Linking tree genetics and stream consumers

Author(s): Zacchaeus G. Compson, Bruce A. Hungate, Thomas G. Whitham, George W. Koch, Paul Dijkstra, Adam C. Siders, Todd Wojtowicz, Ryan Jacobs, David N. Rakestraw, Kiel E. Allred, Chelsea K. Sayer and Jane C. Marks

Source: *Ecology* , AUGUST 2018, Vol. 99, No. 8 (AUGUST 2018), pp. 1759-1770

Published by: Wiley on behalf of the Ecological Society of America

Stable URL: https://www.jstor.org/stable/10.2307/26626144

Ecology, 99(8), 2018, pp. 1759–1770

Linking tree genetics and stream consumers: isotopic tracers elucidate controls on carbon and nitrogen assimilation

Zacchaeus G. Compson [1,2,3,4,5] Bruce A. Hungate [1,2,3] Thomas G. Whitham,[2,3] George W. Koch,[1,2,3] Paul Dijkstra,[1,2,3] Adam C. Siders,[1,2,3] Todd Wojtowicz,[2,3] Ryan Jacobs,[2,3] David N. Rakestraw,[2,3] Kiel E. Allred,[2,3] Chelsea K. Sayer,[2,3] and Jane C. Marks[1,2,3]

[1]*Center for Ecosystem Science and Society, 800 S. Beaver Street, P.O. Box 5620, Flagstaff, Arizona 86011-5620 USA*
[2]*Merriam-Powell Center for Environmental Research, 800 S. Beaver Street, P.O. Box 6077, Flagstaff, Arizona 86011-6077 USA*
[3]*Department of Biological Sciences, Northern Arizona University, 617 S. Beaver Street, P.O. Box 5640, Flagstaff, Arizona 86011-5640 USA*

Abstract. Leaf litter provides an important nutrient subsidy to headwater streams, but little is known about how tree genetics influence energy pathways from litter to higher trophic levels. Despite the charge to quantify carbon (C) and nitrogen (N) pathways from decomposing litter, the relationship between litter decomposition and aquatic consumers remains unresolved. We measured litter preference (attachments to litter), C and N assimilation rates, and growth rates of a shredding caddisfly (*Hesperophylax magnus*, Limnephilidae) in response to leaf litter of different chemical and physical phenotypes using *Populus* cross types (*P. fremontii*, *P. angustifolia*, and F_1 hybrids) and genotypes within *P. angustifolia*. We combined laboratory mesocosm studies using litter from a common garden with a field study using doubly labeled litter (^{13}C and ^{15}N) grown in a greenhouse and incubated in Oak Creek, Arizona, USA. We found that, in the lab, shredders initially chose relatively labile (low lignin and condensed tannin concentrations, rapidly decomposing) cross type litter, but preference changed within 4 d to relatively recalcitrant (high lignin and condensed tannin concentrations, slowly decomposing) litter types. Additionally, in the lab, shredder growth rates were higher on relatively recalcitrant compared to labile cross type litter. Over the course of a three-week field experiment, shredders also assimilated more C and N from relatively recalcitrant compared to labile cross type litter. Finally, among *P. angustifolia* genotypes, N assimilation by shredders was positively related to litter lignin and C:N, but negatively related to condensed tannins and decomposition rate. C assimilation was likewise positively related to litter C:N, and also to litter %N. C assimilation was not associated with condensed tannins or lignin. Collectively, these findings suggest that relatively recalcitrant litter of *Populus* cross types provides more nutritional benefit, in terms of N fluxes and growth, than labile litter, but among *P. angustifolia* genotypes the specific trait of litter recalcitrance (lignin or tannins) determines effects on C or N assimilation. As shredders provide nutrients and energy to higher trophic levels, the influence of these genetically based plant decomposition pathways on shredder preference and performance may affect community and food web structure.

Key words: *aquatic consumers; assimilation; carbon; condensed tannins; intraspecific variation; isotopic tracer; leaf litter; lignin; litter decomposition; nitrogen;* Populus; *tree genetics.*

Introduction

Differences among plant species, hybrids, and genotypes within a species strongly affect biological communities and ecosystem processes (Hobbie 1992, Whitham et al. 2006, Vilà et al. 2011). Further, plant genetic variation influences associated communities, both among hybrids (Haloin and Strauss 2008, Crutsinger et al. 2010) and genotypes (Johnson and Agrawal 2005, Zytynska et al. 2011), as well as ecosystem processes, such as decomposition, nutrient cycling, and trophic dynamics (Whitham et al. 2006, Rudman et al. 2015). Heritable litter traits, including phytochemistry, the amount of litter generated, and the timing of leaf fall, can determine how litter affects ecosystem and food web properties in adjacent aquatic ecosystems. In streams, intraspecific variation in litter traits can affect decomposition rate, macroinvertebrate assemblages, insect emergence, and microbial communities (LeRoy et al. 2007, Wymore et al. 2013, Jackrel and Wootton 2014, Compson et al. 2016). Similarly, in lentic ecosystems, genetic differences in litter traits of *Populus trichocarpa* affect decomposition rate, phytoplankton concentrations, nutrient dynamics, and the relative strength of top-down effects (Crutsinger et al. 2014, Rudman et al. 2015, Rodriguez-Cabal et al. 2016).

Despite the charge to go beyond measuring mass loss of litter and move toward quantifying carbon (C) and nitrogen (N) pathways from litter (Gessner et al. 1999), the association between litter decomposition rate and aquatic consumers remains unclear, likely because decomposition rate is an integrative metric of ecosystem function (Gessner and Chauvet 1994, Hieber and Gessner 2002) that responds to a suite of chemical and structural litter traits (Fogel and Cromack 1977, Melillo et al. 1982). Aquatic shredders, which feed on leaf litter and transfer energy from terrestrial litter up the aquatic food web, prefer litter that supports higher

Manuscript received 31 December 2016; revised 30 January 2018; accepted 9 February 2018. Corresponding Editor: Amy D. Rosemond.
[4] Present address: Canadian Rivers Institute, University of New Brunswick, 10 Bailey Drive, P.O. Box 4400, Fredericton, New Brunswick E3B 5A3, Canada.
[5] E-mail: Zacchaeus.Greg.Compson@gmail.com

growth rates (Canhoto and Graça 1995). Litter and plant properties that prevent or slow a shredder's ability to access litter nutrition (e.g., toughness, secondary compounds) are expected to be avoided by shredders and lead to lower growth rates (Graça 2001, but see Friberg and Jacobsen 1994). Additionally, microbes are thought to enhance the nutritional quality of litter (reviewed by Graça 2001). Fungi, for example, enhance litter quality either by digesting cell walls to free simple compounds that are more palatable to shredders (Jenkins and Suberkropp 1995, Rodrigues and Graça 1997) or by concentrating nitrogen in tissues like hyphae that shredders can directly consume (Slansky and Scriber 1985). Radiolabeled studies have demonstrated that fungi provide between 0.05% and 57% of the C needs of aquatic shredders (Findlay et al. 1986a,b), while bacteria contributed much less (<1%, Findlay et al. 1986b).

There is also growing evidence that slowly decomposing, recalcitrant litter supports higher abundances of aquatic insects in their larval (Grubbs and Cummins 1994) and emergent forms (Kominoski et al. 2012, Compson et al. 2013) and yields higher rates of C and N assimilation to aquatic insects compared to rapidly decomposing, labile litter (Compson et al. 2015). Recalcitrant litter often loses less mass as leachate (Magill and Aber 2000, Wymore et al. 2015), persists longer in the stream (Cortes et al. 1994, Canhoto and Graça 1996), and may provide more structural complexity to litter packs (Hansen 2000), which leads to higher abundance and richness of some arthropods (Bultman and Uetz 1982). The persistence of recalcitrant litter in streams is also responsible for slowing decomposition in litter mixtures (Swan and Palmer 2004), likely enhancing the nutritional benefit of labile litter to consumers by extending its temporal footprint (Palmer et al. 2000).

This research links plant genes to ecosystems, tracing how C and N in genetically distinct litter move through aquatic food webs. Exploring how variation in riparian trees influences energy movement through adjacent detrital food webs is important for understanding the links between terrestrial and aquatic ecosystems. Headwater streams represent important systems to study these cross-ecosystem linkages because they are predominantly fueled by terrestrial litter inputs (Fisher and Likens 1973) that support multiple trophic levels (Wallace et al. 1997). Here, we test how leaf litter chemistry (% lignin, % soluble condensed tannins, %N, %C, C:N) and decomposition rate are associated with the preference, C and N assimilation, and growth of a shredding caddisfly, *Hesperophylax magnus* (Limnephilidae). Because rapidly decomposing litter generally has higher concentrations of labile substrates and lower concentrations of recalcitrant substrates compared to slowly decomposing litter (Chapin et al. 2011), we refer to rapidly decomposing litter types with low lignin and condensed tannin concentrations as "relatively labile" and slowly decomposing litter types with high concentrations of lignin and condensed tannins as "relatively recalcitrant." We combined laboratory studies where we measured shredder preference and growth with a field study using doubly labeled (^{13}C and ^{15}N) litter to test how litter traits influence shredder C and N assimilation.

