Oecologia
DOI 10.1007/s00442-014-3063-y

ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species

Ada Pastor · Zacchaeus G. Compson · Paul Dijkstra · Joan L. Riera · Eugènia Martí · Francesc Sabater · Bruce A. Hungate · Jane C. Marks

Received: 25 February 2014 / Accepted: 21 August 2014

Abstract Leaf litter decomposition plays a major role in nutrient dynamics in forested streams. The chemical composition of litter affects its processing by microorganisms, which obtain nutrients from litter and from the water column. The balance of these fluxes is not well known, because they occur simultaneously and thus are difficult to quantify separately. Here, we examined C and N flow from streamwater and leaf litter to microbial biofilms during decomposition. We used isotopically enriched leaves (^{13}C and ^{15}N) from two riparian foundation tree species: fast-decomposing *Populus fremontii* and slow-decomposing *Populus angustifolia*, which differed in their concentration of recalcitrant compounds. We adapted the isotope pool dilution method to estimate gross elemental fluxes into litter microbes. Three key findings emerged: litter type strongly affected biomass and stoichiometry of microbial assemblages growing on litter; the proportion of C and N in microorganisms derived from the streamwater, as opposed to the litter, did not differ between litter types, but increased throughout decomposition; gross immobilization of N from the streamwater was higher for *P. fremontii* compared to *P. angustifolia*, probably as a consequence of the higher microbial biomass on *P. fremontii*. In contrast, gross immobilization of C from the streamwater was higher for *P. angustifolia*, suggesting that dissolved organic C in streamwater was used as an additional energy source by microbial assemblages growing on slow-decomposing litter. These results indicate that biofilms on decomposing litter have specific element requirements driven by litter characteristics, which might have implications for whole-stream nutrient retention.

Keywords *Populus* · Recalcitrant compounds · Immobilization · Nutrient cycling

Communicated by Robert O. Hall.

A. Pastor (✉) · J. L. Riera · F. Sabater
Department d'Ecologia, Universitat de Barcelona, Barcelona, Spain
e-mail: adapastor@ub.edu

Z. G. Compson · P. Dijkstra · B. A. Hungate · J. C. Marks
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA

Z. G. Compson · P. Dijkstra · B. A. Hungate · J. C. Marks
Ecosystem Science and Society Center, Northern Arizona University, Flagstaff, AZ, USA

E. Martí
Integrative Freshwater Ecology Group, Center for Advanced Studies of Blanes, CEAB-CSIC, Spanish Council for Scientific Research, Blanes, Girona, Spain

Introduction

Decomposition of organic matter makes available the products of primary photosynthesis to detritus-based food webs and releases inorganic components into the environment. Multiple studies have examined detrital mass-loss rates, showing generally positive relationships with nutrient content and negative relationships with concentrations of recalcitrant compounds in the litter, relationships that tend to hold across many ecosystems (Melillo et al. 1984; Taylor et al. 1989; Enriquez et al. 1993; Parton et al. 2007; Cornwell et al. 2008).

In stream and rivers, decomposition rates are rapid due to constant water availability and nutrient supply (Tranvik et al. 2009; Battin et al. 2009). Leaf litter inputs are essential resources for forested headwater streams (Vannote et al. 1980), which strongly affect food webs (Wallace et al.

Published online: 12 September 2014

1997; Gessner et al. 2010) and ecosystem function (Fisher and Likens 1973; Mulholland et al. 1985, 2000; Meyer et al. 1998; Argerich et al. 2008). Increasing stream nutrient concentrations accelerate detrital mass loss (Meyer and Johnson 1983; Suberkropp and Chauvet 1995; Gulis and Suberkropp 2003; Ferreira et al. 2014), although this effect can be reversed at high nutrient concentrations (Carreiro et al. 2000; Woodward et al. 2012). Variation in relationships among decomposition rates, leaf characteristics (litter quality), and stream nutrient concentrations has been partially explained by different responses in biomass accrual or activity of microbial assemblages (hereafter referred to as 'biofilms') on leaf litter (Gessner and Chauvet 1994; Gessner 1997; Gulis and Suberkropp 2003; Stelzer et al. 2003).

Litter decomposition in streams is usually measured as net loss of litter mass and net changes in its element content over time (Tank et al. 2010). However, changes in element content are the result of simultaneous gross fluxes of elements released from and retained in the litter. Processes driving litter element loss include chemical leaching, microbial mineralization of organic matter, physical fragmentation, and breakdown by stream animals. Additionally, biofilms growing on litter assimilate organic C and N from the leaf and from streamwater, which can be further lost from the biofilm-litter system through respiration and deamination (i.e., N mineralization). Concurrently, C and N gross fluxes from streamwater into the biofilm-litter system take place due to silt deposition and abiotic adsorption (Bott et al. 1984; Webster and Benfield 1986).

Despite the common empirical observations of the positive effects of stream nutrient concentrations on decomposition rates (Ferreira et al. 2014), studies quantifying gross fluxes entering the biofilm-litter are scarce. Moreover, most of the available data on immobilization is for bulk detrital samples and for a single point in time (e.g., Tank et al. 2000; Dodds et al. 2004). How immobilization varies throughout decomposition and in response to different leaf litter types is not known, even though the chemical properties of leaf litter vary strongly among species and over time (Cornwell et al. 2008). Finally, most studies focus on one element (i.e., N), even though biosynthesis couples N with C in specific ratios with effects scaling to the ecosystem (Sterner and Elser 2002; Gruber and Galloway 2008). In this study, we aim to fill some of these gaps in our knowledge by evaluating the immobilization fluxes into the biofilm-litter during decomposition of two closely related tree species, which differ strongly in phytochemical characteristics.

