Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs

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Abstract: Dissolved organic C (DOC) leached from leaf litter contributes to the C pool of stream ecosystems and affects C cycling in streams. We studied how differences in leaf-litter chemistry affect the optical properties and decomposition of DOC. We used 2 species of cottonwoods (*Populus*) and their naturally occurring hybrids that differ in leaf-litter phytochemistry and decomposition rate. We measured DOC and nutrient concentration in leaf leachates and determined the effect of DOC quality on heterotrophic respiration in 24-h incubations with stream sediments. Differences in DOC composition and quality were characterized with fluorescence spectroscopy. Rapidly decomposing leaves with lower tannin and lignin concentrations leached ~40 to 50% more DOC and total dissolved N than did slowly decomposing leaves. Rates of heterotrophic respiration were 25 to 50% higher on leachate from rapidly decomposing leaf types. Rates of heterotrophic respiration were related to metrics of aromaticity. Specifically, rates of respiration were correlated negatively with the Fluorescence Index and positively with Specific Ultraviolet Absorbance ($SUVA_{254}$) and T280 tryptophan-like fluorescence peak. These results reveal that leaf-litter DOC is distinctly different from ambient streamwater DOC. The relationships between optical characteristics of leaf leachate and bioavailability are opposite those found in streamwater DOC. Differences in phytochemistry among leaf types can influence stream ecosystems with respect to DOC quantity, composition, and rates of stream respiration. These patterns suggest that the relationship between the chemical structure of DOC and its biogeochemistry is more complex than previously recognized. These unique properties of leaf-litter DOC will be important when assessing the effects of terrestrial C on aquatic ecosystems, especially during leaf fall.

Key words: dissolved organic carbon, leaf litter, fluorescence spectroscopy, *Populus*

The largest source of dissolved organic C (DOC) in streams originates in soils and enters streams through ground water, surface run-off, and snowmelt (Allan and Castillo 2008). Leaf fall also provides a large, but seasonal, source of DOC to streams as leaves are leached (Cummins 1974) and contributes 30 to 42% of the total DOC pool during autumn (McDowell and Fisher 1976, Meyer et al. 1998). The quantity and bioavailability (ease of heterotrophic mineralization) of DOC derived from leaf litter is likely to vary with the solubility of the leaf tissue, which differs among tree species (Strauss and Lamberti 2002, Cleveland et al. 2004).

DOC from different sources of organic matter has distinct chemical characteristics (McDowell and Likens 1988, McKnight et al. 2001, Wickland et al. 2007) and varies in its bioavailability to heterotrophic bacteria (Meyer et al. 1987, Fellman et al. 2009). DOC comprises, in part, low-molecular-weight amino acids and carbohydrates, which are mineralized quickly (Amon et al. 2001, Balcarczyk et al. 2009) and support high rates of bacterial productivity (Meyer et al. 1987, Moran and Hodson 1990). DOC also consists of humic-like compounds with higher molecular weights, which tend to have longer water-column residence times (Fellman et al. 2009) and support less bacterial productivity (Meyer et al. 1987).

DOC derived from plants and soils differs chemically from DOC originating from autochthonous microbial sources (McKnight et al. 2001), and chemical differences in DOC can influence stream ecosystem processes (McArthur and Richardson 2002, Strauss and Lamberti 2002, Balcarczyk et al. 2009). The ecosystem effects of fresh leaf-litter DOC warrant particular focus. Leaf-litter DOC may be compositionally unique compared to the DOC found in ambient stream water because of its lack of microbial processing. Thus, leaf-litter DOC may exert strong influence on stream ecosystem processes.

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Optical properties of DOC can be used to characterize sources and infer its functional properties (Coble et al. 1990, Murphy et al. 2010, Cory et al. 2011). Quantitative metrics can be generated from fluorescence spectroscopy to characterize and compare DOC pools and to link dissolved organic matter (DOM) composition to its function in aquatic ecosystems. The fluorescence index (FI) is negatively correlated with aromaticity (McKnight et al. 2001) and positively correlated with bioavailability (Johnson et al. 2011). Specific Ultraviolet Absorbance (SUVA) is positively correlated with aromaticity (Weishaar et al. 2003) and negatively correlated with bioavailability (McDowell et al. 2006, Wickland et al. 2012). Neither FI (McKnight et al. 2001) nor SUVA (Weishaar et al. 2003) measure aromaticity directly. However, both are correlated with aromaticity based on ^{13}C nuclear magnetic resonance (NMR) data. The T280 fluorescence peak generated from excitation and emission matrices is associated with the abundance of certain labile amino acids, especially tryptophan and tyrosine (Coble 1996, Leenheer and Croué 2003, Stedmon et al. 2003, Stedmon and Markager 2005) and is positively correlated with DOC-uptake rates and bioavailability (Baker and Inverarity 2004, Wickland et al. 2007, Fellman et al. 2009).