This study addressed three a priori hypotheses. (1) Shredders will initially prefer relatively labile compared to relatively recalcitrant litter types. Through time, however, we expected preference to switch from relatively labile to relatively recalcitrant litter types as chemical concentrations in litter shifted. (2) Shredders will have higher growth rates on litter types low in tannins, but high in N concentrations. We predicted this since N is often a limiting resource in headwater streams (Biggs 2000) and tannins can interfere with digestion (Graça 2001). (3) Shredders will assimilate more C and N from litter types with high lignin concentrations. The rationale for this hypothesis was that lignin can act as a structural component that retains nutrients (Berg 1986), and releases them slowly over time, making them available to shredders longer. In contrast, we expected tannin concentrations to deter shredders and lower assimilation rates. To our knowledge, this is the first study to test how intraspecific variation in riparian trees affects shredder preference, growth rates, and nutrient assimilation. Because these litter traits are genetically based, our findings further extend our understanding of how the evolution of traits in plants link terrestrial and aquatic ecosystems.

Methods

Study system

We capitalized on a leaf litter-detritivore system occurring in headwater streams of northern Arizona. This is a model system for measuring energy transfer through aquatic food webs for several reasons. First, litter was collected from the *Populus* hybridizing system, which represents two tree species (*Populus angustifolia* and *P. fremontii*), their naturally occurring F_1 hybrids (*P. angustifolia* × *P. fremnotii*), and genotypes within these species and hybrids, which exhibit considerable phenotypic variation in litter chemistry traits (Whitham et al. 2006, Holeski et al. 2012, Appendix S1: Table S1). In the southwest United States, riparian zones are dominated by deciduous tree species that vary predictably in decomposition rate (LeRoy and Marks 2006). Litter from the cottonwood hybrid complex spans this range of decomposition rates, with *P. fremontii* decomposing relatively rapidly and *P. angustifolia* and backcross hybrids decomposing relatively slowly (Driebe and Whitham 2000, LeRoy et al. 2006). For the purposes of our study, we define "labile" litter as those cross types and genotypes that decompose rapidly and have low lignin and condensed tannin concentrations (e.g., *P. fremontii* litter), and "recalcitrant" litter as those litter types that decompose slowly and have significantly higher lignin and/or condensed tannin concentrations (e.g., *P. angustifolia* litter). We recognize that this variation represents only a subset of all leaf types and use the terms recalcitrant and labile with respect to variation within cottonwoods. Second, litter came from trees growing in a common garden, enabling us to isolate genetic from environmental differences in litter traits. Third, *P. angustifolia* genotypes vary significantly in both lignin and tannin concentrations, allowing us to decouple these two traits (Schweitzer et al. 2008, LeRoy et al. 2007, Appendix S1: Table S1). Finally, the caddisfly we examined, *H. magnus*, belongs to the family Limnephilidae, which is an important group of shredders, with a wide distribution from northern Mexico to central Canada. In our studies, these shredders were very large (~25 to ~35 mm), reached high peak densities of ~70 individuals/m^2, and had high litter processing rates (Z. Compson, *unpublished data*). Additionally, *H. magnus* is a hardy species that is easy to maintain and rear in the lab.

Common garden leaf litter and cuttings

Litter and cuttings were taken from the Ogden Nature Center (ONC) common garden in Ogden, Utah, that was planted in 1991. This common garden contains *Populus fremontii, P. angustifolia, P. fremontii* × *P. angustifolia* F_1 hybrids, and genotypes of these cross types that were collected in the wild within the Ogden River watershed. The common garden reduces environmental variation and isolates the influence of plant genetics. Genetically mediated differences in litter chemistry, decomposition (LeRoy et al. 2006, 2007), and trophic dynamics (Bailey et al. 2006) have been well documented in the *Populus* system.

For the laboratory preference and growth experiments, litter was collected from individual branch nets (two or three per tree and later aggregated into a single sample) to prevent it from being colonized by soil microbes. Litter came from clones of replicated genotypes of *P. fremontii*, *P. angustifolia*, and F_1 hybrids in the common garden (n = 41 genotypes total, each replicated 3–11 times). Litter for each clone of each genotype was kept separate and treated as a replicate. Nets were placed on trees in late October 2010 and leaf litter was collected in early December 2010. Litter was air dried and then stored in cardboard boxes in the lab.

For the field assimilation experiment, cuttings were collected by taking 10-cm sections of live tree branches in February 2008 before bud break. Cuttings were taken from single tree clones of each genotype of each cross type (n = 46 genotypes total, each replicated 6–22 times) and planted in book planters. Trees were grown in the greenhouse for 2 yr. Trees were transplanted to larger pots after the first year and transferred to pools with a nutrient solution in the second year. Plants were placed randomly on greenhouse benches (and later in pools) and rotated periodically to minimize edge effects from watering and light. Greenhouse air temperature was ~24°C during the day and ~18°C during the night throughout the growing season and was reduced to ~10°C during the day and ~4.4°C during the night in late October to promote leaf senescence. Plants were watered every other day in the summer and one to two times per week in the winter. In the second and third years, plants were fertilized with 60 ppm Peters Professional Water Soluble 20-20-20 (NPK) fertilizer with micronutrients (The Scotts Company, Marysville, Ohio, USA) to supplement nutrients from the greenhouse potting soil, which was nutrient poor (P. Patterson, *personal communication*).

Shredder litter preference lab experiments

Shredder preference studies were conducted separately for cross types (Appendix S2: Fig. S1a) and *P. angustifolia* genotypes (Appendix S2: Fig. S1b) and used litter from the common garden. Litter was placed in mesocosms and incubated for 48 h before shredders were added. Mesocosms consisted of 1.5 L of stream water (16.3° ± 0.1°C [here and throughout reported as mean ± SE]), 50 g heat-sterilized (500°C) gravel substrate, surface-area-standardized whole pieces of leaf litter, and 10 fifth-instar shredders (case length range = 21–25 mm). Shredders were acclimated to the lab for approximately one week before the start of the experiment. During this period, shredders were given a mixture of litter from the three cross types (*P. fremontii*, F_1 hybrid, and *P. angustifolia*), after which they were removed from their food source to clear their guts for 48 h before the start of the experiment. For the cross-type preference experiment, mixed litter of genotypes from each of the three cross types (*P. fremontii*, F_1 hybrid, and *P. angustifolia*) was placed in mesocosms (n = 12 mesocosms). For the genotype preference experiment, litter from clones of *P. angustifolia* genotypes (n = 25 genotypes replicated 3–11 times) was placed in mesocosms with five randomly selected genotypes per mesocosm (n = 29 mesocosms), and individual pieces of litter (for a clone of a given genotype) were tracked in mesocosms using tags attached to leaf litter petioles. Although randomly pairing only five genotypes together in a mesocosm did not expose all shredders to all litter types, replicating these mixtures at the clonal level (i.e., replicates of a genotype) meant that no single genotype was ever paired with the same other four genotypes. This likely added variation to mean shredder preference for a given genotype, since preference was relative to the subset of genotypes within a mesocosm. Consequently, we contend that our results are conservative, biasing against our ability to detect preference patterns among genotypes. For both experiments, litter was standardized to surface area using length–area regressions. This was necessary because it was impossible to accurately measure litter area when it was dry because of how it curled; however, litter length could easily be measured using digital calipers, and so we could standardize for area using these regressions for each litter type. Litter length–area regressions were calculated by placing litter in humidifiers and then flattening, digitizing, and measuring it using Image-J software (Abramoff et al. 2004). Preference was measured as attachment frequency (no. attachments·individual^{-1}·d^{-1}), or the number of times a shredder's mouthparts attached to leaf litter, totaled across four surveys per day (n = 14 d). We acknowledge that measuring litter preference in this way is limited, as insects could feasibly use their mouthparts for behaviors not related to nutrient acquisition (i.e., to anchor themselves to litter), but the limited flow in mesocosms created by aerators meant that shredders did not need to anchor themselves and could instead move about freely. During surveys, we could clearly differentiate between shredders that were stationary on leaf litter and those with mouthparts attached to the litter. Additionally, another lab trial demonstrated that this preference metric correlates with litter decomposition (R^2 = 0.31, $F_{1,45}$ = 19.8, $P = 5.5 \times 10^{-5}$), which was measured as the instantaneous decomposition rate constant, k(d^{-1}), and the mass loss attributed to insect shredding (R^2 = 0.46, $F_{1,45}$ = 38.4, $P = 1.6 \times 10^{-7}$), which was calculated by subtracting the mass loss attributed to microbes and leaching (no shredders) from the total mass loss (including shredders) in paired mesocosms. Decomposition was modeled for all experiments using an exponential decay model (Benfield 2006). Though litter was only added at the beginning of these preference experiments, we found no evidence to suggest that litter became limiting (percent mass remaining: *P. angustifolia* = 19.7% ± 3.2%, F_1 hybrid = 11.5% ± 3.0%, *P. fremontii* = 10.8% ± 1.4%).

