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The Influence of Leaf Type on Carbon and Nitrogen Assimilation by Aquatic Invertebrate Communities: A New Perspective on Trophic Efficiency

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Abstract

Despite abounding evidence that leaf litter traits can predict decomposition rate, the way these traits influence trophic efficiency and element transfer to higher trophic levels is not resolved. Here, we used litter labeled with ^{13}C and ^{15}N stable isotopes to trace fluxes of litter C and N from four leaf types to freshwater invertebrate communities. We measured absolute (mg C or N) and relative assimilation (percentage of litter C or N incorporated into invertebrate biomass relative to C and N lost during decomposition). Four patterns emerged: (1) Invertebrate communities assimilated more C and N from slowly decomposing litter than communities feeding on rapidly decomposing litter; (2) absolute assimilation of both C and N in leaf packs was positively correlated with the relative biomass of invertebrate taxa in leaf packs; (3) Chironomidae larvae, which colonize packs in the early decomposition stages, assimilated the most C and N by the end of the 35-day experiment; and (4) most taxa, spanning five functional feeding groups (collector–gatherers, shredders, collector–filterers, scrapers, and predators), showed similar patterns in both absolute and relative assimilation across leaf types. These results challenge traditional views of litter quality by demonstrating that trophic efficiency is negatively associated with decomposition rate across these four leaf types.

Key words: Carbon; Nitrogen; Stable isotopes; Leaf litter; Decomposition; Invertebrates; Assimilation; Trophic efficiency.

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Highlights

- We traced elements from litter to invertebrates with labeled leaves (^{13}C and ^{15}N).

- Element assimilation was higher on slowly decomposing litter types.
- Assimilation of litter C and N was consistent across diverse invertebrate taxa.

Introduction

Trophic efficiency is the fraction of production of a trophic level that is converted into new production in the next trophic level, and can be partitioned into ingestion, assimilation, respiration, and production (Lindeman 1942). Trophic efficiency varies depending on resource quantity, quality, and metabolic properties, as well as the population size of consumers (Humphreys 1979; Dickman and others 2008; Marcarelli and others 2011; Halvorson and others 2017; Vadeboncoeur and Power 2017). As headwater streams are largely fueled by leaf litter inputs, understanding how litter traits affect trophic efficiency is central to understanding conditions that maintain macroscopic food webs in these ecosystems.

Litter type is an important determinant of decomposition rate (Cornwell and others 2008; Makkonen and others 2012) and likely is a driving factor in trophic efficiency (Evans-White and Halvorson 2017). Rapidly decomposing litter often has high concentrations of nitrogen (N) and phosphorus (P) and low concentrations of compounds such as condensed tannins, lignin, and phenols, which inhibit litter breakdown (Triska and Sedell 1976; Webster and Benfield 1986; LeRoy and others 2007). In contrast, slowly decomposing litter tends to have high concentrations of complex carbohydrates and defensive compounds that inhibit breakdown by microbes (Gessner and Chauvet 1994; Ostrofsky 1997; Driebe and Whitham 2000; LeRoy and others 2007). Decomposition rate alone, however, does not capture pathways of element flow and may be a poor proxy for trophic efficiency (Marks 2019). Litter traits that accelerate microbial decomposition may have mixed effects on element transfer to higher trophic levels. In detrital food webs, microbes play complex ecological roles, both facilitating and inhibiting organic matter transfer to invertebrates. Microbes can increase ingestion and assimilation by ''conditioning'' detritus and functioning as prey (Webster and Benfield 1986; Suberkropp 1992; Steffan and others 2015; Steffan and others 2017) but can also decrease total ingestion by competing with invertebrates for detrital resources through mineralization mass loss (Barlöcher 1980; Kinzig and Harte 1998). Differences in the relative importance of these pathways and processes (facilitation, prey, competition) across leaf types could be manifested as differences in elemental transfer from leaves to invertebrates.

Stream ecologists have taken multiple approaches to understanding how litter quality affects macroinvertebrates, including many feeding studies measuring the effect of leaf type on assimilation and growth of invertebrates (Golladay and others 1983; Perry and others 1987; Graça and others 2001; Halvorson and others 2015), field studies comparing breakdown rates and colonization of macroinvertebrates in leaf packs with different litter types (Hladyz and others 2009), and field studies comparing invertebrates in watersheds with different riparian species (Gonçalves and others 2006; LeRoy and Marks 2006). Collectively, these studies have yielded mixed results (Graça 2001), showing that rapidly decomposing litter types are better for invertebrate growth (Golladay and others 1983; Canhoto and Graça 1995; Motomori and others 2001), slowly decomposing litter types are better for invertebrate growth and emergence (Fuller and others 2015; Halvorson and others 2015; Kominoski and others 2012; Compson and others 2013; Compson and others 2016), or litter type had no effect on invertebrate growth (Alonso and others 2010; Fugère and others 2012; Compson and others 2015; Fogelman and others 2018). Despite decades of studying leaf litter utilization in streams, we still cannot predict how differences in litter type affect macroscopic food webs.

To resolve these mixed results in understanding how litter type affects macroscopic food webs, we used leaf litter labeled with ^{13}C and ^{15}N stable isotopes to measure transfer of C and N from detritus to macroinvertebrates to test how litter type and decomposition rates are related to the C and N assimilated by invertebrate communities. Stable isotopes offer a sensitive and direct measure of element assimilation, transforming our ability to compare C and N assimilation of detritus by invertebrates across litter types (Compson and others 2015; Compson and others 2018; Siders and others 2018). We used a factorial design that crossed litter type (four plant species) with three colonization treatments designed to capture invertebrates at different stages of colonization. Colonization treatments included large mesh litter packs that were placed in small mesh bags, functioning as ''cages,'' after day 14 and day 28, and uncaged large mesh controls allowing for immigration and emigration. All treatments were harvested after 35 days. Given that invertebrate communities change over time, treatments were designed to capture invertebrate assemblages at

different time points to establish whether time at which invertebrates colonize leaf packs influences C and N assimilation. Two of the plant species used here decompose rapidly (*Fraxinus velutina* and *Populus fremontii*) and two decompose slowly (*Quercus gambelii* and *Platanus wrightii*; LeRoy and Marks 2006; Siders and others 2018). We measured absolute assimilation as the total mass of C or N assimilated by insect communities and relative assimilation, which standardizes absolute assimilation by the mass C or N lost from the litter. Because relative assimilation accounts for litter mass remaining in the leaf pack, it provides a more direct measure of the fate of C and N lost during decomposition and is a proxy for trophic efficiency for individual elements (Siders and others 2018).

We predicted: (1) invertebrate communities feeding on recalcitrant litter would have higher assimilation of C and N than those feeding on labile litter types. This is consistent with patterns observed with a large shredding caddisfly feeding on these four litter types (Siders and others 2018) as well as invertebrates feeding on different cross types and genotypes of *Populus* litter (Compson and others 2015, 2018). (2) Invertebrates colonizing litter packs in the early stages of decomposition would assimilate more C and N compared to later colonizers, because they feed on litter over a longer time period. (3) The proportion of the absolute assimilation of C and N for each taxon of the summed total absolute assimilation for all taxa in a leaf pack would be positively correlated with their relative biomass in the litter pack. This prediction reflects the null hypothesis that the biomass of individual taxa is related to their functional roles in assimilating leaf litter. Alternatively, if some taxa have higher assimilation irrespective of their biomass, such as through increased assimilation efficiency, then we would not see a relationship between assimilation and biomass. Results from this experiment will expand our understanding of detrital food web dynamics by coupling measurements of mass loss with C and N assimilation by invertebrates. This approach integrates ecosystem and community ecology by demonstrating how biogeochemical cycling of C and N, especially regarding their fate within aquatic ecosystems, depends on basal resource traits and the associated invertebrate community composition.