The optical properties of leaf-litter DOC can illustrate how variation in leaf phytochemistry affects C cycling and heterotrophic activity in streams. These effects may be overlooked when DOC is measured in bulk (Findlay and Sinsabaugh 1999). We used fluorescence spectroscopy to characterize the variation in composition of leachate from 4 closely related leaf types that dominate riparian areas in the western part of North America and contribute up to 93% of the leaf litter to aquatic systems (Driebe and Whitham 2000). We used leaf litter from replicated genotypes of 2 species of cottonwood (*Populus fremontii* and *Populus angustifolia*) and their naturally occurring hybrids (cross types) that show genetically based inter- and intraspecific differences in phytochemistry (Whitham et al. 2003, Rehill et al. 2006, LeRoy et al. 2007, Holeski et al. 2012, Wymore et al. 2013), and that vary in rates of leaf-litter decomposition (Driebe and Whitham 2000, LeRoy et al. 2007). Among cross types and genotypes, decomposition rates decrease with higher concentrations of tannins and lignin in both terrestrial (Schweitzer et al. 2004) and aquatic systems (Driebe and Whitham 2000, LeRoy et al. 2007). Therefore, our study extends beyond studies comparing leaf-litter chemistry and decomposition in aquatic ecosystems (e.g., Driebe and Whitham 2000, LeRoy et al. 2007), and our goal was to connect leaf-litter chemistry to DOC composition, optical properties, and its bioavailability at the cross type and genotype scales. The *Populus* model system enables us to partition variation in measures of leachate composition and DOC bioavailability at the cross type and genotype levels. We addressed 3 primary questions: 1) What is the range in DOC quantity, composition, and bioavailability across a complex of leaf litter that varies in its initial leaf-litter phytochemistry? 2) Is leaf-litter-leachate DOC composition, measured via its optical properties, correlated with rates of heterotrophic respiration? 3) How does the variation in optical properties of DOC from this complex of cottonwood leaf litter compare to DOC in ambient stream water?

We predicted that the quantity and quality of DOC leached and its bioavailability, measured as respiration rate, would be correlated with leaf-litter chemistry. We also predicted that rates of heterotrophic respiration of DOC would be correlated positively with FI (Johnson et al. 2011) and T280 (Coble 1996, Fellman et al. 2009) and negatively with $SUVA_{254}$ (Wickland et al. 2012). Heterotrophic respiration also is influenced by the availability of leachate-derived N and P, so we hypothesized that leachate from fast-decomposing leaf types would also generate more nutrients, enhancing rates of respiration. We also investigated the co-metabolism of leachate C and N on rates of heterotrophic respiration. We predicted that the ratio of DOC to dissolved organic N (DOC : DON) would be negatively correlated with heterotrophic respiration. Sources of terrestrial DOC are often grouped together as a single source entering stream ecosystems (e.g., McKnight et al. 2001). Nevertheless, variation within this terrestrial source is likely to be large, a situation that motivates our desire to understand its influence on stream ecosystems.

METHODS

Leaf collection and leaching

Multiple genotypes of known pure (Fremont cottonwood: *P. fremontii*, narrowleaf cottonwood: *P. angustifolia*) or hybrid (F_1-hybrids: *P. fremontii* × *P. angustifolia*, backcross hybrids: F_1-hybrids × *P. angustifolia*) cottonwoods were grown in a common garden that standardized for environmental effects. Genetic identities were determined with 35 *P. fremontii* molecular markers (Keim et al. 1989, Martinsen et al. 2001). The garden was planted in 1991 in Ogden, Utah (lat 41°14′48″N, long 112°00′00″W) with cuttings taken from individual trees along a 105-km transect adjacent to the Weber River, Utah. Cross types differ in phytochemistry and decomposition (Rehill et al. 2006, LeRoy et al. 2007; Table 1). We developed a nested experimental design with genotypes nested within cross type. We collected naturally abscised leaf litter in 2008 with bridal-veil nets from multiple genotypes/cross type (*P. fremontii*: $n = 5$, F_1 hybrids: $n = 4$, backcross hybrids: $n = 5$, *P. angustifolia*: $n = 6$) and 3 replicated trees/genotype (i.e., genetic clones grown in different locations within the common garden) except for 1 *P. fremontii* genotype that was replicated only twice.

We air-dried leaf litter and stored it in the laboratory. We standardized the leaf-leaching protocol. We placed 1 g

Table 1. Mean (±1 SE) values of dissolved organic C optical properties and leaf-litter phytochemistry among the 4 cottonwood cross types. Fluorescence Index (FI), Specific Ultraviolet Absorbance at 254 nm ($SUVA_{254}$), and T280 are from our study. Leaf litter % tannin, % lignin, and C:N ratios were calculated from LeRoy et al. (2007). Means with the same superscript letter are not significantly different among cross types (α = 0.05).