Researchers have provided several lines of evidence to explain biofilm immobilization of dissolved organic C (DOC) and dissolved inorganic and organic N from streamwater. First, C and N stoichiometry of litter frequently does not fulfill the elemental requirements of biofilms because microorganisms have lower C:N ratios than the litter substrate (Sterner and Elser 2002; Parton et al. 2007). Second, recalcitrant compounds in litter decrease microbial enzymatic activity (Sinsabaugh et al. 1993) and the proportion of less readily resources available for biofilms (Gessner and Chauvet 1994). Thus, shortage of suitable resources might enhance biofilm DOC and N uptake from the water column. Third, DOC and N in the water could occur in readily available forms and are thus easily assimilated (Wiegner et al. 2005; Kaplan et al. 2008). Therefore, differences in litter characteristics may influence nutrient immobilization from streamwater during decomposition. Finally, biofilms may vary in microbial biomass and composition depending on litter types (Wymore et al. 2013; Frossard et al. 2013). The accumulation of microbial biofilm on the decomposing leaf increases the capacity to assimilate elements from the surrounding environment by presenting a higher assimilating surface area to the water column. Furthermore, the nature of this assimilating surface could influence the stoichiometry of microbial biosynthesis and the need to import elements from streamwater into the leaf-biofilm complex.

In this study, we quantified the relative importance of the C and N fluxes from streamwater into biofilm on litter. We used ^{13}C- and ^{15}N-enriched leaf litter and applied a variation of the isotope pool dilution method (Kirkham and Bartholomew 1954), which has been widely used in soil biogeochemistry to study nutrient dynamics during decomposition in soils (Murphy et al. 2003). This method quantifies the rate at which the isotopic value of an artificially enriched element pool declines due to the mass fluxes from an unlabeled pool (Kirkham and Bartholomew 1954).

We used ^{13}C- and ^{15}N-labeled litter from two foundation riparian tree species, *Populus fremontii* and *Populus angustifolia*. Phytochemical differences (Table 2) between these species, especially tannin content, drive changes in their decomposition rates with implications for adjacent terrestrial and aquatic ecosystems (Driebe and Whitham 2000; LeRoy et al. 2006; Whitham et al. 2006; Holeski et al. 2012). We expected that microbes growing on litter with a higher content of recalcitrant compounds would show a relatively greater reliance on C and N from streamwater than those growing on leaves with a lower content of recalcitrant compounds because these compounds are a less-accessible resource for heterotrophic microbes. Understanding the relative importance of C and N sources for biofilms on litter and how they vary during decomposition and among litter types will yield insights on the mechanisms driving litter decomposition, how decomposition controls the flux of C and N to the microbial food web, and the basic microbial and chemical controls on stream biogeochemical cycling.

Table 1 Physical and chemical parameters measured at Oak Creek during the experimental period (range of values or SE *in parentheses*)

Parameter	Mean (range or SE)
Discharge ($m^3\ s^{-1}$)	1.0 (0.9–1.7)
Temperature (°C)	11.4 (11.3–11.5
pH	7.1 (7.0–7.3)
SpC ($\mu S\ cm^{-1}$)	295.7 (294.4–297.8)
DO ($mg\ L^{-1}$)	8.6 (8.3–8.8)
DO (%)	94.2 (91.6–95.1)
NH_4 ($mg\ N\ L^{-1}$)	0.05 (±0.00)
NO_3 ($mg\ N\ L^{-1}$)	0.06 (±0.00)
DOC ($mg\ C\ L^{-1}$)	0.52 (±0.03)
DOC-^{13}C (atom %)	1.08 (±0.00)
NO_3^--^{15}N (atom %)	0.37 (±0.00)
NH_4^+-^{15}N (atom %)	0.37 (±0.00)

SpC Specific conductivity, *DO* dissolved O_2, *DOC* dissolved organic C

Materials and methods

Study site

This study was conducted in upper Oak Creek (1600 m a.s.l) on the southern edge of the Colorado Plateau (35°02′N, 111°43′W; Arizona). Oak Creek is a first-order stream in a deep narrow canyon. It drains a 77,450-km^2 catchment extensively covered by ponderosa pine (*Pinus ponderosa*). This area is characterized by steep topography and limestone and sandstone bedrock (LeRoy et al. 2006; Wymore et al. 2013). The riparian vegetation is predominately deciduous, including Fremont cottonwood (*Populus fremontii*), narrowleaf cottonwood (*Populus angustifolia*), Arizona alder (*Alnus oblongifolia* Torr.), Arizona sycamore (*Platanus wrightii* S. Wats.), coyote willow (*Salix exigua* Nutt.), and Goodding's willow (*Salix gooddingii* Ball) (LeRoy et al. 2006). This experiment was conducted from November to December 2011. During this time, discharge, streamwater temperature, pH, O_2 concentration and specific conductivity were relatively constant and concentrations of stream nutrients and isotopic values of dissolved N and DOC were low (Table 1).

Field experiment with labeled leaf litter

Tree cuttings of *P. fremontii* and *P. angustifolia,* from the Ogden Nature Center common garden (Ogden, UT) were grown at the Northern Arizona University Arboretum Research Greenhouse. Plants were isotopically labeled in a hydroponic nutrient solution by dripping $(^{15}NH_4)_2SO_4$ in aqueous solution into pots twice a week and pulsed with 99 atom % $^{13}CO_2$ for 4 h twice a week for 6 months (Compson et al. 2014, in revision). Variability in litter chemistry is lower for *P. fremontii* compared to *P. angustifolia* (Wymore et al. 2013; Table 2), so we selected five genotypes of *P. fremontii* and ten of *P. angustifolia.* Naturally senesced leaf litter was collected, air dried and stored. The variability of the stable C isotope composition ($\delta^{13}C$) and stable N isotope composition ($\delta^{15}N$) label in different leaf litter tissues was tested by boiling individual pieces of leaf litter ($n = 10$ per species) for 1 h to remove nearly all the soluble compounds and then taking the difference between pre- and post-leached samples. No differences between litter species (*P. fremontii* and *P. angustifolia*) for $\delta^{13}C$ (*t*-test $t_{1,18} = 0.16$, $P = 0.87$) or $\delta^{15}N$ (*t*-test $t_{1,18} = -0.16$, $P = 0.87$) were found (Compson et al. 2014, in revision).