Methods

Study Site

This study took place in Oak Creek, Arizona, USA (1800 m asl, 35° 0′ 12.55″ N, 111° 44′ 8.06″ W), between April 6 and May 11, 2014. Oak Creek is a perennial headwater stream with a mean annual discharge of 368 l/s (LeRoy and Marks 2006). Cobbles are the dominant substrate in Oak Creek, and the riparian vegetation is dominated by the four species used in this study as well as by *Alnus oblongifolia*, *Salix gooddingii*, and *Salix exigua*. Mean (± 1 SE) stream physical and chemical variables are presented in Supplemental Table 1. Mean water temperature during the study was 13.9 (± 0.07) °C, pH was 7.93 (± 0.02), specific conductivity was 286 (± 1.9) µS/cm, and dissolved oxygen concentration was 8.88 (± 0.03) mg/l (Supplemental Table 1). Nutrient concentrations in Oak Creek are low and Pastor and others (2014) present mean (± 1 SE) NH_4–N, NO_3–N, and DOC–C concentrations (mg/l) as 0.05 (± 0.00), 0.06 (± 0.00) and 0.52 (± 0.03), respectively, in the same stream reach during another study.

Leaf labeling

Leaf labeling followed methods described in Siders and others (2018). We grew and labeled *F. velutina*, *Q. gambelii*, and *P. wrightii* trees in 19-liter pots, and *P. fremontii* in 4-liter pots at the Northern Arizona University Research Greenhouse. *Populus fremontii* trees were grown in 4-liter pots due to their smaller size and the need for more trees of this species to have an adequate amount of litter. During C labeling, trees were sealed in two 1.22 × 1.52 × 2.44 m^3 airtight, steel-framed acrylic growth chambers and exposed to 0.27 l/m^3 99 atom% $^{13}CO_2$ twice weekly for 4 h. Nitrogen labeling was conducted by watering pots with approximately 13.2 mg of 98 atom% ^{15}N aqueous $(NH_4)_2SO_4$ twice per week. We watered trees twice per week to capacity immediately before and after N labeling to help evenly disperse ^{15}N ammonium sulfate throughout the pots. All leaves were removed prior to the start of labeling, and we began labeling before new leaves emerged to ensure leaves were uniformly labeled. Trees were labeled from July 9, 2013, through November 26, 2013. The greenhouse was subsequently cooled, and litter was harvested after natural senescence. Chemical variables measured on the initial dry litter are published in Siders and others (2018) and summarized in Table 1.

Table 1. Mean (± 1 SE) Leaf Litter %C, %N, C:N Ratios, and Mass Loss Across Litter Species.

Leaf type	Initial %C	Initial %N	Initial C:N	Final %C	Final %N	Final C:N	% Mass loss
P. fremontii	37 (0.37)^{C}	0.64 (0.04)^{AB}	63 (5.1)^{C}	28 (1.86)^{B}	2.14 (0.10)^{A}	13 (0.5)^{D}	77 (1.2)^{C}
F. velutina	44 (0.17)^{AB}	0.56 (0.03)^{B}	84 (5.8)^{B}	35 (1.36)^{A}	2.24 (0.03)^{A}	15 (0.7)^{C}	62 (1.2)^{B}
P. wrightii	44 (0.15)^{B}	0.39 (0.03)^{C}	124 (11)^{A}	34 (0.88)^{A}	1.51 (0.04)^{C}	22 (0.9)^{A}	18 (1.7)^{A}
Q. gambelii	45 (0.59)^{A}	0.75 (0.03)^{A}	62 (2.5)^{C}	33 (0.86)^{AB}	1.78 (0.05)^{B}	18 (0.3)^{B}	13 (1.2)^{A}

Initial litter was dried prior to incubation (n = 15). Final litter mass and chemistry loss are calculated from litter incubated in Oak Creek, AZ for 35 days across the uncaged treatments (n = 10) and across the three colonization treatments (n = 30), respectively. Letters indicate significant differences across leaf types from one-way ANOVAs.

Field Experiment

We created 30 litter packs for each of the four litter types by placing 2 g (± 0.01) of litter in 20 × 20 cm litter packs using 4 × 10 mm Vexar mesh. This mesh size allowed most aquatic invertebrates to colonize the litter, but excluded fish. We used a randomized block design and placed leaf packs in shallow riffle–run habitats over a relatively homogenous study reach, approximately 100 m in length and averaging 23.9 ± 1.3 (mean ± 1 SE) cm depth. Blocks were placed several meters apart from one another, which likely precluded particulate organic matter from settling on downstream leaf packs. This study included three ''cage'' treatments (n = 10 leaf packs per leaf type per treatment) to determine how distinct invertebrate communities, colonizing after 14, 28, and 35 days, assimilated litter C and N. Our goal was to isolate two groups of invertebrates: (1) invertebrates that colonize packs during the first two weeks of decomposition (day 14) and (2) invertebrate communities that develop in packs over 28 days. We placed fine mesh enclosures (mesh size < 0.5 mm) over one-third of the litter packs on days 14 (Treatment 1) and 28 (Treatment 2). These ''cage'' treatments prevented invertebrates from immigrating into or emigrating from leaf packs, but this exclusion and the mesh size did not alter decomposition rates. We also had a set of litter packs that were never caged so that invertebrates could move into or out of the packs throughout the duration of the experiment (Treatment 3). We anticipated that the packs caged at day 28 and the uncaged packs would have more similar invertebrate assemblages compared to the packs caged at day 14. All packs were harvested on day 35. This design served two purposes: (1) It captured invertebrate assemblages at different stages of development, but prevented changes due to colonization or emigration, thus capturing community assimilation rates at distinct time points and (2) it allowed us to test whether the amount of isotopic label incorporated into invertebrates was a function of the time in which invertebrates were confined to litter packs. This second objective is important for developing protocols using labeled leaves to study macroinvertebrate communities in field settings, as this is a relatively new technique only used in one other community-level study (Compson and others 2015).

Leaf Pack Processing

All litter packs were removed from the stream after 35 days, sealed in plastic bags, placed on ice, and returned to the laboratory. We rinsed leaf litter of sediment and invertebrates with deionized water and oven-dried litter at 60°C. We obtained final dry mass using an AG135 analytical balance (Mettler Toledo, Greifensee, Switzerland) and present decomposition as percent litter mass loss. Elemental analysis (%C, %N, ^{13}C, ^{15}N) of the harvested litter and insect tissue was carried out using a Carlo Erba NC 2100 elemental analyzer (CE Instruments, Milan, Italy) with a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo-Electron Corp., Bremen, Germany). All invertebrates that were retained in a 250-µm sieve were placed in 120-ml specimen containers with 80 ml of deionized water and frozen. Samples were thawed and split to one-fourth to count and measure small, common taxa. Prior to sample splitting, invertebrate samples were placed into a tray and larger individuals from less common taxa (that is, Trichoptera, Odonata, and Lepidoptera) were retained so that they would not be missed during sample splitting. All invertebrates were identified to genus except for two taxa, Chironomidae and Simuliidae, which were identified to family. We recorded lengths and abundances of all insects to calculate biomass using published length–mass regressions (Benke and others 1999) and created our own regressions for *Atopsyche* sp. and *Oplonaeshna* sp. and dried invertebrate samples in an oven at 60°C.

Community and Stable Isotope Analysis

We focused on the representative invertebrate community consisting of taxa that were found in at least 15% of all packs and prepared one isotope sample per taxon per pack. Rare taxa that were not included in our analyses typically accounted for less than 1 mg or about 2–3% of the total invertebrate biomass. Stable isotope sample preparation followed Compson and others (2015). Invertebrate tissue was ground and weighed in 4 × 6 mm tin capsules (Costech Technologies, Inc., Montreal, QC). When invertebrate dry mass was below 0.6 mg, we added acetanilide standard (Fisher Scientific) so that the total mass of the insect plus acetanilide sample was 1.0 ± 0.1 mg. This was done so total N in samples was at the ideal concentration for detection by the mass spectrometer. For samples to which we added acetanilide, we calculated atom % ^{13}C and ^{15}N using equation (1).

$$\mathrm{Atom\%}X_{\mathrm{al}} = \frac{(\mathrm{Atom\%}X_{\mathrm{mix}} * M_{\mathrm{mix}}) - (\mathrm{Atom\%}X_{\mathrm{acet}} * M_{\mathrm{acet}})}{M_{\mathrm{al}}} \quad (1)$$

where atom % of element X of the labeled animal tissue is X_{al}, and the mass (mg) of the labeled animal tissue is M_{al}. Atom % of element X of the mixture of labeled animal tissue plus acetanilide is represented by X_{mix}, and the mass (mg) of this mixture is M_{mix}. Atom % of element X of the acetanilide is X_{acet} and the mass (mg) of the acetanilide is M_{acet}.