Cross type	FI	$SUVA_{254}$	T280	% tannin	% lignin	C : N
Fremont	1.39 ± 0.04[a]	0.3 ± 0.01[a]	0.51 ± 0.06[a]	0.2 ± 0.1	5.2 ± 0.4	90.0 ± 13.4
F_1-hybrid	1.60 ± 0.22[ab]	0.1 ± 0.02[b]	0.22 ± 0.08[a]	3.3 ± 0.6	9.1 ± 2.1	93.7 ± 17.3
Backcross	1.86 ± 0.06[c]	0.2 ± 0.02[b]	0.06 ± 0.01[b]	14 ± 3.2	23 ± 2.2	103 ± 11.4
Narrowleaf	1.80 ± 0.05[bc]	0.1 ± 0.01[b]	0.11 ± 0.02[b]	9.8 ± 2.0	21 ± 1.0	89.6 ± 5.6

of whole leaf litter from each tree in 400 mL of deionized water in acid-washed glass beakers and allowed the material to leach for 24 h at room temperature. We used a 24-h leaching period based on methods used by Strauss and Lamberti (2002) and based on reports in multiple studies that a high degree of mass loss occurs during the first 24 h of immersion (Petersen and Cummins 1974, McDowell and Fisher 1976, Webster and Benfield 1986, and references therein). We syringe-filtered leachate (Whatman GF/F) into amber glass vials, stored at 4°C and protected from ultraviolet (UV) light until analysis. After leaching, we removed the leaves from the beakers, dried them at room temperature, and reweighed them to calculate mass loss.

Leachate chemistry and composition

We analyzed DOC concentration in leachate solutions on an OI Analytical Model 1010 Total Carbon Analyzer (OI Analytical, College Station, Texas) and measured total dissolved N (TDN) on a Shimadzu TOC-V (Shimadzu Instruments, Marlboro, Massachusetts) with total N mode. For the DOC concentration portion of the experiment, we used 3 *Populus* genotypes, each replicated 3× except for 1 *P. fremontii* genotype, which was replicated twice. We subsampled each genetic clone (i.e., tree) in triplicate for a total of 177 measurements of DOC and TDN. We measured NH_4^+ (phenate method), soluble reactive P (SRP; molybdate blue), and $NO_3^- + NO_2^-$ (NO_3^-; Cd–Cu reduction) content of leachate with a Westco SmartChem robotic colorimetric analyzer (Westco Scientific, Brookfield, Connecticut). We calculated DON as TDN – ($NO_3^- + NH_4^+$). For the leachate chemistry portion of the experiment, we replicated each of the 20 genotypes 2 to 3× for a total of 49 measurements of dissolved NH_4^+, SRP, NO_3^-, and DON.

We assessed DOM composition with fluorescence spectroscopy using a Horiba Jobin Yvon Fluoromax 3 scanning fluorescent spectrophotometer (Horiba Scientific, Edison, New Jersey) and UV absorbance using a Shimadzu SPD-M20A photo diode array detector with HPLC (200–700 nm in 1-nm intervals) interfaced with a Shimadzu LC VP Series Control. Raw Excitation and Emission Matrices (EEMs) were collected at excitation wavelengths of 240 to 450 nm in 5-nm intervals and emission wavelengths of 300 to 600 nm in 2-nm intervals. EEMs were corrected for blanks (Milli-Q water; EMD Millipore, Billerica, Massachusetts), Raman scans (excitation = 350 nm, emission = 365–450 nm in 0.5-nm intervals of Milli-Q water), and inner-filter effect using protocols outlined by Murphy et al. (2010). These data allowed us to conduct a suite of analyses to characterize the bioavailability and dynamics of DOC including FI, T280 (Ex/EM: 280/350 nm), and $SUVA_{254}$ (reviewed by Cory et al. 2011 and references therein). Experimental FI values were checked against the 2 microbial and terrestrial endmember fulvic acid standards (International Humic Substances Society) following methods outlined by Cory et al. (2010) and a dilution series. We calculated $SUVA_{254}$ by dividing the UV absorbance at 254 nm measured in inverse meters (/m) by DOC concentration (mg/L). $SUVA_{254}$ is reported in units of L mg^{-1} C m^{-1}. For the leachate composition portion of the experiment, we used 3 genotypes replicated 3×, except for 1 *P. fremontii* genotype and 2 F_1-hybrid genotypes, which were replicated twice for a total of 57 measurements of DOC optical properties.

