Contributions of autochthonous, allochthonous, and aged carbon and organic matter to macroinvertebrate nutrition in the Susquehanna River basin

Amber R. Bellamy[1,3], James E. Bauer[1,4], and Andrea G. Grottoli[2,5]

[1]Aquatic Biogeochemistry Laboratory, Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, Ohio 43210 USA
[2]School of Earth Sciences, Ohio State University, Columbus, Ohio 43210 USA

Abstract: Previous research suggests that rivers transport large amounts of aged allochthonous material, but it is unclear how much of this material contributes nutritionally to macroinvertebrate biomass. Furthermore, little research has investigated allochthonous vs autochthonous nutritional resource use by macroinvertebrates in the tributaries and low-order streams of major river systems. The primary goal of this study was to quantify both the sources and ages of organic matter (OM) that contributes to the nutrition of stream macroinvertebrates in different functional feeding groups (FFGs). We used natural abundance stable (δ^{13}C, δ^{15}N, and δ^2H) and radiogenic (Δ^{14}C) isotopes to assess resource use in a network of 7 subwatershed streams in the Susquehanna River basin. Isotope mixing models revealed that autochthonous OM contributed as much as 80% of macroinvertebrate biomass for scraper, filtering collector, and collector–gatherer FFGs, whereas allochthonous sources contributed much less (6–44%) to macroinvertebrate biomass of these FFGs. However, allochthonous OM contributed up to 73% of shredder FFG biomass. Contributions of aged (ranging from 75–11,700 y B.P.) OM to macroinvertebrate biomass varied by macroinvertebrate group. Chironomids assimilated the most aged OM (43%) of any macroinvertebrate group, most of which was from soil and sediment sources. We also found that macroinvertebrates in a stream with active methane seepage had the oldest C in their tissues of the streams in this study (5600–4200 y B.P.), which was probably a result of the macroinvertebrates consuming C derived from this methane seep. Our findings show that the sources and ages of OM assimilated by macroinvertebrates largely varied as a function of FFG and that these differences probably resulted from FFG habitats and diet preferences, as well as the presence or absence of ancient forms of C in streams.

Key words: macroinvertebrates, stable isotopes, radiocarbon, nutritional sources, autochthony, aged organic matter

Our understanding of the roles of allochthonous vs autochthonous organic matter (OM) to stream food webs and the roles that watershed land use, hydrology, and temporal variability play in the relative importance of these OM types has advanced significantly over the past few decades. Accurate assessment of nutritional resource use by stream macroinvertebrates is challenging because of heterogeneity in physical characteristics of streams, materials they transport (Muehlbauer et al. 2014), and land use and other anthropogenic activities (Lambert et al. 2017, Page et al. 2017). Habitat heterogeneity can influence the amount of autochthonous and allochthonous OM available to aquatic consumers, as can consumer functional feeding group (FFG) characteristics

based on behavioral and morphological adaptations associated with mechanisms of acquiring nutrition (Cummins and Klug 1979, Cummins 2016). Classifying stream macroinvertebrates by FFGs may therefore facilitate predictions of the relative importance of allochthonous vs autochthonous nutritional resources to these organisms.

Much of the recent research conducted in streams and tributaries of large rivers has focused on the roles of presumably contemporary or 'young' autochthonous (i.e., aquatic) production, allochthonous terrestrial leaf litter, and dissolved OM (DOM) to consumer (e.g., bacteria, invertebrates, and fish) secondary production (Wallace et al. 1997, 2015, Tank et al. 2010, Guo et al. 2016). Relatively little research has

studied whether old or ancient (hundreds to thousands of years old) OM, derived from soils and weathered sedimentary rocks, contributes to aquatic consumer nutrition either directly (i.e., via direct consumption) or indirectly (e.g., via microbial 'repackaging'). This dearth of research is likely a result of the assumption that aged materials are low quality food sources (Kleber et al. 2011, Marín-Spiotta et al. 2014, Guillemette et al. 2017). However, old and ancient forms of carbon (C) and OM dominate global C reservoirs and are transported to aquatic systems where they can be incorporated into stream and river DOM, particulate OM (POM), and dissolved inorganic carbon (DIC) (Hedges 1992, Hossler and Bauer 2012, Marwick et al. 2015). Thus, aged C and OM pools can potentially contribute significantly to consumer biomass and secondary production (see recent reviews by Bellamy and Bauer 2017, Guillemette et al. 2017, Larsen et al. 2018).

The simultaneous use of multiple stable isotopes and natural abundance ^{14}C allows for a robust assessment of OM sources, their ages, and their contributions to secondary production in aquatic systems. The natural abundances of ^{13}C and ^{15}N stable isotopes are frequently used as integrative and quantitative tracers of nutritional contributions to aquatic food webs (Finlay and Kendall 2007, Fry 2007). In addition, the stable isotope ratio of H (δ^2H) has a much larger dynamic range (up to 100 ‰ or more) than δ^{13}C and δ^{15}N values (typically a few to tens of ‰). Thus, δ^2H often better differentiates between terrestrial vegetation and algal primary producer biomass and detritus (Doucett et al. 2007). When δ^{13}C, δ^{15}N, and δ^2H are used simultaneously, estimates of consumer nutritional sources are typically more accurate and precise (Doucett et al. 2007, Finlay et al. 2010, Wilkinson et al. 2015). In comparison to stable isotopes, natural abundance radiocarbon (^{14}C), the radioactive isotope of C ($t_{1/2}$ = 5568 y), has an even greater dynamic range (i.e., Δ^{14}C range ≥ ~1000‰) than stable isotopes. However, ^{14}C also allows the measurement of ages (modern to ≥ 50,000 y B.P.) of different OM nutritional sources used by organisms (Stuiver and Polach 1977, Schell 1983, Caraco et al. 2010, Bellamy and Bauer 2017). Assimilated materials can range in age from modern (≥~0 y B.P.; i.e., terrestrial vegetation), to old (~100–1000 y B.P.; i.e., soil-derived OM), to ancient (≥~1000 y B.P.; i.e., shale-derived OM) (Bellamy and Bauer 2017). The ^{14}C age of aquatic primary producers reflects the age of the dissolved CO_2 (CO_2 [aq]) and DIC at the time of C fixation. Terrestrial vegetation is modern because it relies on present-day atmospheric CO_2 (Randerson et al. 2002), in contrast to soils and sediments that are derived from old terrestrial vegetation (~100–1000 y B.P.) (Schuur et al. 2016).