The mass (mg) of element X assimilated (absolute assimilation; A.A.) by each invertebrate taxon on each pack is calculated as,

$$\mathrm{A.A.} = \left(\frac{(\mathrm{Atom\%}X_{\mathrm{al}} - \mathrm{Atom\%}X_{\mathrm{as}})}{(\mathrm{Atom\%}X_{\mathrm{ll}} - \mathrm{Atom\%}X_{\mathrm{as}})}\right) * \left(M_{\mathrm{al}} * \left(\frac{\%X_{\mathrm{al}}}{100}\right)\right) \quad (2)$$

where X_{al} is the labeled animal tissue of element X, X_{as} is the natural abundance animal tissue, and X_{ll} is the labeled litter. M_{al} is the total mass (mg) of the labeled animals. We used the average atom % of 15 individual leaves of each species not placed in the stream for X_{ll}. We collected three invertebrate samples for each taxon upstream of the study reach to estimate natural abundance isotopes. For all taxa, we found invertebrates in the litter packs with lower isotopic values than those collected for natural abundance. Therefore, we used the lowest isotopic values observed for each taxon in the study as natural abundance. This probably occurred because we had a substantially larger sample size of invertebrates that we measured in the litter packs (typically $n > 50$ for most taxa) than those sampled for natural abundance upstream of the study reach ($n = 3$ per taxon).

We determined the mass (mg) C and N lost from the litter using equation (3),

$$M_{Xl} = 2000 * P_{Xs} * PM_l \quad (3)$$

where M_{Xl} is the mass of element X that was lost during decomposition. Two thousand mg was the initial mass of each litter pack. The initial proportion of element X was represented by P_{Xs}, and PM_l was the proportion of the total leaf mass lost at the end of the 35-day experiment. This method may overestimate N loss because N is imported into the detrital matrix by microbes (Cheever and others 2013; Pastor and others 2014). We do not believe this affects our comparisons across leaf types because another study in the same stream found that the percent N in litter packs acquired by microbes did not differ across leaf types (Pastor and others 2014). Finally, we added the masses of element X assimilated (A.A.; equation 2) for all taxa found on a given pack and divided this by the mass of element X lost during decomposition (M_{Xl}; equation 3) and multiplied this by 100 to calculate relative assimilation, or the percentage of C and N lost from the litter that was assimilated by the invertebrate community in each pack. Relative assimilation takes into account that litter is a finite resource and rapidly decomposing litter may not be available for consumers throughout their entire larval stage.

Data Analysis

We used one-way analysis of variance (ANOVA) to compare differences in initial and final %C, %N, and C:N across litter types and to compare absolute assimilation of C and N of individual insect taxa among leaf types for the uncaged treatment. We used two-way ANOVA to test whether litter mass remaining, community biomass, C and N mass assimilated (absolute assimilation), and the percentage of litter C and N lost from the leaf and assimilated (relative assimilation) varied across litter types and the three colonization treatments (14-day caged, 28-day caged, and uncaged packs). When we detected a significant difference, Tukey's honestly significant difference (HSD) was used to compare differences. Data were log10-transformed as needed to meet assumptions of normality and equal variance.

We used non-metric multidimensional scaling (NMDS) ordinations with Bray–Curtis distance measures and multiresponse permutation procedures (MRPP) to visualize and test for differences in invertebrate community composition across leaf species and colonization treatments using PC ORD version 6.0 (McCune and Mefford 2011). Before conducting NMDS ordinations and MRPP, we relativized data by maximum abundances (McCune and others 2002). When MRPP tests revealed significant differences, we used indicator species analysis to determine which taxa showed significant fidelity for a specific treatment. Indicator species analysis calculates indicator values based on a taxon's relative frequency and relative abundance within sampling units and is a measure of the exclusiveness of a taxon to a specific treatment (McCune and Mefford 2011).

To test whether invertebrate biomass drove C or N assimilation, we regressed the proportion of a taxon's contribution to the community C or N assimilation against the taxon's proportion of community biomass. We calculated the average proportion of C and N that each taxon assimilated by dividing the average mass C or N assimilated by each taxon by the total mass C or N assimilated for the whole community in each pack. We focused this analysis on the uncaged packs because they best represent natural leaf packs. Alpha = 0.05 for all tests.

Results

Leaf Litter Mass Loss and Chemistry

As expected, at the end of the 35-day study, *P. fremontii* litter decomposed the most rapidly (77% mass loss; Figure 1A, Table 1) followed by *F. velutina* (62% mass loss). *Platanus wrightii* and *Q. gambelii* litter decomposed slowly and did not differ in mass loss (18% and 13%, respectively; Figure 1A, Tables 1 and 2). Litter mass loss did not differ among colonization treatments (Table 2). At day 35, the percent C decreased and the percent N increased in all leaf types, which was reflected in lower C:N in decomposed litter relative to initial litter (Table 1). The increase in N and reduction in C:N ratios are likely due to immobilization of N from the water column by microbes (Pastor and others 2014). Final C:N ratios differed among all litter types with slowly decomposing *P. wrightii* and *Q. gambelii* having higher C:N ratios than rapidly decomposing *F. velutina* and *P. fremontii* (Tables 1 and 2, Figure 1B).

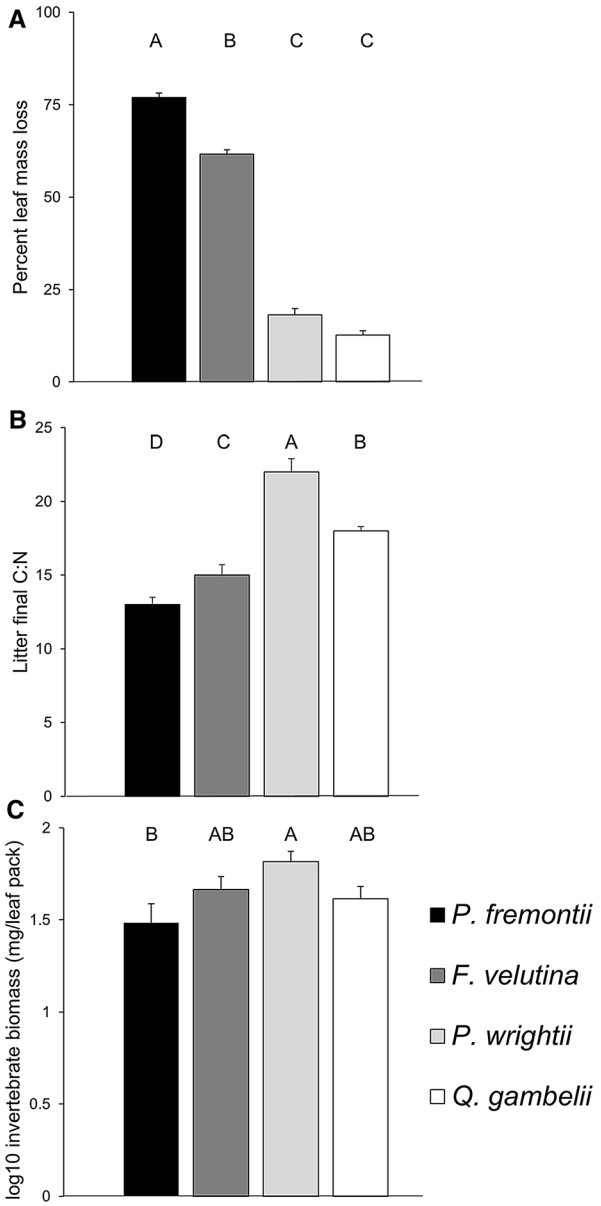

Figure 1. Percent litter mass loss (**A**), litter C:N ratios (**B**), and invertebrate biomass (**C**) from four riparian tree species incubated in Oak Creek, AZ for 35 days. Data presented are means (± SE; n = 30 for each leaf species). The above letters denote significant differences among leaf types.