Respiration

We tested the effect of DOC quality on heterotrophic respiration in stream microcosms amended with equal concentrations of DOC. For this portion of the experiment, leachates were created from each of the 20 genotypes and replicated 2 or 3×. We filled fifty-nine 500-mL Mason jars with 200 mL stream water and 5 g of sediments collected from Pump House Wash, a small 1st-order tributary of Oak Creek in north-central Arizona. We measured dissolved O_2 (DO) in 5 randomly chosen jars with a YSI Professional Pro Plus Dissolved Oxygen Probe (Yellow Springs Instruments, Yellow Springs, Ohio). We then amended 54 jars with 20 mg C/L from 24-h leachates and immediately capped and sealed the jars. We also ran 5 control jars with no DOC addition to account for the decrease in DO consequent to metabolism of ambient DOC. We incubated jars at room temperature and in the dark to limit photosynthesis. To calculate DO consumption resulting from the DOC addition, we subtracted the mean control DO con-

centration after 24 h from the mean initial DO. We then subtracted experimental DO concentrations after 24 h from control DO to calculate the net increase in respiration associated with DOC addition.

Statistical analyses

We compared DOC concentration, mass loss, and respiration among cottonwood cross types with a 1-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference post hoc comparisons. Each genotype could be classified as belonging to a cross type, so we used a nested ANOVA approach to test for differences among genotypes nested within cross type (LeRoy et al. 2007). For the nested ANOVAs, cross type was considered a fixed factor and genotype a random factor. When the nested ANOVA produced a significant *F*-statistic for differences among genotypes, we used a subsequent 1-way ANOVA and Tukey's Honestly Significant Difference post hoc test to compare means among genotypes within each cross type.

We used linear regression to estimate the amount of variation in respiration rate explained by fluorescence characteristics (FI, T280, and $SUVA_{254}$) and leachate TDN, and to test the DOC : DON co-metabolism hypothesis. We ran these analyses in SPSS for Windows (version 21.0; IBM SPSS, Armonk, New York). Because of multicollinearity among optical measurements, we used partial least-squares regression (PLSR) (Carrascal et al. 2009) to assess whether a predictive model could be developed using multiple leachate optical properties to explain rates of respiration. PLSR is a multivariate technique and is an extension of multiple regression that uses latent variables (i.e., factors) to establish a relationship between predictor variables and response variables. PLSR performs well (identifies relevant variables) when there is a high degree of collinearity among predictor variables, the number of observation to predictor variables is relatively small, or sample size is small (Carrascal et al. 2009; see e.g., Wortman et al. 2012, Smith and Cox 2014). We followed the methods outlined by Carrascal et al. (2009) and retained only those factors that explained >5% of the original variation in the response variable. Consequently, we retained the 1st factor in the PLSR model. The PLSR analysis used the NIPALS algorithm and FI, T280, and $SUVA_{254}$ were loaded as predictor variables. We used a Variable Influence of Projection (VIP) threshold of 0.8 to determine if a predictor variable contributed significantly to the model. The PLSR analysis was performed in JMP (version 11; SAS Institute Inc., Cary, North Carolina).

RESULTS

DOC concentration, mass loss, and respiration

As hypothesized, the fast-decomposing cross types leached more DOC (Fig. 1A) and lost more mass after 24 h (Fig. 1B) than did slow-decomposing cross types. Fremont and F_1-hybrids leached ~45% more DOC than backcross hybrids and narrowleaf (F = 6.61, df = 3, p = 0.004) and lost, on average, 60% more mass after 24 h of leaching (F = 9.91, df = 3, p = 0.001). Respiration rates were higher in jars amended with leachate than in unamended controls. Heterotrophic respiration differed significantly among cross types (Fig. 2). More DO was consumed in jars with Fremont leachate than in jars with backcross or narrowleaf leachates. The F_1-hybrid leachate was intermediate (F = 4.82, df = 3, p = 0.014), and ~50% more DO was consumed in jars with Fremont leachate than in jars with narrowleaf leachate.

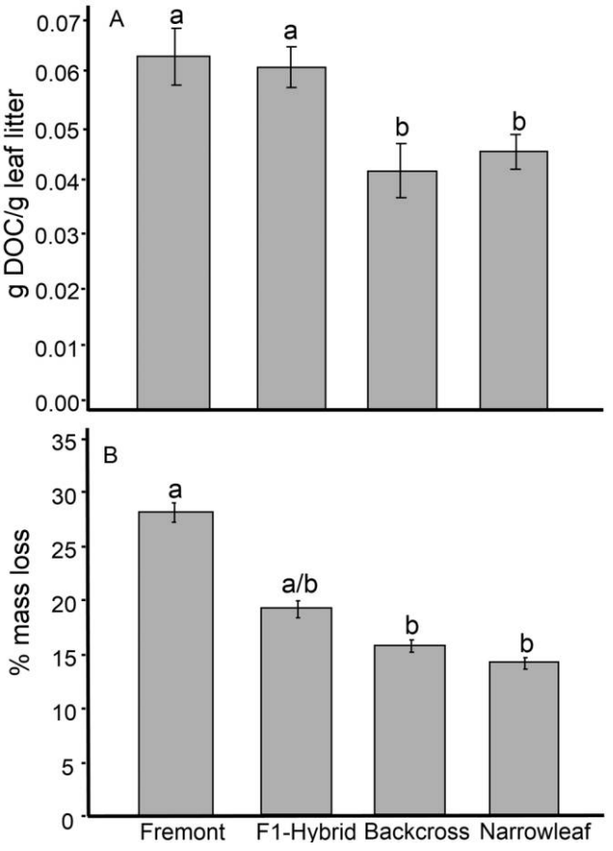

Figure 1. Mean (±1 SE) dissolved organic C (DOC) leached (A) and % mass loss (B) from fresh leaf litter from cottonwood cross types after 24 h. Bars with the same letters are not significantly different (α = 0.05).