The sources and ages of OM and DIC within the main stem of the Susquehanna River have been studied previously and found to range from ~50 to −200‰ (DOM; modern– 1790 y B.P.), ~−60 to −170‰ (DIC; 500–1500 y B.P.), and ~−100 to −220‰ (POM; 850–2000 y B.P.) (Raymond et al.

2004, Hossler and Bauer 2013a, b). These variably-aged materials are transported by tributaries from various points within the watershed, but the role of autochthonous, allochthonous, and aged materials in the aquatic food webs of tributaries of the Susquehanna River have not yet been explored. We hypothesized that aged forms of OM may be both ingested and assimilated by macroinvertebrates in the tributaries of the Susquehanna River, as they are in other freshwater systems (Bellamy and Bauer 2017). In addition to providing information about the capacity of macroinvertebrates to use available nutritional resources, FFG classification may also help identify means for the handling and consumption of aged OM sources. The goals of the present study were to: 1) estimate the basal contributions of allochthonous and autochthonous OM sources to macroinvertebrate consumers in low-order tributaries of the Susquehanna River basin, 2) assess how these contributions and their ages may vary in different FFGs of stream macroinvertebrates, and 3) highlight the utility of measuring natural abundance ^{14}C in stream food web studies.

METHODS
Site description

We conducted this study in the Susquehanna River basin. We sampled 7 low- to mid-order (2nd–5th order) streams in different watersheds (Fig. 1) once per year in August 2011 and 2012, May 2013, and June 2014. Watersheds varied in size, canopy cover, and the percentage of agricultural land use (Table 1). Most of the streams were located in the temperate broadleaf and mixed forest ecoregion. Dominant tree genera included *Betula* spp. (birch), *Alnus* spp. (alder), *Fagus* spp. (beech), and *Acer* spp. (maple) (Zimmerman et al. 2012). Dominant soil types within these watersheds included inceptisols and ultisols (Blumberg and Cunningham 1982, NRCS USDA 2017). The methods we used to determine % agricultural land use (17–85%) are detailed in Appendix S1.1 and Table S1.1. We selected streams to represent the broad range of conditions within the Susquehanna River basin and sampled at 1 site in each stream (Table 1). We used a multi-year sampling approach to capture potential annual variability in the isotope signatures of sources and macroinvertebrates (Appendix S1.2).

Field sampling

We collected macroinvertebrates that belong to different FFGs from riffles, runs, and pools during base flow conditions at each site. Individual macroinvertebrates were collected both by hand from rocks and logs and with a kick net in areas with relatively abundant sediments and aquatic vegetation. We attempted to collect as many of the same macroinvertebrate genera and FFGs as possible across all sites at each sampling time. We separated predators and primary consumers, placed them in filtered (47-mm quartz fiber QMA
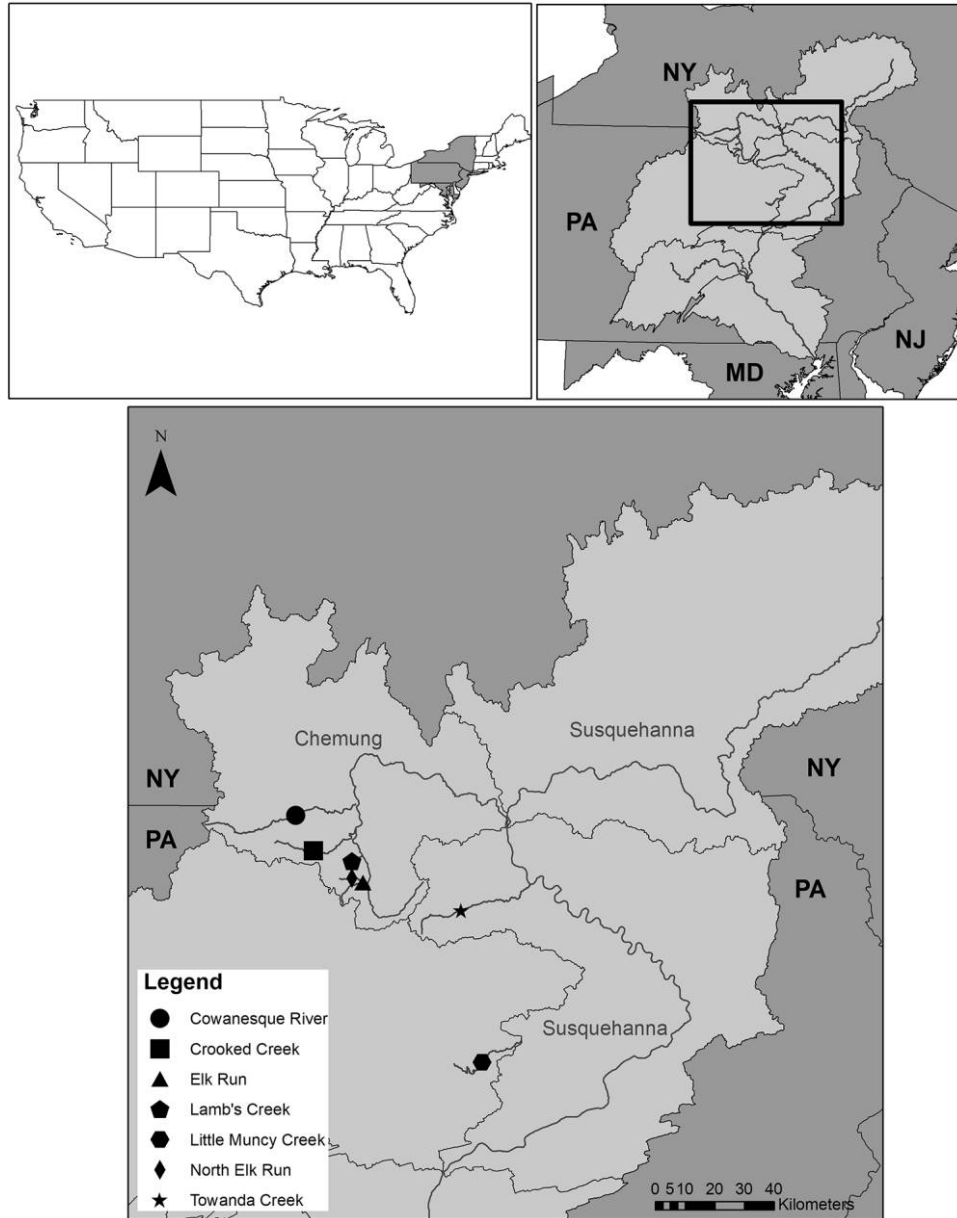
Figure 1. Sampling locations in the Susquehanna River basin (shown in light gray) in Pennsylvania (PA), USA. The main stems of the Chemung and Susquehanna Rivers as well as the tributary streams that were sampled are shown (heavy lines). The boundaries for 5 of the 6 major watersheds that comprise the Susquehanna River basin are also shown (light lines). NY = New York, NJ = New Jersey, MD = Maryland.

filter baked at 500°C) streamwater from their respective sites, and allowed them to void their guts for 24 h (Brooke et al. 1996). After 24 h, organisms were placed in baked (500°C) aluminum foil pouches and sealed plastic bags and frozen on dry ice until processing.

