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ECOSPHERE

Litter identity affects assimilation of carbon and nitrogen by a shredding caddisfly

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Abstract. Ecologists often equate litter quality with decomposition rate. In soil and sediments, litter that is rapidly decomposed by microbes often has low concentrations of tannin and lignin and low C:N ratios. Do these same traits also favor element transfer to higher trophic levels in streams, where many insects depend on litter as their primary food source? We test the hypothesis that slow decomposition rates promote element transfer from litter to insects, whereas rapid decomposition favors microbes. We measured carbon and nitrogen fluxes from four plant species to a leaf-shredding caddisfly using isotopically labeled litter. Caddisflies assimilated a higher percentage of litter carbon and nitrogen lost from slowly decomposing litters (*Platanus wrightii* and *Quercus gambelii*). In contrast, rapidly decomposing litters (*Fraxinus velutina* and *Populus fremontii*) supported higher microbial biomass. These results challenge the view that rapidly decomposing litter is higher quality by demonstrating that slowly decomposing litters provide a critical resource for insects.

Key words: carbon; decomposition; detritivore; insects; leaf litter; nitrogen; stable isotopes.

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INTRODUCTION

Most primary production is not consumed by herbivores and enters the detrital pool (Polis and Strong 1996, Cebrian 2004, Moore et al. 2004). The amount of energy moving through the detrital pathway can equal or exceed the amount moving through the grazed pathway (Moore et al. 2004), such that leaf litter is the dominant energy source for microbes and invertebrates in most headwater streams (Fisher and Likens 1973, Vannote et al. 1980). Detrital inputs can increase species diversity and predator biomass and create longer food chains (Hairston and Hairston 1993). The rate at which litter decomposes varies predictably with physical and chemical litter traits, declining with lignin and tannin concentrations, and increasing with sugars and specific leaf area (Cornwell et al. 2008, Makkonen et al. 2012). The rate of litter decomposition is well studied because it is an important ecosystem process, and its controls can be consistently detected, with global-scale patterns emerging (Boyero et al. 2011). Yet, the simplicity of a single rate to describe litter disappearance masks the variety of fates of the elements the litter contains and the multiple pathways through which they flow.

Rapidly decomposing litter is often considered to be a higher quality resource than slowly decomposing litter (Golladay et al. 1983, Graça

2001, Fierer et al. 2005, Rubino et al. 2007, Marcarelli et al. 2011) because it has fewer chemically recalcitrant compounds (Triska and Sedell 1976, Webster and Benfield 1986, LeRoy et al. 2007), which inhibit breakdown by microbes and invertebrates (Cameron and LaPoint 1978, Gessner and Chauvet 1994, Rahman et al. 2013). Low carbon:nitrogen (C:N) ratios characteristic of rapidly decomposing litter are considered a more optimal stoichiometry for detritivores in both terrestrial and aquatic ecosystems (Enríquez et al. 1993, Ostrofsky 1997, Berg 2000). Microbial biomass and abundance can be higher and peak more quickly on rapidly decomposing litter compared with slowly decomposing litter (Gessner and Chauvet 1994, Bardgett and Shine 1999, Gulis and Suberkropp 2003, Wymore et al. 2013, Pastor et al. 2014), and invertebrates prefer litter colonized by microbes over uncolonized or sterile litter (Golladay et al. 1983, Arsuffi and Suberkropp 1984, Graça et al. 2001).

Many studies testing how litter quality affects invertebrates provide unlimited litter resources to decouple litter quality from quantity (Iversen 1974, Golladay et al. 1983, Graça et al. 2001). In the field, where litter quantity may be limiting, compounds lost during leaching or broken down by microbes may not be available to invertebrates. Despite a large body of research on decomposition, few studies test how litter traits affect pathways of element flow to leaching, microbial and invertebrate assimilation, and respiration (Gessner et al. 1999). In both terrestrial and aquatic literature, faster decomposing leaves have been considered to be of higher quality (Melillo et al. 1982, Hobbie 2000, Graça 2001, Marcarelli et al. 2011), such that the apparent value of litter increases with its rate of disappearance, regardless of the elemental fate: to higher trophic levels, to sediment or soil organic matter reservoirs, to microbial biomass, to dissolved organic matter, or to the atmosphere as carbon dioxide (CO_2).

Prior work on *Populus* has examined elemental fluxes of carbon (C) and nitrogen (N) to higher trophic levels and mechanisms of mass loss. Invertebrates assimilated more N from slowly decomposing *Populus angustifolia* litter compared with rapidly decomposing *Populus fremontii* litter (Compson et al. 2015), and insect emergence was higher on slowly decomposing *P. angustifolia* (Compson et al. 2013). In contrast, high decomposition rates of *P. fremontii* were correlated with higher microbial biomass and leaching of dissolved compounds (Pastor et al. 2014, Wymore et al. 2015). Although microbes living on litter can be an important food source for leaf-shredding insects, some compounds that retard microbial decomposition might increase element transfer to invertebrates (Compson et al. 2018). In addition to the *Populus* research, recent studies of detritus in temperate stream ecosystems also show higher invertebrate growth on slowly decomposing litter (Fuller et al. 2015, Halvorson et al. 2015), suggesting that decomposition rate alone may not be a good predictor of food quality for detritivores.