Shredder growth lab experiment

The growth experiment (Appendix S2: Fig. S1c) also used litter from the common garden. Fifth-instar shredders (case length range = 21–25 mm) were acclimated to the lab in the

same way as for the preference experiments. After one week of acclimation, shredders were allowed to clear their guts for 48 h, and then were gently removed from their cases and blotted dry prior to measuring wet mass on a Metler-Toledo microbalance (Columbus, Ohio, USA). Shredders were gently reinserted into their cases and placed into experimental mesocosms at a density of three individuals per mesocosm. Very few insects failed to return to their cases after only a few seconds. Each mesocosm contained 1.5 L of stream water (temperature 18.4° ± 0.1°C), 50 g heat-sterilized (500°C) gravel substrate, and litter from a single, replicate clone of a genotype of its respective cross type (n = 41 genotypes total, replicated 3–11 times). Litter was incubated for 48 h before shredders were introduced to allow the litter to become neutrally buoyant.

Mesocosms were maintained for 14 days. At the end of the experiment, wet mass measurements were taken in the same way initial wet masses were measured, and shredders were frozen, dried, and reweighed. Growth (mg dry mass·individual^{-1}·d^{-1}) was calculated using a wet-dry mass regression of 100 individuals across the wet-mass range observed from our experimental data ($y = 0.28x - 12.90$, $R^2 = 0.68$, $F_{1,98} = 206.7$, $P < 2.2 \times 10^{-16}$). Our approach of using wet masses to estimate growth rates has been extensively documented in the literature for larval caddisflies (e.g., Hutchens et al. 1997) and other detritivores (e.g., Cummins et al. 1973, Tuchman et al. 2002). For both growth and preference laboratory experiments, mesocosms were replenished with stream water from Oak Creek, Arizona, and changed twice a week to remove nitrogenous waste.

Isotopically labeled leaf litter for shredder assimilation field experiment

Populus fremontii, F_1 hybrid, and *P. angustifolia* genotypes (n = 46 total, 4–8 replicate clones per genotype) were isotopically labeled for C and N during the summer of 2010. Labeling for C was done by placing plants in clear acrylic chambers inside the greenhouse and exposing them to 99 atom% ^{13}C-CO_2 twice a week for 4 h. The N label was added by growing plants in pools with a constant supply of 99 atom% ^{15}N ammonium sulfate.

Shredder assimilation field experiment using labeled litter

The field experiment (Appendix S2: Fig. S1d) occurred in a natural *Populus* hybrid zone in Oak Creek, Arizona, from March to April 2011, when the abundance of *H. magnus* was high. Each fine-mesh litter pack (1 mm mesh) was filled with 1 g of isotopically labeled litter from a single replicate clone of a *P. fremontii,* F_1 hybrid, or *P. angustifolia* genotype (46 total genotypes, replicated 4–8 times per genotype; n = 714 total litter packs across three harvests). Despite deploying 714 total litter packs (~238 packs per harvest), several packs were compromised during the experiment due to animal or human disturbance, which left them out of the water; consequently, only 659 litter packs were processed and analyzed for this experiment. Litter packs were randomly affixed to rebar at Oak Creek. Shredders, collected locally hours before the start of the experiment, were added to litter packs (n = 1 per pack). To standardize for size and life history stage, we used only fifth-instar individuals with case lengths ranging between 21 and 25 mm. Litter packs were positioned randomly along a ~100-m riffle-run reach of Oak Creek. Upon each harvest (day 7, 14, 21), litter packs were removed from the creek, placed on ice, and taken to the lab to process. Shredders were frozen, removed from cases, and dried. Litter and insect samples were dried at 60°C for 96 h and weighed to obtain dry mass. Tissues were ground with a mortar and pestle to homogenize the sample and weighed into tin cups for stable isotope analysis. Subsamples of initial litter were taken before the experiment to determine isotope values for each litter pack. Remaining litter was harvested, rinsed, and dried to determine mass loss. Litter did not become limiting during this experiment (percent mass remaining: *P. angustifolia* = 21.1% ± 1.6%, F_1 hybrid = 17.7% ± 1.3%, *P. fremontii* = 17.1% ± 1.2%).

Leaf litter chemistry and isotope analysis

Initial litter chemistry is presented in Appendix S1: Table S1. Subsamples of litter from each genotype were taken for chemical analysis of lignin (percentage of dry mass), soluble condensed tannins (percentage of dry mass), %C (100 × g C total/g), and %N (100 × g N total/g). Dried litter was ground with a Wiley mill (mesh size #40), freeze-dried, and stored at −20°C. Fiber (acid detergent fiber) and lignin (acid detergent lignin) were assayed using an Ankom 200 Digester (ANKOM Technology Corporation, New York, New York, USA) to sequentially extract fiber (lignin + cellulose) in hot cetyl-trimethyl-ammonium bromide acidified with H_2SO_4, followed by digestion in 72% H_2SO_4 for lignin. Soluble condensed tannin content was assessed using the acid butanol assay (Porter et al. 1986) with purified *P. angustifolia* condensed tannins as standards (Hagerman and Butler 1989). This procedure involved performing an extraction on ground litter with 70% acetone with ascorbic acid. The product was then reacted with ferric ammonium sulfate in acidic media to produce a product quantified via colorimetry. Litter %C, % N, $\delta^{13}C$, and $\delta^{15}N$ were analyzed at the Colorado Plateau Stable Isotope Laboratory (CPSIL) using a Thermo Finnigan Flash 1112 element analyzer and isotope ratio mass spectrometer (Thermo Finnigan, San Jose, California, USA).

Isotope compositions were expressed in standard delta notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand (‰) relative to VPDB (Vienna PeeDee Belemnite) for C and air for N:

$$\delta = 1000 \times \left(\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right)‰ \quad (1)$$

where R is the molar ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Atom% was calculated as follows:

$$\text{atom\%} = \left(\frac{R_{\text{sample}}}{1 + R_{\text{sample}}} \right) \times 100\%. \quad (2)$$

Using a mass balance approach, we calculated element assimilation rates of C and N (A_X) from litter by shredders as

$$A_X = \frac{\left(\frac{(\text{atom\% } X_{\text{sl}} - \text{atom\% } X_{\text{su}})}{(\text{atom\% } X_{\text{ll}} - \text{atom\% } X_{\text{su}})} \right) \times \left(M_{\text{sl}}\ (\mu\text{g}) \times \left(\frac{\%X_{\text{sl}}}{100} \right) \right)}{T\ (d)} \quad (3)$$

where X_{su} is unlabeled shredder tissue, X_{sl} is labeled shredder tissue, and X_{ll} is labeled litter for a given element (i.e., C or N), M_{sl} is the mass of the labeled shredder (μg), $\%X_{sl}$ is the percentage of element X in the tissue of the labeled shredder, and T is time (days). This measure of assimilation rate determines the rate at which C or N flows from litter to the insects. We calculated a second metric of assimilation that quantifies the mass of C or N assimilated by the insect as a percentage of the mass of C or N lost during decomposition. Here we computed the total C or N assimilated by the insect (the numerator in Eq. 3), divided by the amount of each element that was lost by leaf litter during decomposition. We calculated the amount of C and N lost by leaf litter by multiplying mass loss by the % of C or N in the initial litter.

Data analysis

We examined insect preference data using repeated-measures MANOVA (rmMANOVA) models, with genotype or cross type as the between-subjects factors and time and time × genotype or time × cross type as the within-subjects factors. Wilk's lambda was used as the test statistic for hypothesis testing in rmMANOVA. We opted for this multivariate approach, rather than the traditional univariate approach (rmANOVA), because our data sets violated the assumption of sphericity, which is not an assumption of MANOVA (O'Brien and Kaiser 1985). Additionally, rmMANOVA has more power than rmANOVA to resolve treatment differences, especially when samples sizes are large (Maxwell and Delaney 2004). Low replication of many *P. fremontii* and F_1 hybrid genotypes prevented us from examining genotype patterns of these cross types, and so we analyzed cross type and genotype growth data using different one-way ANOVA models and only examined genotype patterns within *P. angustifolia*. Nested ANOVAs, with genotype nested within cross type (genotype[cross type]), were used for the assimilation experiments, as they were designed with replicated genotypes of each of the three cross types. Time (harvest day), cross type, and genotype were treated as fixed effects in our models. Treating genotype as a fixed effect limits our ability to make inferences beyond the genotypes used in our study, but this was necessary for two reasons: (1) rmMANOVA cannot accommodate random variables and (2) genotypes of leaf litter and cuttings were selected to maximize chemical differences. Because litter preference experiments were more technically complex, requiring litter to be standardized to surface area to allow equal probability of a shredder selecting a leaf surface based on chance alone, the cross type and genotype experiments were conducted separately, and only *P. angustifolia* genotypes were examined. Prior to each ANOVA analysis, response variables were $\log_{10}$-transformed as necessary to meet the assumptions of normality and homogeneity of variance. We generated 95% bootstrapped confidence intervals using the reshape2 package in R (R Core Team 2017) and visualized differences among litter types through time using ggplot2.