We also collected the dominant aquatic and terrestrial nutritional sources (i.e., aquatic and terrestrial vegetation, terrestrial soils, and stream sediments) available to macroinvertebrates at each stream every year, with the exception of algae in 2011 because it was not present in adequate quantities for isotope analyses (Appendix S1.3). We collected 3 to 4 samples each of aquatic and terrestrial veg-

etation (i.e., primarily filamentous algae and leaves from riparian trees and shrubs), 2 to 3 biofilm samples, and 2 stream sediment samples during each sampling period. We also collected 2 terrestrial soil samples (1 each from surface and ~20-cm depths) from each site in 2011, 2012, and 2013 (see details in Appendix S1.3). After collection we immediately placed potential nutritional sources in baked aluminum foil pouches and plastic bags and froze them on dry ice until we processed them in the lab. More specific details regarding the timing of sampling and the collection of additional samples and field measurements are described in Appendices S1.2–3.

Table 1. Sampling site and subwatershed characteristics of study streams within the Susquehanna River watershed. Means ± standard deviation (SD) for all years.

Sampling site	Dates sampled	Stream order[a]	Watershed area (km^2)	pH	Temperature (°C)	DO (mg/L)	Agricultural cover (%)	2014 Canopy cover (%)
Crooked Creek	2011–2014	4	110	8.1 ± 0.3	20.5 ± 2.6	9.3 ± 1.1	71	10
Cowanesque River	2011–2014	5	556	8.2 ± 0.4	24.6 ± 2.3	8.7 ± 0.2	52	0
Lamb's Creek	2011–2014	2	6	7.3 ± 0.3	16.3 ± 1.5	8.1 ± 0.2	17	80
North Elk Run	2011–2014	2	17	7.4 ± 0.2	21.0 ± 1.0	6.7 ± 2.6	85	66
Elk Run	2011–2014	3	65	8.5 ± 0.5	24.0 ± 2.6	8.9 ± 0.7	76	43
Little Muncy Creek	2011, 2013, 2014	5	113	7.5 ± 0.6	18.6 ± 1.3	8.9 ± 0.4	41	28
Towanda Creek	2012–2014	5	169	8.5 ± 0.4	23.7 ± 1.9	10.0 ± 1.0	61	31

[a] From the Susquehanna River Basin Coalition Water Resource Portal (available from: http://gis.srbc.net/wrp/)

Sample preparation and analyses

After returning to the lab, we sorted and identified macroinvertebrates to genus or to family when genus identification was not possible. When possible, organisms were assigned to the appropriate FFG following Merritt et al. (2008) (Table S1.2). However, chironomids were only identified to family and kept as a separate group because their FFG classification varies across subfamilies and genera (i.e., predatory Tanypodinae and non-predatory non-Tanypodinae chironomids [Reuss et al. 2013]). Macroinvertebrates, terrestrial vegetation, and aquatic vegetation were dried and ground to a fine powder with solvent-cleaned mortars and pestles in preparation for stable isotope and radiocarbon analyses. Small (<~1 mg dry tissue) macroinvertebrates from the same genus, site, and sampling period were pooled to ensure adequate sample amounts for isotope analysis (Table S1.2). Algae, biofilm, terrestrial soil, aquatic sediment samples, and POM-containing filters were acid-fumed with fresh concentrated HCl in a clean glass desiccator prior to homogenization to dissolve any carbonates within the samples. A subsample of each was set aside prior to acid fuming for δ^2H analysis. Each potential nutritional resource sample was analyzed (i.e., samples were not combined) for its isotope composition to ensure that the natural isotope variability of nutritional resources was optimized in the mixing model.

We measured the stable isotope content (as δ^{13}C, δ^{15}N, and δ^2H) of macroinvertebrates and their potential nutritional resources so we could quantitatively model nutritional source contributions to organisms. In preparation for analysis, we packed ground, homogenized samples and filters into tin capsules for δ^{13}C and δ^{15}N analyses. The δ^{13}C and δ^{15}N samples collected in 2011 and 2012 were measured at the Ohio State University (OSU) Stable Isotope Laboratory (SIBLab) with a Costech elemental analyzer connected to a Delta IV stable isotope ratio mass spectrometer (IRMS) via a ConFlo III interface. The δ^{13}C and δ^{15}N samples collected in 2013 and 2014 were measured at the University of California (UC) at Davis Stable Isotope Facility (SIF) with a PDZ Europa ANCA-GSL elemental analyzer

interfaced with a PDZ Europa 20-20 IRMS. Stable isotope values for δ^{13}C and δ^{15}N are reported relative to international standards V-PDB and air, respectively. The OSU SIBLab had an average standard deviation for replicate analyses of the USGS24 and IAEA-N2 standards of ±0.06 for δ^{13}C and ±0.15‰ for δ^{15}N. The average standard deviation for replicate analyses of sample duplicates at OSU SIBLab were ±0.10 for δ^{13}C and ±0.16‰ for δ^{15}N. The UC Davis SIF reported the maximum standard deviations for replicate analyses of bovine liver, USGS41, Nylon 5, glutamic acid, and peach leaf standards, which were ±0.28‰ for δ^{13}C and ±0.59‰ for δ^{15}N. The standard deviations for replicate analyses of selected duplicate samples analyzed by the SIF were ±0.09‰ for δ^{13}C and ±0.13‰ for δ^{15}N.

For δ^2H analysis we packed non-acidified, homogenized samples of macroinvertebrates, aquatic and terrestrial vegetation, soil, and sediments into silver capsules. We stored stream water samples in baked scintillation vials in a refrigerator for bulk δ^2H analysis. δ^2H samples were analyzed at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. To determine the δ^2H of the non-exchangeable H of solid samples, we used a bench-top equilibration method to estimate the exchange of H between the samples and local water vapor (Wassenaar and Hobson 2003, Doucett et al. 2007). Solid samples were analyzed for δ^2H on a Thermo–Finnigan TC/EA and DELTAplus-XL and stream water samples were analyzed for δ^2H with a Los Gatos Research DLT-100 Liquid Water Isotope Analyzer. The average standard deviation for replicate analyses of the keratin, caribou hoof, and kudo horn normalization standards ranged from ±1.1 to 3.0‰ depending on the reference material and the date samples were analyzed. The average standard deviation for replicate analyses of sample duplicates was ±2.7‰.

