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RESEARCH ARTICLE

Influence of land use and lithology on sources and ages of nutritional resources for stream macroinvertebrates: a multi-isotopic approach

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Abstract Terrestrially-derived carbon (C) and organic matter (OM)—often of significant age—dominate in many streams and rivers, yet little is known about their potential nutritional contributions to aquatic macroinvertebrate consumers. Impacts of watershed characteristics (e.g., land use and lithology) on the sources and ages of C and OM utilized by aquatic consumers are also poorly understood. To assess these factors, macroinvertebrates were collected from six headwater streams having different watershed lithologies and land uses in the Hudson-Mohawk River system (New York, USA) and analyzed for natural $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\Delta^{14}C$. A Bayesian stable isotopic mixing model revealed that autochthonous primary production dominated (62–92%) the biomass of all functional feeding groups (FFGs) across all sites, with allochthonous sources being of secondary but still significant (21–31%) importance. Macroinvertebrates collected from streams in watersheds having low vs. high agricultural land use were estimated to assimilate 0–13 and 4–31% soil-derived C and OM, respectively. $\Delta^{14}C$ values and apparent ages of macroinvertebrates from shale-rich and shale-poor sites were also significantly different (mean $\Delta^{14}C = -75$ and $-34‰$; equivalent ^{14}C ages = 630 and 280 years B.P., respectively). Inclusion of $\Delta^{14}C$ data in mixing models confirmed the importance of autochthonous primary production, and also demonstrated indirect lithological control of nutritional resource utilization by influencing stream substrate type and potential retention of allochthonous C and OM. Findings from this study further showed that the relative magnitudes of autochthonous vs. allochthonous contributions to macroinvertebrates were dependent on FFG, land use type, and lithology.

Keywords Macroinvertebrates · Isotopes · Nutritional subsidies · Land use · Lithology

Introduction

Nutritional resource utilization by aquatic macroinvertebrates can vary as a function of morphological and behavioral adaptations [i.e., functional feeding group (FFG); Cummins 2016], characteristics of the organisms' habitats, and the relative availability of different nutritional resources (Cummins and Klug 1979; Rosi-Marshall and Wallace 2002; Vannote et al. 1980; Wallace et al. 2015). Potential nutritional sources to aquatic systems comprised of living and non-living carbon (C) and organic matter (OM) can be generally classified as autochthonous (produced within the system, e.g., algae and macrophytes) or allochthonous (produced outside the system, e.g., living and senesced leaves, woody debris, and soils) in origin (Ishikawa et al. 2014; McCutchan and Lewis 2002; Vannote et al. 1980). Morphological and ecological characteristics of macroinvertebrates have also been used in previous studies to predict the forms of C and OM contributing to macroinvertebrate nutrition (Cummins 2016; Roach 2013; Vannote et al. 1980).

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Natural abundance isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$) are increasingly used to provide quantitative estimates of autochthonous and allochthonous contributions to aquatic consumer nutrition (Bunn and Boon 1993; McCutchan and Lewis 2002; Middelburg 2014). In some aquatic systems there may be significant overlap in the $\delta^{13}C$ and $\delta^{15}N$ values of different sources of nutritional C and OM, making it difficult to accurately assess resource contributions to organism biomass (Moore and Semmens 2008; Phillips et al. 2014). $\delta^{2}H$ is increasingly used for establishing the contributions of terrestrial vs. aquatic OM to consumer biomass because of the large isotopic separation (~100‰) between the two (Doucett et al. 2007; Finlay et al. 2010; Wilkinson et al. 2015). Natural abundance radiocarbon (^{14}C) is a novel tracer of C sources and nutrition in aquatic food web studies and has a much greater dynamic range (≥1000‰) than $\delta^{13}C$ and $\delta^{15}N$ (tens of ‰ at most) or $\delta^{2}H$ (~100‰). Natural abundance ^{14}C also uniquely allows for determination of the ages of potential C and OM nutritional resources utilized by consumer organisms.

Examination of the ^{14}C ages of C and OM pools in streams and rivers has revealed that in many cases highly aged forms such as soils, weathered sedimentary rock, groundwater, and both natural and anthropogenic petroleum-derived hydrocarbons contribute to aquatic particulate and dissolved OM (POM and DOM, respectively) and dissolved inorganic C (DIC) pools (Butman et al. 2015; Hossler and Bauer 2013a, b; Longworth et al. 2007; Marwick et al. 2015; Raymond and Bauer 2001). Aged aquatic C and OM therefore contains materials that were fixed and stored in soils and/or sedimentary rocks for between decades to millions of years or more (Hedges 1992; Tourtelot 1979; Trumbore 1997) before mobilization to aquatic systems. These aged aquatic forms of C and OM may serve as primary or secondary nutritional sources to bacterial and metazoan consumers. A recent review of this topic suggests that nutritional support of aquatic consumers by aged C and OM may be the rule rather than the exception across a range of inland water systems (Bellamy and Bauer 2017).

In the present study we evaluated the relative contributions of allochthonous and autochthonous sources of nutrition to stream macroinvertebrate consumers belonging to different FFGs in six geographically proximate temperate subwatersheds. The roles of watershed land use and lithology on the ages of nutritional resources utilized by macroinvertebrate consumers were also examined. We predicted (1) greater contributions of allochthonous sources of nutrition to shredders, collector–gatherers and chironomids, and of autochthonous materials to filtering collectors and scrapers due to differences in invertebrate feeding strategies and morphologies (Cummins 2016; Cummins and Klug 1979), and (2) depletion in stream macroinvertebrate ^{14}C (i.e., decreasing apparent age) in watersheds containing significant amounts of fossil shale OM.

Methods

Site description

Individual sites from six streams in subwatersheds of varying size (21–149 km^2; Table 1) were sampled in the Mohawk-Hudson River watershed, New York, USA (Fig. 1). These sites were previously studied by Longworth et al. (2007) and found to vary in the amounts of OM-rich shale [2–4% total organic C (TOC) and OM-poor shale (<0.5% TOC)] in their watershed lithologies (Table 1).

Table 1 Site and watershed characteristics of the six study streams in the Mohawk-Hudson River watershed sampled in June 2014

Site	Watershed area (km^2)[a]	pH	Temp. (°C)	DO (mg/l)	OM-Rich Shale (%)[a]	OM-Poor Shale (%)[a]	Agriculture by area (%)[a]	Canopy Cover (%)	Stream Width (m)[b]
Nowadega Creek (NC)[c]	82	8.22	20.6	9.7	45	44	49	3	25.3
Fly Brook (FB)	31	8.14	17.4	8.7	0	7	46	66	5.8
Couch Hollow Branch (CH)	24	6.77	17.4	8.8	0	0	6	92	5.7
Schoharie Tributary (ST)	21	8.31	15.9	9.6	0	95	19	6	7.9
Wampecack Creek (WC)	50	8.33	18.9	9.8	0	8	61	0	8.5
Otsquago Creek (OC)	149	8.20	21.0	9.5	55	37	65	7	17.7

DO dissolved oxygen

[a] From Longworth et al. (2007)