To examine how intraspecific variation in litter traits (i.e., percent lignin, percent soluble condensed tannins, %N, %C, C:N, and decomposition rate, $k(d^{-1})$) influenced our response variables (i.e., shredder attachments to litter, growth rates, C and N assimilation rates, and percent litter C and N assimilated), we performed a series of multiple regression analyses using mean values of *P. angustifolia* genotypes; we restricted these analyses to *P. angustifolia* genotypes because different genotypes of this species were well represented in the common garden, while other cross types were fewer in number, making intraspecific analyses of these cross types less robust. Variables were rescaled to z scores prior to multiple regression analyses, and variable importance was assessed using the relaimpo package in R. Unless noted, all analyses were performed using JMP Pro version 11.0 (SAS Institute, Cary, North Carolina, USA).

Results

Shredder litter preference lab experiments

Consistent with our first hypothesis, among cross types we found that shredders initially preferred *P. fremontii* litter, but this preference switched after approximately 4 d, when F_1 hybrid litter was preferred; by the end of the experiment, *P. angustifolia* litter was preferred (rmMANOVA; cross type $F_{2,33} = 0.87$, $P = 0.43$; time × cross type $F_{28,40} = 3.06$, $P < 0.0006$; Fig. 1a). Within *P. angustifolia*, a similar pattern occurred, where shredders preferred litter from relatively labile genotypes initially and switched to relatively

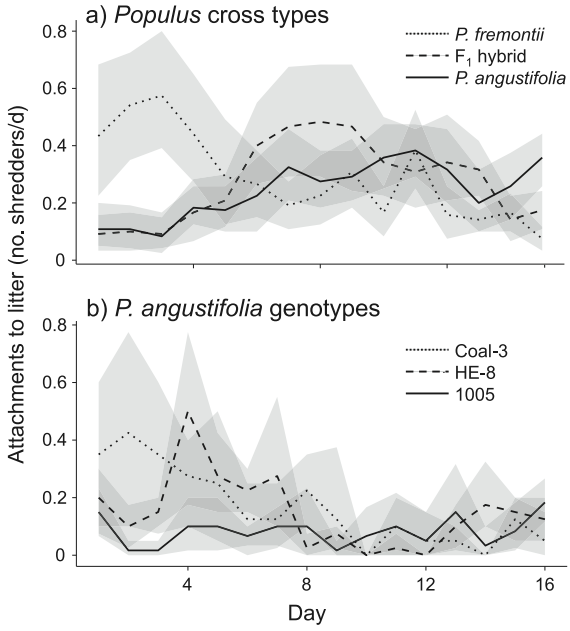

Fig. 1. Shredder attachments to leaf litter of (a) *Populus fremontii* (dotted line), F_1 hybrids (dashed line), and *P. angustifolia* (solid line) cross types through time and (b) a relatively labile (Coal-3: low lignin and condensed tannin concentrations, rapidly decomposing; dotted line), intermediate (HE-8: medium lignin and condensed tannin concentrations, intermediate decomposing; dashed line), and recalcitrant (1005: high lignin and condensed tannin concentrations, slowly decomposing; solid line) *P. angustifolia* genotype through time. We present only three of the *P. angustifolia* genotypes analyzed for simplicity (panel b) and chose genotypes representative of the range of recalcitrance in our system. Gray bands around lines represent 95% bootstrapped confidence intervals. For both *Populus* cross types and *P. angustifolia* genotypes, shredders preferred relatively labile litter types initially, but switched to more recalcitrant litter types later in the experiment.

recalcitrant genotypes after approximately 4 d (rmMANOVA; genotype $F_{24,107} = 1.05$, $P = 0.41$; genotype × time $F_{450,1308.9} = 1.15$, $P = 0.036$; Fig. 1b; Appendix S2: Fig. S2); however, by the end of the experiment (after approximately 8 d), there wasn't clear preference among litter types (Fig. 1b; Appendix S2: Fig. S2). Despite these temporal patterns, preference did not differ among cross types ($F_{2,33} = 0.87$, $P = 0.43$) or genotypes ($F_{24,107} = 1.16$, $P = 0.29$) when litter attachments were totaled across each two-week experiment (Appendix S2: Fig. S3).

Litter chemistry (percent lignin and percent soluble condensed tannins) for the three cross types changed during the preference experiment (whole model; lignin $F_{5,22} = 12.16$, $P < 0.0001$; condensed tannins $F_{5,26} = 131.16$, $P < 0.0001$; cross type × time; lignin $F_{2,22} = 9.19$, $P = 0.0013$; condensed tannins $F_{2,22} = 77.82$, $P < 0.0001$). At the beginning of the experiment, litter chemistry patterns were like those documented by other studies of *Populus* (LeRoy et al. 2006, 2007), where fast-decomposing litter had low concentrations of tannins and lignin compared to medium- and slow-decomposing cross types (Appendix S1: Table S1, Appendix S2: Fig. S4). By the end of the experiment, litter in mesocosms did not differ among litter types for tannins (day 14, $F_{2,14} = 1.84$, $P = 0.20$) or lignin (day 14, $F_{2,10} = 0.080$, $P = 0.92$; Appendix S2: Fig. S4).

Shredder growth lab experiment

Our second hypothesis, about growth rates, was partially supported. Among cross types, litter type influenced shredder growth rates ($F_{2,44} = 3.54$, $P = 0.038$; Fig. 2a; Appendix S2: Fig. S1c). *P. angustifolia* litter yielded the highest growth rates of the three cross types: shredder growth rates were three times faster on *P. angustifolia* compared to F_1 hybrid litter and growth rates on *P. fremontii* were intermediate but not statistically different from growth on *Populus angustifolia*. Within *Populus*, litter genotype was also a strong predictor of shredder growth rates (genotype [cross type] $F_{34,131} = 1.91$, $P = 0.0076$; Appendix S2: Fig. S1c, *P. angustifolia* genotypes only). However, contrary to our second hypothesis, no litter traits of *P. angustifolia* genotypes predicted shredder growth rates in our multiple regression analysis (multiple regression; full model, $F_{6,23} = 1.48$, $P = 0.23$).

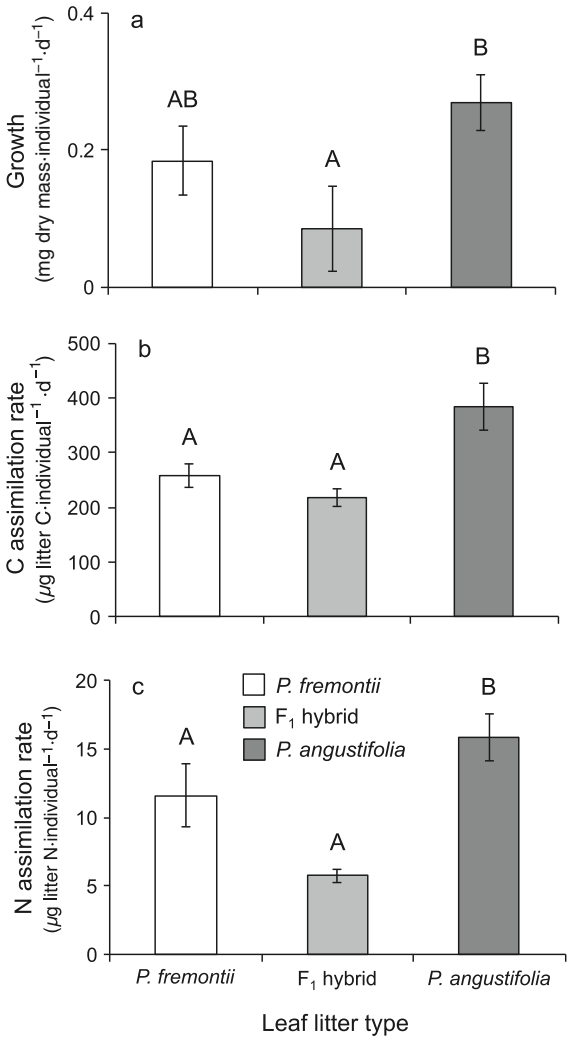

FIG. 2. Shredder (a) growth rates and element assimilation rates for (b) C and (c) N from leaf litter of three cottonwood cross types (*Populus fremontii*, F_1 hybrid, and *P. angustifolia*). Shredder growth rates were measured in the laboratory after 14 days using litter from common gardens and assimilation rates were measured in the field after 14 days using isotopically labeled litter. Different letters above bars designate statistical differences among groups (Tukey's HSD, $\alpha = 0.05$). Shredder assimilation and growth rates were higher on relatively recalcitrant (high lignin and condensed tannin concentrations, slowly decomposing) compared to relatively labile (low lignin and condensed tannin concentrations, rapidly decomposing) litter types.