Invertebrate Responses

Invertebrate biomass differed across leaf types but not colonization treatments (Table 2), while community composition differed across both leaf type and colonization treatment (Figure 2). The most abundant invertebrates in leaf packs spanned five orders and included nine taxa representing five functional feeding groups (Table 3; Merritt and others 2008). Invertebrate biomass was highest on

Table 2. Results from Two-Way ANOVAs for Litter Mass Loss, Invertebrate Biomass, Mass Carbon Assimilated, Mass Nitrogen Assimilated, Percent Carbon Assimilated, and Percent Nitrogen Assimilated.

Response variable	Source	*DF*	*F* ratio	*p* value
Litter mass loss	Leaf type	3	531	< **0.0001**
	Colonization treatment	2	1.57	0.21
	Leaf type × treatment	6	1.18	0.32
Invertebrate biomass	Leaf type	3	3.13	**0.03**
	Colonization treatment	2	0.67	0.52
	Leaf type × treatment	6	0.69	0.66
Mass C assimilated	Leaf type	3	5.33	**0.002**
	Colonization treatment	2	1.93	0.15
	Leaf type × treatment	6	0.27	0.95
Mass N assimilated	Leaf type	3	2.33	0.08
	Colonization treatment	2	0.67	0.52
	Leaf type × treatment	6	0.84	0.54
Percent C assimilated	Leaf type	3	40.0	< **0.0001**
	Colonization treatment	2	1.10	0.34
	Leaf type × treatment	6	0.16	0.99
Percent N assimilated	Leaf type	3	32.6	**0.0001**
	Colonization treatment	2	0.30	0.74
	Leaf type × treatment	6	0.81	0.56

The two independent variables were leaf type (four plant species) and colonization treatment (leaf packs caged at day 14, leaf packs caged at day 28, and uncaged packs). All litter packs were removed from Oak Creek, AZ after 35 days.
Significant p values are in bold.

Table 3. Invertebrate Taxa and Their Functional Feeding Groups Found in Litter Packs and Used for Analysis

Order	Family	Genus	Functional feeding group
Diptera	Chironomidae	–	Collector–gatherer
Diptera	Simuliidae	–	Collector–filterer
Ephemeroptera	Baetidae	Baetis	Collector–gatherer
Ephemeroptera	Leptohyphidae	Tricorythodes	Collector–gatherer
Odonata	Aeshnidae	Oplonaeschna	Predator
Lepidoptera	Crambidae	Petrophila	Scraper; facultative shredder
Trichoptera	Brachycentridae	Micrasema	Shredder
Trichoptera	Hydrobiosidae	Atopsyche	Predator
Trichoptera	Hydropsychidae	Hydropsyche	Collector–filterer

We included all taxa that were found in at least 15% of all packs. Together these taxa accounted for ~ 97% of total invertebrate biomass.

P. wrightii and slightly, but significantly, higher than *P. fremontii*. Invertebrate biomass on *F. velutina* and *Q. gambelii* did not differ from the other two litter types (Figure 1C). In contrast, leaf type had less of an effect on invertebrate assemblages than colonization treatment, with no significant differences among leaf types in the 14-day ($A = -0.02$, $p = 0.96$; Figure 2A) and 28-day caged treatments ($A = -0.002$, $p = 0.53$; Figure 2B). Communities, however, differed significantly across leaf types in the uncaged packs ($A = 0.03$, $p = 0.04$) with *P. fremontii* and *Q. gambelii* communities being similar, while *F. velutina* and *P. wrightii* had distinct communities (Figure 2C). The NMDS and MRPP revealed distinct invertebrate communities in packs that were caged after 14 days compared with packs that were caged after 28 days and the uncaged packs ($A = 0.03$, $p < 0.0001$, Figure 2D). This finding is important for testing prediction (2) that time of invertebrate colonization influences assimilation of C and N since we expected invertebrate communities to change over time, potentially altering the amounts of C and N assimilated. Indicator species among colonization treatments included Chironomidae (a generalist midge, $p = 0.0002$), Simuliidae (blackflies, $p = 0.0006$),

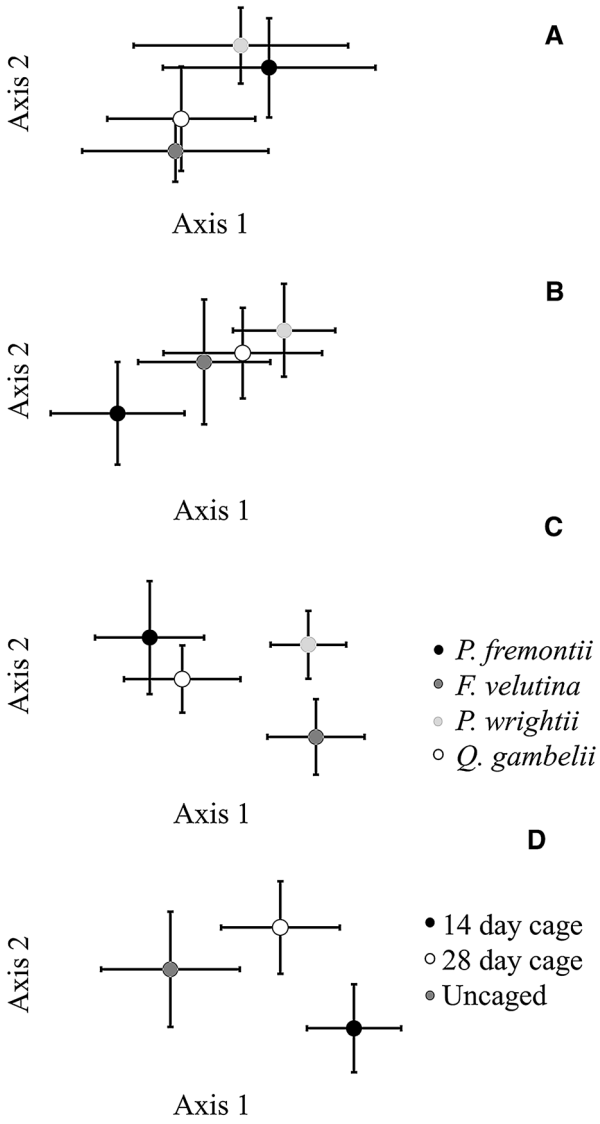

Figure 2. Results from non-metric multidimensional scaling (NMDS) analysis of macroinvertebrate assemblages found in leaf litter packs across four leaf species in 14-day caged (**A**), 28-day caged (**B**) and uncaged controls (**C**) and across colonization treatments for all litter types combined (**D**). All packs were harvested after 35 days. Data presented are means (± SE; n = 10 in Panels **A**–**C**; n = 40 in Panel **D**).

and *Hydropsyche* (a net-spinning caddisfly, $p = 0.02$). Collector–gatherer taxa usually accounted for the largest proportion of the total biomass (Figure 3). Packs caged after 14 days were dominated by Chironomids and *Baetis* mayflies, whereas relative biomass was more evenly distributed in uncaged packs and was higher for the two filter feeders: Simuliidae and *Hydropsyche* (Figure 3). There were no indicator species associated with different leaf types.

Trophic Efficiency: C and N Assimilation

Absolute and relative assimilation of both elements varied among litter types but not by colonization treatments (Table 2). As predicted, absolute assimilation of C was significantly higher for communities feeding on recalcitrant *P. wrightii* and *Q. gambelii* litter than on *P. fremontii* litter, supporting prediction (1) (Table 2, Figure 4A). In contrast, there was only a marginal difference ($0.1 > p > 0.05$) in absolute assimilation of N (Table 2, Figure 4B), such that communities feeding on *Q. gambelii* assimilated more N than the other three litter types. As predicted, trophic efficiency, measured as relative element assimilation, was significantly higher on slowly decomposing *P. wrightii* and *Q. gambelii* litter relative to more rapidly decomposing *F. velutina* and *P. fremontii* litter for both C (eight times more C assimilated, Figure 4C) and N (six times more N assimilated, Figure 4D). Communities assimilated on average 1.06% (± 0.17) of the C lost and 1.36% (± 0.17) of the N lost from the leaves across all litter types and colonization treatments. Prediction (2) that invertebrates colonizing litter packs in the early stages of decomposition would assimilate more C and N compared to later colonizers was not supported because there were no differences in absolute assimilation or relative assimilation of C or N across cage treatments (Table 2).