[b] Width of stream at sampling location

[c] Abbreviations of sites are given in parentheses after each site name

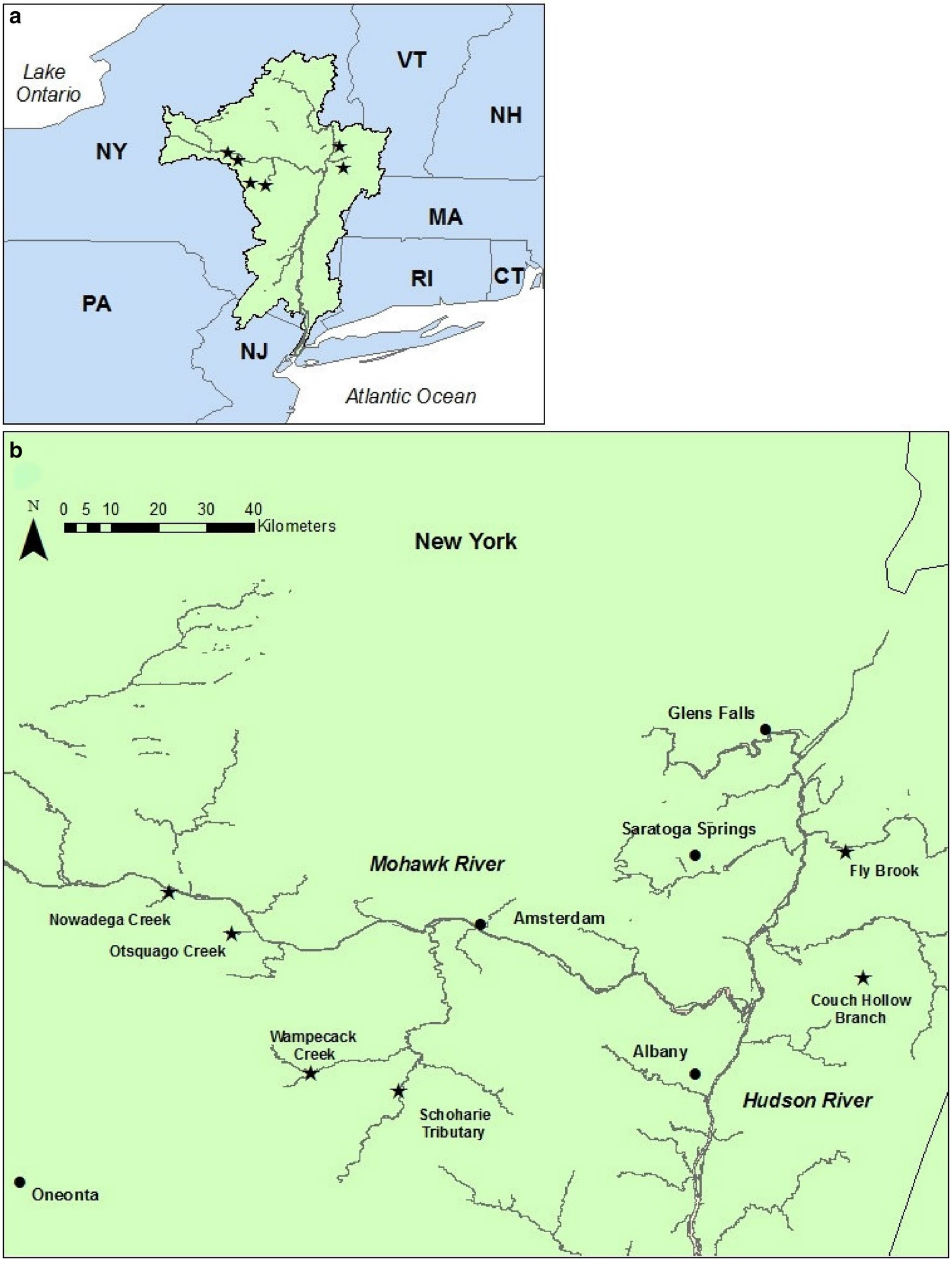

Fig. 1 **a** The Hudson-Mohawk River drainage basin (New York, USA) and **b** the sampling region of the present study in the Hudson-Mohawk watershed. *Stars* in panels **a** and **b** represent sampling sites and *filled dots* represent major regional population centers

OM-rich shales in this region are a part of the Utica shale formation, whereas OM-poor shales consisted of Grey and Frankfort shales and Gneiss (Longworth et al. 2007). The six subwatersheds also varied by over an order of magnitude in the relative amounts of agricultural activity (pasture and row crop; 6–65%; Table 1).

Field sampling

Macroinvertebrates belonging to different FFGs (filtering collectors, scrapers, collector–gatherers, shredders, predators, and chironomids) and their potential nutritional sources were collected from each stream in June 2014. Organisms were collected by hand-picking them from rocks and logs and using a kick net where sediments and aquatic vegetation dominated. Primary consumers and predators were separated from each other into quartz fiber-filtered (type QMA, 0.8 µm nominal pore size) stream water and allowed to void their guts for 24 h at ambient temperature (Brooke et al. 1996). After gut voidance, organisms were placed in baked (525 °C) aluminum foil pouches and frozen in Ziplock bags on dry ice in the field and at −20 °C in the lab until processing (note that all other sample types, unless otherwise noted, were stored in the same manner).

The dominant potential nutritional sources to macroinvertebrates, including both aquatic and terrestrial OM, were also collected from the six individual sites. Stream sediment samples were collected using a 60 ml syringe corer. Where cleanly eroded stream banks were present within a sampling reach, terrestrial soil samples were collected by inserting syringe corers horizontally into the stream bank at shallow (surface) and deep (~20 cm) depths. When eroded stream bank was not present, terrestrial soil samples were collected by excavating a hole of ~20 cm depth on level ground within 10 m of the stream. Soil samples were collected at ~1 and ~20 cm depths using a baked spatula. When available, shale shards were also collected.

Terrestrial vegetation from riparian trees and aquatic vegetation samples were collected by hand using clean disposable nitrile gloves and preserved as for soil and sediment samples. Stream biofilm samples were collected from 2 to 3 cobbles of similar size (15–20 cm in diameter) by scraping the surface of a ~25 cm^2 area with a new toothbrush or a baked razor blade, and rinsing the scraped material from the cobble surface using ultra-pure Labconco water into acid-cleaned (10% HCl) polycarbonate bottles. Bulk suspended POM was collected by filtering water through a baked 47 mm QMA filter.

DIC samples from each site were QMA-filtered into baked, crimp-sealed 125 ml serum bottles containing 200 µl of a saturated $HgCl_2$ solution and sparged with ultra-high purity N_2 gas; bottles were stored in the dark at ambient room temperature until processing. DOM and nutrients (N and P) from the same filtrations were collected in acid-cleaned polycarbonate bottles. Stream water samples from each site were also collected in baked 20 ml scintillation vials for δ^2H analysis of H_2O and stored at ambient temperature. Basic water properties including temperature, pH, dissolved oxygen (DO), and conductivity were measured at each site using a Model AP110 Accumet Portable pH/ORP meter, a YSI ProODO handheld DO meter, and a YSI Model 30/10 FT multiparameter probe, respectively.

Sample processing and analysis

Macroinvertebrates were sorted and identified to genus whenever possible and assigned to their appropriate FFG according to Merritt et al. [2008; see Supplementary Information [SI] Table S1.1]. Chironomids were identified to family only (Chironomidae) and were treated as such in the mixing models. Chironomids were not assigned to a specific FFG because their FFG and trophic position can vary across subfamily and genus (Reuss et al. 2013). Organisms, terrestrial vegetation, and aquatic vegetation were thawed, dried at 60 °C, and homogenized to a fine powder in preparation for stable isotope and ^{14}C analysis. In some cases, multiple individuals of small macroinvertebrates were pooled to increase sample sizes for isotopic (especially Δ^{14}C) analysis (SI Table S1.1). Samples of aquatic vegetation, terrestrial soil, aquatic sediment, shale, biofilms, and POM filters were acid-fumed with fresh concentrated HCl in a clean glass desiccator prior to homogenization to ensure that carbonates were removed. With the exception of POM filters and biofilms, subsamples of each sample type were set aside prior to acid fuming for δ^2H analysis. POM and biofilm samples were not analyzed for δ^2H because of interference from the quartz filters (POM) and large uncertainties in the organically bound H content of the biofilms.