In addition to stable isotopes, we analyzed the natural abundance radiocarbon (^{14}C) content of a subset of organisms and potential nutritional resources as a separate tracer in our quantitative models. These analyses were used to

estimate the ages of nutritional sources assimilated by consumer organisms. For Δ^{14}C analyses, homogenized subsamples of selected macroinvertebrates and potential nutritional sources were placed in evacuated quartz tubes with Cu and CuO and combusted to CO_2 at 750° C for 4 h. Within 24 h of combustion, the CO_2 from each sealed tube was purified and quantified on a vacuum extraction line and sealed in a baked 6 mm Pyrex tube (Raymond and Bauer 2001). DIC samples were acidified and the CO_2 was collected and purified as above. Purified sample CO_2 was reduced to graphite and analyzed for Δ^{14}C at the NSF Arizona Accelerator Mass Spectrometry (AMS) Facility at the University of Arizona and at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) laboratory at Woods Hole Oceanographic Institution. Δ^{14}C was measured in selected samples collected from all sites except Lamb's Creek.

Statistical analyses

We used a non-parametric multivariate statistical approach to determine whether the isotope values of macroinvertebrates in different groups (FFGs and chironomids) were significantly different. We did these analyses in PRIMER with the PERMANOVA+ software package (Clarke and Gorley 2006, v. 6, PRIMER-E Ltd). Macroinvertebrate stable isotope values (δ^{13}C, δ^{15}N, and δ^2H) were normalized and patterns of variation among macroinvertebrate groups were visually evaluated with non-metric multidimensional scaling (NMDS). The stress value associated with NMDS plots is a measure of the goodness of fit between the true dissimilarity and the ordination distances of points, where stress <0.1 suggests an excellent fit and stress >0.3 suggests a poor fit to the data (Clarke 1993). The NMDS plot reflects the degree of isotopic similarity among macroinvertebrates as represented by distance in 2-dimensional space. Stable isotope values were correlated (Pearson's) with NMDS axes to facilitate interpretation of NMDS results by showing the direction and magnitude of influence for each isotope driving differences in the positions of macroinvertebrates in NMDS space. We did a 1-way permutational analysis of variance (PERMANOVA) on macroinvertebrate stable isotope values to establish whether macroinvertebrate groups had significantly different isotope values and we applied a Bonferroni correction for pairwise comparisons between macroinvertebrate groups.

Natural abundance radiocarbon (Δ^{14}C) measurements were not possible for all macroinvertebrate and potential nutritional resource samples because of sample size and other constraints. Therefore, a subset of macroinvertebrate isotope data that also included radiocarbon data (i.e., in addition to the stable isotopes) was normalized and evaluated visually with NMDS. We did an additional 1-way PERMANOVA to determine whether macroinvertebrate groups had significantly different isotope values in this subset of data and applied a Bonferroni correction for pairwise comparisons between macroinvertebrate groups. The 2 oldest macroinvertebrate tissue samples collected from the site at Little Muncy Creek in 2011 were extreme outliers, and one of these δ^2H measurements was of poor quality. We, therefore, excluded both samples from the statistical analyses and mixing models.

Isotope mixing models   We used the Bayesian isotope-mixing model *MixSIAR* (Stock and Semmens 2013) in R Statistical software (R Development Core Team 2014) to estimate the relative contributions of different potential nutritional sources to macroinvertebrate biomass. These models incorporate uncertainty in potential nutritional sources that results from isotopic variability of sources and consumers. Sources of uncertainty associated with isotope values include isotope fractionation, isotopic variability in potential nutritional sources, and consumer use of multiple nutritional sources (Finlay et al. 2002, Moore and Semmens 2008, Phillips et al. 2014). To determine the relative contributions of different potential nutritional sources to macroinvertebrate groups across the Susquehanna basin, we calculated yearly averages for each macroinvertebrate group collected from each site so that each macroinvertebrate group was equally weighted in the model. Potential nutritional resource isotope values used in the models were averaged across sites and years and included algae (modern–old), terrestrial vegetation (modern), and soil and sediment-derived OM (old–ancient) (Bellamy and Bauer 2017). We used isotope values from filamentous algae as the representative algal source in the mixing model. Filamentous algae are often considered unpalatable to macroinvertebrates, but their isotope values are similar to more palatable epilithic algae and the relationship of δ^{13}C fractionation to the availability of CO_2 is similar for filamentous and epilithic algae (Finlay 2004).

We used 2 mixing models for this analysis. The first mixing model used only the stable isotope data (δ^{13}C, δ^{15}N, and δ^2H). The 2nd model used a subset of the data with stable isotope and radiocarbon (Δ^{14}C) values from scrapers, filtering collectors, and predators. Chironomids and shredder and collector–gatherer FFGs were not included in this 2nd model because the number of Δ^{14}C values for these macroinvertebrate groups was too small to provide reliable results (i.e., $n = 3$, 2, and 3, respectively). For further details about mixing model procedures, including trophic fractionation and dietary water corrections, see Appendix S2.

RESULTS
Stable isotope and radiocarbon compositions of macroinvertebrate consumers