Table 2 Initial litter characteristics and decomposition dynamics for *Populus fremontii* and *Populus angustifolia* (mean and SE)

	P. fremontii (low-tannin litter)	*P. angustifolia* (high-tannin litter)	Statistical significance
Leaf litter label			
^{13}C (atom %)	2.20 ± 0.98	2.02 ± 0.64	
^{15}N (atom %)	3.57 ± 1.60	3.13 ± 0.99	
Initial leaf litter characteristics			
Soluble condensed tannin (%)[a]	0.11 ± 0.06	1.94 ± 0.49	$t_8 = 3.69$; $P < 0.01$
Bound condensed tannin (%)[a]	0.17 ± 0.02	2.91 ± 0.34	$t_8 = -6.53$; $P < 0.01$
Lignin (%)[a]	9.58 ± 0.18	23.05 ± 1.39	$t_8 = -7.72$; $P < 0.001$
% C	38.0 ± 0.6	41.2 ± 0.5	$t_{13} = -3.77$; $P < 0.005$
% N	3.3 ± 0.5	3.0 ± 0.2	n.s.
C:N	12.6 ± 1.7	15.0 ± 1.0	n.s.
Decomposition dynamics			
Decomposition rate constant (k; day^{-1})	0.063 ± 0.002	0.037 ± 0.004	$t_{13} = 4.70$, $P < 0.001$

n.s. Not statistically significant at α 0.05

[a] Data from Wymore et al. (2013)

For each genotype, litter was mixed and three composite samples were analyzed for initial C and N content and isotope composition using a Carlo Erba NC 2,100 Elemental Analyzer (CE Instruments, Milan) interfaced with a Thermo-Finnigan Delta Plus XL (Thermo-Electron, Bremen, Germany) isotope ratio mass spectrometer (IRMS) at the Colorado Plateau Stable Isotope Laboratory (CPSIL) (http://www.isotope.nau.edu). *P. angustifolia* litter (high-tannin litter), had higher % C values than *P. fremontii* litter (low-tannin litter), but % N and C:N did not statistically differ (Table 2).

Litter was incubated in the stream using fine-mesh litterbags (10.5 × 10.5 cm^2, 0.5-mm mesh), which were deployed in the river zip-tied to rebar on 10 November 2011. Each litterbag contained 1 g of leaf litter. After 6, 13, 20 and 27 days of the experiment, 15 litterbags of *P. fremontii* and 30 litterbags of *P. angustifolia* (3 replicates × genotype) were collected from the stream (only *P. angustifolia* litterbags were collected for the final harvest). Upon harvest, litterbags were placed into zip-lock bags, and transported on ice to the laboratory where they were processed within 24 h.

For each harvest, dissolved O_2 (DO), conductivity, pH and water temperature were determined using a Hydrolab Minisonde (Hydrolab-Hach, Loveland, CO) in a five-point transect along the experimental reach. Stream discharge data were obtained from the United States Geological Survey Oak Creek weather station. Three replicate water samples (~4 L each) were collected upstream from the experimental reach on day 13 of litterbag incubation and analyzed for NO_3^- and DOC concentration and isotope composition. Water was filtered through a 0.2-µm Acrodisk filter and analyzed colorimetrically for NH_4^+ and NO_3^- concentration using an autoanalyzer (Lachat Quickchem FIA+ 8000; Lachat Instruments, Milwaukee, WI). DOC concentration was analyzed by the persulfate oxidation method with an OI Analytical Model 1,010 Total Carbon Analyzer connected to a Delta Plus Advantage IRMS. The $\delta^{15}N$ of NO_3^- was determined by reduction to N_2O followed by coupled gas chromatography (Thermo Finnigan Precon and Delta Advantage IRMS), using the bacteria denitrification method (Casciotti et al. 2002) at CPSIL. The $\delta^{15}N$ of NH_4^+ was determined using the ammonia-diffusion method (Holmes et al. 1998).

Laboratory analysis

Litter was removed from the litterbags, rinsed with deionized water, and wet mass was recorded. At each harvest date, litter content from the three replicate bags was pooled and homogenized, resulting in five composite samples for *P. fremontii* and ten for *P. angustifolia*. Each composite sample was subsequently split into two subsamples, one for bulk-litter elemental analysis (~1 g wet weight), and the rest for determination of microbial biomass.

Percent moisture of litter was determined for bulk litter by weighing subsamples before and after oven drying at 60 °C for 24 h. Dried litter was ground with a mortar and pestle to a fine powder and was analyzed for C and N content and isotopic composition at CPSIL, as described above. Subsamples for microbial biomass determination were processed using an adaptation of the chloroform fumigation-extraction technique, originally developed for soils (Brookes et al. 1985; Vance et al. 1987) and later modified for stream detritus (Mulholland et al. 2000; Sanzone et al. 2001; Cheever et al. 2013). Labile organic material was extracted from the litter with 50 mL of 0.05 M K_2SO_4, stored on ice overnight, shaken for 1 h, and centrifuged at 9,800 *g* for 10 min, after which the supernatant was poured off and discarded. Litter samples were then placed in glass beakers in a desiccator and fumigated with alcohol-free chloroform. The desiccator was 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 then removed from the desiccator, extracted with 50 mL of 0.05 M K_2SO_4, shaken for 1 h, and centrifuged at 9,800 *g* for 10 min. The supernatant was filtered through 1.2-µm filters (Supor Membrane; PALL Live Sciences, NY) and placed in a ventilated oven (60 °C) for 48 h. Dried K_2SO_4 salts from both pre- and post-fumigation samples were ground with a mortar and pestle to a fine powder, weighed, and analyzed for C and N elemental and isotope composition as described above. K_2SO_4 blanks routinely yield non-detectable C and N peaks using this method. To calculate immobilization rates, ^{15}N and ^{13}C isotopic values were expressed in atom % excess, correcting for the natural abundance of C and N isotopes, respectively; that is, ^{13}C atom (atom % excess) = $100 \times [^{13}C/(^{13}C + {}^{12}C)] - 1.1112$ % and ^{15}N atom (atom % excess) = $100 \times [^{15}N/(^{15}N + {}^{14}N)] - 0.3663$ %. For salt samples, the precision of the international standard NIST 2711 MT soil was $\pm 5.0 \times 10^{-7}$ atom % excess for ^{13}C and $\pm 6.9 \times 10^{-7}$ atom % excess for ^{15}N (SD of six replicate samples). The precision of the NIST peach leaves standard, when bulk litter samples were run, was $\pm 6.1 \times 10^{-6}$ atom % excess for ^{13}C and $\pm 2.5 \times 10^{-4}$ atom % excess for ^{15}N (SD of 32 replicate samples).