Here, we use isotopically labeled leaf litter to measure C and N fluxes from litter to an aquatic insect. This technique advances our understanding of detrital food webs by quantifying multiple pathways and fates of C and N bound in litter, to test how litter traits affect higher trophic levels. Our overarching hypothesis is that caddisflies gain a larger proportion of the C and N lost in slowly decomposing litter than in rapidly decomposing litter (Hypothesis 1). Additionally, we hypothesize that a larger proportion of the C and N bound in rapidly decomposing litter is either leached as dissolved organic matter (Hypothesis 2a) or consumed by microbes (Hypothesis 2b) compared with that in the slowly decomposing litter. We use two metrics to measure assimilation of litter by insects. The first metric, absolute assimilation, is the amount (mg) of C or N the insect derived from the litter. The second metric, relative assimilation, standardizes absolute assimilation by litter mass loss (Fig. 1). The second metric more directly tests hypothesis 1 as it only considers carbon and nitrogen that have already decomposed at a given point in the decomposition process.

These hypotheses challenge the prevailing view that rapidly decomposing litter is a high-quality resource and provides a framework for understanding how litter traits affect mass loss and pathways of element flow to structure detrital-based stream food webs. We maintain that litter that is more easily decomposed by microbes may not be a better food resource for higher trophic levels and argue for replacing concepts of high vs. low quality with a more comprehensive

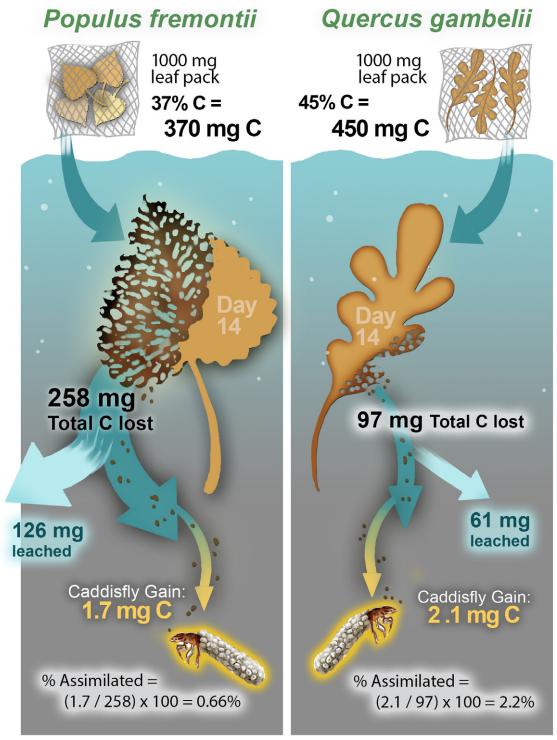

Fig. 1. A conceptual diagram demonstrating how we calculated relative assimilation rates for C for the most rapidly (*Populus fremontii*) and most slowly (*Quercus gambelii*) decomposing litters. C loss due to leaching was measured in 24-h laboratory incubations. We estimated the initial mass of C in the leaf pack using the % C of dry litter prior to field incubation. We calculated total C mass loss at day 14, from litter packs incubated in the river, by multiplying total mass loss (mg litter) by the proportion of C derived from the leaf (Eq. 3). Absolute C assimilation or the amount of C (mg) from the leaf incorporated into the insect was measured using an isotope mixing model (Eq. 2). Relative C assimilation was measured as the percent of total C loss that was assimilated by the insect (graphic by Victor Leshyk).

understanding of the rates and pathways of element flux from leaves through ecosystems and food webs.

Methods

Study Site

This study took place from 6 April 2014 to 20 April 2014 in Oak Creek, Arizona, USA (1800 m above sea level 35°0′12.55″ N, 111°44′ 8.06″ W). Oak Creek is a perennial headwater stream flowing off the southern edge of the Colorado Plateau, with an annual average flow of 368 L/s (LeRoy and Marks 2006). Riparian vegetation includes *Platanus wrightii*, *Quercus gambelii*, *P. fremontii*, *P. angustifolia*, *Fraxinus velutina*, *Alnus oblongifolia*, *Salix gooddingii*, and *Salix exigua*. We measured stream temperature, pH, conductivity, and salinity at the start, middle, and conclusion of the experiment using a Hydrolab minisonde (Hydrolab-Hach Corporation, Loveland, Colorado, USA) by taking five measurements across three transects (Table 1).

Leaf labeling

We used litter from four species common to riparian streams in the southwestern United States that differ in decomposition rates. Two of the species, *P. fremontii* and *F. velutina*, decompose relatively quickly, whereas the other two species, *Q. gambelii* and *P. wrightii*, decompose more slowly (LeRoy and Marks 2006, Table 2). We grew and labeled trees in the Northern Arizona University Research Greenhouse. We used 24 *P. fremontii* trees, grown from cuttings collected in 2008 from the Ogden Nature Center in Ogden, Utah, USA. Twelve small trees of the other species were purchased from a local garden shop. Trees were labeled from 9 July 2013 through 26 November 2013. To label with ^{13}C, trees were placed in airtight chambers (1.22 × 1.52 × 2.44 m) in which 0.27 L/m^3 99 atom percent $^{13}CO_2$ was added. Plants were

Table 1. Water physical and chemical variables (mean ± 1 standard error [SE], n = 45) measured in Oak Creek, AZ, USA.

Variables	Mean (±1 SE)
Temperature (°C)	13.94 (0.07)
pH	7.93 (0.02)
Specific conductivity (μS/cm)	286 (1.9)
Salinity (ppt)	0.14 (0.00)
Dissolved oxygen (% saturation)	102.38 (0.18)
Dissolved oxygen (mg/L)	8.88 (0.03)
Barometric pressure (mmHg)	664.10 (0.26)

Note: Measurements were taken using a Hydrolab minisonde (Hydrolab-Hach Corporation) at five points along three transects during three time points during the experiment.

Table 2. Initial and final litter chemistry, percent litter mass loss, and mass (mg) C and N leached for the four litter types used in this study.