Homogenized samples and filter portions were packed in tin capsules for δ^{13}C and δ^{15}N analysis. δ^{13}C and δ^{15}N analyses were conducted at the University of California at Davis Stable Isotope Facility, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer. Stable isotope values for δ^{13}C and δ^{15}N are reported relative to the V-PDB and air international standards, respectively. For δ^2H analysis, homogenized samples of macroinvertebrate, aquatic and terrestrial vegetation, soil, and sediment were packed in silver capsules. In order to determine δ^2H of the nonexchangeable H fraction for solid samples, a bench-top equilibration method was used to allow for the exchange of H in the samples with H in the local water vapor (Doucett et al. 2007; Wassenaar and Hobson 2003). Solid samples were analyzed for δ^2H on a Thermo-Finnigan TC/EA and Delta PLUS-XL and stream water samples were analyzed using a Los Gatos Research DLT-100 Liquid Water Isotope Analyzer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University.

For Δ^{14}C analyses, selected homogenized and acid-fumed samples and filters from two of the streams (Fly Brook and Nowadega Creek) were placed in pre-baked 9 mm diameter quartz tubes containing cleaned CuO and elemental Cu and combusted to CO_2 at 750 °C for 4 h.

Within 24 h of combustion, CO_2 from each sealed tube combustion was purified and quantified on a vacuum extraction line and sealed in pre-baked 6 mm Pyrex tubes. The purified CO_2 was reduced to graphite and analyzed for $\Delta^{14}C$ at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Laboratory at Woods Hole Oceanographic Institution. DIC samples were acidified and sparged using ultra-high purity He gas and the CO_2 was collected and purified cryogenically on a vacuum extraction line, and stored and analyzed for $\Delta^{14}C$ as above. Dissolved inorganic N and P nutrient concentrations were analyzed using a Lachat QuikChem 8500, and DOC concentrations were analyzed using a Shimadzu 5000 TOC Analyzer.

Statistical analyses

Multivariate statistical analyses were conducted using the PRIMER software package (Clarke and Gorley 2006; v. 6, PRIMER-E Ltd) in order to assess relationships between the composite isotope data of organisms from different sampling sites and FFGs. Macroinvertebrate $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\Delta^{14}C$ values were normalized and visually evaluated using non-metric multidimensional scaling (NMDS). One-way analysis of similarity (ANOSIM) was used for macroinvertebrate isotopic data in order to determine whether significant isotopic differences existed between (a) individual sites, (b) FFGs, (c) percent of agricultural land use in each watershed, and (d) the presence or absence of OM-rich shale in watershed lithologies. Agricultural land use in the study watersheds fell into two categories: <20% ("low") and >40% ("high"; Table 1). The ANOSIM pairwise test statistic, R, is considered a better indicator of separation between groups than the p value because it is not influenced by sample size, which was relatively small in this study (Clarke and Gorley 2006). In cases where the overall p values for the ANOSIM model were significant ($p < 0.05$), all pairwise comparisons within the model were examined, including those where $p > 0.05$, but global $R > 0.8$. We used a nonparametric Wilcoxon/Kruskal–Wallis rank test to assess differences in the individual isotopic compositions ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$) of macroinvertebrates across FFGs and site, and a Wilcoxon/Mann–Whitney test to assess the same differences in high and low agricultural streams using JMP software (JMP, Version 11, SAS Institute Inc., Cary, NC, USA).

Isotopic mixing models

Proportional contributions of potential nutritional sources to macroinvertebrate biomass were estimated using MixSIAR (Stock and Semmens 2013), an isotopic mixing model that employs a Bayesian approach (Moore and Semmens 2008) via a graphical user interface (GUI) and R Statistical Software (R Core Team 2014). Use of a Bayesian approach allows for the incorporation of uncertainty in source contribution estimates, as there are multiple sources of variation that could impact source contribution estimates, including, but not limited to, use of multiple nutritional sources by consumers, isotopic fractionation, and spatial and temporal variability of isotopic signatures (Finlay et al. 2002; Moore and Semmens 2008). In order for the model to better incorporate uncertainties in isotope values and fractionation, means and standard deviations of the isotope values of potential nutritional resources and trophic fractionation were included in the model. MixSIAR then provides posterior probability distributions for proportional resource contributions to provide a measure of the variability around the estimates provided. Details of the isotopic signatures of potential nutritional sources, trophic fractionation factors, methods associated with the Bayesian model, and how the models were run are described in SI Sect. 2.

Results

Isotopic values of potential nutritional resources and macroinvertebrate consumers

Algae had the lowest $\delta^{13}C$ and $\delta^{2}H$ values of potential nutritional resources collected from each site, while soil OM had the greatest $\delta^{13}C$ and $\delta^{2}H$ values (Fig. 2a, b). Algae were higher in $\delta^{15}N$ in high-agriculture than in low-agriculture streams, but both were higher in $\delta^{15}N$ than other potential nutritional resources (Fig. 2a, b). Overlap in the $\delta^{13}C$ and $\delta^{15}N$ values of algae and terrestrial vegetation were observed for samples collected from each system (Fig. 2a, b).

The uncorrected (i.e., for trophic fractionation) stable and radiocarbon isotopic values for all macroinvertebrates and FFGs were used for statistical analyses and are presented in SI Sect. 3 and Table S3.1. NMDS analysis did not reveal any obvious clustering by FFG (SI Fig. 3.1), but one-way ANOSIM analysis revealed significant differences in composite isotopic compositions ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$) between all FFGs (SI Table S3.2A). Significant differences were found between almost all pairwise comparisons of FFG isotopic composition. Examination of individual isotopes revealed significant differences in $\delta^{13}C$ values between FFGs (Kruskal–Wallis, $p < 0.0001$) but not for $\delta^{15}N$ or $\delta^{2}H$ values, suggesting that significant differences in the composite isotopic composition of FFGs were primarily driven by $\delta^{13}C$ values. Scraper FFG macroinvertebrates were lower in $\delta^{13}C$ and $\delta^{2}H$ than other FFGs across all sites, with the exception of Fly Brook (Fig. 2a, b; SI Table S3.1). In contrast, chironomids, collector–gatherers, and shredders were elevated in $\delta^{13}C$ and $\delta^{2}H$ compared to