Parameter calculations

To characterize decomposition rates for litter types, we calculated the leaf litter decomposition rate constant (*k*, day^{-1}) as the slope of the log-transformed percentage of remaining mass over time for each *Populus* genotype (Benfield 2006). Microbial biomass, in terms of C (MB_C) and N (MB_N), was estimated using the C and N content in the fumigated

litter samples for each date. MB_C and MB_N were expressed per unit of litter mass (i.e., mg C or N g^{-1} litter) to compare results among harvest dates and between the two litter types. The change in isotopic and N isotope composition in the fumigated litter samples was used in a two-end member mixing model to quantify the relative contribution of leaf litter and water column C and N to microbial biomass (Phillips and Gregg 2003; Boecklen et al. 2011). For each harvest date, the percentage of C or N in microbial biomass derived from streamwater was calculated using the following equations:

$$\%\,C\text{streamwater source} = \frac{^{13}C\,\text{microorganisms} - ^{13}C\,\text{leaf}}{^{13}C\,\text{DOC} - ^{13}C\,\text{leaf}} \times 100 \quad (1)$$

$$\%\,N\text{streamwater source} = \frac{^{15}N\,\text{microorganisms} - ^{15}N\,\text{leaf}}{^{15}N\,NO_3^- - ^{15}N\,\text{leaf}} \times 100 \quad (2)$$

where ^{13}C and ^{15}N in microorganisms are the isotopic values (in atom %) of the chloroform-extracted fraction, ^{13}C and ^{15}N in leaves are the initial isotope values (in atom %) measured in the leaves, and ^{13}C DOC and ^{15}N NO_3^- are the isotope values (in atom %) measured in the streamwater samples (Table 1). Thus, the percentages of C and N in microbial biomass derived from leaf sources are the remaining percentages, i.e., % C leaf source = 100 % C streamwater source and % N leaf source = 100 % N streamwater source. Equation 2 assumes that the isotopic values of NO_3^- are similar to those of other streamwater N sources in relation to the high isotopic label of the leaf litter. This assumption is in agreement with the results of Eq. 2 using NH_4^+-^{15}N instead of NO_3^--^{15}N, which did not significantly alter the results.

To quantify C and N fluxes from the water column into the biofilm-litter system on each harvest date, gross immobilization (GI) rates of C (GI_C; mg C g^{-1} litter day^{-1}) and N (GI_N; mg N g^{-1} litter day^{-1}) were calculated using the isotope pool dilution method (Kirkham and Bartholomew 1954; Hart et al. 1994):

$$GI_{(\Delta t)} = \frac{M_i - M_f}{t_f - t_i} \times \frac{\log_{10}\frac{M_{i\ at\%\ excess}}{M_{f\ at\%\ excess}}}{\log_{10}\frac{M_i}{M_f}} \quad (3)$$

where M is the mass of C or N in the biofilm-litter system at the initial (t_i) and final (t_f) time (in mg C or N g^{-1} litter) and $M_{i\ at\%\ excess}$ and $M_{f\ at\%\ excess}$ are the ^{15}N or ^{13}C atom % excess of the biofilm-litter system for the same interval. Therefore, the measurement of GI is based on the dilution of the isotope composition of the biofilm-litter system over time as a reflection of the import of C and N from the unlabeled water column pools. In order to integrate the isotopic data from all harvests, we generated linear models of decay of ^{15}N and ^{13}C isotope for each replicate, and then used the measured isotopic values of the litter (atom % excess) for the initial and final pool, for the interval of time studied, as input into Eq. 3. Finally, GI rates of C and N were standardized by MB_C and MB_N, respectively, as a measurement of microbial efficiency for GI of these elements, which can be compared between litter types and among sampling dates.

Data analysis

Parameters were calculated for each cottonwood genotype; each genotype at each sampling date was treated as a replicate for each species. Two-group Student's *t*-tests were used to compare values of k between litter types. Analyses of covariance (ANCOVAs) were used to analyze the effect of litter type, with harvest days as an additive covariate, on MB_C and MB_N, % of microbial mass derived from the leaf source for both C and N, and GI_C and GI_N. To test for differences between litter types in the GI_C:GI_N, we bootstrapped the difference of these ratios between litter types (1,000 iterations for each time point and species), determined the 95 % confidence intervals (CIs) for this difference, and determined whether it overlapped with zero. The correlation between the ratio GI_C:GI_N and microbial C:N was tested using Spearman correlation. The Resample Stat add-in for Excel software was used for the bootstrapping procedure (http://www.resample.com/excel/). All other statistical analyses were conducted using R, version 2.15.1 (R Development Core Team 2012).

Results

Decomposition rates and microbial biomass

As expected, leaf litter decomposition rate was 1.5 times higher for low-tannin litter (*P. fremontii*) than high-tannin leaf litter (*P. angustifolia;* Table 2). For the two litter types, microbial biomass, either measured as C or N biomass, increased until day 13, but then leveled off (Fig. 1a, b). Microbial C:N showed the opposite pattern, decreasing sharply at the second harvest, then leveled off. Microbial biomass (both as MB_C and MB_N) per gram of litter was higher in litter with a low tannin content than in litter with a high tannin content (ANCOVA, MB_C $F_{1,42} = 32.09$, $P < 0.0001$; MB_N $F_{1,42} = 38.38$, $P < 0.0001$; Fig. 1a, b). In contrast, microbial C:N was higher in *P. angustifolia* litter than in *P. fremontii* litter (ANCOVA, $F_{1,42} = 12.39$, $P < 0.01$; Fig. 1c). Microbial C accounted for 2.7–8.4 % of the total litter C pool in *P. fremontii* litter and between 1.1 and 5.3 % for *P. angustifolia* litter. Microbial N represented