In support of prediction (3), the proportion of invertebrate biomass in each pack was correlated with the proportion of C ($F_{1,187} = 300$, $p < 0.0001$, $r^2 = 0.62$; Figure 5A) and N assimilated ($F_{1,187} = 50.6$, $p < 0.0001$, $r^2 = 0.21$; Figure 5B), although the relationship was stronger for C (Figure 5). Chironomids almost always assimilated a higher proportion of litter C and N relative to this taxon's biomass as indicated by a higher ratio (Supplemental Table 2). *Petrophila*, *Baetis*, and *Tricorythodes* tended to assimilate C and N proportionally to their biomass, whereas all other taxa including the two filter feeders (Simuliidae and *Hydropsyche*) mostly assimilated C and N in a lower proportion than their relative biomass.

Taxon-specific assimilation across leaf types generally reflected assimilation patterns of the entire communities (Tables 4 and 5, Figure 6). The three collector–gatherers (Chironomids, *Baetis*, and *Trichorythodes*), and the facultative shredder (*Petrophila*), all had higher relative assimilation of C and N on slowly decomposing litter types. Only Chironomids displayed a significant difference in absolute assimilation of C among leaf types (Supplemental Table 3), and there were no differences

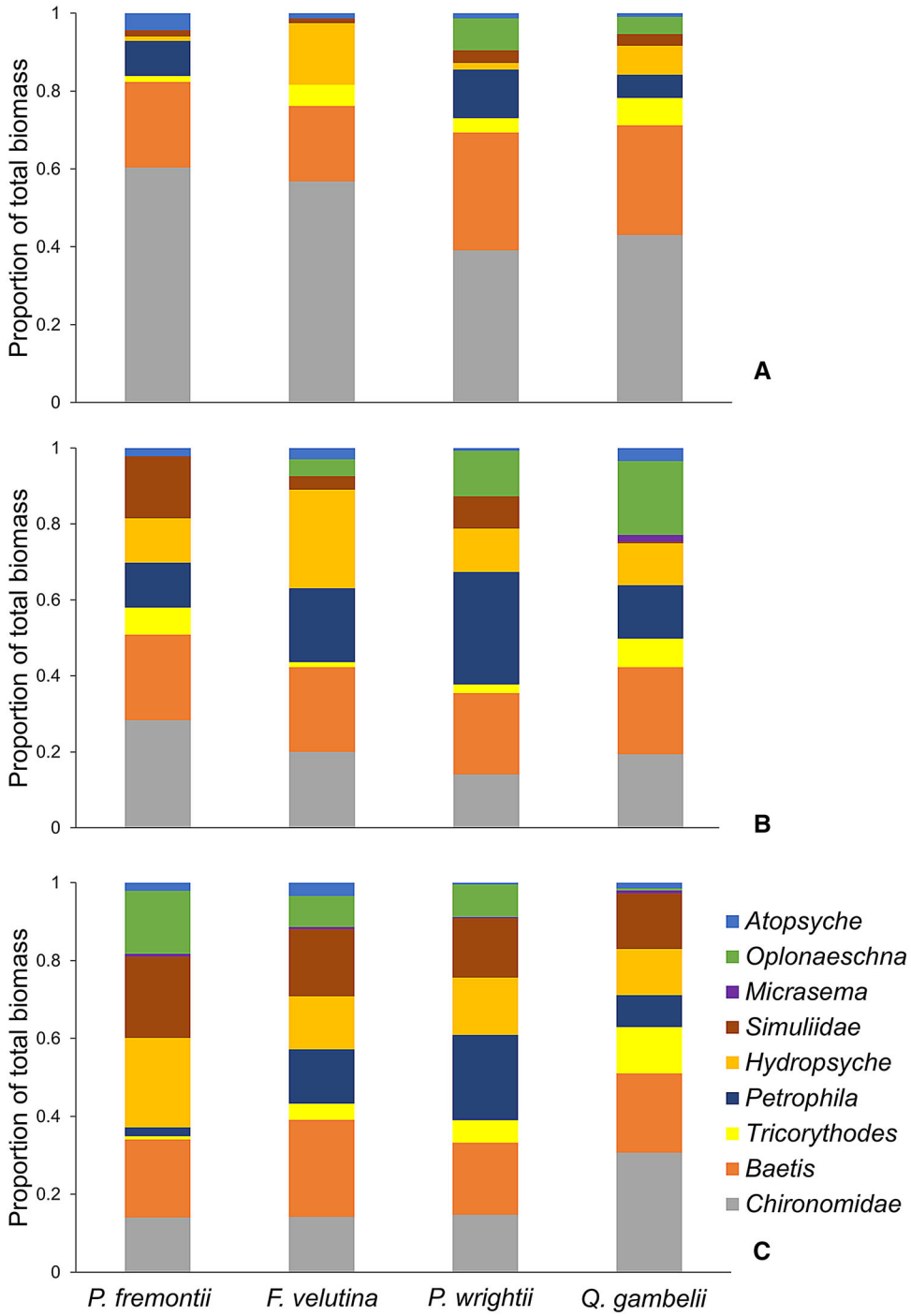

Figure 3. Relative proportions of the total macroinvertebrate biomass for dominant taxa that colonized four leaf types in the 14-day caged (**A**), 28-day caged (**B**), and uncaged (**C**) treatments. All leaf packs were incubated in Oak Creek, AZ and harvested after 35 days. Rare taxa were excluded from this analysis. Mean values are shown for each leaf type (n = 10).

in absolute assimilation of N for any taxa (Table 5). In general, differences in relative assimilation among taxa were similar to those at the community level, such that taxa feeding on recalcitrant litter had higher relative assimilation. Similarly, C and N assimilation for predators followed the community pattern, but sample sizes were too small to conduct statistical tests as predators only colonized a subset of packs. Relative assimilation of Simuliidae, one of the filter feeders (Table 5), was also higher on slowly decomposing litter than rapidly decomposing litter types, but the other filter feeder, *Hy-*

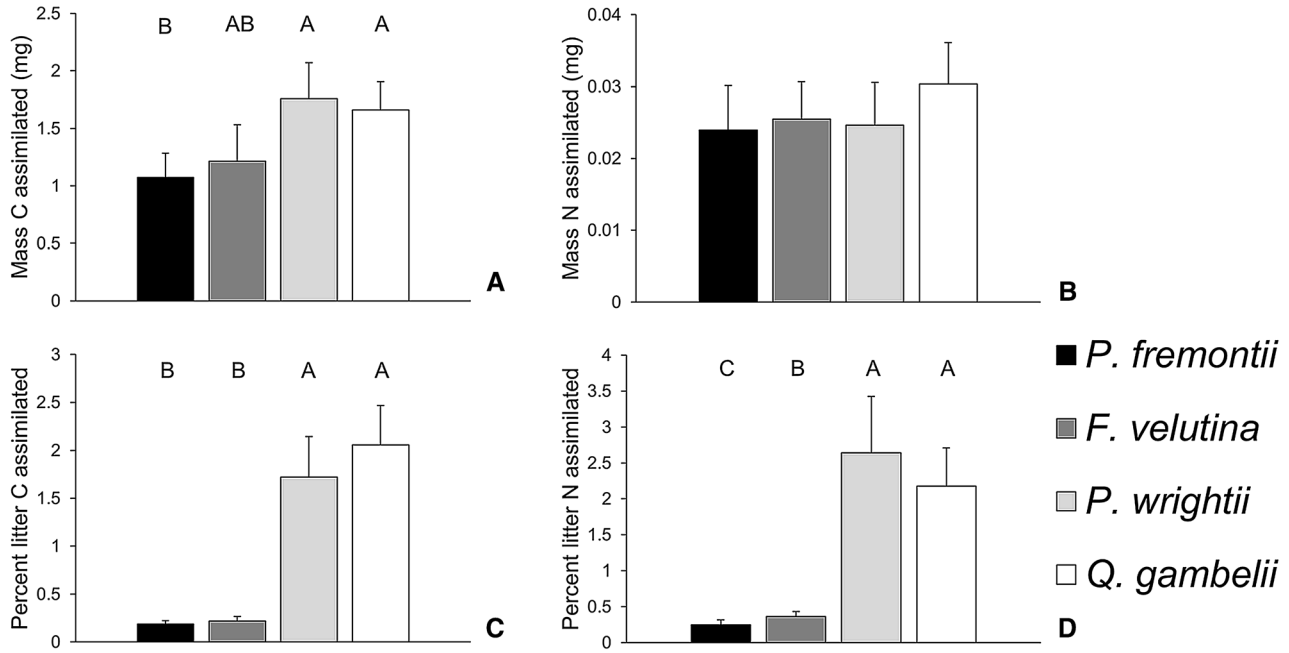

Figure 4. Mass (mean ± 1 SE) carbon (**A**) and nitrogen (**B**) and the percentage of litter carbon (**C**) and nitrogen (**D**) that was lost and assimilated by invertebrate communities across leaf types following 35-day incubations in Oak Creek, AZ. Colonization treatment packs are combined for each leaf type as there was no significant difference across colonization treatments or a significant interaction between colonization treatment and leaf type (Table 2; n = 30). Letters denote significant differences among leaf types.

dropsyche, showed no significant differences (Supplemental Table 3). Absolute assimilation for the two filter feeders did not follow the overall trends, but the variance among replicates was too high to discern patterns among leaf types.