Variables	*Populus fremontii*	*Fraxinus velutina*	*Platanus wrightii*	*Quercus gambelii*
Initial %C	37 (0.37)^{C}	44 (0.17)^{AB}	44 (0.15)^{B}	45 (0.59)^{A}
Initial %N	0.64 (0.04)^{AB}	0.56 (0.03)^{B}	0.39 (0.03)^{C}	0.75 (0.03)^{A}
Initial C:N	63 (5.1)^{C}	84 (5.8)^{B}	124 (11)^{A}	62 (2.5)^{C}
Final %C	43 (0.49)^{A}	43 (0.73)^{A}	35 (1.54)^{B}	39 (0.89)^{A}
Final %N	2.13 (0.07)^{A}	1.83 (0.03)^{B}	1.00 (0.03)^{D}	1.26 (0.03)^{C}
Final C:N	20 (0.57)^{C}	23 (0.58)^{B}	35 (1.52)^{A}	31 (1.09)^{A}
% mass loss	73 (1.6)^{A}	47 (2.2)^{B}	11 (2.4)^{C}	7.7 (1.5)^{C}
C leached (mg)	126 (12)	59 (7)	57 (0.8)	61 (1.8)
N leached (mg)	0.9 (0.09)	0.9 (0.07)	1.1 (0.09)	1.3 (0.03)

Notes: Litter was incubated for in Oak Creek, AZ, USA, for 14 d. Litter chemistry variables (%C, %N, C:N) were measured prior to incubation (mean ± 1 standard error [SE], n = 15) and at day 14 (mean ± 1 SE, n = 10). Masses of carbon and nitrogen leached were measured after leaching litter for 24 h in distilled water (mean ± 1 SE, n = 2). Differing letters indicate significant differences across litter types. The masses of C and N leached were not compared statistically due to low sample size (n = 2).

exposed to $^{13}CO_2$ for four hours twice per week. Trees were labeled with ^{15}N by top-watering using a Dosmatic Advantage A20–2.5% mixer-proportioner attached to a reservoir containing an aqueous mix of enriched ammonium sulfate ($(NH_4)_2SO_4$) and Peters Professional Water Soluble 20-20-20 (NPK) fertilizer with micronutrients (The Scotts Company, Marysville, Ohio, USA); this translated to 60 ppm fertilizer and approximately 13.2 mg of 98 atom percent ^{15}N ammonium sulfate to the soil of each tree twice per week. In November, the greenhouse temperature was lowered, promoting leaf senescence. Litter was collected daily during senescence.

Litter isotope and chemistry analysis

We analyzed $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C:N ratios of the initial litter (pre-incubation) and final litter (post-incubation). We ground initial litter (n = 15 leaves per litter type) to 425 μm in a Wiley Mill and weighed 4 ± 0.1 mg of each sample in 5 × 9 mm tin capsules (Costech Analytical Technologies, Valencia, California, USA). Samples were analyzed using a Carlo Erba NC 2100 Elemental Analyzer (CE Instruments, Milan, Italy) connected to a Thermo-Finnigan Delta Plus XL (Thermo-Electron, Bremen, Germany) isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory.

Litter chemistry was analyzed using pyrolysis–gas chromatography and mass spectrometry (py-GCMS) following Grandy et al. (2009) and Wickings et al. (2012). We analyzed 10 mg litter samples (n = 3 replicates per litter type) taken from seven leaves. Samples were pyrolyzed at 600°C for 20 s on a CDS Pyroprobe 5150 pyrolyzer (CDS Analytical, Oxford, Pennsylvania, USA), and volatiles were transferred to a Thermo Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Austin, Texas, USA) and then a Polaris Q mass spectrometer (Thermo Fisher Scientific). Peaks were analyzed using Automated Mass Spectral Deconvolution and Identification System (AMDIS, V 2.65, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) and the National Institute of Standards and Technology compound library. Abundances of each compound were compared relative to the total ion signal from all detected and identified peaks and are reported as percentages (Grandy et al. 2009, Wickings et al. 2012).

Leaching

We determined the mass of C and N lost to leaching by placing 0.5 g of each litter type in 200 mL of deionized water in acid-washed glass beakers (Wymore et al. 2015, 2018). After 24 h, leachate was filtered through pre-combusted glass fiber filters (Whatman GF/F) and frozen until analysis. We chose this time period because most leaching occurs within the first 24 h (Webster and Benfield 1986). We analyzed dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) using high-temperature catalytic oxidation on a Shimadzu TOC-VSCH (Shimadzu Instruments, Marlboro, Massachusetts, USA) with TNM-1 nitrogen unit. Due to limited quantities of litter, we only had two replicates per litter type and did not perform statistical analyses.

Field experiment

We created 10 replicate litter packs for each of the four species by placing 1.00 g of litter into fine mesh packs (mesh size 0.5 × 0.5 mm). We collected *Hesperophylax magnus* (Trichoptera: Limnephilidae) individuals, a leaf-shredding caddisfly that is abundant in Oak Creek (Blinn and Ruiter 2009). In the field, we determined the wet weight of the caddisflies in their cases, measured case dimensions, and selected caddisflies with a case length in the range of 23–27 mm. The average initial dry mass of all caddisflies used for the experiment was 49.1 ± 1.50 mg, which was calculated from a wet–dry mass regression using 30 individuals ($y = 0.1189x - 0.0286$, $R^2 = 0.72$).

We enclosed one caddisfly into each litter pack. The mesh size was small enough that the caddisfly could not escape and other invertebrates could not colonize the packs. We attached packs to rebar with cable ties and placed the rebar across the streambed, perpendicular to stream flow. After 14 d, we removed litter packs from the stream, sealed them in plastic bags, and returned them to the laboratory on ice.