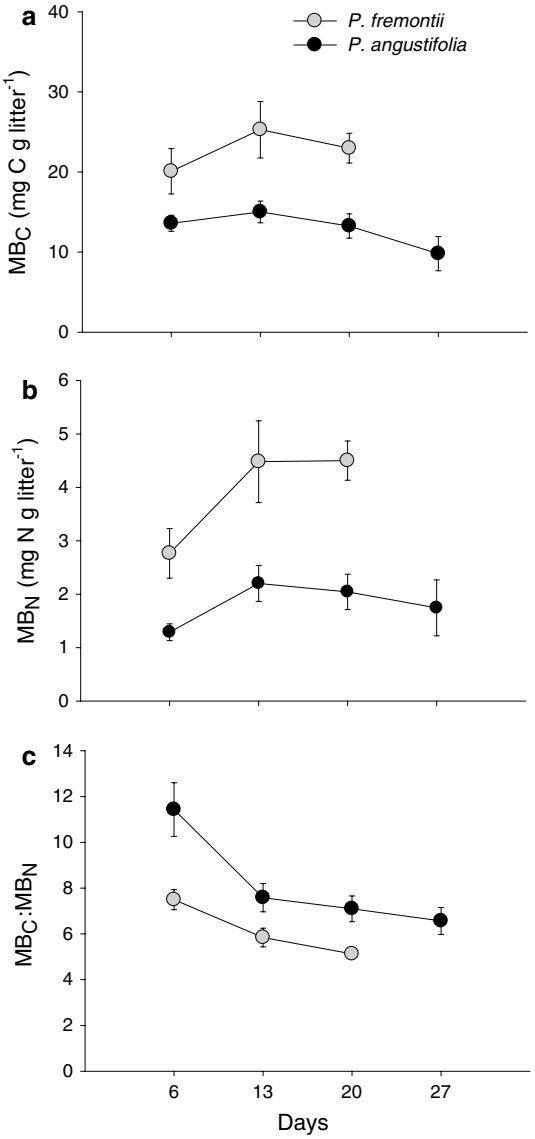

Fig. 1 Temporal variation of microbial biomass (MB) C (MB_C; **a**), N (MB_N; **b**) and C:N mass ratio (**c**) during the leaf litter decomposition period for *Populus fremontii* (*grey circles*; $n = 5$) and *Populus angustifolia* (*black circles*; $n = 10$). *Data points* are means and *vertical bars* represent SEs

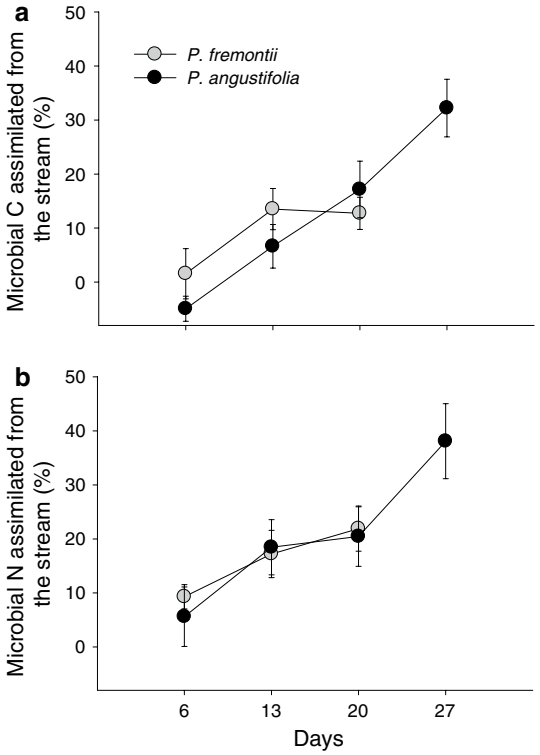

Fig. 2 Temporal variation of the percentage of C (**a**) and N (**b**) in the microbial assemblage that is derived from the streamwater for *P. fremontii* (*grey circles*; $n = 5$) and *P. angustifolia* (*black circles*; $n = 10$). Due to method error, some percentages are lower than 0 %. *Data points* are means and *vertical bars* represent SEs

between 4.7 and 18.5 % for *P. fremontii* litter and between 2.1 and 15.0 % for *P. angustifolia* litter.

Contribution of microbial C and N from the streamwater

The proportion of C and N derived from streamwater increased during the incubation, and at the last harvest (27 days) accounted for 32 % for C and 38 % for N (average values for *P. angustifolia*) of the microbial biomass (Fig. 2). For both litter types, and averaged over the duration of the experiment, leaf litter was the major source of C and N for the growth of microbial assemblages (average over the two litter types: 89 ± 2 % for C, 81 ± 3 % for N). We did not find significant differences between leaf types in the percentages of C and N in microbial mass that were derived from the streamwater (ANCOVA: $P > 0.05$).

GI_C and GI_N from streamwater into the biofilm-litter system

GI_C was, on average, almost two times higher for *P. angustifolia* litter ($GI_C = 3.79 \pm 0.41$ mg C g^{-1} litter day^{-1}) than for *P. fremontii* litter ($GI_C = 1.94 \pm 0.59$ mg N g^{-1} litter day^{-1}; Fig. 3a; ANCOVA, $F_{1,42} = 5.55$, $P < 0.05$). The pattern reversed for GI_N, which was, on average, two times higher for *P. fremontii* litter ($GI_N = 0.08 \pm 0.02$ mg N g^{-1} litter day^{-1}) compared to *P. angustifolia* litter ($GI_N = 0.16 \pm 0.02$ mg C g^{-1} litter day^{-1}; Fig. 3b; ANCOVA, $F_{1,42} = 6.82$, $P < 0.05$). The ratio between GI_C and GI_N was significantly higher for *P. angustifolia* litter (on average 35.2) than for *P. fremontii* litter (on average 13.9) (95 % CI for the difference, 6.5–59.6; Fig. 3c). GI_C standardized by the microbial C content was nearly three times higher for *P. angustifolia* litter (mean 0.40 ± 0.06 mg C mg^{-1} MB_C day^{-1}) than for *P. fremontii*