Shredder assimilation field experiment using labeled litter

Consistent with our third hypothesis, among cross types we found higher fluxes of C and N to shredders from slow-decomposing *P. angustifolia* litter compared to intermediate- and fast-decomposing *P. fremontii* and F_1 hybrid litter. For C, shredder assimilation rates (A) were highest from *P. angustifolia* litter compared to *P. fremontii* and F_1 hybrid litter (A_C; cross type $F_{2,302} = 6.54$, $P = 0.0017$; Fig. 2b). C assimilation was ~1.5-times higher on *P. angustifolia* litter compared to *P. fremontii* and F_1 hybrid litter. For N, the same pattern emerged: shredder assimilation rates were higher from *P. angustifolia* compared to the other litter types (A_N; cross type $F_{2,303} = 5.94$, $P = 0.0029$; Fig. 2c). We also detected temporal effects on C (time, $F_{1,302} = 293.70$, $P < 0.0001$) and N (time, $F_{2,303} = 78.80$, $P < 0.0001$) assimilation rates, with assimilation rates generally decreasing through time for all cross types. There was also a marginal interaction between litter type and time for C assimilation (cross type × time, $F_{2,302} = 2.77$, $P = 0.065$), reflecting more rapidly declining shredder C assimilation rates over time in *P. fremontii* and F_1 hybrid compared to *P. angustifolia* litter. Among cross types, the percentages of C and N assimilated by shredders from decomposing litter were also significantly higher for *P. angustifolia* compared to *P. fremontii* and F_1 hybrid litter (C, $F_{2,302} = 6.84$, $P < 0.0012$; N, $F_{2,303} = 3.84$, $P = 0.0227$; Fig. 3a, b), with a significant temporal effect for C (time, $F_{1,302} = 19.01$, $P < 0.0001$); these patterns, however, were quite variable, and only day 7 for C (Fig. 3a) and day 14 for N (Fig. 3b) differed statistically among cross types. After

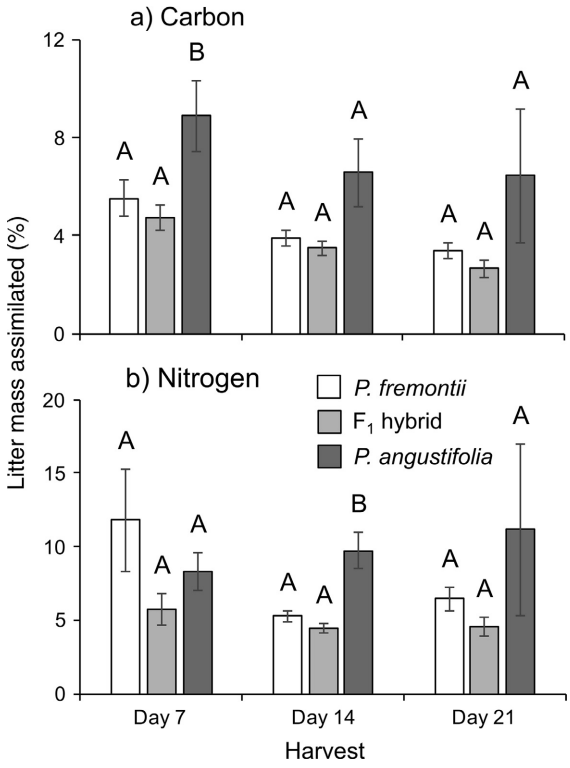

FIG. 3. The percentages of (a) C and (b) N mass lost during decomposition that was incorporated into shredder tissue from leaf litter of three *Populus* cross types (*P. fremontii*, F_1 hybrid, and *P. angustifolia*) harvested on days 7, 14 and 21 (calculated as [μg litter C or N assimilated]/[μg litter C or N lost] × 100). Different letters above bars designate statistical differences among groups for a given harvest (Tukey's HSD, $\alpha = 0.05$). There was a trend for shredder C assimilation rates to be higher from relatively recalcitrant (high lignin and tannin concentrations, slowly decomposing) compared to labile (medium and low lignin and tannin concentrations, rapidly decomposing) litter (statistically significant on day 7). Shredder N assimilation rates were higher for recalcitrant litter on day 14.

21 d, approximately 6% of C lost from *P. angustifolia* litter was assimilated by shredders, compared to only 3% of C lost from *P. fremontii* litter. Similarly, approximately 8% of N lost was assimilated from *P. angustifolia* litter, compared to only 4% of N lost in F_1 hybrid and 6% in *P. fremontii* litter. Together, these results indicate that, despite temporal patterns, over time shredders assimilated more total C and N, and a higher percentage of the litter C and N lost during decomposition, from slowly decomposing *P. angustifolia* litter compared to more rapidly decomposing F_1 hybrid and *P. fremontii* litter (Figs. 2, 3).

As predicted, rates of C (A_C; genotype[cross type], $F_{43,302} = 2.25$, $P < 0.0001$) and N (A_N; genotype[cross type], $F_{43,303} = 3.40$, $P < 0.0001$) assimilation by shredders differed among genotypes of *P. angustifolia* and correlated with litter traits (Fig. 4). C assimilation rates were positively associated with the %N and C:N of initial litter (Fig. 4a, Appendix S3: Table S1). Similarly, N assimilation rates were positively associated with the C:N of initial litter; however, N assimilation rates were also positively associated with percent lignin and negatively associated with percent soluble condensed tannins and litter decomposition rate (Fig. 4b, Appendix S3: Table S1). For every 1% increase in litter lignin concentration, a shredder is expected to assimilate, on average, 3.6 μg N/d, given that all other variables are held constant (Appendix S3: Table S2). Conversely, for every 1% decrease in litter condensed tannin concentration, a shredder is expected to assimilate 9.0 μg N/d (Appendix S3: Table S1). The percent of mass assimilated by shredders from decomposing litter also differed among genotypes, for both C (genotype[cross type], $F_{43,302} = 2.51$, $P < 0.0001$) and N (genotype[cross type], $F_{43,303} = 2.21$, $P < 0.0001$). The percent of C that was assimilated from decomposing litter was positively associated with the %N and C:N of initial litter (Fig. 4c, Appendix S3: Table S1). Similarly, the percent of N that was assimilated from decomposing litter was positively associated with the C:N of initial litter; however, the percent of N assimilated was also positively associated with percent lignin and negatively associated with percent soluble condensed tannins and litter decomposition rate (Fig. 4d, Appendix S3: Table S1).

DISCUSSION

Litter traits and shredder performance

Our results demonstrate that genetically derived traits of litter affect shredder preference, growth, and nutrient assimilation. Overall, across plant cross types, shredders performed better on more recalcitrant litter. Although recalcitrant litter is generally thought to be a low-quality resource for freshwater consumers (Canhoto and Graça 1995, Graça and Bärlocher 1999), other studies have shown that insects are more abundant on recalcitrant litter (Grubbs and Cummins 1994, Kominoski et al. 2012). These results also complement prior studies on cottonwood species that showed higher nutrient assimilation and emergence rates of aquatic insects on *P. angustiofolia* compared to *P. fremontii* litter (Compson et al. 2013, 2015). At the watershed scale, recalcitrant litter can increase aquatic shredder species richness, likely because it supports the establishment of spring and summer shredders by persisting longer and slowing down decomposition in litter patches (Grubbs and Cummins 1994, Swan and Palmer 2004).

Our experimental design allowed us to decouple the effects of condensed tannins and lignin on shredder nutrient assimilation from litter of *Populus angustifolia* genotypes in the field. Tannin and lignin concentrations can be correlated in plant tissues because they are both partially biosynthesized from the metabolic products of the shikimic acid pathway (Hagerman and Butler 1991), and both retard litter decomposition (e.g., Wardle et al. 2002). Across a variety of systems, however, lignin and tannins or phenolics are not always correlated, especially in leaf litter systems (Appendix S4: Table S1). In our study, within *P. angustifolia*, lignin and condensed tannins were not correlated, for either garden or greenhouse litter (Appendix S4: Table S1). Our results show that lignin and tannins have opposite effects on N assimilation by shredders feeding on decomposing litter. Lignin concentrations correlated positively with N assimilation, whereas soluble condensed tannins correlated negatively with N assimilation. On average, a 1% increase in litter lignin content is expected to increase shredder N assimilation by 23% of its mean daily value