Macroinvertebrate δ^{15}N values ranged from 2.2 to 14.2‰ (Fig. 2A). Macroinvertebrate δ^{15}N values tended to be higher in streams with watersheds that had more agricultural cover (Table S3.1). Macroinvertebrate δ^{13}C and
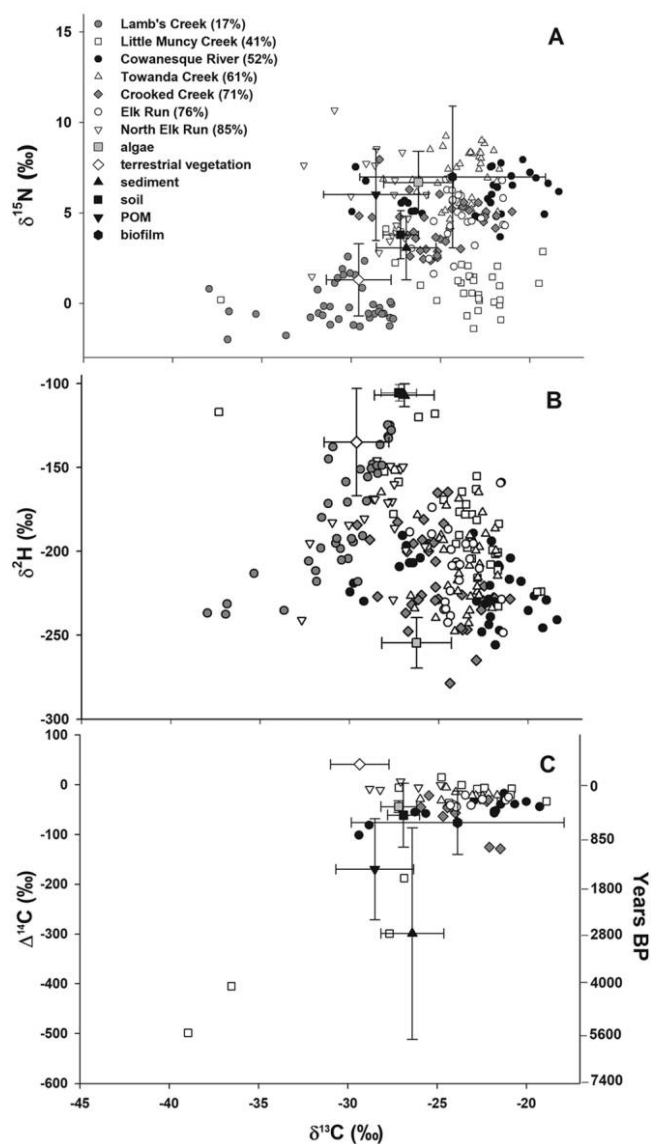
Figure 2. Biplots of $\delta^{15}N$ (A), δ^2H (B), and $\Delta^{14}C$ (C) vs $\delta^{13}C$ values of macroinvertebrate individuals and their potential nutritional sources (means ± SD) collected from 7 Susquehanna River basin streams. $\delta^{13}C$ and $\delta^{15}N$ values were corrected for trophic fractionation (Post 2002), and δ^2H values were corrected for the influence of dietary water (Wilkinson et al. 2015). In panel C, the 2 most ^{14}C-depleted macroinvertebrates from Little Muncy Creek are shown here but were not included in statistical or mixing model analyses. In the legend, percent agricultural land use in the watershed is shown in parentheses after stream name. POM = particulate organic matter.

δ^2H values ranged from −38.9 to −18‰ and −278.5 to −116.9‰, respectively, across all sites within the Susquehanna watershed (Fig. 2A, B). For the subset of samples with radiocarbon measurements, $\Delta^{14}C$ values ranged from −499 to 6‰ (Fig. 2C; Table S3.1). The lowest $\Delta^{14}C$ values measured in macroinvertebrates (equivalent ages of 5600 and 4200 y B.P.) were from Little Muncy Creek in 2011

when methane ebullition was observed, presumably because of extensive and ongoing hydraulic fracturing activities in this region (Entrekin et al. 2011, Osborn et al. 2011, Jackson et al. 2013). The most ^{14}C-enriched macroinvertebrates (equivalent to modern ^{14}C ages) were predators from North Elk Run in 2014 (Fig. 2C, Table S3.1). Within sites, predators were consistently the most ^{15}N-enriched macroinvertebrates (Table S3.1), whereas filtering collectors, scrapers, and in some cases collector–gatherers, had the lowest $\delta^{13}C$ and δ^2H values compared with the other FFGs (Table S3.1). There were no visually apparent patterns in $\Delta^{14}C$ values for different macroinvertebrate groups except that shredders ($n = 2$) were the most ^{14}C-enriched.

The NMDS plot showed that samples from different macroinvertebrate groups, especially shredders, clustered together in multiple isotope ($\delta^{13}C$, $\delta^{15}N$, and δ^2H) space (Fig. 3A). This pattern was consistent with the results of a 1-way PERMANOVA (Table S4.1). Stable isotope vectors
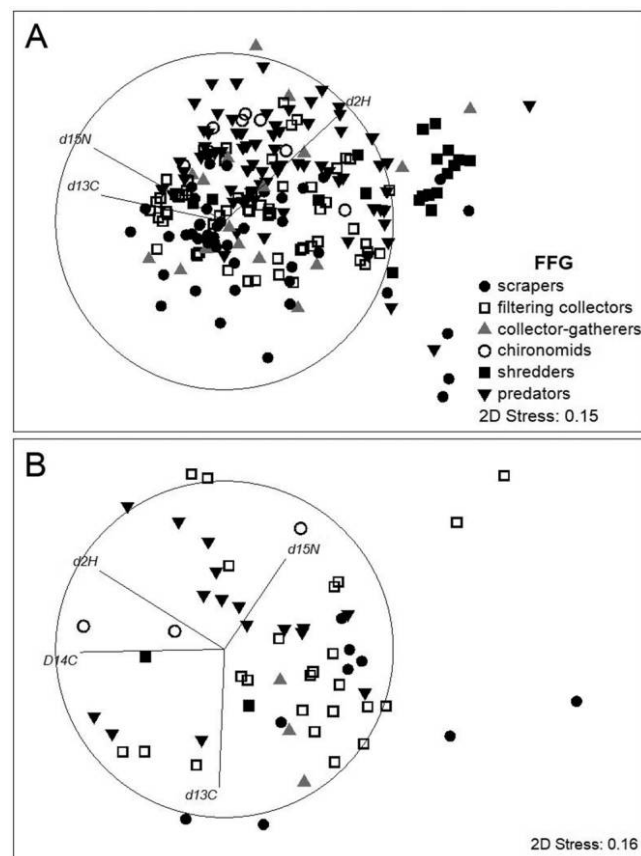

Figure 3. NMDS results showing joint variation in $\delta^{13}C$, $\delta^{15}N$, and δ^2H values in tissues of all macroinvertebrate individuals, which are identified by their functional feeding group (FFG) (A). Corresponding PERMANOVA results are given in Table S4.1. NMDS results for the subset of macroinvertebrate individuals with $\delta^{13}C$, $\delta^{15}N$, δ^2H, and $\Delta^{14}C$ values by FFG (B). Corresponding PERMANOVA results are given in Table S4.2. Direction and strength of variation in isotope values in ordination space are shown with vectors. The circle provides a reference for the vectors.

plotted onto the NMDS space suggested that separation of predators and shredders from other macroinvertebrate groups was primarily associated with variation in δ^2H values. Pairwise comparisons of isotope values of macroinvertebrate groups indicated that shredders differed from all other groups and that predators differed from all other groups except chironomids and collector–gatherers (Table S4.1). Additionally, filtering collectors and chironomids differed significantly in isotope values, as did scrapers and chironomids (Table S4.1). Inclusion of Δ^{14}C values with stable isotopes further showed that macroinvertebrate groups differed from each other isotopically and that scrapers were slightly more clustered than other groups (Fig. 3B, Table S4.2). Pairwise comparisons within the 1-way PERMANOVA analysis including δ^{13}C, δ^{15}N, δ^2H, and Δ^{14}C revealed significant differences between scrapers and filtering collectors and scrapers and predators (Table S4.2).