Discussion

This study demonstrated that invertebrate communities assimilated more of the C and N that was lost from litter packs from slowly decomposing compared to rapidly decomposing litter types. Rapidly decomposing litter is generally thought to be a higher-quality resource for decomposers than slowly decomposing litters (Melillo and others 1982; Golladay and others 1983; Spain and Le Feuvre 1987). Our results challenge this and suggest that rapidly decomposing litters may be a ''higher''-quality resource for microbes but not necessarily for higher trophic levels. For example, rapidly decomposing litter which often has high leaching and microbial respiration rates may be a high-quality resource for specific microbes, but the mass loss from leaching would only be directly available to downstream invertebrates during flocculation, or possibly indirectly through downstream microbial uptake, whereas C that is respired is not available to invertebrates. Studies that have found higher invertebrate growth rates or preference on labile litter have not always considered that leaf litter is a finite resource and have not viewed assimilation from a mass balance perspective, where rapidly decomposing litter will persist for a shorter duration in streams (Marks 2019). Other recent studies have shown higher growth rates of insects feeding on slowly decomposing litter types (Fuller and others 2015; Halvorson and others 2015), which tends to support our overall conclusions that recalcitrant litter can be an important resource for invertebrates and that litter quality may differ for microbial decomposers and invertebrate detritivores.

Relative assimilation approximated 1% for both C and N across all leaf types and treatments. If relative assimilation of C and N mimics energy efficiency, averaging 10% between trophic levels (Lindeman 1942), our results are consistent with two trophic links (leaves to microbes to insects). Little work has been done on trophic transfer efficiencies in detrital-based freshwater food webs. Whole-stream ^{15}N additions showed trophic transfer efficiencies of N from primary uptake compartments such as biofilms and detritus to primary consumers to be 11.5% (Norman and others 2017). The mean transfer efficiency for scrapers,

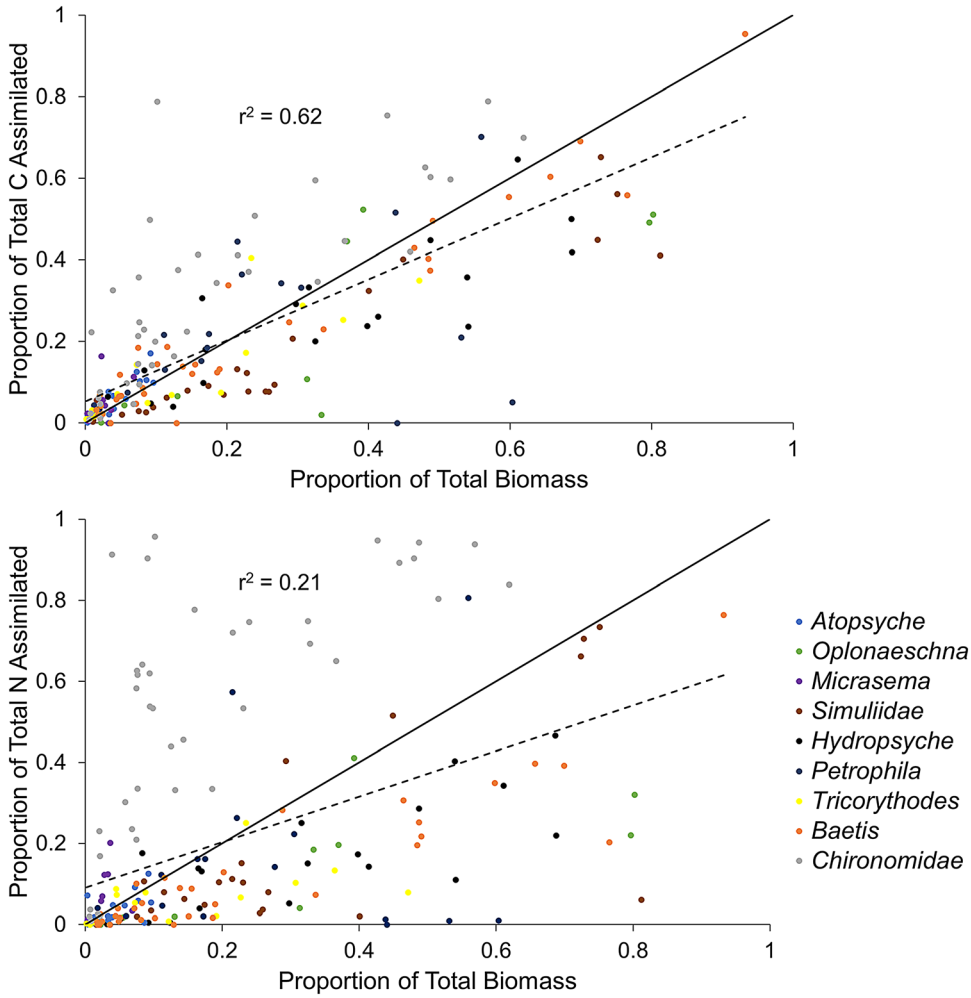

Figure 5. Proportion carbon (**A**) and nitrogen (**B**) assimilated in each leaf pack regressed against the proportion of the total biomass that each taxon contributed to the total leaf pack. Data plotted are from the uncaged treatment harvested at day 35. The dashed black line represents the regression line, and the solid black line represents the 1:1 ratio line.

which feed primarily on algae, was five times higher than all other functional feeding groups such as shredders and collectors, which can feed on both algae and detritus, indicating the scrapers were likely driving these patterns of high efficiency. Therefore, our efficiency results from detritus tend to be in close agreement with the shredders, collectors, and filterers in Norman and others (2017), which tended to have low N transfer efficiencies of about 1%. Microbes are not always considered in trophic hierarchies, but studies using natural abundance ^{15}N isotopes demonstrate their importance (Steffan and others 2015; Steffan and others 2017). We cannot measure trophic fractionation in this study because differences due to isotopic fractionation are small relative to the enrichment values and variation in isotope concentrations in the leaves.

Leaf mass loss and the different pathways of mass loss drove relative assimilation patterns, and multiple mechanisms likely underlie differences in mass loss. First, soluble compounds that are rapidly lost from litter are available to microbes, but probably not invertebrates (Petersen and Cummins 1974; McDowell and Fisher 1976; Webster and Benfield 1986; Meyer 1994). Mass loss during leaching tends to be higher in faster decomposing litter types (Webster and Benfield 1986; Wymore and others 2015; Siders and others 2018) and can be up to 30% of the initial mass (Wymore and others 2015). Leaching rates measured for these four litter types show significantly higher mass loss from *P. fremontii* leaves relative to the other three leaf types (Siders and others 2018), so this mechanism can only partially account for the pattern since leaching rates of *F. velutina* were similar to *Q.*

Table 4. Mass Carbon Assimilated (mean ± 1 SE—µg/pack—upper section) and Nitrogen Assimilated (µg/pack—lower section) by the Nine Dominant Invertebrate Taxa Found in Uncaged Leaf Packs Incubated in Oak Creek, AZ for 35 Days.