We deployed a second set of litter packs, interspersed with the caddisfly litter packs, to estimate microbial biomass using the same mesh size (without caddisflies). Nine packs of each litter type were harvested on day 14. We used the same litter for *F. velutina*, *Q. gambelii*, and *P. wrightii*. We did not have sufficient litter for *P. fremontii*, so we used litter that was grown in the greenhouse in 2008 as part of another experiment (Compson et al. 2015). The *P. fremontii* litter grown in 2008 had a C:N ratio of 20, whereas the *P. fremontii* grown in 2013 had a C:N of 63.

Litter pack processing

In the laboratory, we separated caddisflies from the litter. The remaining litter was rinsed and dried at 60°C for 48 h and then weighed. We calculated decomposition rates using an exponential decay model (Benfield 2006). We extracted caddisflies from their cases before freezing them for 24 h. Frozen caddisflies were dried at 60°C and placed in a desiccator before measuring final dry mass. We ground caddisflies and weighed between 0.9 and 1.1 mg of tissue in 4 × 6 mm tin capsules for isotope analysis. We obtained natural abundance $\delta^{13}C$ and $\delta^{15}N$ values from unenriched caddisflies to be used as end-members for the isotope mixing model.

Microbial biomass—C and N

Microbial biomass was estimated using a chloroform fumigation-extraction technique (Brookes et al. 1985, Vance et al. 1987) with modifications for stream detritus described in Pastor et al. (2014). Litter was extracted with 50 mL of 0.05 mol/L K_2SO_4, stored on ice overnight, shaken for one hour, and centrifuged at 9800 *g* for 10 min, after which the supernatant was poured off and discarded. Remaining litter was fumigated in desiccators with alcohol-free chloroform, and desiccators were evacuated until chloroform boiled. Samples were vented three times, and then sealed under vacuum and kept in the dark for 24 h. Fumigated samples were removed from desiccators, extracted with 50 mL of 0.05 mol/L K_2SO_4 shaken for one hour, and centrifuged at 9800 *g* for 10 min. The supernatant was filtered through 1.2-μm filters (Supor Membrane; PALL Life Sciences, New York, USA) and placed in an oven (60°C) for 48 h. Dried K_2SO_4 salt with extracted C and N from microbial biomass was ground with a mortar and pestle to a fine powder, weighed, and analyzed for C and N elemental and isotopic composition.

Data analysis

We compared differences in initial and final litter %C, %N, C:N, litter mass remaining, and chemical classes across the litter types using one-way analysis of variance (ANOVA) tests and Tukey's honestly significant difference (HSD) tests when differences were significant. We used a non-metric multidimensional scaling (NMDS) ordination consisting of the most abundant 25 compounds found in all litter types to visually compare differences in litter chemistry across litter types (Wickings et al. 2012, Frey et al. 2014) and a multiple response permutation procedure (MRPP) to test for differences among groups.

We calculated instantaneous growth rates as:

$$\text{Instantaneous growth rate, \% per day} = \frac{\ln(\frac{M_f}{M_i}) \times 100}{14} \quad (1)$$

where M_f is the final mass of the caddisfly (mg) after the 14-d experiment, M_i is the initial

caddisfly mass (mg) at the beginning of the experiment, and 14 is the duration of the experiment in days.

We converted all isotope values from $\delta^{13}C$ and $\delta^{15}N$ to atom percent ^{13}C and ^{15}N (Fry 2006) and calculated absolute assimilation (A.A.) as the C and N mass (mg) assimilated in each caddisfly:

$$\text{A.A.} = \left(\frac{\text{Atom \% } X_{\text{al}} - \text{Atom \% } X_{\text{as}}}{\text{Atom \% } X_{\text{ll}} - \text{Atom \% } X_{\text{as}}}\right) \times (M_{\text{al}} \times P_X) \quad (2)$$

where X_{al} represents the labeled animal tissue of element X, X_{as} represents the unlabeled natural abundance of animal tissue, and X_{ll} represents the labeled litter; M_{al} is the final mass (mg) of the labeled caddisfly, and P_X is the proportion of element X in the labeled caddisfly. For the initial litter, we used the average atom percent (X_{ll}) of 15 leaf litter samples for each species. Eq. 2 uses a mixing model to measure the proportion of C or N that the insect acquired from the labeled leaves and then computes the total amount of C or N assimilated based on the mass of the caddisfly and the percent of C or N in its tissue.

We calculated relative assimilation for C and N by dividing absolute assimilation by total element loss for each pack (Fig. 1). We calculated the mass (mg) of C loss by subtracting the final mass C in the leaf pack–microbial matrix derived from the litter from the initial C in the pack. The initial mass of carbon was measured for each litter type based on the mass of the initial leaf pack multiplied by the proportion C for each litter type (n = 15). We were able to use the decrease in the isotope label during decomposition to estimate the amount of C in the pack that came from the leaf vs. the water column. We calculated the final mass C in the leaf pack (Eq. 3) using a mixing model to estimate the proportion of C in the pack derived from the initial litter (Appendix S1: Table S1) multiplied by the mass of C remaining in the pack.

$$\text{C mass remaining} = \left(\frac{\text{Atom \% } C_{\text{lf}} - \text{Atom \% } C_{\text{w}}}{\text{Atom \% } C_{\text{ls}} - \text{Atom \% } C_{\text{w}}}\right) \times (M_{\text{f}} \times P_C) \quad (3)$$

where C_{lf} represents the final C enrichment of the litter, C_{w} represents the natural abundance C isotope value of the stream water, and C_{ls} represents the initial C enrichment of the litter. The final mass of the litter is M_{f}, and P_C is the proportion of C in the leaf pack.

There was too much variance in the initial ^{15}N values used in the mixing model to measure mass loss of N using Eq. 3. Because mass gain of N from the water column was high, it was difficult to estimate changes in %N of the litter using the mixing model (Appendix S1: Table S1). Alternatively, we calculated mass N loss by multiplying total mass loss by the proportion of N in the initial litter.