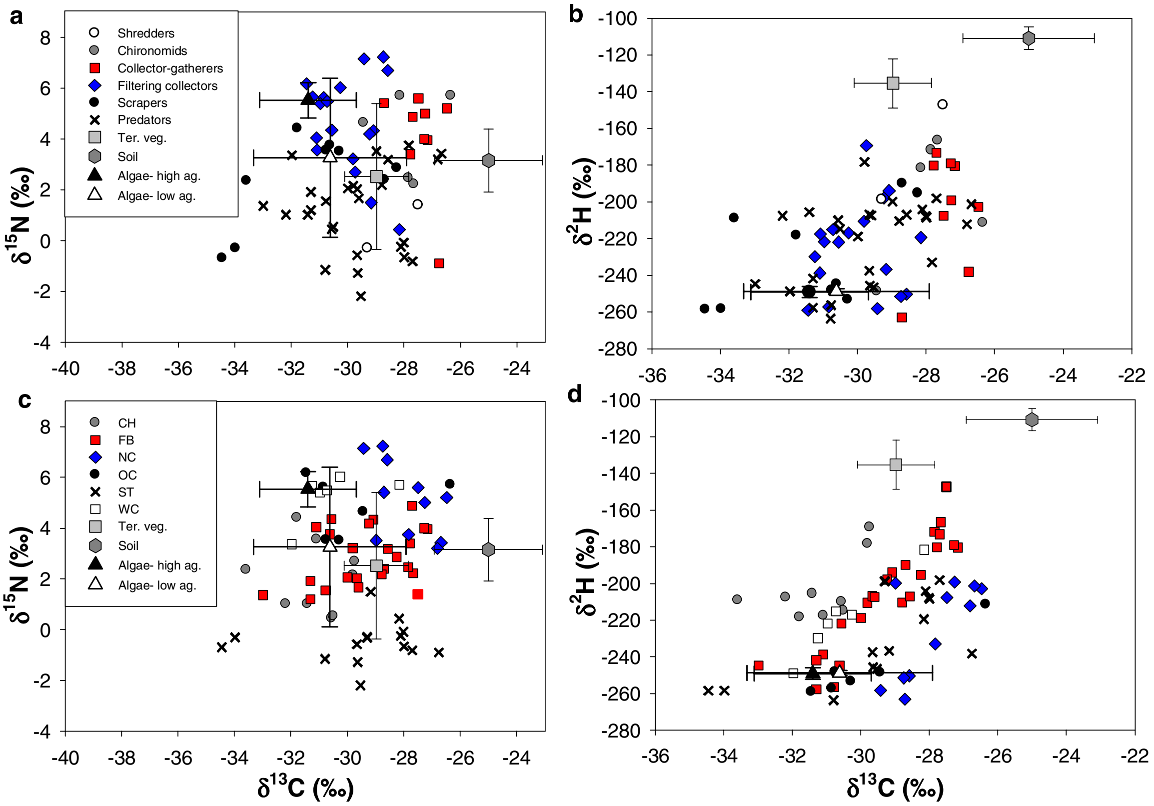

Fig. 2 $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of macroinvertebrates and their potential nutritional sources collected from the six study streams in the Mohawk-Hudson River watershed (mean ± SD). **a** $\delta^{15}N$ vs. $\delta^{13}C$ of macroinvertebrates and potential nutritional resources grouped by FFG, **b** $\delta^{2}H$ vs. $\delta^{13}C$ of macroinvertebrates and potential nutritional resources grouped by FFG, **c** $\delta^{15}N$ vs. $\delta^{13}C$ of macroinvertebrates and potential nutritional resources grouped by site, **d** $\delta^{2}H$ vs. $\delta^{13}C$ of macroinvertebrates and potential nutritional resources grouped by site. Macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values are corrected for trophic fractionation (Post 2002) and macroinvertebrate $\delta^{2}H$ values are corrected for the influence of dietary water (Wilkinson et al. 2015). The shredder FFG is included in our figures even though sample size was too low to include it in statistical and mixing model analyses. *CH* Couch Hollow, *FB* Fly Brook, *NC* Nowadega Creek, *OC* Otsquago Creek, *ST* Schoharie Tributary, *WC* Wampecack Creek

other FFGs at all sites (Fig. 2a, b; SI Table S3.1). Filtering collectors from Otsquago Creek were lower in $\delta^{13}C$ and $\delta^{2}H$ relative to filtering collectors for the other sites. Predators (uncorrected for trophic fractionation) were the most elevated in $\delta^{15}N$ of all FFGs (SI Table S3.1).

NMDS of combined isotopic data revealed some clustering of macroinvertebrates by site (SI Fig. 3.2). One-way ANOSIM also indicated significant differences in macroinvertebrate composite isotopic compositions between sites, and all pairwise site comparisons were significant (SI Table S3.2B). Stable isotope data showed that Nowadega Creek macroinvertebrates were highest in $\delta^{15}N$ (Fig. 2c; SI Table 3.1), whereas those collected from the Schoharie Tributary were lowest in $\delta^{15}N$ (Fig. 2c; SI Table 3.1). Couch Hollow Branch macroinvertebrates were generally lower in $\delta^{13}C$ compared to those collected from the other five sites (Fig. 2c, d). In contrast to $\delta^{13}C$ and $\delta^{15}N$, macroinvertebrate $\delta^{2}H$ values did not show any site-specific patterns (Fig. 2d). Further inspection of individual isotopes revealed significant differences in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values between sites (Kruskal–Wallis, $p = 0.0006$, $p < 0.0001$, and $p = 0.0077$, respectively).

NMDS showed that macroinvertebrate individuals clustered according to high vs. low agriculture in their respective watersheds (SI Fig. S3.3), and the differences between macroinvertebrate stable isotopic values from the high and low agriculture watersheds were significant ($R = 0.251$, $p < 0.001$). Macroinvertebrates collected from streams with high agriculture were elevated in $\delta^{15}N$ compared to macroinvertebrates collected from streams with low agriculture (Fig. 3a). There were no apparent differences in macroinvertebrate $\delta^{13}C$ or $\delta^{2}H$ values relative to the amount of watershed agriculture (Fig. 3a, b). A Mann–Whitney test revealed significant differences in the $\delta^{15}N$ compositions of macroinvertebrates collected from high and low agriculture streams ($p < 0.0001$), but not for $\delta^{13}C$ and $\delta^{2}H$.

Soil OM had the lowest $\Delta^{14}C$ values of all potential nutritional resources at both Fly Brook and Nowadega

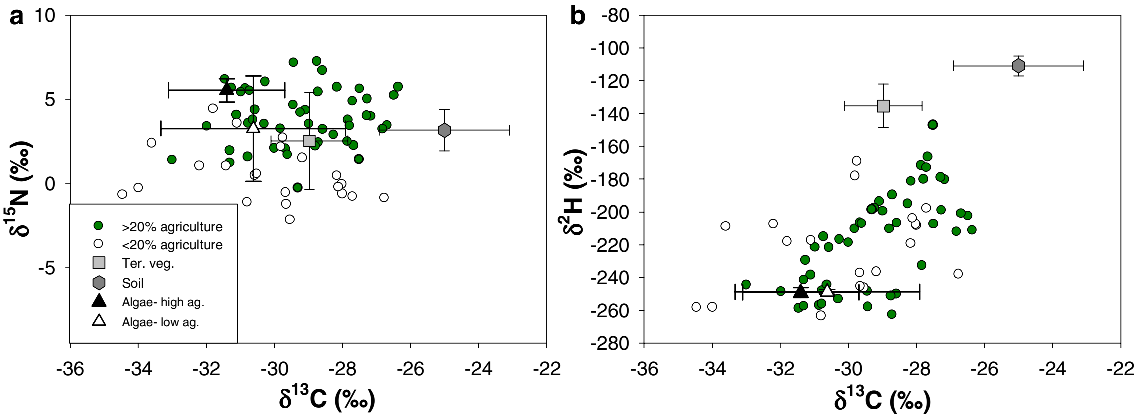

Fig. 3 **a** δ^{15}N vs. δ^{13}C values of macroinvertebrates and their potential nutritional sources from high agriculture and low agriculture watersheds (mean ± SD), **b** δ^{2}H vs. δ^{13}C of macroinvertebrates and their potential nutritional sources from high agriculture and low agriculture watersheds (mean ± SD). Macroinvertebrate δ^{13}C and δ^{15}N values are corrected for trophic fractionation (Post 2002) and macroinvertebrate δ^{2}H values are corrected for the influence of dietary water (Wilkinson et al. 2015)

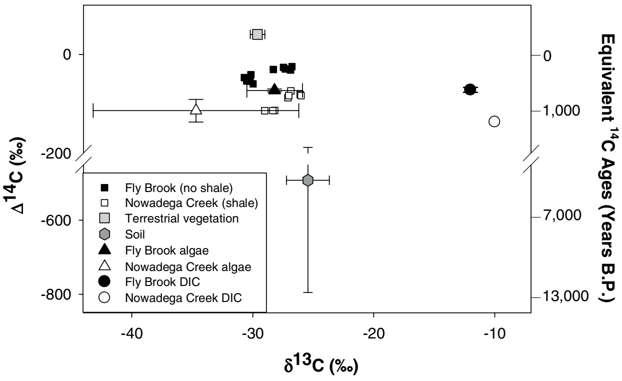

Fig. 4 Δ^{14}C values and equivalent ^{14}C ages vs. δ^{13}C of macroinvertebrate individuals and their potential nutritional sources (means ± SD) collected from Fly Brook (no shale in watershed) and Nowadega Creek (shale in watershed)