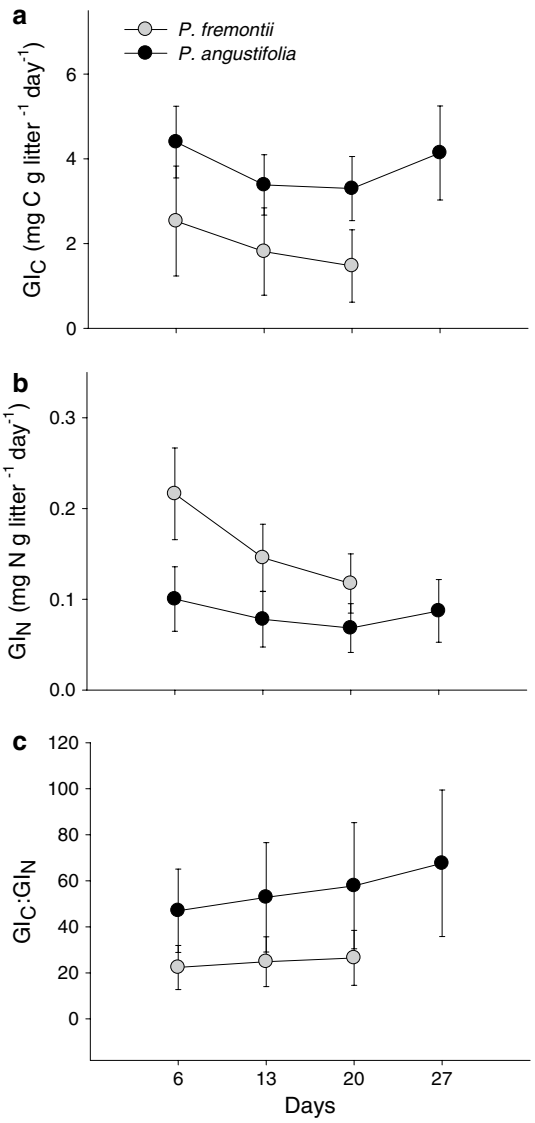

Fig. 3 Temporal variation of gross immobilization (GI) rates for C (GI_C; **a**) N (GI_N; **b**) and their stoichiometric relationship (**c**) for *P. fremontii* (*grey circles*; $n = 5$) and *P. angustifolia* (*black circles*; $n = 10$). *Data points* are means and *vertical bars* represent SEs

litter (mean 0.12 ± 0.05 mg C mg^{-1} MB_C day^{-1}; ANCOVA, $F_{1,42} = 7.09$, $P < 0.05$). In contrast, there was no difference between litter types for GI_N standardized by the microbial N content (average for both species 0.07 ± 0.01 mg N mg^{-1} MB_N day^{-1}; ANCOVA, $P > 0.05$). Finally, microbial GI_C:GI_N was not related to microbial C:N for either litter species (Spearman correlation; $P > 0.05$).

Discussion

The main goal of this study was to understand the biogeochemical interactions between the biofilm litter system and the streamwater during litter decomposition. Our results indicated that litter phytochemical characteristics had a strong effect on the biomass and stoichiometry of microorganisms growing on litter. The strong species differences in C and N immobilization from the water column into the litter biofilm (Fig. 3) suggest that microbial assemblages on these species differ in C and N demand, and that these differences are strongly influenced by leaf litter characteristics.

Litter decomposition and microbial biomass

Decomposition rates were higher for *P. fremontii* than *P. angustifolia*, as previously shown (Driebe and Whitham 2000; LeRoy et al. 2006; Holeski et al. 2012). Leaf litter with a lower content of recalcitrant compounds (*P. fremontii*) accrued more microbial biomass compared to litter with a higher content of recalcitrant compound (*P. angustifolia*). This finding is in agreement with results from previous studies where recalcitrant litter types showed low microbial biomass accrual (Gulis and Suberkropp 2003; Talbot and Treseder 2012; but see LeRoy et al. 2007). In addition, elemental stoichiometry of biofilms differed between litter types, with higher C:N values for high-tannin litter. Differences in C:N ratio among microbial biofilms on litter might be explained by differences in the composition of the microbial assemblage, as fungal mycelia often have higher C:N ratios than bacteria (Sterner and Elser 2002; Strickland and Rousk 2010). Leaf-associated fungal biomass typically exceeds bacterial biomass (Gessner 1997; Findlay et al. 2002), and our results suggest that this pattern is stronger for biofilms on high-tannin compared to biofilms on low-tannin litter. This fact is further supported by a related study on decomposing cottonwood litter in the same stream reach where quantitative polymerase chain reaction results revealed a higher fungi:bacteria gene abundance ratio for *P. angustifolia* than for *P. fremontii* (Wymore et al. 2013). Fungi may be better competitors than bacteria in more recalcitrant leaves due to their hyphal networks and enzymatic capabilities to break down recalcitrant materials compared to bacteria (Kohlmeier et al. 2005; De Boer et al. 2005; Moorhead and Sinsabaugh 2006; Romaní et al. 2006).

The relative contribution of C and N from streamwater during decomposition

The reliance on elemental resources from streamwater by biofilms was low at the beginning of the decomposition process and increased with time for both litter types, probably as labile compounds were consumed by microbes or leached out of the leaves. The fact that immobilization fluxes and microbial biomass remained fairly constant

over time would explain the linear increase of the contribution of C and N from the streamwater. Previous studies reported similar patterns. For example, Cheever et al. (2013) showed that microorganisms colonizing decomposing leaves acquired more N from the streamwater during late decomposition stages compared to early stages, deriving up to 80–90 % of N from the water column by the end of the decomposition experiment (i.e., 12–15 weeks). In other systems, such as a N-rich estuary, microbial assimilation of DIN into particulate organic material also increased with time, reaching nearly 70 % by the end of the experiment (Caraco et al. 1998). Information about microbial reliance on streamwater for C is mostly limited to microorganisms in sediments, where DOC has been estimated to support up to half of their metabolism (Findlay et al. 1993; Fischer et al. 2002; Sobczak and Findlay 2002; Wiegner et al. 2005). Considering C and N together, our data suggest that N derived from streamwater is an important supplement for microbial growth, supporting the common assumption that stoichiometric constraints faced by microorganisms growing on litter would require N from streamwater (Sterner and Elser 2002). Our data also indicate that streamwater organic C is an important source for microbial communities, almost as important as N. The importance of stream C supplements during leaf litter decomposition is surprising (Cheever et al. 2013) and suggests high microbial reliance on multiple streamwater resources during decomposition.

Immobilization of C and N into the biofilm-litter system: contrasting patterns between litter types

Leaf species differed in the stoichiometry of C and N fluxes from the streamwater to the biofilm-litter system. Gross immobilization rates of N were higher on low-tannin litter compared to litter with high-tannin content, contrary to our expectations, probably because a higher content of recalcitrant compounds in the latter slowed down microbial growth and consequently reduced N demand from the streamwater. This argument is supported by specific rates of N immobilization (per unit microbial biomass) which did not differ between litter types.