We compared differences in caddisfly instantaneous growth rates, caddisfly C:N, C and N mass assimilated by caddisflies (absolute assimilation), percent litter C and N lost from litter and assimilated by caddisflies (relative assimilation), and microbial biomass C and N across litter types using one-way ANOVAs and Tukey's HSD when differences were significant. We natural-log transformed final litter %C, C:N ratios, microbial biomass C and N, and the percentages of litter C and N assimilated by caddisflies to meet assumptions of normality and equal variance. All analyses were conducted in R version 3.1.2 (R Core Team 2014), except for NMDS and MRPP analyses, which were conducted in PC ORD version 6.0 (McCune and Mefford 2011).

Results

Overview

Our results demonstrate significant differences in pathways of element fluxes across litter types, showing litter traits that accelerate microbial decomposition can also limit C and N assimilation by insects (Fig. 2). First, caddisflies assimilated a higher percentage of the C and N that was released during decomposition from slowly decomposing *Q. gambelii* and *P. wrightii* leaves relative to rapidly decomposing *P. fremontii* and *F. velutina*, partially supporting hypothesis 1 (Fig. 2e, f). In contrast, absolute assimilation of N was significantly higher for faster decomposing *P. fremontii,* suggesting that fast-decomposing litter provides a larger pulse of N to insects early in the decomposition process (Fig. 2d). Second, patterns in leaching were partially consistent with hypothesis 2a. Leaching of C was higher for *P. fremontii,* but there were no differences in N losses during leaching across plant species

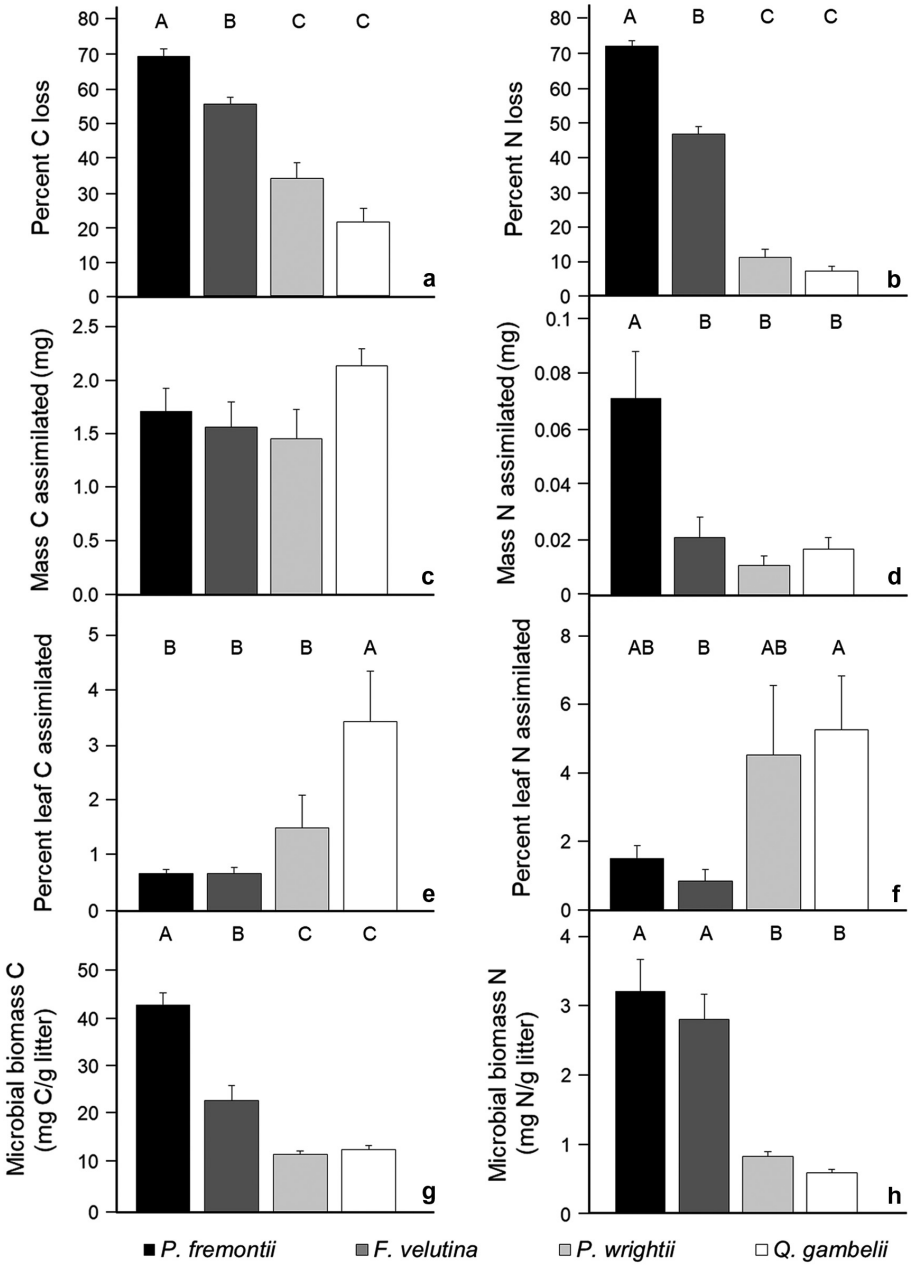

Fig. 2. Carbon and nitrogen mass loss from leaves, assimilation of carbon and nitrogen by caddisflies, and microbial biomass carbon and nitrogen across four leaf species incubated in Oak Creek, AZ, USA, for 14 d. Percent mass losses of carbon and nitrogen (panels a and b, mean + 1 standard error [SE], $n = 10$). Absolute assimilation (panels c and d, mean + 1 SE, $n = 10$) by caddisflies is the mass of carbon or nitrogen from leaves incorporated into individual caddisflies. Relative assimilation (panels e and f, mean + 1 SE, $n = 10$) standardizes absolute assimilation by total element loss. Microbial biomass carbon and nitrogen (panels f and g, mean + 1 SE, $n = 9$).