Leachate composition and nutrient chemistry

Optical properties of cottonwood leaf-litter leachate also varied among the cross types (Table 1). FI values increased in the order Fremont, F_1-hybrid, narrowleaf, and backcross leachate (F = 10.533, df = 3, p < 0.001), whereas $SUVA_{254}$ decreased in the order Fremont, backcross, F_1-hybrid, and narrowleaf (F = 7.973, df = 3, p < 0.005). T280 values decreased in the order Fremont, F_1-hybrid, narrowleaf, and backcross (F = 14.253, df = 3, p < 0.001).

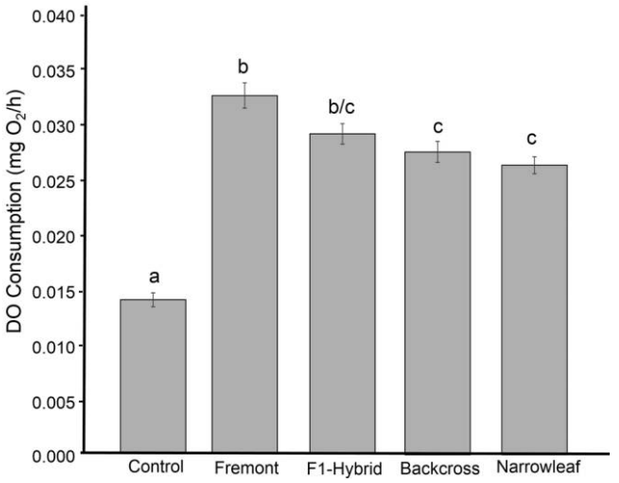

Figure 2. Mean (±1 SE) respiration rate (measured as dissolved O_2 [DO] consumption) in response to amendment of microcosm water with leaf leachate from fresh leaf litter from cottonwood cross types after 24 h and an unamended control. Bars with the same letters are not significantly different ($\alpha = 0.05$).

Leachate nutrient content differed among cross types, but significant differences were found only for TDN and DON (Table 2). Fremont released significantly more TDN ($F = 6.92$, df = 3, $p = 0.003$) and DON ($F = 6.91$, df = 3, $p = 0.003$) than the other cross types. NO_3^-, NH_4^+, and SRP did not differ among cross types.

Bioassay respiration and leachate composition

As predicted, O_2 consumption was positively correlated with T280 ($R^2 = 0.29$, $p = 0.013$; Fig. 3A). However, contrary to our prediction, O_2 consumption was negatively correlated with FI ($R^2 = 0.47$, $p = 0.016$; Fig. 3B) and positively correlated with $SUVA_{254}$ ($R^2 = 0.41$, $p = 0.002$; Fig. 3C). TDN was positively correlated with O_2 consumption ($R^2 = 0.16$, $p = 0.04$). Leachate DOC : DON ratios tended to be negatively correlated with O_2 consumption ($R^2 = 0.07$, $p = 0.13$).

The PLSR analysis indicated that the 3 optical predictor variables contributed significantly to the model (VIP > 0.8). The 3 optical measurements loaded equally onto the 1st factor with T280 and SUVA loading positively and FI loading negatively. The first PLSR factor accounted for 76% of the variation in the 3 predictor variables and explained 49.5% of the variation in respiration rates (Table 3).

Genotype-level differences

DOC concentration, mass loss, and FI differed significantly among genotypes, and a significant amount of variation in these variables was explained by genotype nested within cross type. DOC concentration differed among Fremont ($F = 4.2$, df = 4, $p < 0.05$) and backcross ($F = 41.1$, df = 4, $p < 0.001$) genotypes, mass loss differed among F_1-hybrid ($F = 4.9$, df = 3, $p < 0.05$) and backcross ($F = 10.7$, df = 4, $p < 0.001$) genotypes, and FI differed among Fremont ($F = 3.8$, df = 4, $p < 0.05$) and backcross ($F = 6.0$, df = 4, $p < 0.01$) genotypes.