Creek, while terrestrial vegetation, based on the value of atmospheric CO_2 (Levin et al. 2013), had the highest Δ^{14}C values (Fig. 4). Δ^{14}C values of algae and DIC for each respective site were similar (Fig. 4). Macroinvertebrate Δ^{14}C values at Fly Brook ranged from −59 to 23‰ (equivalent ^{14}C age = 430 years B.P. to modern-aged, respectively; Fig. 4; SI Table S3.1), with the highest Δ^{14}C organism being a shredder. Macroinvertebrate Δ^{14}C values at Nowadega Creek ranged from −114 to −1‰ (910 years B.P. to modern-aged, respectively; Fig. 4). Inclusion of Δ^{14}C data in NMDS for the two sites for which it was available showed further separation between sites both with and without OM-rich shale in their watersheds (Nowadega Creek and Fly Brook; R = 0.572, $p < 0.001$; SI Figur. S3.4).

Potential nutritional source contributions to macroinvertebrate biomass

Mixing model estimates of nutritional source contributions (5, 50, and 95% posterior probabilities) for the different macroinvertebrate FFGs are provided in SI Sect. 4. Model results using mean δ^{13}C, δ^{15}N, and δ^{2}H values indicated that algae were the primary nutritional resource (63–92% contribution) for all FFGs in the streams studied (Fig. 5; SI Table S4.1). However, soil OM and terrestrial vegetation (i.e., allochthonous OM) comprised up to 21 and 31% of consumer biomass, respectively (Fig. 5; SI Table S4.1).

When organisms were grouped by the percent agriculture in the watershed, mixing model results again indicated that algae were the largest contributor to macroinvertebrate biomass, particularly in the sites with <20% agriculture (Fig. 6). Algal material comprised nearly the entirety of stream collector–gatherer and scraper biomass (≥96%; Fig. 6; SI Table S4.3) in low agriculture watersheds according to our mixing models. While filtering collectors, scrapers, and predators from high agriculture streams were still primarily reliant on algae (≥76%; Fig. 6; SI Table S4.2), there were also large contributions (4–31%) from allochthonous OM in the forms of soil-derived OM and terrestrial vegetation (Fig. 6; SI Table S4.2). Soil OM contributed up to 31% of the biomass of macroinvertebrates collected from the >40% agriculture streams but did not exceed 13% for macroinvertebrates from the <20% agricultural streams (Fig. 6; SI Tables S4.2, S4.3).

Inclusion of Δ^{14}C data in the mixing model for the two sites for which it was measured showed a slight decrease (37–92%) in the range of algal assimilation by macroinvertebrates compared to models using stable isotopes alone (62–92%; Fig. 7a, b; SI Tables S4.4, S4.5). This was most

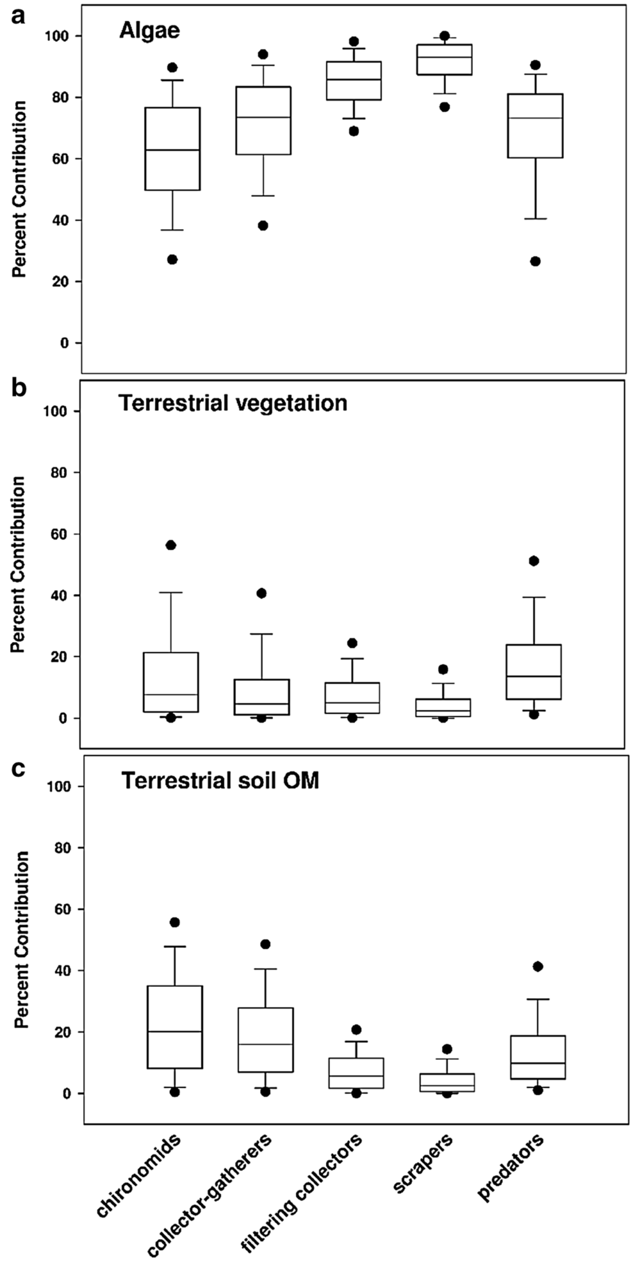

Fig. 5 Posterior distributions of the percent contributions of **a** algal, **b** terrestrial vegetation, and **c** terrestrial soil organic matter (OM) sources of potential nutrition to the biomass of macroinvertebrates using average isotopic values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ across all individuals belonging each FFG for all sites. n = 5 for scrapers, n = 6 for filtering collectors, n = 3 for collector–gaherers, n = 3 for chironomids, and n = 5 for predators. The averages of all potential nutritional sources across all sites were used in the model and algal isotope values were estimated (see "Methods" section for details). The 5th, 25th, 50th, 75th, and 95th percentiles are shown

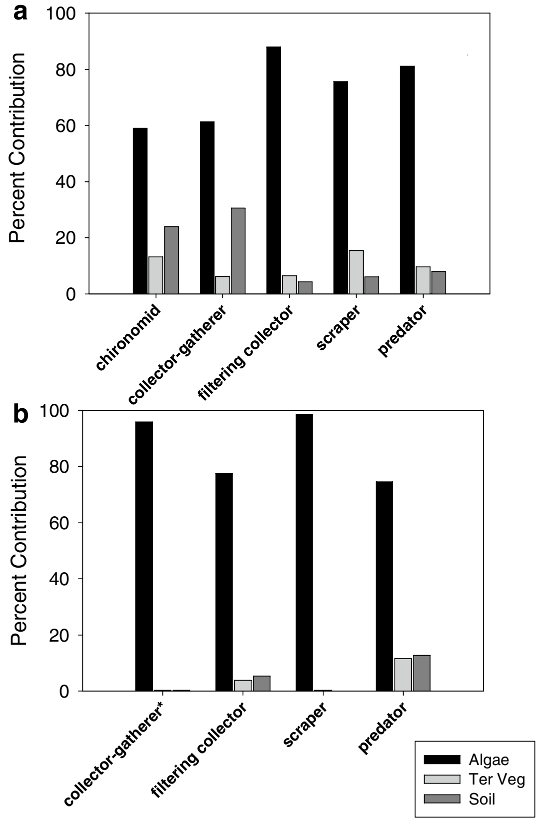

Fig. 6 Comparison of median proportional nutritional source contributions to macroinvertebrate biomass from mixing models in **a** sites with >20% watershed agriculture and **b** sites with <20% watershed agriculture. *n = 1