(Table 2). Third, microbial biomass C and N were significantly higher on rapidly decomposing *P. fremontii* and *F. velutina* relative to slowly decomposing *P. wrightii and Q. gambelii* (Fig. 2g, h—hypothesis 2b).

Litter chemistry

Populus fremontii litter differed in chemical composition from *F. velutina*, *Q. gambelii*, and *P. wrightii* litter (MRPP: $A = 0.388$, $P = 0.0001$; Fig. 3). *Populus fremontii* litter contained the lowest relative abundance of lignin, whereas the other three litter types did not differ in relative abundance of lignin ($F_{3,8} = 19.5$, $P = 0.0005$; Table 3). *Populus fremontii* litter also contained a higher relative abundance of phenols than the other litter types ($F_{3,8} = 30.5$, $P = 0.0001$; Table 3). *Quercus gambelii* litter contained a significantly lower relative abundance of lipids than all other litter types ($F_{3,8} = 8.75$, $P = 0.0066$; Table 3). There were no differences in the relative abundances of aromatics ($F_{3,8} = 0.50$, $P = 0.70$, Table 3), polysaccharides ($F_{3,8} = 0.49$, $P = 0.70$), N-bearing compounds ($F_{3,8} = 1.31$, $P = 0.16$), or proteins ($F_{3,8} = 0.10$, $P = 0.96$) among litter types.

Leaf litter mass loss

As expected, *P. fremontii* litter decomposed the fastest (73% mass loss; Table 2). *Fraxinus velutina* also decomposed rapidly, with 47% mass loss (Table 2). Total mass loss for *Q. gambelii* (7.7% mass loss) and *P. wrightii* (11% mass loss) were lower than the other two litter types ($F_{3,35} = 243$, $P < 0.0001$; Table 2). Mass losses of C followed decomposition patterns and were considerably higher for *P. fremontii and F. velutina* (Fig. 2a). Mass loss N was higher for *P. fremontii* and *F. velutina* relative to the two slowly decomposing species (Fig. 2b). Leaching of C over 24 h was also higher for *P. fremontii* than the other three species (Table 2). Mass N leached over 24 h was similar across litter types. Nitrogen loss due to leaching was similar to total N loss for *Q. gambelii* and *P. wrightii*. Elemental loss due to leaching is likely overestimated as litter was leached in distilled water where osmotic pressure is higher than in stream water. In the two slowly decomposing litter types, N loss was low and variable preventing us from differentiating between losses due to leaching and overall decomposition (Table 2). Additionally, estimating N loss in the river is compounded by N gain into the litter packs from microbial immobilization. The proportion of C in the litter packs derived from the water column on day 14 was low, ranged from 0.03 to 0.12, and was highest for *F. velutina* (Appendix S1: Table S1). In contrast, the proportion of N in the litter packs derived from the water column on day 14 ranged from 0.23 to 0.47 and was highest for *P. wrightii* (Appendix S1: Table S1).

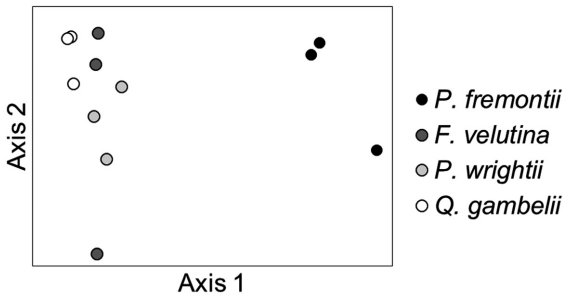

Fig. 3. A non-metric dimensional scaling ordination of the 25 chemical compounds with the highest relative abundances found in the four litter species used in this study. Data were generated using pyrolysis–gas chromatography and mass spectrometry analysis. *Populus fremontii* litter was significantly different from the other three litter species (multiple response permutation procedure; $A = 0.388$, $P = 0.0001$).

Shredder element assimilation and growth rates

The total mass C assimilated by caddisflies from litter did not differ among litter types ($F_{3,35} = 0.96$, $P = 0.17$; Fig. 2c), but caddisflies feeding on *P. fremontii* litter assimilated a higher mass N than all other litter types ($F_{3,35} = 7.57$, $P = 0.0005$; Fig. 2d). As hypothesized, when the C and N mass assimilated was standardized to litter mass loss (Fig. 1), both C ($F_{3,34} = 16.6$, $P < 0.0001$; Fig. 2e) and N ($F_{3,34} = 4.93$, $P = 0.006$; Fig. 2f) assimilation was higher from slowly decomposing *Q. gambelii* and *P. wrightii* litter compared with more rapidly decomposing *P. fremontii* and *F. velutina*. Specifically, caddisflies assimilated five times more litter C lost in decomposition from *Q. gambelii* than *P. fremontii*. Similarly, caddisflies assimilated six times

Table 3. The relative abundances of aromatics, lignin, lipids, polysaccharides, N-bearing, proteins, phenols, and unknown origin obtained through pyrolysis–gas chromatography and mass spectrometry of dried litter from the four litter types (mean ± 1 standard error, $n = 3$).