Nutritional source contribution estimates to macroinvertebrates from isotope mixing models

The Bayesian isotope mixing models that used only stable isotopes showed that autochthonous primary production (i.e., algae) contributed most to scraper, filtering collector, and collector–gatherer biomass (median of 68–74%) (Fig. 4, Table S5.1). Algae contributed a lower percent to chironomid and predator biomass (median of 44–51%) and even less to shredder biomass (median of 17%) (Fig. 4A). The variability associated with the estimates of algal contributions to macroinvertebrate biomass was smaller than that of terrestrial vegetation soil and sediment-derived OM (Fig. 4A–C). Allochthonous OM (i.e., terrestrial vegetation) substantially contributed to the biomass of shredders (73%) and predators (39%) (Fig. 4B, D). Additionally, soil and sediment-derived OM contributed substantially to chironomid biomass (43%) (Fig. 4C, D).

DIC δ^{13}C and Δ^{14}C values were significantly depleted compared to all other stream samples because of the active methane seepage in Little Muncy Creek in August 2011. Little Muncy DIC had δ^{13}C (−20.0 ± 0.2‰) and Δ^{14}C (−253 ± 4‰; equivalent ^{14}C age of 2340 y B.P.) values that were more depleted than the δ^{13}C (−10.7 ± 0.6‰) and Δ^{14}C (−18 ± 8‰; equivalent ^{14}C age of 150 y B.P.) DIC values we found in May 2013 and June 2014. The August 2011 DIC δ^{13}C and Δ^{14}C values from Little Muncy Creek were also lower by 10 and 171‰, respectively, compared to DIC collected from the Cowanesque River where no methane ebullition was observed. Unfortunately, algae was sparse in Little Muncy Creek in 2011, so we were unable to collect enough algae to determine if the depleted δ^{13}C and Δ^{14}C DIC values propagated to the algae contributed to macroinvertebrate biomass.

We found similar results for scrapers, filtering collectors, and predators when we repeated the mixing models on the subset of the samples that had ^{14}C measurements
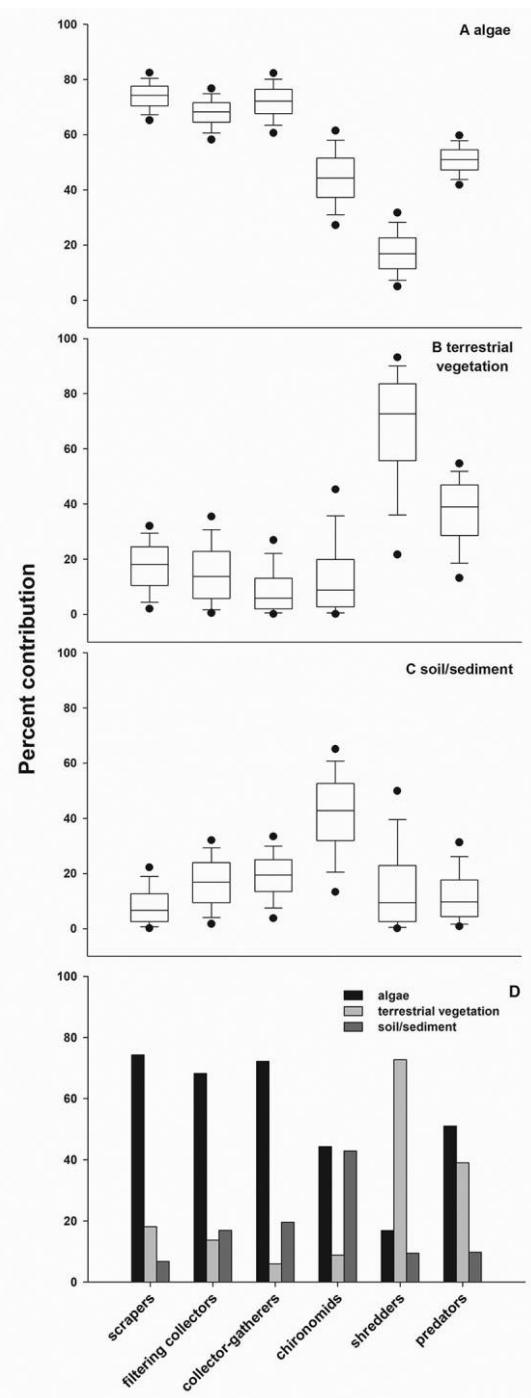

