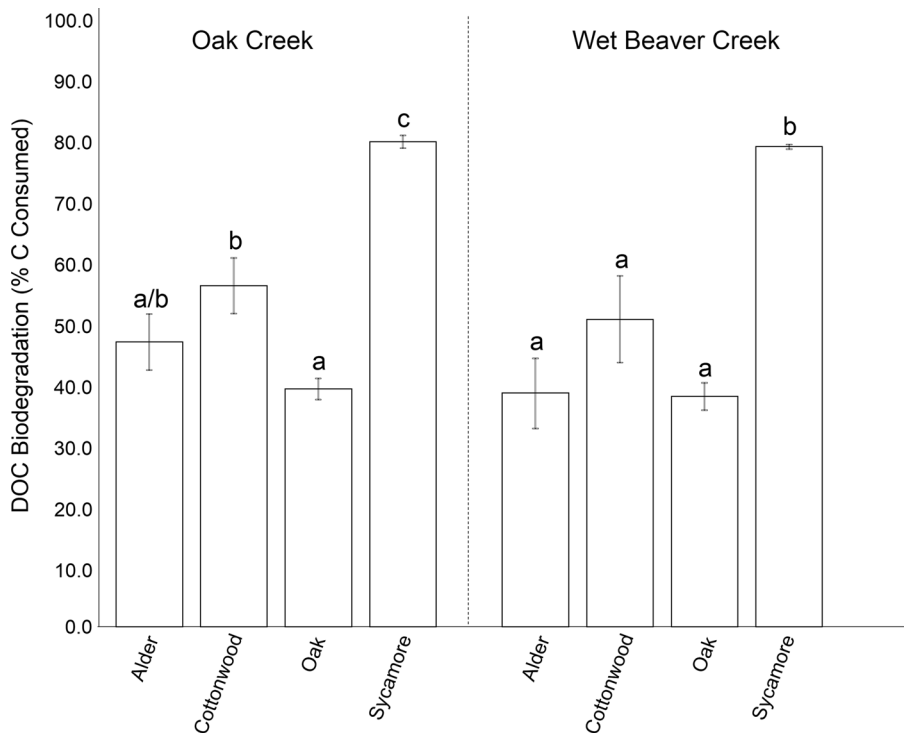

Fig. 4 Biodegradation of dissolved organic carbon leached from four species of leaf litter in water and sediment microcosms from two Arizona streams over a 24-h period. Values are means (± 1SE). Letters represent statistically significant differences among leachates within a stream environment

priming effect especially in explaining the high rates of DOC biodegradation associated with sycamore leachate. Highly available leachate may stimulate further decomposition of the ambient DOM pool leading to elevated rates of DOC consumption.

The four species used in this study produced a greater range in leachate optical properties than

previously reported for leaf litter leachates (Strauss & Lamberti, 2002; Jaffé et al., 2004; Wickland et al., 2007). Compositional variation among leachates can explain why we saw more profound differences in community composition than other studies (Wu et al., 2009; Leff et al., 2012). Although these FI values fall within the range of ambient stream water samples (1.0–2.8; McKnight et al., 2001; Balcarczyk et al., 2009; Yamashita et al., 2011) they also extend beyond previously reported values from leaf litter leachate (1.15–2.0; Jaffé et al., 2004; Wickland et al., 2007). And while our reported $SUVA_{254}$ values for this assortment of leaf litter (0.5–4.8) fall within the range of other leachate studies from across distinct biomes (0.1–4.7; Strauss & Lamberti, 2002; Wickland et al., 2007; Balcarczyk et al., 2009; Wymore et al., 2015), we were able to capture this range within a single watershed. The third optical parameter, T280, has yet to be used widely in leaf litter leachate studies. However, values reported here are in line with a *Populus*-based leachate study (Wymore et al., 2015).

Inconsistent relationships between leaf litter leachate optical properties and bioavailability (e.g., high sycamore $SUVA_{254}$ values and high rates of DOC biodegradation) highlight the large degree of variation in leachate chemistry among species. The use of a highly labile and unprocessed form of DOC may explain why this study observed so much interspecific variation with respect to leachate chemistry and very strong plant species effects. Much of the previous stream research describing the chemical composition and optical properties of DOC comes from bulk water samples (e.g., McKnight et al., 2001; Yamashita et al., 2011; Wickland et al., 2012). This DOC pool has undergone multiple microbial transformations during its transit through both soil and aquatic flow paths, and the contribution and variation of the initial labile forms may have gone previously unmeasured. Species-specific compounds may also drive certain patterns. For example, oak leachate which had low DOC:DON ratios (relative to cottonwood and alder) was associated with lower bacterial gene abundance and lower rates of DOC biodegradation which is in contrast to low DOC:DON sycamore leachate which had both high bacterial gene abundances and the highest rates of DOC biodegradation. These patterns may indicate the presence of inhibitory and antibacterial compounds that are able to suppress bacterial growth (Gunnarsson et al., 1988; Schlief & Mutz, 2007) specific to oak.

Species can affect ecosystem processes (Tilman et al., 1997; Hooper et al., 2005) as strongly as abiotic factors (Hooper et al., 2012). While extrapolating our results to the ecosystems, scale must be done in caution, our results highlight how the presence and abundance of certain riparian plant species can structure aquatic bacterial communities and influence ecosystem respiration. Because this work was not performed in situ it carries with it inherent limitations and potential artifacts. Scaling up such work and understanding and quantifying these riparian-stream interactions is needed as we expect plant communities to shift with a drying and warming climate (Allan & Breshears, 1998). These interactions are especially relevant during leaf fall when large amounts of DOC are leached. Changes in the composition of both the leachate and bacterial community (including potential changes in the phyllosphere community) will have important implications for stream ecosystem functioning. Future research focusing on indirect effects of the DOC input such as priming (Guenet et al., 2010, 2014) will reveal the temporal and spatial dynamics of labile DOC inputs.

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