Variable	*Populus fremontii*	*Fraxinus velutina*	*Platanus wrightii*	*Quercus gambelii*
% Aromatics	6.84 (0.97)^{A}	6.06 (1.82)^{A}	5.55 (1.33)^{A}	4.79 (0.11)^{A}
% Lignin	15.1 (2.95)^{B}	39.1 (4.97)^{A}	33.1 (1.13)^{A}	45.8 (0.95)^{A}
% Lipids	13.6 (6.02)^{A}	13.9 (3.87)^{A}	8.60 (1.26)^{A}	2.50 (0.13)^{B}
% Polysaccharides	23.5 (1.23)^{A}	20.7 (0.63)^{A}	22.7 (3.64)^{A}	20.9 (0.58)^{A}
% N-Bearing	3.41 (1.35)^{A}	6.39 (1.66)^{A}	5.81 (1.28)^{A}	3.93 (0.34)^{A}
% Proteins	1.21 (0.69)^{A}	1.63 (0.76)^{A}	1.86 (1.30)^{A}	1.41 (0.18)^{A}
% Phenols	12.68 (2.56)^{B}	1.05 (0.29)^{B}	1.21 (0.35)^{B}	0.51 (0.09)^{B}
% Unknown origin	23.6 (1.56)^{A}	11.2 (2.52)^{B}	21.2(0.98)^{A}	20.2 (1.46)^{B}

Note: Differing letters indicate significant differences across litter types.

more litter N lost in decomposition from *Q. gambelii* than *F. velutina*. There were no differences in caddisfly growth rates across litter types ($F_{3,35} = 0.96$, $P = 0.42$; Appendix S1: Table S2) nor were there correlations among caddisfly masses and mass assimilated of either element (Appendix S1: Fig. S1). Growth rates overall were low (0.35 ± 0.26% per day, $n = 39$; Appendix S1: Table S2) likely due to the short duration of the experiment and the inherent variation in estimating initial dry weights of caddisflies from wet weights of caddisflies in their cases. We used this method, however, to minimize stress on caddisflies. Mortality was low (one caddisfly), suggesting that caddisflies tolerated the experimental conditions. Caddisfly stoichiometry did not differ among litter types, suggesting that observed differences in assimilation were not related to changes in body C:N ($F_{3,35} = 1.26$, $P = 0.30$; Appendix S1: Fig. S2).

Microbial biomass C and N

As hypothesized, microbial biomass was higher on the two rapidly decomposing litter types, *P. fremontii* and *F. velutina*, compared with slowly decomposing *Q. gambelii* and *P. wrightii* (Fig. 2g, h). Microbial biomass C was approximately two to three times higher on *P. fremontii* and *F. velutina* than *P. wrightii* and *Q. gambelii* ($F_{3,32} = 27.8$, $P < 0.0001$; Fig. 2g). Microbial biomass N was three to five times higher on *P. fremontii* and *F. velutina* than on *P. wrightii* and *Q. gambelii* ($F_{3,32} = 35.4$, $P < 0.0001$; Fig. 2h). C:N ratios of microbes did not differ across plant species.

Phytochemistry and correlations among element assimilation rates

Initial C:N ratios of litter were poor predictors of C and N assimilation by caddisflies. C:N ratios of litter at day 14 were significantly lower than initial C:N ratios for all species, reflecting microbial assimilation of N from the water column and respiratory loss of C. Final litter C:N ratios were lower for *P. fremontii* and *F. velutina* than for *Q. gambelii* and *P. wrightii* (Table 2).

Discussion

This study challenges the commonly held view that slowly decomposing litter is "poor quality" by demonstrating that slowly decomposing litter disproportionately promotes C and N transfer to higher trophic levels, whereas rapidly decomposing litter supports the microbial pathway. This research advances our understanding of detrital food webs by demonstrating that (1) litter mass loss alone does not reflect element fluxes in aquatic food webs, (2) microbes and insects have opposite patterns of element assimilation, and (3) litter traits that have similar effects on microbial decomposition can have contrasting effects on element fluxes to insects. We advocate moving away from characterizing litter as low or high quality based on rates of decomposition and argue for conceptual models that focus on how litter traits determine the magnitude of element fluxes to different pools in detrital food webs.

Caddisflies assimilated a higher percentage of the C and N bound in slowly decomposing litter types (*P. wrightii* and *Q. gambelii*) than from rapidly decomposing litter types (*F. velutina* and

P. fremontii). This result runs counter to findings, often from laboratory studies, that aquatic invertebrates grow more quickly on rapidly decomposing litter and perform better on litter conditioned by microbes (Mackay and Kalff 1973, Golladay et al. 1983, Graça et al. 2001, but see Fuller et al. 2015 and Halvorson et al. 2015). We offer three mechanisms for the apparent contradictions between our results and studies that suggest rapidly decomposing leaf litter is of higher quality than slowly decomposing litter.

(1) Rapidly decomposing litter has high leaching rates, resulting in compounds largely unavailable to invertebrate shredders. Dissolved organic carbon leached from litter can be respired by microbes within 24 h, with little energy transfer to the macroscopic food chain (Cummins et al. 1972, Kaplan and Bott 1983, Meyer 1994, Wymore et al. 2015). In contrast, in slowly decomposing litter, more C and N are bound in complex compounds that are not water-soluble (Rahman et al. 2013). In cases where DOC and microbes aggregate to form particulates, dissolved compounds may be an important food source for filter feeders, although not for shredders (Petersen and Cummins 1974). Leaching of C in deionized water was higher for *P. fremontii* than for the other three litter types. *Fraxinus velutina* litter, however, leached the same amount as *Q. gambelii* and *P. wrightii,* indicating that leaching alone does not explain the observed differences in elemental assimilation. In contrast to C, loss of N due to leaching was similar across species. This might explain why absolute N assimilation was higher on *P. fremontii* leaves, which leached relatively lower amounts of N than C. Our technique did not allow us to differentiate N loss to leaching vs. total N loss for the two slowly decomposing litter types because total N losses were relatively low compared with estimated leaching losses in distilled water.