DISCUSSION

Within a complex of related tree species, their natural hybrids, and genotypes, we found a range in DOC and leachate optical properties and composition that captures much of the variability found among a much wider taxonomic breadth of trees species (Strauss and Lamberti 2002, Jaffé et al. 2004, Wickland et al. 2007). Subtle shifts in the species or genetic composition of riparian deciduous forests could have substantial effects on stream C cycling during leaf fall as a result of differences in the quantity and bioavailability of leaf-litter leachate. Both cross type and genotype differences in leachate quantity and quality can cause substantial differences in stream metabolic rates. For example, at the cross-type scale, Fremont and F_1-hybrid litter leached ~45% more DOC than backcross cross types (Fig. 1A), whereas at the genotype scale certain backcross genotypes leached 90% more DOC than other genotypes (0.059–0.031 g DOC/g leaf litter). Significant effects at both the cross-type and genotype scales are consistent with other studies of leaf-litter decomposition in streams (Driebe and Whitham 2000, LeRoy et al. 2006, 2007). Differences among species and cross types in rates of leaf-litter decomposition are reflected in rates of DOC decomposition. Plants that produce leaves that decompose rapidly (*P. fremontii*) also generate DOC that decomposes rapidly, whereas plants that produce leaves that decompose slowly (*P. angustifolia*) produce DOC that decomposes

Table 2. Mean (±1 SE) values of cottonwood leachate nutrients after a 24-h leaching. Data are presented as mass/g leaf-litter leached. TDN = total dissolved N, DON = dissolved organic N. Means with the same superscript letter are not significantly different among cross types ($\alpha = 0.05$).

Cross type	TDN (mg N/g)	$NO_3^- + NO_2^-$ (mg N/g)	NH_4^+ (mg N/g)	DON (mg N/g)	PO_4^{3-} (μg P/g)
Fremont	0.74 ± 0.03^a	0.0018 ± 0.0003^a	0.010 ± 0.002^a	0.73 ± 0.03^a	178 ± 50^a
F_1-hybrid	0.54 ± 0.04^b	0.0012 ± 0.0002^a	0.009 ± 0.001^a	0.53 ± 0.04^b	275 ± 40^a
Backcross	0.48 ± 0.04^b	0.0024 ± 0.0002^a	0.006 ± 0.001^a	0.47 ± 0.04^b	188 ± 32^a
Narrowleaf	0.49 ± 0.03^b	0.0019 ± 0.0001^a	0.020 ± 0.015^a	0.47 ± 0.03^b	345 ± 45^a

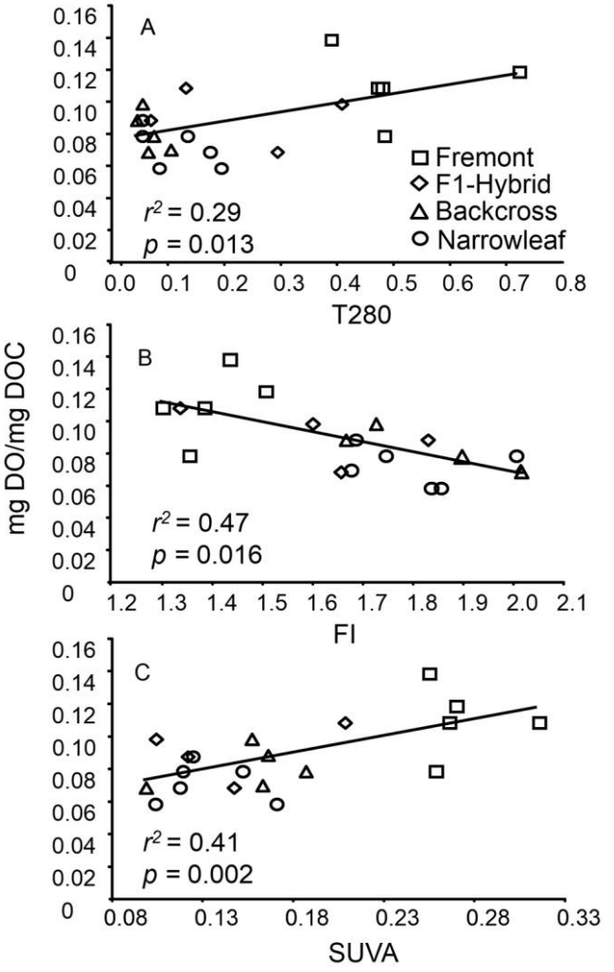

Figure 3. Regression analyses of heterotrophic respiration (measured as dissolved O_2 [DO] consumption) vs T280 (A), Fluorescence Index (FI) (B), and Specific Ultraviolet Absorbance (SUVA) at 254 nm (C) metrics of dissolved organic C (DOC) composition measured by fluorescence and absorbance spectroscopy. Points represent genotype means. Shapes represent cottonwood cross types.

slowly. Hybrids show intermediate values (Driebe and Whitham 2000, LeRoy et al. 2006, 2007).