Figure 4. Posterior probability distributions of percent contribution estimates for algal (A), terrestrial vegetation (B), and soil/sediment organic matter (C) to macroinvertebrate biomass based on the isotope-mixing model across all study sites using δ^{13}C, δ^{15}N, and δ^2H. The box boundaries are the 25th and 75th percentiles, the line in the center of the box is the median, the whiskers are the 10th and 90th percentiles, and the dots are the 5th and 95th percentiles. Actual values are provided in Table S5.1. Panel D shows median values only from the isotope mixing model estimates of percent contributions from the 3 different sources to different functional feeding groups.
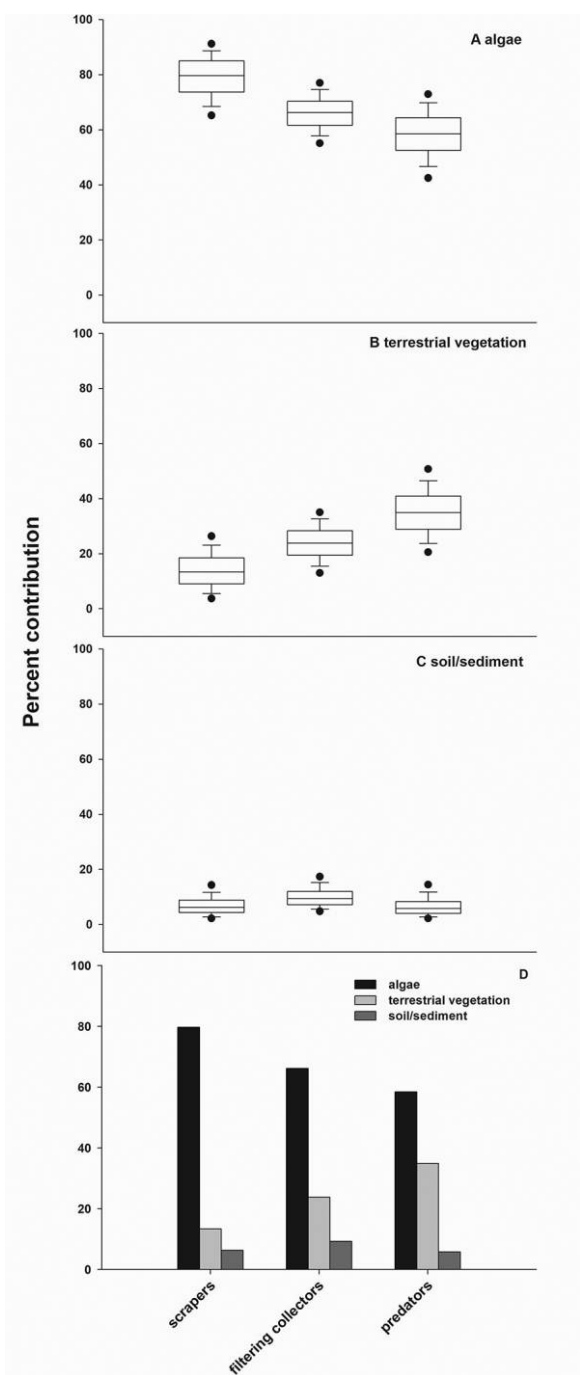
Figure 5. Posterior probability distributions of percent contribution estimates for algal (A), terrestrial vegetation (B), and soil/sediment OM (C) nutritional sources to macroinvertebrate biomass from the isotope-mixing model of a subset of macroinvertebrate individuals where all four isotopes ($\delta^{13}C$, $\delta^{15}N$, δ^2H, and $\Delta^{14}C$) were measured. The box boundaries are the 25th and 75th percentiles, the line in the center of the box is the median, the whiskers are the 10th and 90th percentiles, and the dots are the 5th and 95th percentiles. Actual values are provided in Table S5.2. Panel D shows isotope mixing model estimates of median (50th percentile only from A, B, and C) % nutritional source contributions to macroinvertebrate functional feeding groups.

in addition to stable isotope measurements (Fig. 5A–D; Table S5.2). For these macroinvertebrate groups, autochthonous algae remained the primary source of OM (median contribution of 59–79%) (Fig. 5A, D). The ranges of posterior probability distributions associated with the estimates of terrestrial vegetation and soil and sediment-derived OM were smaller than when $\Delta^{14}C$ was not part of the mixing model: difference between 5th and 95th percentiles across FFGs = 27–71% vs 22–30% for terrestrial vegetation and 20–52% vs 12–13% for soil and sediment OM (Figs 4B vs 5B and Figs 4C vs 5C, respectively).

DISCUSSION

Previous work in the main stem of the Susquehanna River and other temperate east coast US rivers has shown that much of the C and OM transported by these systems is terrestrially-derived and varies in age (Hossler and Bauer 2012, 2013a, b). However, watershed-stream exchanges (Gomi et al. 2002, Lowe et al. 2006), stream heterogeneity, and in-stream processing (Power and Dietrich 2002, Campbell Grant et al. 2007) can all affect the specific sources and ages of the OM available to macroinvertebrates in headwater streams. These factors may result in differences in nutritional resource utilization across headwater streams, as well as between headwater streams and river main stems.

Assessing the assimilation of allochthonous vs autochthonous OM by consumers by concurrently measuring stable isotopes and natural abundance ^{14}C in stream food web studies has been relatively limited to date (see Bellamy and Bauer 2017 for review). However, multiple isotope approaches generally provide higher resolution of the different nutritional sources used by consumers and the ages of those resources than traditional approaches that use only 1 or 2 isotopes or gut content analysis (Middelburg 2014). The overarching goal of the present study was to assess the relative importance of allochthonous and autochthonous nutritional resources to different macroinvertebrate groups (FFGs and chironomids) across the Susquehanna River basin. We were able to obtain robust representative measurements for the Susquehanna River basin as a whole by examining nutritional resources across a broad spatial and temporal scale coupled with the use of multiple isotope tracers. Potential nutritional resources collected during 1 sampling event will not necessarily isotopically match the resources an organism has assimilated. Thus, our approach potentially reduced the confounding factors associated with shifts in isotope signatures of nutritional resources on short time scales and with organism tissue turnover time (Jardine et al. 2014).

Nutritional source contributions to macroinvertebrates

We found that algae was the primary nutritional resource that contributed to macroinvertebrate biomass for

scrapers, filtering collectors, and predators in the Susquehanna River watershed irrespective of the mixing model we used (Figs 4, 5). Several other recent studies also suggest that algae play a more important role in low-order stream food webs, where light can be limiting, than previously thought (Guo et al. 2016, Neres-Lima et al. 2016, Rosi-Marshall et al. 2016). These findings do not agree with some aspects of the River Continuum Concept (RCC), which states that inputs of allochthonous organic matter (OM) into streams and their use by stream macroinvertebrates decreases with increasing stream order and decreasing canopy cover (Vannote et al. 1980). Most of the streams we sampled were ≦4th-order (Table 1), so the RCC predicts that the macroinvertebrate groups present in these streams would primarily consume terrestrial vegetation. Several older studies in larger floodplain systems also suggest that the RCC model is too simple for prediction of nutritional resource use by macroinvertebrates (Thorp and Delong 1994, Bunn et al. 2003). In some of the streams in our study, algae were in relatively low abundance, possibly because of light limitation from canopy cover. However, algae are generally considered to be more nutritious than terrestrially-derived materials and may be preferentially selected for consumption and assimilation (McCutchan and Lewis 2002, Thorp and Delong 2002, Guo et al. 2016, Rosi-Marshall et al. 2016). OM quality may therefore be more important than quantity when assessing nutritional needs of some consumers (Marcarelli et al. 2011, Brett et al. 2017).