(2) Litter that is more rapidly decomposed by microbes is less available to aquatic invertebrates. In our study, microbial biomass was higher on rapidly decomposing litter, suggesting more C may be lost to microbial respiration in these litter types. Many invertebrate growth and assimilation studies were conducted in the laboratory, where litter was not a limiting resource (Cummins et al. 1973, Iversen 1974, Golladay et al. 1983, Graça et al. 2001). In the field, however, litter can be a limiting resource (Wallace et al. 1997, Eggert and Wallace 2003, Wallace et al. 2015) and both litter stoichiometry and quantity affect invertebrate growth rates (Halvorson et al. 2017). We did not test whether litter was limiting, but at the end of the experiment, *P. fremontii* had 113 $\pm$ 7.5 mg (mean $\pm$ 1 standard error [SE]) of carbon remaining. Carbon lost to microbial respiration is unavailable to invertebrates regardless of its nutritional quality. While we measured microbial biomass at a single time point providing only a snapshot of microbial activity during decomposition, results from a similar long-term study with three sampling dates showed consistently higher microbial biomass on rapidly decomposing litter (Pastor et al. 2014), suggesting that the patterns we observed would persist throughout decomposition.

(3) Microbes simultaneously facilitate and compete with detritivorous invertebrates. Microbes improve the nutritional quality of litter directly by serving as a food source and indirectly by breaking down complex organic compounds (Triska 1970, Mackay and Kalff 1973, Suberkropp 1992). Our results, however, suggest that microbes may also compete with invertebrates for detrital resources. Simultaneously, mutualistic and competitive relationships between microbes and plants have been documented in soils (Harte and Kinzig 1993, Kinzig and Harte 1998) and may also be prevalent between microbes and invertebrates in streams (Bärlocher 1980). Microbes enhance food quality for invertebrates but also may be responsible for significant C and N loss from the food web through a microbial loop (Meyer 1994), and the strength of competitive interactions may be stronger in some litter types than others. Although we are unable to differentiate if labeled C and N in insects came directly from litter or from microbes that assimilated the C and N, this approach, which accounts for element losses and gains, can help elucidate how litter traits affect the repackaging of detrital C and N to compare the relative fluxes of elemental loss to the microbial vs. invertebrate pathways (sensu Evans-White and Halvorson 2017).

Our results suggest that rapidly decomposing litter can provide a pulse of nutrients soon after litter fall but that slowly decomposing litter types

provide more nutrients to insects over the course of decomposition. Comparing absolute vs. relative assimilation allows for an estimate of the temporal pulse of nutrient acquisition (absolute assimilation) vs. the overall contribution (relative assimilation) of C and N in litter to insects. During this experiment, which focused on the early stages of decomposition, caddisflies assimilated roughly the same amount of C from all litter types, perhaps because invertebrates can alter assimilation efficiencies depending on the stoichiometry of the food resource (Fuller et al. 2015, Halvorson et al. 2016, Santonja et al. 2018). However, caddisflies assimilated a much larger proportion of the C that was broken down during this time period from slowly decomposing *Q. gambelii* and *P. wrightii*. If we extrapolate these results to several months of decomposition, insects could continue to assimilate C and N from slowly decomposing *P. wrightii* and *Q. gambelii* after the more rapidly decomposing species no longer remain in the river. Because slowly decomposing litter can persist in rivers into the spring and summer months when many insects emerge, it may be a particularly important resource. Longer experiments (3–5 weeks) have shown similar trends, indicating that these results are not specific to early stages of decomposition (Compson et al. 2018; Siders et al., *in prep*).

The higher absolute assimilation of N by caddisflies feeding on *P. fremontii* indicates that this litter provides a pulse of N to invertebrates during the early stages of decomposition and might be explained by the higher microbial biomass on *P. fremontii*. Invertebrates prefer litter colonized by microbes (Golladay et al. 1983, Arsuffi and Suberkropp 1984, Graça et al. 2001), and *P. fremontii* also had the highest final percent N. The contrasting patterns between C and N assimilation could be because relatively more C is leached than N and that in remaining litter (post-leaching) less N is bound to complex compounds.

Litter traits that predictably affect decomposition rates may have contrasting effects on element assimilation by insects. These results are consistent with patterns seen among genetically distinct *Populus* leaves, where lignin concentrations were positively correlated with C and N assimilation, but tannin concentrations were negatively correlated (Compson et al. 2018). Although both aromatics and lignin slow decomposition (Gessner and Chauvet 1994, Almendros et al. 2000, Driebe and Whitham 2000), they had opposite effects on assimilation of C and N, suggesting that specific compounds influence assimilation (Fuller et al. 2015). Litter stoichiometry (C:N) can drive litter breakdown rates (Enríquez et al. 1993, Ostrofsky 1997, Hladyz et al. 2009) and the perception of litter quality (Cross et al. 2005, Hladyz et al. 2009, García-Palacios et al. 2016). Across these four litter types, initial C:N ratios were not good predictors of decomposition rate or C and N assimilation by invertebrates, further suggesting that specific types of carbon compounds are an important component of element cycling (Bray et al. 2012, Fuller et al. 2015, Haddix et al. 2016, Evans-White and Halvorson 2017).

By using stable isotope tracers to study element flow, we have demonstrated the importance of understanding both decomposition rates and pathways of energy flow from detritus through stream food webs. Although aquatic ecosystems are ideal for testing general concepts about detrital food webs because decomposition rates are high and invertebrates are abundant, this approach is equally applicable to terrestrial food webs. Future studies using labeled litter across a broader range of litter types, invertebrate decomposers, and environmental conditions and ecosystems will enable ecologists to develop a more comprehensive framework for understanding how detrital inputs flow through food webs and ecosystems.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2340/full