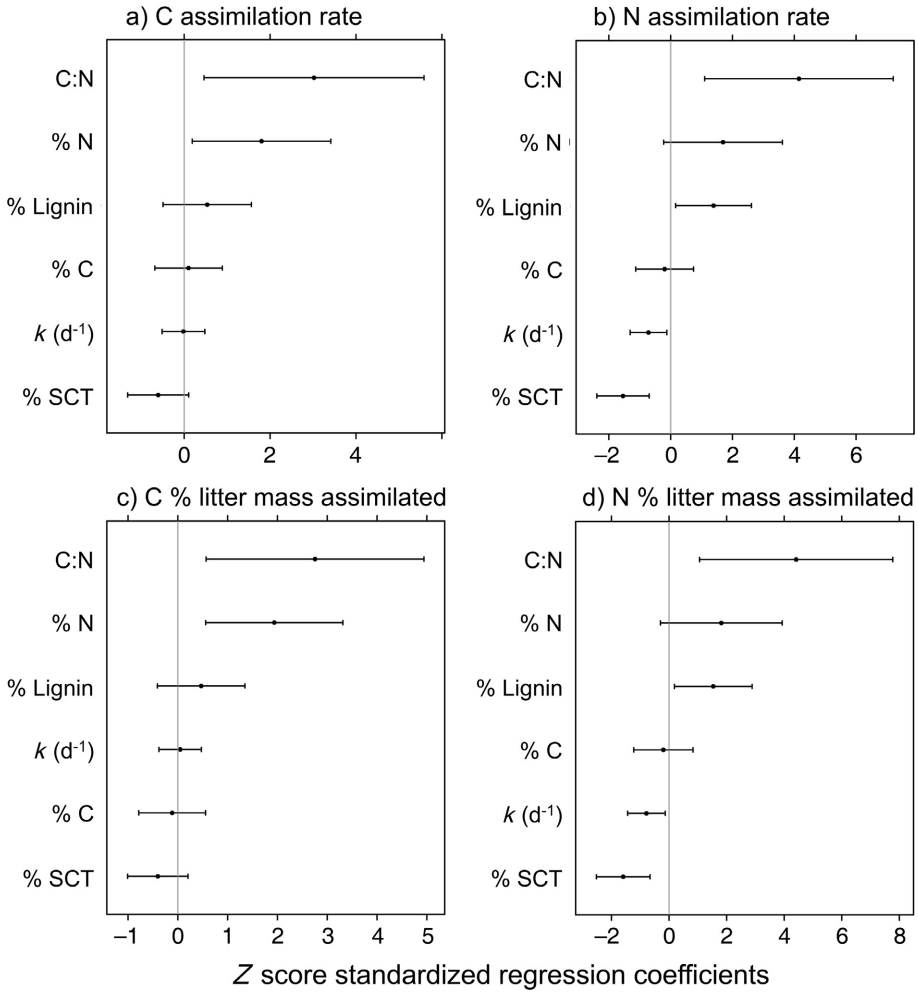

FIG. 4. Standardized regression coefficients (based on z score standardized variables) for multiple regression analysis of the association of litter soluble condensed tannins (% SCT), % lignin, % C, % N, C:N, and decomposition, $k(d^{-1})$, on shredder (a) assimilation rate of C (μg litter C individual^{-1} d^{-1}), (b) assimilation rate of N (μg litter N individual^{-1} d^{-1}), (c) the percent of C that was lost in decomposition and assimilated by the shredder, and (d) the percent of N that was lost in decomposition and assimilated by the shredder. Positive values represent positive slopes, negative values represent negative slopes, and 95% confidence intervals that do not overlap with zero depict significant predictors. Litter C:N, % N, and % lignin were generally positively correlated predictors of both C and N shredder assimilation rates, while % condensed tannins and litter decomposition were negatively correlated predictors of N shredder assimilation.

from *P. angustifolia* litter, while a 1% increase in tannin litter content is expected to decrease shedder N assimilation by 57% of its daily mean. Across the variation in lignin and tannin content of *P. angustifolia* genotypes used in our study, this translates to a range of 32.4 μg N·shredder^{-1}·d^{-1} for lignin and 47.9 μg N·shredder^{-1}·d^{-1} for condensed tannins, or two and three times the mean daily assimilation rate per shredder for lignin and tannins, respectively. This means that both lignin and condensed tannins were strong regulators of N pathways from leaf litter to higher trophic levels in our system. Lignin can bind N (Berg 1986), preventing it from being leached from litter before shredders can utilize it. Conversely, tannins are often defense compounds that likely deter insect feeding (Canhoto and Graça 1995, Graça and Bärlocher 1999). If the patterns we describe are seen in other systems where lignin and tannins are correlated, the effects of these compounds on shredder nutrient assimilation could negate each other; however, in our system, where they were not correlated, this likely means N fluxes to higher trophic levels from some litter genotypes are regulated by lignin, while N fluxes from other litter genotypes are regulated by tannins.

Aquatic shredders exhibit one of the greatest stoichiometric mismatches in C:N with their food resource (Cross et al. 2003). Our results suggest that shredders may regulate their internal stoichiometry by assimilating more C from leaves when N is more available (Manzoni et al. 2010). Because N is often a limiting resource in headwater streams (Biggs 2000), these findings underscore the importance of shredders as ecosystem links, concentrating and aggregating N from a relatively N-poor, high-biomass resource (terrestrial leaf litter) and making it available at higher concentrations in a relatively low-biomass subsidy (secondary production) to aquatic and terrestrial predators (Cross et al. 2005, Bartels et al. 2012). High C:N ratios are often viewed as an indication of low litter quality (e.g., Aerts 1997). Our results, however, show that C and N assimilation are positively correlated with C:N, underscoring that the type of C compound

(e.g., tannins compared to lignin) may be more important than C:N in determining food quality.

Because we examined the influence of chemical traits on a narrow range of litter types (i.e., *P. angustifolia* genotypes), we acknowledge that the patterns we describe may only apply to the *Populus* system; further studies are needed in other systems to test whether the differences in how lignin and tannins influence C and N fluxes to shredders are a general phenomenon that occurs across a wider range of riparian species. Additionally, it is possible that the large, fifth-instar shredders used in this study could have disproportionately high C and N assimilation rates from relatively recalcitrant litter compared to other shredders. *H. magnus* reached peak densities in Oak Creek in late April to mid-May, just prior to emergence, when much of the remaining leaf litter in the stream was likely recalcitrant litter from leaf drop in the previous autumn, and so these shredders are likely adapted to utilize slowly decomposing riparian litter types. Patterns of C and N flow from leaf litter to insects could be different for other shredders and in other systems where shredders are adapted to more labile litter types, especially since shredders can be locally adapted to riparian litter (Jackrel and Wootton 2014).

Although we did not measure other pathways of C and N flow in this study, we have previously shown that fast decomposing litter loses more C and N to leaching (Wymore et al. 2015) and supports higher microbial biomass (Pastor et al. 2014) than slowly decomposing litter. While microbes can increase the nutritional quality of leaf litter for detritivores (Graça 2001), they contribute a fraction of the total C respired by stream detritivores (Findlay et al. 1986a,b), indicating that the C requirements of shredders could largely come directly from leaf litter. Another study that examined a limnephilid shredder (*Pycnopsyche gentilis*) found that fungal C accounted for 50% of the daily growth rate of the insect in its fifth instar stage (the same life stage as the limnephilids used in our study), indicating that the shredder had to assimilate detrital mass to meet its nutritional demand (Chung and Suberkropp 2009). Because total bacterial and fungal C biomass makes up only a small proportion of the microbe–detritus complex (Methvin and Suberkropp 2003), leaf litter might be the greatest contributor to shredder C demand despite 5–50 times higher consumer assimilation efficiencies from microbes compared to leaf litter (Findlay 2010, Halvorson et al. 2016). Moreover, while microbes can be an important food resource for shredders, they also likely compete with shredders for leaf nutrients (Bärlocher 1980, Gessner et al. 1999). Because nutrient enrichment can accelerate aquatic litter decomposition and increase the proportion of leaf C channeled through the microbial pathway (Gulis and Suberkropp 2003, Cross et al. 2007), microbes could potentially outcompete shredders in systems where nutrient levels are high (e.g., streams affected by agricultural runoff). For example, nutrient enrichment can increase bacterial and fungal biomass on CPOM and increase respiration rates, leading to C loss from this resource (Tant et al. 2013); if nutrient enrichment in streams is high enough, then this pathway, coupled with rapid losses from leaching, could mean microbes outcompete shredders in these systems. Consequently, our findings that litter with high lignin concentrations is important to aquatic shredder nutrient assimilation and growth indicate that recalcitrant litter might play an even greater role in supporting the macroscopic food web in nutrient enriched systems. Specifically, this could mean that litter lignin content could potentially exert an even greater control on N movement to higher trophic levels in these systems by slowing litter decomposition and allowing it to persist long enough for shredders to access.