Differences in the assimilation of nutritional resources and the importance of autochthonous OM among macroinvertebrate groups (Figs 4, 5) may be a result of the different habitats used by macroinvertebrate groups or their different abilities to selectively consume autochthonous OM. Most of the scrapers, filtering collectors, and collector–gatherers we included in our study were collected from riffles or runs, whereas many chironomids, shredders, and predators were collected from areas with slower-moving or pooled water. Macroinvertebrates collected from areas with increased water flow may have had access to more autochthonous OM such as periphyton and diatoms, whereas detrital-based resources may have been more accessible to macroinvertebrates inhabiting slow-moving or standing water (Sullivan 2013, Page et al. 2017). It may also be easier for scrapers, filtering collectors, and collector–gatherers to forage on and assimilate algae because these groups can be more selective about algal consumption given their feeding mechanisms (Rasmussen 2010). Many of the filtering collectors included in our analyses were hydropsychid caddisflies, which are thought to be generalist consumers (Benke and Wallace 1980). However, fatty acid analyses have suggested that hydropsychids must get essential fatty acids from algal resources or animal prey (Torres-Ruiz et al. 2010), and there is some evidence that they selectively assimilate algae (Finlay et al. 2002). Scrapers are also known to selectively consume and assimilate algae, but this can vary by genera and

availability of algal resources (Finlay 2001, McNeely et al. 2006).

Algae was not the dominant source of OM for shredders and chironomids, which primarily relied on terrestrial vegetation as well as soil and sediment-derived OM. We observed that chironomids assimilated more OM from aged soil and sediment than any other macroinvertebrate group (Fig. 4D), which is unsurprising because we primarily collected chironomids from stream sediments. However, the mixing model suggested that chironomids assimilated more soil and sediment-derived OM (an aged OM source) than the other macroinvertebrate groups, but the chironomid tissue was modern in age (Table S3.1). These results indicate that chironomids may have selectively assimilated the younger fraction of soil and sediment-derived OM (i.e., fresh detrital OM). The contradiction between mixing model results and the ^{14}C age of chironomids could be a result of the variability in $\Delta^{14}C$ and associated age of soil and sediment-derived OM, which ranged from modern to ancient (Fig. 2C). Unfortunately, we did not have enough $\Delta^{14}C$ data to include chironomids in the mixing model with radiocarbon data, which may have clarified their assimilation of aged OM. Further, we did not separate chironomids in the subfamily Tanypodinae (which are typically predatory; Baker and McLachlan 1979) from non-predaceous chironomids when we combined them for isotope analysis, which probably confounded our results.

None of the habitat types or feeding mechanisms alone fully explains the differences in the proportional contribution of autochthonous and allochthonous OM to the macroinvertebrate groups, but these characteristics probably interact. However, recent studies suggest that OM quality probably influences the incorporation and assimilation of OM into aquatic food webs (Guo et al. 2016, Hayden et al. 2016, Brett et al. 2017), so the FFG classification may not be strongly predictive of allochthonous nutritional resource use (Mihuc 1997, Collins et al. 2015).

Age of OM assimilated by macroinvertebrates

Many of the algal samples contained old or ancient C (Fig. 2C, Table S2.2). Algae was the primary nutritional resource used by most macroinvertebrate groups in the Susquehanna River basin, and DIC is the main source of C for algal photosynthesis. Thus, consumption of algae that fixed aged DIC was likely partly responsible for the incorporation of old C into macroinvertebrate tissues in our study.

Macroinvertebrate tissues from Little Muncy Creek in 2011 were particularly old. Little Muncy Creek had active methane seepage (i.e., ebullition) in the study reach during our 2011 sampling (see Appendix S1.2). We did not measure the isotope signatures of the methane directly, but methane seepage did affect the isotope signature and apparent age of DIC, both of which were much lower in Little Muncy Creek than in the other streams we sampled. These

results suggest that, in August 2011, thermogenic methane, biogenic methane, or both were emitted from the stream bed, oxidized to CO_2 by methane-oxidizing bacteria, and then taken up by local algae during photosynthesis. Inputs of old methane C and its incorporation into the DIC pool is the most likely explanation for the ^{13}C and ^{14}C-depleted macroinvertebrates collected from Little Muncy Creek (Fig. 2C) and is a plausible mechanism by which ancient methane C can propagate into the stream food web. Evidence of methane contributions to macroinvertebrate biomass has also been observed in other lotic systems including the River Lambourn (Trimmer et al. 2009) and the Nyack floodplain (DelVecchia et al. 2016).

Utility and value of measuring natural abundance ^{14}C in stream food web studies

Stable isotope mixing models are useful for assessing the relative contributions of nutritional resources to consumer tissues, but there is still a large amount of uncertainty associated with mixing model outcomes (Phillips et al. 2014). Use of $\delta^{13}C$ alone is often not enough to differentiate between allochthonous and autochthonous sources, so δ^2H has become increasingly common in aquatic food web studies (Finlay et al. 2010, Collins et al. 2015, Wilkinson et al. 2015). However, assumptions about the influence of dietary water on the δ^2H isotope composition of aquatic consumers must be considered when interpreting δ^2H-based analyses (Brett et al. 2018).

By incorporating 3 and 4 isotopes in our mixing models, we reduced the variation among mixing model estimates (i.e., Figs 4A–C vs 5A–C). Large overlap of $\delta^{13}C$ signatures of terrestrial vegetation and soil and sediment-derived OM (Fig. 2A, B) probably led to larger variability in the estimates of the contributions of those sources. However, our median estimates of nutritional resource contributions for the scraper, filtering collector, and predator FFGs were similar in the 3- and 4-isotope mixing models. Use of $\Delta^{14}C$ provided better mixing model outcomes for our particular data set by reducing the uncertainty around contribution estimates, in addition to providing important insight into the ages of the OM and C that comprised consumer biomass. However, unless researchers are specifically interested in assessing the ages of C and OM used by consumers, use of 3 stable isotopes is more cost-effective than radiocarbon and can still provide reliable contribution estimates.

Findings from the present study demonstrate that incorporation of both old or ancient C and OM by aquatic food webs is probably a general feature of streams within a large watershed. Additionally, these results are similar to findings from a number of other inland water systems (see Bellamy and Bauer 2017 for review). Our findings further show that the assimilation of aged C varies by macroinvertebrate group. Aged sources of C and OM are potential sources of nutrition that have been traditionally considered unavailable to consumers (Kleber et al. 2011, Marín-Spiotta et al. 2014). The use of aged sources of nutrition (e.g., soil and sediment-derived OM) that were biosynthesized by primary producers in the distant past by aquatic consumers has implications for our conceptual and quantitative models of aquatic elemental and energy flow and may influence both community and ecosystem structure and function. Future studies should seek to establish whether aged nutrition sources displace or augment younger, more recently formed nutrition sources in aquatic food webs.

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