	Atopsyche	*Baetis*	Chironomidae	*Hydropsyche*	*Micrasema*	*Oplonaeschna*	*Petrophila*	Simuliidae	*Tricorythodes*
P. fremontii	61 (42)	191 (70)	207 (76)*	448 (181)	19.2	208 (42)	1113 (1001)	297 (149)	34 (21)
F. velutina	123 (68)	223 (60)	164 (26)	1174 (647)	59.8 (37.6)	347	364 (101)	690 (647)	103 (69)
P. wrightii	46 (32)	305 (70)	560 (147)	352 (180)	42.7 (11.3)	423 (414)	527 (219)	1350 (1233)	139 (50)
Q. gambelii	62 (22)	302 (84)	455 (96)	413 (165)	45.8	102	856 (498)	306 (160)	257 (109)
P. fremontii	0.25 (0.14)	0.89 (0.31)	14.4 (10.3)	2.71 (1.31)	0.10	1.52 (0.78)	17.5 (16.5)	2.39 (1.16)	0.18 (0.09)
F. velutina	1.30 (0.71)	2.12 (0.56)	6.07 (1.17)	10.3 (5.15)	0.57 (0.25)	2.12	5.30 (2.00)	12.5 (11.7)	1.16 (0.78)
P. wrightii	1.01 (0.95)	2.07 (0.47)	19.7 (6.53)	6.32 (4.97)	1.79 (0.58)	5.58 (4.09)	1.92 (1.27)	26.4 (24.3)	1.47 (0.87)
Q. gambelii	0.62 (0.31)	2.06 (0.56)	8.97 (2.40)	2.74 (1.11)	3.41	0.57	6.96 (1.54)	5.49 (2.83)	1.79 (0.68)

Taxa with an asterisk at the top of the column differed significantly in absolute assimilation of carbon or nitrogen among leaf types. Values without a standard error did not have replication within a leaf type.

gambelii and *P. wrightii*. Second, the slower release of C and N in recalcitrant litter might coincide better with invertebrate dispersal and colonization. Microbial decomposition can result in over 50% mass loss in the initial weeks, which may occur before invertebrates can colonize the litter. Invertebrate species richness typically takes 10 – 25 days to plateau, and it can take 10 – 30 days to reach maximum invertebrate densities (Wise and Molles 1979; Lake and Doeg 1985; Minshall and others 1985; Peckarsky 1986). Competition between microbes and invertebrates for detrital resources may be the dominant interaction on rapidly decomposing leaves because microbes are able to colonize more quickly than invertebrates. In contrast, the role of microbes as both mutualists and prey may be the dominant interactions in slowly decomposing litter types where C and N is bound in complex, recalcitrant compounds (Rahman and others 2013). Recalcitrant compounds often require fungal degradation to repackage long-chain C compounds into compounds that are more readily assimilated by invertebrates (Webster and Benfield 1986; Suberkropp 1992; Gessner and Chauvet 1994; Kohlmeier and others 2005). Fungi are also a high-quality food resource for invertebrates (Suberkropp 1992; Chung and Suberkropp 2009), have lower C:N relative to leaf litter (Cross and others 2005), and may play a more important functional role as prey in slowly decomposing litter. In this study, we were unable to estimate how much of the assimilated leaf litter is directly consumed or is transferred through the microbial pathway, but further work on this topic would unravel the complex interactions between leaf litter, microbes, and invertebrates. Slowly decomposing litter is important for supporting long-lived shredders in the later seasons (late winter to midsummer; Webster and Waide 1982; Grubbs and Cummins 1994; Hutchens and others 1997). Based on the decomposition rates that we observed, if trees shed their leaves in October or November, by February or March the standing stock of *P. fremontii* and *F. velutina* leaves would be almost gone (< 10% mass remaining), while over half of *Q. gambelii* and *P. wrightii* mass would remain. Because larger shredders typically emerge in the summer (Merritt and others 2008), these invertebrates could be food limited for months prior to emergence in the absence of recalcitrant leaf litter (Marks 2019).

Absolute assimilation of C was higher on recalcitrant litter types than labile litter probably because what remained of the labile litter was mostly recalcitrant compounds which can be difficult for

Table 5. Relative Assimilation of Carbon (mean ± 1 SE—upper section) and Nitrogen (lower section) by the Nine Dominant Invertebrate Taxa Found in Uncaged Leaf Packs Incubated in Oak Creek, AZ for 35 Days.

	Atopsyche	*Baetis*	Chironomidae	*Hydropsyche*	*Micrasema*	*Oplonaeschna*	*Petrophila*	Simuliidae	*Tricorythodes*
P. fremontii	0.01 (0.007)	0.03 (0.01)*	0.04 (0.01)*	0.08 (0.03)	0.003	0.04 (0.007)	0.19 (0.17)	0.05 (0.03)*	0.006 (0.004)*
F. velutina	0.02 (0.01)	0.04 (0.01)	0.03 (0.004)	0.19 (0.10)	0.01 (0.008)	0.06	0.07 (0.02)	0.11 (0.10)	0.02 (0.01)
P. wrightii	0.03 (0.02)	0.26 (0.08)	0.59 (0.25)	0.22 (0.09)	0.04 (0.009)	0.31 (0.31)	0.54 (0.21)	0.69 (0.62)	0.27 (0.19)
Q. gambelii	0.05 (0.008)	0.23 (0.05)	0.58 (0.17)	0.22 (0.07)	0.03	0.1	0.50 (0.16)	0.26 (0.12)	0.43 (0.19)
P. fremontii	0.003 (0.001)*	0.009 (0.003)*	0.16 (0.11)*	0.02 (0.01)	0.001	0.02 (0.009)	0.17 (0.16)	0.02 (0.01)*	0.001 (0.0009)*
F. velutina	0.02 (0.01)	0.03 (0.008)	0.09 (0.02)	0.14 (0.07)	0.009 (0.004)	0.03	0.07 (0.03)	0.15 (0.14)	0.02 (0.01)
P. wrightii	0.06 (0.05)	0.20 (0.05)	3.03 (1.92)	0.38 (0.28)	0.16 (0.03)	0.43 (0.35)	0.14 (0.07)	1.51 (1.37)	0.38 (0.32)
Q. gambelii	0.03 (0.01)	0.10 (0.02)	0.71 (0.24)	0.09 (0.03)	0.13	0.03	0.28 (0.03)	0.31 (0.17)	0.23 (0.14)

Relative assimilation is calculated as the percent of carbon or nitrogen lost during decomposition that was assimilated by each invertebrate taxon in a leaf pack. Taxa with an asterisk at the top of the column had significantly different assimilation of carbon or nitrogen among leaf types. Values without a standard error did not have replication within a leaf type.

invertebrates to feed upon. There were no differences in absolute assimilation of N across the four litter types. A potential reason for not observing differences in absolute assimilation of N could be a result of the duration of the study and microbial interactions with the leaf litter. Over time, leaf microbes become more dependent on the water column to meet demands of C and N (Cheever and others 2013; Pastor and others 2014). Over 60% of N can be bound to recalcitrant compounds such as lignin and cellulose (Fioretto and others 2005), which may result in microbes switching to deriving most of their N from the water column to meet metabolic demands. Therefore, the observed pattern of no differences in absolute assimilation of N may be expected if the invertebrates are feeding more on the leaf microbes than the litter itself in the later stages of decomposition. This could be why shorter duration studies using labeled litter in this system did find differences in N assimilation among diverse leaf types for both individual shredders (Compson and others 2018; Siders and others 2018) and invertebrate communities (Compson and others 2015).

We did not observe differences in element assimilation among the colonization treatments, in contrast to our prediction. This is likely due to total invertebrate biomass, rather than timing of colonization, playing a greater role in controlling assimilation, and is supported by two lines of evidence. First, invertebrate biomass did not differ among the three colonization treatments even though the community composition differed among the treatments. Most notably, between days 14 and 28, Chironomids decreased due to either emigration or predation, while Simuliids and *Hydropsyche*, the two filter feeding taxa, immigrated into the packs. Predation would result in decreases in Chironomid biomass, but retention of the litter C and N by predators. Thus, the finding of no differences in assimilation of litter C and N among colonization treatments is likely due to multiple factors including retention of litter C and N through predation and feeding by insects that colonized later. Second, the positive correlations between relative biomass and proportion assimilated indicate that the relative biomass of the invertebrates in litter packs can partially explain assimilation patterns, even when the relative biomass among taxa changes over time. In the uncaged control treatment, Chironomids had high absolute assimilation of both C and N, likely because they colonized early and were feeding on litter longer than other groups, and also because they can have high production rates (Benke 1998).