While the relative contributions of microbial and leaf litter C to the energetic demands of aquatic shredders has been well documented through radio-labeling studies, the proportions of shredder N and phosphorus (P) that come from microbes and leaf litter have not. Studies estimating shredder efficiencies have shown that microbial C is more efficiently assimilated than bulk litter C, while P assimilation efficiencies from microbial and bulk litter were similar (Fuller et al. 2015, Halvorson et al. 2015). These studies, however, did not assess the contribution of C or P directly from leaf litter, and so the lack of differences in P assimilation efficiency from microbes and bulk litter could have been an artifact of the large proportion of the bulk litter P pool that is made up of microbial P (Halvorson et al. 2016). In our study, we could estimate the proportion of litter C and N incorporated into shredders because we used labeled litter. We estimated that ~3–9% of litter C and ~5–12% of litter N lost during decomposition was assimilated by shredders, indicating that, like microbial P (Halvorson et al. 2016), litter N was more efficiently assimilated than C. This is likely because ingested C can be rapidly lost through egestion and respiration (Van Frankenhuyzen et al. 1985) to maintain elemental homeostasis (Sterner and Elser 2002). These findings differ from another study, where C assimilation efficiency was 44% and N assimilation efficiency ranged from 16% to 21% for another shredder feeding on a range of fresh leaf and macrophyte substrates (Jacobsen and Sand-Jensen 1994). We did not measure assimilation effiiciencies for C and N. Our values for the assimilated percent of litter C and N mass loss during decomposition are expected to be lower than assimilation effiiciency values, since mass loss during decomposition will be much higher than shredder consumption because of litter fragmentation, leaching, and mass loss to microbes. However, from a qualitative perspective, it is interesting that the values reported by Jacobsen and Sand-Jensen (1994) demonstrate C assimilation efficiency was higher than N assimilation efficiency, which was the opposite of what we found, likely because we measured shredder assimilation from litter while they measured assimilation from fresh leaves and macrophytes.

Temporal dynamics of shredder performance

We observed temporal dynamics in both preference and nutrient assimilation, suggesting that insects benefit from the pulse of nutrients provided by fast decomposing litter soon after leaf fall. After just two weeks in the river, insects assimilated more total C and N from slowly decomposing litter with high lignin concentrations. One possible explanation for why we observed changing shredder preference and nutrient assimilation patterns through time was because of changing litter chemistry through time. For example, we demonstrated that chemical differences among litter types shifted through time during our litter preference study (Appendix S2:

Fig. S4). Other studies have also documented temporal changes in litter chemistry. When leaf litter falls into the stream, there are initial, rapid losses of N (Canhoto and Graça 1996), P (Cortes et al. 1994, Casas and Gessner 1999), potassium and magnesium (Escudero et al. 1991), and polyphenols (Canhoto and Graça 1996). In contrast, lignin concentrations can increase throughout litter decomposition (Suberkropp et al. 1976, Boulton and Boon 1991) because lignin is disproportionately retained compared to more labile compounds throughout decomposition. Thus, the shifts we observed from high shredder litter preference and N assimilation from *P. fremontii* early in our experiments to *P. angustifolia* later in our experiments could have arisen because the proportion of lignin in remaining leaf litter increased in *P. fremontii* litter and decreased in *P. angustifolia* litter through time (Appendix S2: Fig. S4). This assessment is corroborated by visual observations and photographs of remaining leaf litter showing that *P. fremontii* and F_1 hybrid litter remains as a skeletonized leaf later in decomposition, while all parts of the litter, except sometimes the midvein, disappear throughout *P. angustifolia* litter decomposition (data not shown). Within *P. angustifolia*, changing litter preference patterns were also observed, as indicated by significant genotype × time interactions, but patterns were generally less pronounced (Fig. 1b), which could have been due to less behavioral selection at finer genetic scales or due to the limitations of our experimental design (i.e., not all genotypes were paired with all other genotypes). Additionally, the low shredder attachment rates observed in our preference studies (<1 attachment·shredder^{-1}·d^{-1}) could indicate that shredder activity was low for these lab studies, potentially underestimating actual preference under natural conditions.

Ecological implications of litter types with varying genetic footprints

Leaf litter that feeds and structures the stream food web acts as an afterlife effect of the plant (Findlay et al. 1996, Kane et al. 2011). Our research and that of others demonstrate that intraspecific variation in litter traits can strongly alter freshwater ecosystems and food webs (Crutsinger et al. 2014, Rudman et al. 2015, Rodriguez-Cabal et al. 2016). These genetic effects, which begin in terrestrial ecosystems, cascade through aquatic ecosystems and back to terrestrial ecosystems, as plant genotype influences aquatic insect emergence (Compson et al. 2016), which can affect the abundance and biomass of riparian predators that depend on this reciprocal subsidy (Baxter et al. 2005).

Acknowledgments

Members of the Center for Ecosystem Science and Society provided feedback on the manuscript. NSF provided funding through the IGERT (DGE-0549505) and Ecosystem Studies (DEB-1120343) research programs.

Literature Cited

Abramoff, M. D., P. J. Magalhaes, and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics International 11:36–42.

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79:439–449.

Bailey, J. K., S. C. Wooley, R. L. Lindroth, and T. G. Whitham. 2006. Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. Ecology Letters 9:78–85.

Bärlocher, F. 1980. Leaf-eating invertebrates as competitors of aquatic hyphomycetes. Oecologia 47:303–306.

Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik, and H. Hillebrand2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. Ecology 93:1173–1182.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50:201–220.

Benfield, E. F. 2006. Decomposition of leaf material. Pages 125–155 *in* F. R. Hauer, and G. A. Lamberti, editors. Methods in stream ecology. Second edition. Academic Press, Burlington, Massachusetts, USA.

Berg, B. 1986. Nutrient release from litter and humus in coniferous forest soils—a mini review. Scandinavian Journal of Forest Research 1:359–369.

Biggs, B. J. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17–31.

Boulton, A. J., and P. I. Boon. 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: Time to turn over an old leaf? Marine Freshwater Research 42:1–43.

Bultman, T. L., and G. W. Uetz. 1982. Abundance and community structure of forest floor spiders following litter manipulation. Oecologia 55:34–41.

Canhoto, C., and M. A. S. Graça. 1995. Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis*. Freshwater Biology 34:209–214.

Canhoto, C., and M. A. S. Graça. 1996. Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. Hydrobiologia 333:79–85.

Casas, J. J., and M. O. Gessner. 1999. Leaf litter breakdown in a Mediterranean stream characterised by travertine precipitation. Freshwater Biology 41:781–793.

Chapin, F. S. III, P. A. Matson, and P. Vitousek. 2011. Principles of terrestrial ecosystem ecology. Second edition. Springer Science and Business Media, New York, New York, USA.

Chung, N., and K. Suberkropp. 2009. Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). Freshwater Biology 54:2212–2224.

Compson, Z. G., K. J. Adams, J. A. Edwards, J. M. Maestas, T. G. Whitham, and J. C. Marks. 2013. Leaf litter quality affects aquatic insect emergence: contrasting patterns from two foundation trees. Oecologia 173:507–519.

Compson, Z. G., B. A. Hungate, G. W. Koch, S. C. Hart, J. M. Maestas, K. J. Adams, T. G. Whitham, and J. C. Marks. 2015. Closely related tree species differentially influence the transfer of carbon and nitrogen from leaf litter up the aquatic food web. Ecosystems 18:186–201.

Compson, Z. G., B. A. Hungate, T. G. Whitham, N. Meneses, P. E. Busby, T. Wojtowicz, A. C. Ford, K. J. Adams, and J. C. Marks. 2016. Plant genotype influences aquatic-terrestrial ecosystem linkages through timing and composition of insect emergence. Ecosphere 7:e01331.

Cortes, R. M. V., M. A. S. Graça, and A. Monzón. 1994. Replacement of alder by eucalypt along two streams with different characteristics: differences on decay rates and consequences to the system functioning. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 25:1697–1702.

Cross, W. F., J. P. Benstead, A. D. Rosemond, and B. J. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721–732.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50:1895–1912.

Cross, W. F., J. B. Wallace, and A. D. Rosemond. 2007. Nutrient enrichment reduces constraints on material flows in a detritus-based food web. Ecology 88:2563–2575.

Crutsinger, G. M., S. Y. Strauss, and J. A. Rudgers. 2010. Genetic variation within a dominant shrub species determines plant species colonization in a coastal dune ecosystem. Ecology 91:1237–1243.

Crutsinger, G. M., et al. 2014. Testing a 'genes-to-ecosystems' approach to understanding aquatic–terrestrial linkages. Molecular Ecology 23:5888–5903.

Cummins, K. W., R. C. Petersen, F. O. Howard, J. C. Wuycheck, and V. I. Holt. 1973. The utilization of leaf litter by stream detritivores. Ecology 54:336–345.

Driebe, E. M., and T. G. Whitham. 2000. Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia 123:99–107.

Escudero, A., S. Sanz, J. M. Del Arco, and M. V. Garrido. 1991. Leaf litter decomposition in a mountain stream. Internationale Vereinigung fuer Theoretische und Angewandte Limnologie. Verhandlungen 24:1987–1993.

Findlay, S. 2010. Stream microbial ecology. Journal of the North American Benthological Society 29:170–181.