The relationship between leaf-litter phytochemistry and DOC has been explored in other systems, but results have been inconsistent. For example, the solubility of leaf-litter C was linked to the lignin:N ratio of the litter (Neff and Asner 2001). In contrast, no relationship was found between soluble C and lignin:N ratios in leaf litter from multiple species in a tropical rain forest (Cleveland et al. 2004). We found that leaf litter with significantly higher concentrations of recalcitrant compounds, including tannin and lignin, had significantly lower concentrations of DOC and mass loss during leaching. The mass-loss values during leaching in our study are similar to values obtained in other studies with freshly abscised leaf litter. Across a taxonomically wide range of species, 24-h mass-loss values range from 4 to 27% (Kaushik and Hynes 1971, Petersen and Cummins 1974, McDowell and Fisher 1976, Webster and Benfield 1986), and our values spanned the upper half of this range. Some high-tannin and -lignin narrowleaf genotypes lost as little as 11% of their initial mass, whereas low-tannin and -lignin Fremont genotypes lost as much as 31% of their initial mass. The influence of phytochemistry on soluble C concentrations and mass loss may be stronger in our study than in others because we used fresh litter that had experienced no decomposition via soil processes. The weaker relationships between litter chemistry and mass loss reported in other studies (e.g., Cleveland et al. 2004) may be the result of collection of litter samples from the forest floor where a large percentage of soluble C may already have been leached. In contrast, soluble C lost from freshly abscised leaf litter that enters streams directly may significantly affect seasonal fluxes of C. An estimated 80% of the leaf litter that enters a stream falls in directly (McDowell and Fisher 1976).

Some of the relationships between DOC composition and bioavailability of leaf leachate are opposite those found for DOC in bulk water samples. These patterns suggest that the relationship between the chemical structure of DOC, assessed via its optical properties, and its biogeochemistry can vary among DOC sources, such as soils vs fresh leaf litter. FI values from ambient water samples usually are positively correlated with bioavailability (Johnson et al. 2011), but we observed a significant negative correlation across the narrower range of variation generated by leaf-litter leachate. A similar negative correlation has been observed in samples from headwater streams adjacent to old-growth forests (Burrows et al. 2013). However, Burrows et al. (2013) also reported a possible reversal in the relationship of FI and bioavailability around FI values >1.4. The opposite correlation that we observed for FI is not the result of extreme or unusual values in the structural attributes of leaf

Table 3. Results of a partial least-squares regression analysis based on leachate optical properties. Loaded predictor variables were Fluorescence Index (FI), T280, and Specific Ultraviolet Absorbance at 254 nm ($SUVA_{254}$), and the response variable was respiration rate (measured as dissolved O_2 consumption). Values used in analysis were genotype means (n = 20).

Result	Loading scores with factor 1	Variable influence of projection	% variance explained in x	% variance explained in y
Factor 1			76	49.5
FI	–0.60	1.12		
T280	0.58	0.89		
$SUVA_{254}$	0.58	0.98		

leachate. For example, ambient water samples from a variety of aquatic ecosystems have FI values that range from 1.0 to 2.8 (McKnight et al. 2001, Balcarczyk et al. 2009, Yamashita et al. 2011, Burrows et al. 2013), and our values for leaf leachate fall within this range (1.3–2.0). Our values are on the higher end of values typically reported for leaf leachate and related sources (1.15–1.5; Jaffé et al. 2004, Wickland et al. 2007), but they are still within the range for aquatic samples. That leaf litter from this *Populus* hybridizing complex revealed more variation than usual in an optical metric of leaf-litter leachate is not surprising. Tannin and lignin values can vary as much as 10- to 30-fold among *Populus* cross types (e.g., Driebe and Whitham 2000), and rates of decomposition among *Populus* genotypes span the range of decomposition rate coefficients for multiple plant families (LeRoy et al. 2007). Future work would benefit from an examination of genotype-level variation in DOC properties from other riparian tree species or hybrid complexes. For example, genotypes of *Populus tremuloides* exhibit even greater variation in decomposition rates than the *Populus* hybridizing complex we used (LeRoy et al. 2012).

Other widely studied attributes of C composition, such as $SUVA_{254}$, also show opposite patterns in leaf leachate than in bulk water samples. Our high SUVA values in leaf leachates are associated with high decomposition rates, whereas high $SUVA_{254}$ values have been previously related to low decomposition rates in water samples (McDowell et al. 2006, Balcarczyk et al. 2009) and tropical soils (Wieder et al. 2008). This reversal of the $SUVA_{254}$-decomposition relationship in leaf leachate may be related to the low $SUVA_{254}$ values associated with these leachates. Leaf leachate from temperate deciduous tree species ranges from 0.5 to 2.5 when converted to a 1-m path length (Strauss and Lamberti 2002), but our cottonwood complex is on the lower end of this range (0.1–0.3). These lower $SUVA_{254}$ values may reflect variation across biogeoclimatic regions because these cottonwood trees are native to an arid, high-altitude environment. Our leachate values also are below the range for whole-water samples from streams and soils (1.5–4.7; Weishaar et al. 2003, Wickland et al. 2007, Balcarczyk et al. 2009).