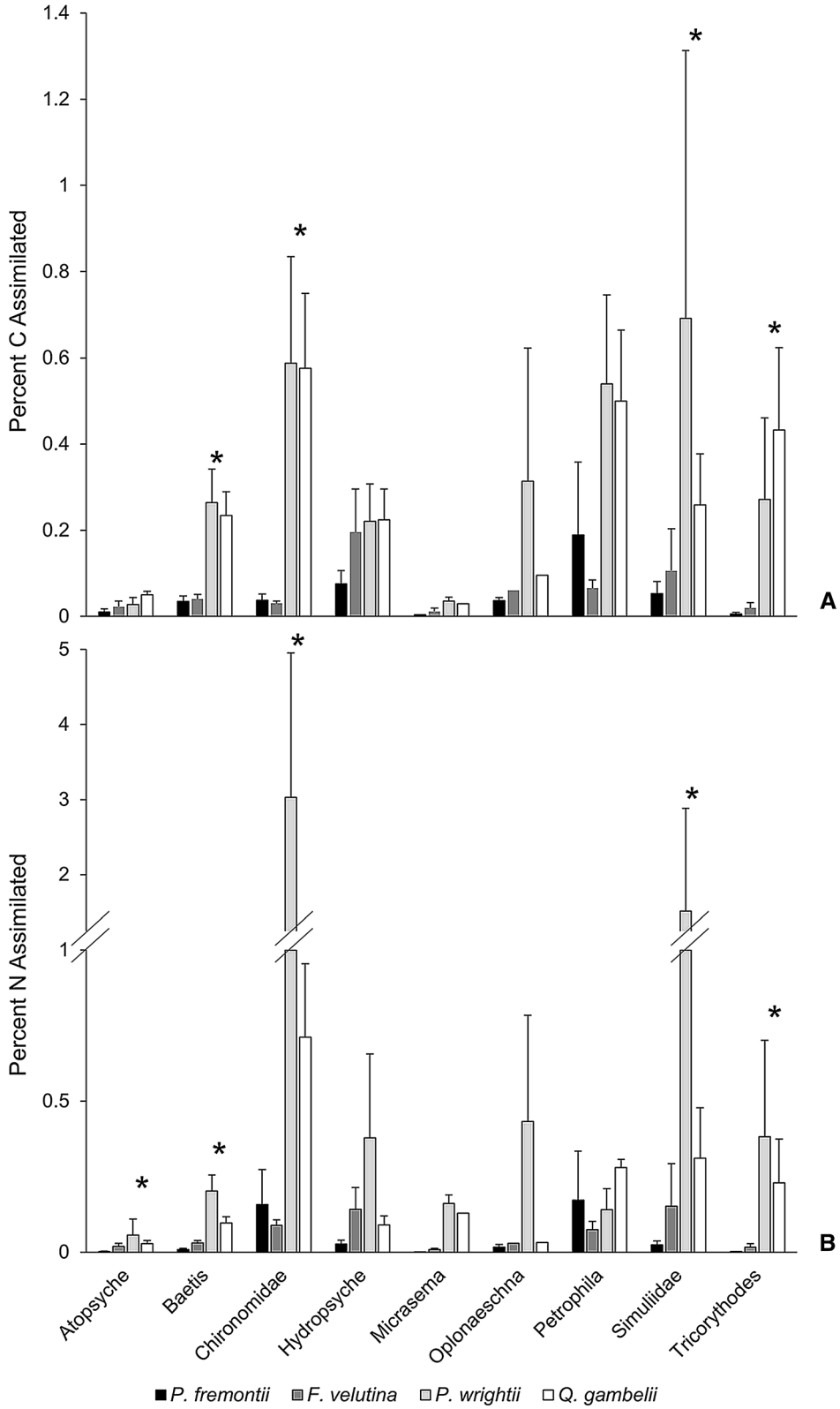

Figure 6. Relative assimilation of carbon and nitrogen across leaf types by the nine dominant macroinvertebrate taxa in the uncaged leaf packs (n = up to 10 depending on taxa presence in leaf packs). All packs were harvested at day 35. Asterisks indicate significant differences across leaf types.

The dominant taxa found in litter packs—Chironomidae, Simuliidae, *Baetis*, and *Hydropsyche*—all feed on particles less than 1 mm in size and are rarely used in assimilation studies. Our results show that both collector–gatherers feeding on settled particles and filter feeders acquire detrital resources in leaf packs, suggesting that their acquisition of small particles may be tightly coupled with microbial activity. Chironomids were the only group that assimilated C and N in proportions greater than their relative biomass, in part because they colonized early and remained in packs, highlighting their importance in transforming litter C and N into new biomass. Chironomids have high dispersal rates in drift (Anderson and Lehmkuhl 1968; Brittain and Eikeland 1988) and dominated the invertebrate assemblage that developed by day 14. Chironomid larvae are considered inefficient feeders that rapidly process organic matter, emptying their guts up to 20 times per day, but they also recycle organic matter from their own fecal pellets and tubes (Hirabayashi and Wotton 1998; Romito and others 2010). The low feeding efficiency observed in laboratory experiments might be offset by continual recycling of organic material in the leaf packs, explaining their high assimilation rates in this and other field experiments (Compson and others 2015). In contrast, *Baetis* mayflies, which also colonized early, had slightly lower absolute assimilation than predicted based on their biomass, suggesting that they feed more on particles entering the litter packs or grow more slowly. Although filter feeders incorporated slightly lower proportions of enriched leaf litter relative to their biomass, their enrichment levels indicate that some of the small particles generated in leaf packs are retained in the packs. This is probably through the structure of the leaf litter and its biofilm rather than the fine mesh of the cages, as there were no differences in assimilation of C or N between the caged and uncaged litter packs. Retention of small particles may be higher for slowly decomposing litter types, which maintain their physical structure much longer. Large shredders, which can be prevalent in Oak Creek, were not abundant at this site during this experiment, probably due to the lack of pools rather than the timing of our study because they were abundant in other reaches of Oak Creek during this time (Siders and others 2018). Nevertheless, this study shows the important role of leaf litter in supporting diverse invertebrate taxa that are not typically used in growth or assimilation studies due to methodological constraints and demonstrates that entire invertebrate communities can benefit from recalcitrant leaf litter.

The role of resource quantity and quality on consumer performance is not well understood in detrital-based food webs. Elevated nutrients may increase litter quality through changes in stoichiometry that better align food resources with consumer demands (C:N, C:P, N:P), but this can simultaneously reduce quantity for invertebrates due to increased microbial respiration (Rosemond and others 2015; Manning and others 2016). Both litter quantity and nutritional quality can limit growth of detritivores (Halvorson and others 2017). Rapidly decomposing litter coupled with high nutrient concentrations in the water column can lead to high microbial immobilization of water column nutrients, which can increase invertebrate production as demonstrated through long-term stream nutrient enrichment studies (Cross and others 2006; Demi and others 2018). If microbial respiration increases to the point that litter becomes limiting, invertebrate production should ultimately be reduced. Our results suggest that litter quantity will limit invertebrates if most of the litter decomposes quickly. The mass balance approach that we describe shows that recalcitrant litter remains in the stream for longer providing food for invertebrates throughout their larval stages and more C and N that is lost from the litter is transferred up the food chain. Rapidly decomposing litter provides a substantial pulse of nutrients to invertebrates shortly following leaf fall, whereas more slowly decomposing litter provides a more sustained food source. Maintaining diverse riparian zones that include functionally diverse litter types likely help fuel productive aquatic food webs (Marks 2019).

This research presents a novel approach to measuring trophic efficiency using isotope tracers. Our results challenge traditional views of litter quality by demonstrating that trophic efficiency, measured as relative element assimilation, is negatively associated with decomposition rate across four leaf types. We found strong patterns among the four litter types used in this study, and further research using this approach to compare trophic efficiency across a wider range of leaf types will provide a comprehensive framework for understanding how litter traits affect elemental cycling and food web structure in brown food webs.

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