Findlay, S., J. L. Meyer, and P. J. Smith. 1986*a*. Contribution of fungal biomass to the diet of a freshwater isopod (*Lirceus* sp.). Freshwater Biology 16:377–385.

Findlay, S., J. L. Meyer, and P. J. Smith. 1986*b*. Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera). Journal of the North American Benthological Society 5:306–310.

Findlay, S., M. Carreiro, V. Krischik, and C. G. Jones. 1996. Effects of damage to living plants on leaf litter quality. Ecological Applications 6:269–275.

Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43:421–439.

Fogel, R., and K. Jr Cromack. 1977. The effect of habitat and substrate quality on Douglas-fir litter decomposition in western Oregon. Canadian Journal of Botany 55:1632–1640.

Friberg, N., and D. Jacobsen. 1994. Feeding plasticity of two detritivore-shredders. Freshwater Biology 32:133–142.

Fuller, C. L., M. A. Evans-White, and S. A. Entrekin. 2015. Growth and stoichiometry of a common aquatic detritivore respond to changes in resource stoichiometry. Oecologia 177:837–848.

Gessner, M. O., and E. Chauvet. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817.

Gessner, M. O., E. Chauvet, and M. Dobson. 1999. A perspective on leaf litter breakdown in streams. Oikos 85:377–384.

Graça, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams—a review. International Review of Hydrobiology 86:383–393.

Graça, M. A. S., and F. Bärlocher. 1999. Proteolytic gut enzymes in *Tipula caloptera*–interaction with phenolics. Aquatic Insects 21:11–18.

Grubbs, S. A., and K. W. Cummins. 1994. Processing and macroinvertebrate colonization of black cherry (*Prunus serotina*) leaves in two streams differing in summer biota, thermal regime and riparian vegetation. American Midland Naturalist 132:284–293.

Gulis, V., and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48:123–134.

Hagerman, A. E., and L. G. Butler. 1989. Choosing appropriate methods and standards for assaying tannin. Journal of Chemical Ecology 15:1795–1810.

Hagerman, A. E., and L. G. Butler. 1991. Tannins and lignins. Pages 355–388 *in* G. A. Rosenthal, and M. R. Berebaum, editors. Herbivores: their interactions with secondary plant metabolites. The chemical participants. Second edition. Academic Press, San Diego, California, USA.

Haloin, J. R., and S. Y. Strauss. 2008. Interplay between ecological communities and evolution: review of feedbacks from microevolutionary to macroevolutionary scales. Annals of the New York Academy of Sciences 1133:87–125.

Halvorson, H. M., J. T. Scott, A. J. Sanders, and M. A. Evans-White. 2015. A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. Freshwater Science 34:508–518.

Halvorson, H. M., G. White, J. T. Scott, and M. A. Evans-White. 2016. Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. Oecologia 180:567–579.

Hansen, R. A. 2000. Effects of habitat complexity and composition on a diverse litter microarthropod assemblage. Ecology 81:1120–1132.

Hieber, M., and M. O. Gessner. 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83:1026–1038.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7:336–339.

Holeski, L. M., M. L. Hillstrom, T. G. Whitham, and R. L. Lindroth. 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.

Hutchens, J. J. Jr, E. F. Benfield, and J. R. Webster. 1997. Diet and growth of a leaf-shredding caddisfly in southern Appalachian streams of contrasting disturbance history. Hydrobiologia 346:193–201.

Jackrel, S. L., and J. T. Wootton. 2014. Local adaptation of stream communities to intraspecific variation in a terrestrial ecosystem subsidy. Ecology 95:37–43.

Jacobsen, D., and K. Sand-Jensen. 1994. Growth and energetics of a trichopteran larva feeding on fresh submerged and terrestrial plants. Oecologia 97:412–418.

Jenkins, C. C., and K. Suberkropp. 1995. The influence of water chemistry on the enzymatic degradation of leaves in streams. Freshwater Biology 33:245–253.

Johnson, M. T. J., and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenthera biennis*). Ecology 86:874–885.

Kane, J. M., K. A. Meinhardt, T. Chang, B. L. Cardall, R. Michalet, and T. G. Whitham. 2011. Drought-induced mortality of a foundation species (*Juniperus momosperma*) promotes positive afterlife effects in understory vegetation. Plant Ecology 212:733–741.

Kominoski, J. S., S. Larrañaga, and J. S. Richardson. 2012. Invertebrate feeding and emergence timing vary among streams along a gradient of riparian forest composition. Freshwater Biology 57:759–772.

LeRoy, C. J., and J. C. Marks. 2006. Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshwater Biology 51:605–617.

LeRoy, C. J., T. G. Whitham, P. Keim, and J. C. Marks. 2006. Plant genes link forests and streams. Ecology 87:255–261.

LeRoy, C. J., T. G. Whitham, S. C. Wooley, and J. C. Marks. 2007. Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. Journal of the North American Benthological Society 26:426–438.

Magill, A. H., and J. D. Aber. 2000. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology and Biochemistry 32:603–613.

Manzoni, S., J. A. Trofymow, R. B. Jackson, and A. Porporato. 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecological Monographs 80:89–106.

Maxwell, S. E., and H. D. Delaney. 2004. Designing experiments and analyzing data: a model comparison perspective. Second edition. Lawrence Erlbaum Associates, Mahwah, New Jersey, USA.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626.

Methvin, B. R., and K. Suberkropp. 2003. Annual production of leaf-decaying fungi in 2 streams. Journal of the North American Benthological Society 22:554–564.

O'Brien, R. G., and M. K. Kaiser. 1985. MANOVA method for analyzing repeated measures designs: an extensive primer. Psychological Bulletin 97:316–333.

Palmer, M. A., et al. 2000. Linkages between aquatic sediment biota and life above sediments as potential drivers of biodiversity and ecological processes. BioScience 50:1062–1075.

Pastor, A., Z. G. Compson, P. Dijkstra, J. L. Riera, E. Martí, F. Sabater, B. A. Hungate, and J. C. Marks. 2014. Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. Oecologia 176:1111–1121.

Porter, L. J., L. N. Hrstich, and B. G. Chan. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

Rodrigues, A. P. L., and M. A. S. Graça. 1997. Enzymatic analysis of leaf decomposition in freshwater by selected aquatic hyphomycetes and terrestrial fungi. Sydowia 49:160–173.

Rodriguez-Cabal, M. A., M. N. Barrios-Garcia, S. M. Rudman, A. D. McKown, T. Sato, and G. M. Crutsinger. 2016. It is about time: genetic variation in the timing of leaf-litter inputs influences aquatic ecosystems. Freshwater Biology 62:356–365.

Rudman, S. M., M. A. Rodriguez-Cabal, A. Stier, T. Sato, J. Heavyside, R. W. El-Sabaawi, and G. M. Crutsinger. 2015. Adaptive genetic variation mediates bottom-up and top-down control in an aquatic ecosystem. Proceedings of the Royal Society B 282:20151234.

Schweitzer, J. A., et al. 2008. From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems 11:1005–1020.

Slansky, F. Jr., and J. M. Scriber. 1985. Food consumption and utilisation. Pages 87–163 *in* G. A. Kerkut, and L. I. Gilbert, editors. Comprehesive insect physiology. Volume 4. Pergamon Press, Oxford, UK.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry. Princeton University Press, Princeton, New Jersey, USA.

Suberkropp, K., G. L. Godshalk, and M. J. Klug. 1976. Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57:720–727.

Swan, C. M., and M. A. Palmer. 2004. Leaf diversity alters litter breakdown in a Piedmont stream. Journal of the North American Benthological Society 23:15–28.

Tant, C. J., A. D. Rosemond, and M. R. First. 2013. Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. Freshwater Science 32:1111–1121.

Tuchman, N. C., R. G. Wetzel, S. T. Rier, K. A. Wahtera, and J. A. Teeri. 2002. Elevated CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Global Change Biology 8:163–170.

Van Frankenhuyzen, K., G. H. Green, and C. Koivisto. 1985. Direct and indirect effects of low pH on the transformation of detritial energy by the shredding caddisfly, *Clistoronia magnifica* (Banks) (Limnephilidae). Canadian Journal of Zoology 63:2298–2304.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarošík, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pyšek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14:702–708.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104.

Wardle, D. A., K. I. Bonner, and G. M. Barker. 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Functional Ecology 16:585–595.

Whitham, T. G., et al. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics 7:510–523.

Wymore, A. S., Z. G. Compson, C. M. Liu, L. B. Price, T. G. Whitham, and J. C. Marks. 2013. Contrasting rRNA gene abundance patterns for aquatic fungi and bacteria in response to leaf-litter chemistry. Freshwater Science 32:663–672.

Wymore, A. S., Z. G. Compson, W. H. McDowell, J. D. Potter, and J. C. Marks. 2015. Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. Freshwater Science 34:857–866.

Zytynska, S. E., M. F. Fay, D. Penney, and R. F. Preziosi. 2011. Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. Philosophical Transactions of the Royal Society B 366:1329–1336.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2224/suppinfo