In contrast, gross immobilization rates of C were higher in high-tannin litter, even when standardized by microbial biomass, indicating higher import of C from streamwater into the biofilm-litter compartment in high-tannin litter. Because tannin compounds may form relatively recalcitrant complexes, it is reasonable to think that C in the high-tannin litter is a less accessible resource, such that microorganisms obtain C more efficiently from the water column. Thus, observations for the C immobilization rate support our hypothesis that the concentration of recalcitrant compounds in litter would increase the dependence on streamwater by microbial biofilms. Furthermore, the decoupling between immobilization C:N from streamwater and microbial C:N further suggested that the interaction of biofilm-litter with streamwater is dependent on contrasting C and N contributions of the leaf litter resource.

Despite the fact that heterogeneous isotopic label in the leaf litter could have increased uncertainty of our immobilization estimations, differences between species were higher, indicating the robustness of the patterns found. Moreover, our estimations might have also included other inputs of C and N besides active uptake by microbes, such as abiotic chemical adsorption and deposition. Despite these pitfalls, the isotopic dilution observed over time suggested the relevance of the biotic uptake over these other processes. Because algae might also have an active role in litter decomposition (Danger et al. 2013; Rier et al. 2014), photosynthesis activity would have resulted in overestimations of microbial immobilization. Even so, severe shading due to reach geomorphology likely limited algae activity (J. Marks, personal observation), diminishing the influence of algae on our estimates of immobilization. Thus, the application of the isotope pool dilution method with labeled litter proved successful and enabled us to discern contrasting patterns in element immobilization fluxes during the decomposition stages of different leaf litter.

Ecological implications

Cottonwoods are dominant in riparian zones of the western United States, providing more than 80 % of the litter to these streams (Driebe and Whitham 2000). They are often considered foundation species due to their large effects on ecosystem structure and function (Whitham et al. 2006); specifically, the influence of recalcitrant compounds of *Populus* litter has significant impacts on C and N dynamics within terrestrial ecosystems (Schweitzer et al. 2004, 2008). Our findings extend these ideas, demonstrating that closely related tree species differentially modulate elemental fluxes in streamwater during decomposition, with initial litter characteristics likely driving nutrient cycling during decomposition (Parton et al. 2007). Furthermore, these differences are likely to persist as decomposition progresses, because the relative composition of recalcitrant compounds likely increases due to preferential loss of leachable and more labile compounds during decomposition (Z. Compson, unpublished data).

Plant litter is often not a readily available resource for consumers. Therefore, microbial decomposers establish biogeochemical interactions with their environment, whether streamwater or soil, to supplement deficiencies in C and nutrients, especially when the resource is relatively recalcitrant (Parton et al. 2007). Understanding these biogeochemical interactions with litter should provide insights

into nutrient retention, which are responsible for the breakdown, nutrient transfer, and transport of this resource.

Interactions between litter and its environment are especially relevant in forested headwater streams, because these ecosystems are energetically dependent on detrital inputs arriving from the adjacent terrestrial ecosystem (Fisher and Likens 1973; Vannote et al. 1980). Moreover, they are key sites for nutrient retention and transformation along the stream continuum where inorganic N uptake rates often account for more than half of the total input arriving from the watershed (Peterson et al. 2001). Overall, our results indicate that litter characteristics of two cottonwood species drive specific streamwater element requirements of biofilms, suggesting that changes in the proportion of inputs arriving into the streams of these two cottonwood species strongly control stream cycling and export downstream.

Acknowledgments We thank the Marks, Sabater and Martí labs for their support and feedback on this study. Dr Susana Bernal, Dr Bob Hall and two anonymous reviewers provided helpful comments on an early draft of this manuscript. The Coconino Forest Service provided us with access to sites near Oak Creek. The National Science Foundation provided funding through the Frontiers in Integrative Biological Research (DEB-0425908), Integrative Graduate Education and Research Traineeship (DGE-0549505), and Ecosystem Studies (DEB-1120343) research programs. Funding was also provided by the MED-FORESTSTREAMS (CGL2011-30590-C02-01) project. A. P. was supported by a Formación de Personal Investigador Ph.D. fellowship from the Spanish Ministry of Science and Innovation within the context of ISONEF (CGL2008-05504-C02-01).

References

Argerich A, Martí E, Sabater F, Ribot M, von Schiller D, Riera JL (2008) Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall. Limnol Oceanogr 53:631–641

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS, Mart E, Packman AI, Newbold JD, Sabater F (2009) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 2:595

Benfield EF (2006) Decompostion of leaf material. In: Hauer FR, Lamberti GA (eds) Methods in stream ecology. Academic Press, Amsterdam, pp 711–720

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42: 411–440

Bott TL, Kaplan LA, Kuserk FT (1984) Benthic bacterial biomass supported by streamwater dissolved organic matter. Microb Ecol 10:335–344

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Caraco NF, Lampman G, Cole JJ et al (1998) Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies. Mar Ecol Prog Ser 167:59–71

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365

Casciotti KL, Sigman DM, Galanter Hastings M, Böhlke K, Hilkert A (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74:4905–4912

Cheever BM, Webster JR, Bilger EE, Thomas SA (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. Ecology 94:1614–1625

Compson Z, Hungate B, Koch G, Hart S, Maestas J, Adams K, Whitham T, Marks J (2014) Closely related tree species differentially influence the transfer of carbon and nitrogen from leaf litter up the aquatic food web. Ecosystems (in revision)

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Díaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065–1071

Danger M, Cornut J, Chauvet E, Chavez P, Elger A, Lecerf A (2013) Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? Ecology 94:1604–1613

De Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811

Dodds WK, Martí E, Tank JL, Pontius J, Hamilton SK, Grimm NB, Bowden WB, McDowell WH, Peterson BJ, Valett HM, Webster JR, Gregory S (2004) Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. Oecologia 140:458–467

Driebe EM, Whitham TG (2000) Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia 123:99–107

Enriquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457–471

Ferreira V, Castagneyrol B, Koricheva, Gulis JV, Chauvet E, and Graça MAS (2014) A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Biol Rev (in press)

Findlay S, Strayer D, Goumbala C, Gould K (1993) Metabolism of streamwater dissolved organic carbon in the shallow hyporheic zone. Limnol Oceanogr 38:1493–1499

Findlay S, Tank JL, Valett HM, Mulholland PJ, McDowell WH, Johnson SL, Hamilton S, Edmonds J, Dodds WK, Bowden WB (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb Ecol 43:55–66