T280 has not been widely reported in other studies. The fluorescence of tryptophan and tyrosine from streamwater assessments can be strongly correlated with DOC uptake rates (Fellman et al. 2009) and DOC loss (Balcarczyk et al. 2009). The positive correlation we observed between leaf-litter T280 values and respiration is consistent with other observations that this group of amino acids is quickly metabolized (Balcarczyk et al. 2009, Fellman et al. 2009).

Why the bioavailability of DOC from leaf leachates has opposite relationships with optical properties than those found for ambient water samples is not clear. The optical values that we described here are not unique, and they are consistent with those in the literature (Strauss and Lamberti 2002, Jaffé et al. 2004, Wickland et al. 2007, Burrows et al. 2013). However, the range of FI values for our leaf leachates, in particular, are not consistent with previously measured values that are associated with the origins of DOM in aquatic systems (e.g., McKnight et al. 2001, Balcarczyk et al. 2009, Cory et al. 2011). Whether the changes to the standard FI and aromaticity pattern with bioavailability, expressed in this paper as respiration, reflect something specific about the structure and fluorescence of leaf-litter-derived humic acids or a broader pattern inherent to labile organic matter also is not clear. One possible explanation is that terrestrially derived DOC has been thoroughly processed by the soil microbial community, and the DOC that subsequently enters the stream may be a largely recalcitrant DOC pool that follows the broad relationships between optical properties and biological availability. Freshly abscised leaf litter, on the other hand, has been considerably less processed. A proportion of the observed aromaticity in cottonwood leachate may be caused by the presence of labile amino acids with high concentrations of aromatic structures as suggested by the DON data. Tryptophan and tyrosine are 2 of these amino acids that are associated with fluorescence at T280 (Coble 1996, Leenheer and Croué 2003, Stedmon et al. 2003, Stedmon and Markager 2005). Aromatic and bioavailable amino acids may also create a priming effect (Guenet et al. 2010), which enhances metabolism of other DOC components, including the more refractory and ambient DOC found in surface waters. Our respiration bioassays may have included the priming of ambient streamwater DOC, but we are unable to account for the magnitude of metabolism caused by priming.

Respiration rates also may be driven by differences in leaching of N, particularly if microbes mineralizing DOC are N limited (Kroer 1993, Zweifel et al. 1993). Therefore, the higher rate of DOC-based respiration observed with *P. fremontii* and F_1-hybrid leachates relative to *P. angustifolia* and backcross hybrids may be an effect of N concentrations rather than of C quality. N and leaf-litter DOC uptake have been linked previously (Mineau et al. 2013). At the reach-scale, DOC from leaf litter had shorter uptake lengths when N was added (Mineau et al. 2013). The negative relationship between DOC : DON ratios and respiration suggests that leachate with a high dissolved C : N ratio may be of lower quality in a manner similar to patterns associated with high leaf-tissue C : N ratio (Webster and Benfield 1986, Kominoski et al. 2009). However, the lack of a strong DOC : DON effect does agree with results of other studies that demonstrate a lack of predictive power of leaf litter C : N ratios in aquatic-decomposition studies (LeRoy et al. 2007, Wymore et al. 2013). The relationship between various forms of C and N entering streams and stream metabolic rates warrants further research.

Whatever the underlying mechanism, our results suggest that deciphering the linkages between DOC structure and its ecological and biogeochemical significance will require that we understand the unique features of distinct organic-matter pools. Other sources of relatively fresh DOC, such as exudation of recent photosynthate by rooted aquatic macrophytes, leaching of downed wood in streams, or low-molecular-weight organic compounds delivered during spring floods (Berggren et al. 2010), also might show patterns opposite those found for surface-water samples. Ultimately, our study suggests that the terrestrial DOC entering streams should at least be subdivided into 2 distinct types: fresh and unprocessed DOC (e.g., leaf-litter leachate) and processed sources of DOC, such as ground water. Following the processing of fresh DOC as it is integrated into the ambient pool would be a valuable line of research to understand how the bulk and ambient pool of DOC is formed.

Species can profoundly affect ecosystem processes (Hooper et al. 2012 and references therein). Variation in the quality of leaf-litter DOC and nutrients at the species, cross-type, and genotype level can influence photosynthesis : respiration (P : R) ratios in stream ecosystems (Sinsabaugh 1997). The variation shown in our study suggests that finer-scale variation in P : R ratios may exist along a stream's axis, especially adjacent to areas of species transition and hybridization. These differences may be large at times considering the magnitude of leaf litter inputs into streams (Petersen and Cummins 1974) and the quantity of DOC leached (McDowell and Fisher 1976, Meyer et al. 1998). Alteration to the biodiversity and species and genetic composition of riparian forests probably will influence C dynamics in streams by shifting the ratio of labile to recalcitrant C entering streams.

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