evident for Fly Brook, which did not contain OM-rich shale in its watershed. Algae were still the primary nutritional resource for all FFGs (39–93%) at both sites when $\Delta^{14}C$ data were included, with the exception of collector–gathers at Fly Brook which primarily assimilated terrestrial vegetation (55%; Fig. 7b). Use of $\Delta^{14}C$ in mixing models also showed increased utilization (28–55%) of terrestrial vegetation and concomitant decreased utilization (0–51%) of soil OM at by macroinvertebrates from both Nowadega Creek and Fly Brook (Fig. 7a). Soil OM was still nutritionally important to predators (21% at Fly Brook, 13% at Nowadega Creek), but contributed less than 5% to all other FFGs at these two sites (SI Tables S4.4, S4.5).

Discussion

Macroinvertebrates have long been known to play a number of important roles in stream ecosystems, including the processing and decomposition of both aquatic and terrestrial OM and support of higher trophic level aquatic and riparian consumers (Baxter et al. 2005; Carlson et al. 2016; Kautza and Sullivan 2016; Prather et al. 2013). Knowledge of the sources and ages of C and OM contributing to the nutrition of macroinvertebrate species and FFGs in streams and other aquatic systems, and how these nutritional contributions

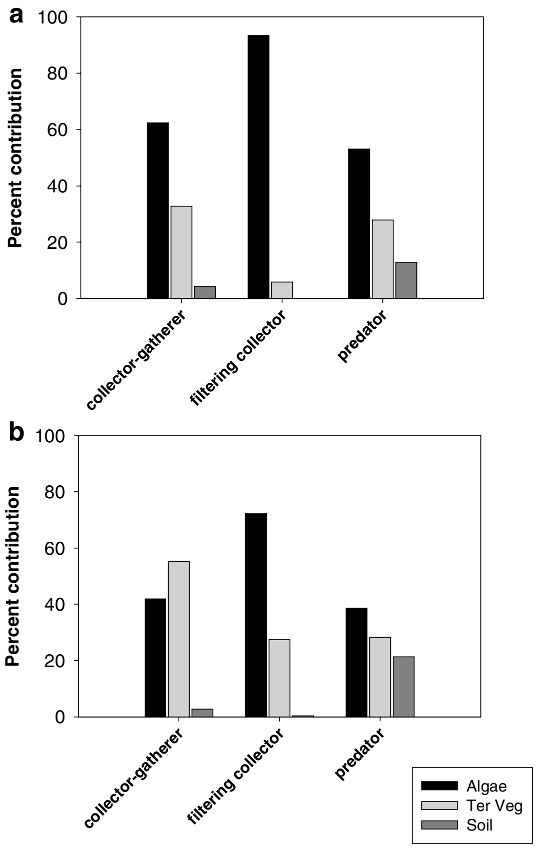

Fig. 7 Comparison of median proportional nutritional source contributions to macroinvertebrate biomass from mixing models for **a** the Nowadega Creek site containing OM-rich shale in its watershed, and **b** the Fly Brook site without shale in its watershed. These sites are the only the two for which natural ^{14}C analyses were conducted on macroinvertebrates and their potential nutritional sources

vary as a function of watershed land use and lithology, are critically important for improving our understanding of the integration of, and connections between, elemental and energetic flows between terrestrial and aquatic systems (Smits et al. 2015; Tanentzap et al. 2017).

Contributions of autochthonous vs. allochthonous primary production to stream consumers

Considering all mixing model scenarios for FFGs examined in our study, with the exception of collector–gatherers from Fly Brook (Fig. 7b), stream algae were the dominant contributor to macroinvertebrate biomass (39–99%). This finding was counter to our initial predictions. The streams we sampled were small subwatersheds of the Hudson-Mohawk basin (all streams had watershed areas <150 km^2; Table 1), and the river continuum concept predicts that allochthonous OM fuels secondary production in headwater and low order streams (Vannote et al. 1980). However, our findings support other recent studies that have highlighted the importance of autochthonous OM to macroinvertebrate nutrition in streams and rivers, despite the fact that allochthonous OM dominates in lotic systems (Brett et al. 2017; Guo et al. 2016; Hayden et al. 2016; Rosi-Marshall et al. 2016).

Aquatic primary production is typically thought to be of higher quality [e.g., lower C:N and higher essential fatty acid (EFA) content] and more bioavailable than allochthonous OM (Guo et al. 2016; Müller-Navarra 2008; Torres-Ruiz et al. 2007). High-quality nutritional resources are important for adequate growth and reproduction in macroinvertebrates (Torres-Ruiz et al. 2007), and macroinvertebrates may be selective in what they consume and assimilate (Goedkoop et al. 2007; Guo et al. 2016).

Of all the FFGs in the present study, scrapers and filtering collectors were the most reliant on algae (≥85%; Fig. 3; SI Table S4.1). Inclusion of ^{14}C data in the mixing models also suggested that algae contributed the most to filtering collector biomass (Fig. 7; SI Tables S4.4, S4.5). Algae-derived materials were also the most important basal nutritional resource to predatory macroinvertebrates in all mixing model scenarios, suggesting that autochthonous nutritional resources may be more readily transferred to higher trophic levels than allochthonous resources (Figs. 5, 6, 7). Previous studies have also shown that fatty acid abundances in aquatic consumers increase with increasing trophic level, which further suggests that some macroinvertebrate predators may have the ability to select higher quality prey (Guo et al. 2016; Lau et al. 2014).

Despite the general dominance of algal material to most of the aquatic consumers in the present study, terrestrial vegetation still contributed measurably, and at times significantly, to macroinvertebrate biomass and is therefore important in macroinvertebrate nutritional and energy budgets. The Bayesian mixing model output indicated that terrestrial vegetation made up 55% of collector–gatherer biomass in Fly Brook (Fig. 7b). Collector–gatherers in Fly Brook consisted of gammarid amphipods and *Ephemera* mayflies, which most likely consume fine particulate OM composed of fragmented terrestrial vegetation and phytoplankton (Cummins 2016; Cummins and Klug 1979). Based on feeding experiments and determination of leaf litter breakdown rates, as well as gut content analysis, terrestrial vegetation is known to be a significant component of the diets of both gammarids and Ephemeridae mayflies (Arsuffi and Suberkropp 1989; Hamilton and Clifford 1983; Piscart et al. 2009).

Shredding macroinvertebrates are also generally thought to depend on terrestrial vegetation, however, other stable isotope studies have shown algae to contribute significantly to their biomass (Leberfinger et al. 2011; Neres-Lima et al. 2016). Unfortunately, limited sample size for the shredder FFG (n = 2) prevented us from assessing nutritional resource utilization using the mixing models. While autochthonous OM was of primary importance to most consumers, it should be noted that the $\delta^{13}C$ values for algae and terrestrial vegetation in our study were similar (Fig. 2a–d) and may have led to increased uncertainty in mixing model outcomes. However, the inclusion of δ^2H data in our mixing model allowed for much better isotopic separation between algae and terrestrial vegetation (>100‰; Fig. 2b, d). Additionally, inclusion of $\Delta^{14}C$ data in our models further supported the importance of autochthonous OM.