Fischer H, Sachse A, Steinberg CEW, Pusch M (2002) Differential retention and utilization of dissolved organic carbon by bacteria in river sediments. Limnol Oceanogr 47:1702–1711

Fisher SG, Likens GE (1973) Energy flow in bear brook, new hampshire: an integrative approach to stream ecosystem metabolism. Ecol Monogr 43:421–439

Frossard A, Gerull L, Mutz M, Gessner MO (2013) Litter supply as a driver of microbial activity and community structure on decomposing leaves: a test in experimental streams. Appl Environ Microbiol 79:4965–4973

Gessner MO (1997) Fungal biomass, production and sporulation associated with particulate organic matter in streams. Limnetica 13:33–44

Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S (2010) Diversity meets decomposition. Trends Ecol Evol 25:372–380

Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. Nature 451:293–296
Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biol 48:123–134
Hart SC, Nason GE, Myrold DD, Perry DA (1994) Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. Ecology 75:880–891
Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. Oecologia 170:695–707
Holmes RM, McClelland JW, Sigman DM, Fry B, Peterson BJ (1998) Measuring $^{15}N-NH_4^+$ in marine, estuarine and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar Chem 60:235–243
Kaplan LA, Wiegner TN, Newbold JD, Ostrom PH, Gandhi H (2008) Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. Freshwater Biol 53:855–864
Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Am Proc 18:33–34
Kohlmeier S, Smits THM, Ford RM, Keel C, Harms H, Wick LY (2005) Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. Environ Sci Technol 39:4640–4646
LeRoy CJ, Whitham TG, Keim P, Marks JC (2006) Plant genes link forests and streams. Ecology 87:255–261
LeRoy CJ, Whitham TG, Wooley SC, Marks JC (2007) Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. J North Am Benthol Soc 26:426–438
Melillo JM, Naiman RJ, Aber JD, Linkins AE (1984) Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bull Mar Sci 35:341–356
Meyer JL, Johnson C (1983) The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. Freshwater Biol 13:177–183
Meyer JL, Wallace JB, Eggert SL (1998) Leaf litter as a source of dissolved organic carbon in streams. Ecosystems 1:240–249
Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76:151–174
Mulholland PJ, Newbold JD, Elwood JW, Ferren LA, Webster JR (1985) Phosphorus spiralling in a woodland stream: seasonal variations. Ecology 66:1012–1023
Mulholland PJ, Tank JL, Sanzone DM, Wollheim WM, Peterson BJ, Webster JR, Meyer JL (2000) Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecol Monogr 70:471–493
Murphy DV, Recous S, Stockdale EA, Fillery IRP, Jensen LS, Hatch DJ, Goulding KWT (2003) Gross nitrogen fluxes in soil: theory, measurement and application of 15 N pool dilution techniques. Adv Agron 79:69–118
Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–364
Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Martí E, Bowden WD, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD (2001) Control of nitrogen export from watersheds by headwater streams. Science 292:86–90
Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261–269
R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org
Rier ST, Shirvinski JM, Kinek KC (2014) In situ light and phosphorus manipulations reveal potential role of biofilm algae in enhancing enzyme-mediated decomposition of organic matter in streams. Freshwater Biol 59:1039–1051
Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569
Sanzone DM, Tank JL, Meyer JL, Mulholland PJ, Findlay SEG (2001) Microbial incorporation of nitrogen in stream detritus. Hydrobiologia 464:27–35
Schweitzer JA, Bailey JK, Rehill BJ, et al. (2004) Genetically based trait in a dominant tree affects ecosystem processes. Ecol Lett 7:127–134
Schweitzer JA, Madritch MD, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG (2008) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a Populus model system. Ecosystems 11:1005–1020
Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, Weiland T (1993) Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74:1586–1593
Sobczak WV, Findlay S (2002) Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpaths. Ecology 83:3194–3209
Stelzer RS, Heffernan J, Likens GE (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshwater Biol 48:1925–1937
Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton
Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in soils—methods, controls, and ecosystem implications. Soil Biol Biochem 42:1385–1395
Suberkropp K, Chauvet E (1995) Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76:1433–1445
Talbot JM, Treseder KK (2012) Interactions among lignin, cellulose, and nitrogen drive litter chemistry-decay relationships. Ecology 93:345–354
Tank JL, Meyer JL, Sanzone DM, Mulholland PJ, Webster JR, Peterson BJ, Wolheim WM, Leonard NE (2000) Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N-tracer addition. Limnol Oceanogr 45:1013–1029
Tank JL, Rosi-Marshall EJ, Griffiths NA, Entrekin SA, Stephen ML (2010) A review of allochthonous organic matter dynamics and metabolism in streams. J North Am Benthol Soc 29:118–146
Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70:97–104
Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ, Dillon P, Finlay K, Fortino K, Knoll LB, Kortelainen PL, Kutser T, Larsen S, Laurion I, Leech DM, McCallister SL, McKnight DM, Melack JM, Overholt E, Porter JA, Prairie Y, Renwick WH, Roland F, Sherman BS, Schindler DW, Sobek S, Tremblay A, Vanni MJ, Verschoor AM, von Wachenfeldt E, Weyhenmeyer GA (2009) Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr 54:2298–2314
Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707
Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137
Wallace JB, Eggert SL, Meyer JL, Webster JR (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277(80):102–104

Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17:567–594

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks JC, Hart SC, Wimp GM, Wooley SC (2006) A framework for community and ecosystem genetics: from genes to ecosystems. Nat Rev Genet 7:510–523

Wiegner TN, Kaplan LA, Newbold JD (2005) Contribution of dissolved organic C to stream metabolism: a mesocosm study using ^{13}C-enriched tree-tissue leachate. J North Am Benthol Soc 24:48–67

Woodward G, Gessner MO, Giller PS, Gulis V, Hladyz S, Lecerf A, Malmqvist B, McKie BG, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graça MAS, Fleituch T, Lacoursière JO, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A, Vought LB-M, Chauvet E (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336:1438–1440

Wymore AS, Compson ZG, Liu CM, Price LB, Whitham TG, Keim P, Marks JC (2013) Contrasting rRNA gene abundance patterns for aquatic fungi and bacteria in response to leaf-litter chemistry. Freshwater Sci 32:663–672