Mixing models that included both stable isotopes and ^{14}C also indicated that algae were the dominant nutritional resource to filtering collectors, collector–gatherers, and predators (42–92%), with the exception of collector–gatherers from Fly Brook (Fig. 7a, b). Other studies that have employed natural ^{14}C in stream and river food web studies have observed equivalent organism apparent ages ranging from ~2000 years B.P. to modern-aged ($\Delta^{14}C = -240$ to 68‰; Caraco et al. 2010; Ishikawa et al. 2016, 2014). While some of these studies contend and/or demonstrate that ^{14}C-depleted consumer biomass is dependent on assimilation of significantly aged OM from allochthonous sources, ^{14}C depletion in aquatic consumers may also be due to consumer utilization of algae that fix ^{14}C-depleted DIC and CO_2(aq) (Bellamy and Bauer 2017; Ishikawa et al. 2014, 2016). Based on our mixing model results and the similarity in $\Delta^{14}C$ values of DIC, algae, and macroinvertebrates, this is likely the case for at least some of our study sites (Fig. 4). There are a number of sources of CO_2 that may contribute to stream DIC, including atmospheric CO_2 exchange, weathering of carbonate rocks, and respired soil and sediment OM, with the most aged sources being carbonate rocks and respired soil CO_2 (Broecker and Walton 1959; Butman and Raymond 2011; Ishikawa et al. 2016; Keaveney and Reimer 2012). The incorporation by aquatic secondary producers of aged (in many cases, highly aged; see review by Bellamy and Bauer 2017) C and OM—regardless of the specific pathway—alters our understanding of terrestrial-aquatic linkages and the sources and ages of C and OM supporting aquatic food webs.

Influence of agricultural activity on macroinvertebrate nutritional resource utilization

Agricultural activity is known to increase exports of young and moderately aged (10^3–10^4 years B.P. in age) soil OM and associated mineral particles to streams and rivers (Burdon et al. 2013; Hossler and Bauer 2012, 2013a, b; Matthaei et al. 2010). These inputs may serve as a source of aged allochthonous nutrition and concomitantly limit light and autochthonous nutritional resources to aquatic consumers by increasing turbidity (Allan 2004; Roach 2013).

Collector–gatherers (*Ephemerella* mayflies) in the low-agriculture Schoharie Tributary were more reliant (96%, Fig. 6b; SI Table S4.3) on algae than in the high-agriculture Nowadega Creek (61%, Fig. 6a), possibly due to the higher quality habitat in streams less impacted by agriculture (Lenat 1984; Lenat and Crawford 1994). Increased sedimentation in our higher agriculture streams may shift nutritional resource utilization by macroinvertebrates inhabiting the sediment–water interface such as *Ephemerella* mayflies from autochthonous to allochthonous materials such as terrestrial vegetation and soils (Yule et al. 2010). The decreased role of autochthonous nutritional resources by this group may be explained by (1) sedimentation inhibiting light availability and aquatic primary production, (2) increased scouring and removal of aquatic autotrophs (Hall et al. 2015; Henley et al. 2000; Horner et al. 1990; Madsen et al. 2001), and/or (3) increased availability and utilization of terrestrial materials (Wang et al. 2014).

Soil OM may also be incidentally ingested by aquatic consumers as they feed on algae or terrestrial vegetation. However, because soil OM is generally considered to be of lower nutritional quality due to its high C:N ratio and molecular composition (Kleber 2010; Kleber and Johnson 2010) it may be ingested and/or assimilated less by consumers. Recent reevaluations of the reactivity and bioavailability of soil OM in aquatic systems (Marín-Spiotta et al. 2014; Schmidt et al. 2011; Weber et al. 2017) suggest that heterotrophic bacteria and fungi may facilitate utilization of soil OM by macroinvertebrates (Hall and Meyer 1998; Wang et al. 2014; Williams et al. 2010). Microbial processing and "repackaging" of soil OM may increase its quality (i.e., lower C:N ~4 to 9) and bioavailability to macroinvertebrate consumers than unaltered OM (C:N ~8 to 25; Finlay and Kendall 2007). Our findings suggest that soil OM may increasingly support macroinvertebrate biomass with increasing inputs of soils under higher agricultural land use. This further suggests that human alteration of watersheds and catchments may play a direct role in the sources and characteristics of C and OM supporting aquatic food webs (de Castro et al. 2016; Docile et al. 2016; Lu et al. 2014).

Potential roles of lithology on nutritional resources and their utilization

Macroinvertebrates from Nowadega Creek (significant amounts of shale in its watershed) and Fly Brook (little to no shale) also showed significant differences in $\Delta^{14}C$ values and ^{14}C ages (Fig. 4). Mixing model results using the $\Delta^{14}C$ data suggest that terrestrial vegetation and soil OM were of greater nutritional importance to Fly Brook than to Nowadega Creek macroinvertebrates (Fig. 7a). We initially predicted that the presence of OM-rich shale in a watershed would potentially provide a more highly aged source of nutrition (Petsch et al. 2001; Schillawski and Petsch 2008). This was based on previous work in the Hudson River showing significant ^{14}C-depletion in zooplankton (mean $\Delta^{14}C = -240‰$, equivalent age of 2000 years B.P.), indicating that they assimilated 57% moderately aged soil OM ($\Delta^{14}C = -350‰$, equivalent age of 3460 years B.P.) or 21% fossil aged OM (>50,000 years B.P.; Caraco et al. 2010). The small but significant decrease in $\Delta^{14}C$ values of Nowadega Creek vs. Fly Brook macroinvertebrates (Fig. 4a) indicates that Nowadega Creek organisms were not assimilating significant amounts of fossil shale-derived OM ($\Delta^{14}C = -1000‰$). Instead, the greater equivalent ^{14}C ages of Nowadega Creek macroinvertebrates are more likely attributable to consumption of algae that are depleted in ^{14}C due to their fixation of ^{14}C-depleted (i.e., aged) DIC and CO_2(aq) (Fig. 4; Ishikawa et al. 2014, 2016).

Lithological factors such as the presence of shale may be overridden by biological and physical factors in certain watersheds. For example, the reach sampled at Nowadega Creek had far lower canopy cover (3%) than the reach sampled at Fly Brook (66%; Table 1). Correspondingly, collector–gatherers and filtering collectors from Fly Brook utilized 21% more terrestrial vegetation than those from Nowadega Creek (Fig. 7; SI Tables S4.4, S4.5). Thus, higher canopy cover, and the greater inputs of terrestrial vegetation mediated by it, may better explain the increased use of terrestrial vegetation than the presence or absence of shale in the watershed (Collins et al. 2015; Doi et al. 2007; England and Rosemond 2004). In addition, previous studies have demonstrated that shale-rich lithologies are not necessarily predictive of the amounts of aged C and OM in associated stream waters (Goñi et al. 2013; Leithold and Blair 2001), possibly due to rapid sedimentation and burial of mineral-associated shale OM.

Internal characteristics such as streambed substrate may also influence nutritional resource utilization by aquatic consumers (Smits et al. 2015; Sullivan 2013). In the present study, the size and type of substrate in our different stream systems varied (SI Table 5.1). Streambeds in shale streams were dominated by large unbroken rock outcrop surfaces and small boulders, whereas substrate in non-shale streams was mixed in composition (SI Table 5.1). Large, unbroken rock streambed surfaces in shale streams may limit the retention of terrestrial OM and detritus in these systems, consequently reducing the availability of allochthonous OM to consumers and increasing the assimilation of aquatic primary production (Smits et al. 2015; Wallace et al. 2015; Walters et al. 2007). Therefore, the presence of shale in our watershed may have indirectly influenced nutritional resource utilization by macroinvertebrates.

Factors affecting the availability of soil and shale-derived OM to macroinvertebrates

Previous studies in the same six subwatersheds of the Hudson-Mohawk system by Longworth et al. (2007) suggested that both land use and lithology were important but independent factors influencing stream suspended POM composition and $\Delta^{14}C$ values. We measured $\Delta^{14}C$ from only Nowadega Creek and Fly Brook samples (Fig. 4; SI Tables S2.6, S2.7, S3.1). Both streams were in high agriculture watersheds, preventing us from evaluating the relative influences of lithology and agriculture separately on nutritional resource availability and utilization by macroinvertebrates. However, Longworth et al. (2007) found that both shallow and deep soils at Nowadega Creek had greater median proportional contributions (52 and 42%, respectively) to the POM pool than ^{14}C-free (i.e., fossil aged) shale OM (6%) using a linear mixing model.

For Nowadega Creek, we employed a mass balance approach using the C:N and $\Delta^{14}C$ values of algae/phytoplankton, soil, and terrestrial vegetation (see SI Sect. 5 for details) to estimate the contribution of each to POM. We used two different combinations of endmembers for our mass balance calculations: (1) algae/phytoplankton, 20 cm-depth soil, and terrestrial vegetation and (2) algae/phytoplankton, shale, and terrestrial vegetation. Mass balance calculations suggested that modern terrestrial vegetation did not contribute to suspended POM, but that POM contained roughly equal contributions (~50:50) from algae and soil OM from 20 cm-depth. When fossil shale was used as the aged end member instead of 20 cm-depth soil OM, our calculations again showed that terrestrial vegetation did not contribute (0%), algae comprised an even greater proportion (81%), and shale-derived OM contributed 19% to suspended POM (SI Sect. 5). Contributions of 20-cm-depth soil-derived OM, shale, or both to the POM pool are required to explain the $\Delta^{14}C$ values of POM from Nowadega Creek, as algae alone were not ^{14}C-depleted enough to explain the ^{14}C-depletion in the POM.

Other possible explanations for the lower-than-expected contributions of shale OM to suspended POM and of soil and shale OM to macroinvertebrate biomass in Nowadega Creek include (1) different delivery mechanisms and input rates of soil- and shale-derived particulates (Leithold et al. 2016) and (2) agricultural tilling increasing inputs of aged soil OM (Boix-Fayos et al. 2009; Lal 2003; Longworth et al. 2007). In some lotic systems, mechanical weathering may be primarily responsible for inputs of shale-derived OM and higher erosive flows may be necessary to mechanically weather shale (Graz et al. 2012; Hilton et al. 2011; Leithold et al. 2006). Shale-derived OM may therefore be less readily mobilized to streams, even when shale outcrops are present due to it being more physically stable than soil-derived OM (Leithold et al. 2006).

Chemical weathering of shales is thought to depend on the specific form(s) of kerogen in them (Durand 1980; Zhu et al. 2015). There is also experimental evidence that solubilization releases fossil DOM from shales to aqueous systems (Schillawski and Petsch 2008). However, only two (from Otsquago Creek) out of 24 $\Delta^{14}C$ values of DOM from Longworth et al. (2007) were even modestly ^{14}C-depleted ($\Delta^{14}C = -40$ and $-22‰$; 330 and 180 years B.P., respectively). Therefore, the input of fossil shale-derived DOM to these and similar systems through chemical weathering is unlikely to be significant. Because of the physical and chemical constraints imposed on the weathering of OM-rich sedimentary rocks, we suggest that any aged allochthonous OM subsidizing macroinvertebrate nutrition in our streams was probably derived from moderately aged soil OM, with agricultural activity facilitating its movement into the adjacent streams.

Use of ^{14}C as a tracer in stream food webs

There is growing interest in assessing the roles and quantitative importance of aged C and OM in aquatic food webs, and the implications that the assimilation of aged C and OM by consumers may have on aquatic community structure and ecosystem dynamics (Bellamy and Bauer 2017; Guillemette et al. 2017). Previous aquatic food web studies employing natural abundance ^{14}C found that highly aged forms of OM, such as weathered sedimentary rock, soils, and peat can contribute significantly (up to 57%) to the nutrition of aquatic consumers (Caraco et al. 2010; Schell 1983; Wang et al. 2014). Fossil shale-derived OM potentially contributed ~20% to Nowadega Creek stream suspended POM (SI Table S5.2). This estimate is larger than that of Longworth et al. (2007; 6%), but we have no direct evidence that a source of fossil shale-derived OM contributed to macroinvertebrate biomass. However, one or more sources of fossil non-shale or moderately aged C (most likely weathered carbonates and/or respired soil CO_2, respectively) contributed to macroinvertebrate biomass via fixation of aged DIC by aquatic primary producers and their subsequent consumption and assimilation. The synthesis of living, ^{14}C-depleted autochthonous OM in aquatic systems has been observed in other lentic and lotic systems (Ishikawa et al. 2013, 2014; Keaveney et al. 2015). Findings from the present study indicate that aged forms of C (both organic and inorganic) can be active components of contemporary stream food webs. Natural abundance ^{14}C can be an important independent tracer in aquatic food web studies, and, as in the present study and others, reveals that modern aquatic food webs can be supported by carbon and OM that was formed thousands to millions of years ago (Bellamy and Bauer 2017; Guillemette et al. 2017).

Utilization of allochthonous vs. autochthonous OM varied by FFG in this study as predicted. Assimilation of aged C and OM was also observed to vary by FFG for the two streams in which natural ^{14}C was measured. Future research should aim to identify the primary mechanisms responsible for the ^{14}C-depletion (i.e., non-modern apparent ages) of the majority of aquatic consumers studied to date (Bellamy and Bauer 2017), i.e., either through direct assimilation of aged OM (e.g., fossil and/or soil-derived OM) or via utilization ^{14}C-depleted algae from fixation of aged DIC/CO_2(aq). Utilization and cycling of aged C and OM by aquatic food webs could lead to significant revisions in our conceptual and quantitative elemental and energetic models and budgets for aquatic systems.

The present findings and those of a similar previous study by Wang et al. (2014) suggest that human land alteration in the form of agriculture may influence the inputs, availability and aquatic consumer utilization of aged OM. The present study provides one example of how land use may directly impact contributions of aged OM to stream ecosystems and the subsequent assimilation of this aged OM by stream consumers. Consideration of these two studies together suggests a potential causative relationship between the amount of agriculture in a watershed and the contributions of aged, soil-derived OM to aquatic consumer biomass (SI Fig. S5.1). We also found that an internal stream characteristic (substrate type) controlled the sources and ages of C and OM available to consumers (SI Table S5.1). Future research should therefore also seek to address the importance of different watershed-scale factors and internal characteristics of aquatic systems on the ages of C and OM pools present in stream waters and sediments, and explore the potential consequences of this for associated food webs.

Contrary to the traditional paradigm that young materials are more readily consumed and assimilated by aquatic organisms (Berggren et al. 2009; Mayorga et al. 2005; Sobczak et al. 2005), findings from the present and other recent studies (see Bellamy and Bauer 2017, for review) suggest that aged forms of C and OM are more often than not important to the nutrition of aquatic consumers. It is increasingly thought that the ages of different sources of naturally occurring OM are alone not indicative of their nutritional value or bioavailability (Guillemette et al. 2017; Marin-Spiotta et al. 2014; McCallister and del Giorgio 2012). Future studies that employ natural ^{14}C as a tracer in aquatic food webs should therefore seek to evaluate (1) the relationship between OM substrate age and its nutritional availability to consumers, and (2) contributions of ^{14}C-depleted algal and macrophytic biomass. Approaches including the use of compound class and compound-specific isotope analyses may also prove valuable for better differentiating between aged allochthonous and aged autochthonous OM utilization by aquatic consumers (Ishikawa et al. 2015; Kruger et al. 2016).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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