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Impact of *Corbicula fluminea* (Asian clam) on seston in an urban stream receiving wastewater effluent

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Abstract. We hypothesized that *C. fluminea* could remove anthropogenic N at a rate sufficient to affect particulate N in the water column of North Buffalo Creek, North Carolina, USA, a 4th-order stream that receives treated urban wastewater. We used stable-isotope analysis to evaluate trophic relationships between seston and *C. fluminea* and conducted field sampling and laboratory experiments to evaluate the potential qualitative and quantitative effects of *C. fluminea* on seston. *Corbicula fluminea* $\delta^{15}N$ was 3 to 5‰ enriched compared to seston along a longitudinal transect downstream of the wastewater treatment plant (WWTP), a result consistent with use of seston N. However, seston $\delta^{13}C$ declined and *C. fluminea* $\delta^{13}C$ showed no pattern with distance downstream, a result that was inconsistent with a trophic relationship between seston and *C. fluminea*. In 2 laboratory experiments designed to measure filter-feeding rate and qualitative effects on seston, seston ash-free dry mass and chlorophyll *a* data indicated that *C. fluminea* either was not filtering or was filtering at a rate insufficient to affect seston concentration over the course of the experiments. $\delta^{15}N$ data showed that the sediment was an N source for *C. fluminea*, but $\delta^{13}C$ and C:N data from the same experiments indicated that *C. fluminea* probably affected seston quality by suspending benthic algae and returning settled algae to the water column. These results illustrate that the food sources for *C. fluminea* and implications of *C. fluminea* activity in stream ecosystems should be evaluated more fully.

Key words: *Corbicula fluminea*, anthropogenic nitrogen, seston quality, urban stream, wastewater treatment plant effluent.

Anthropogenic N addition to terrestrial systems is increasing worldwide and, in turn, is increasing N loading to freshwaters (Peterson et al. 2001, Mulholland et al. 2008). Most urban streams receive both point and nonpoint sources of anthropogenic N. Point sources, such as wastewater treatment plants (WWTP), are highly regulated, but they continue to affect urban streams by contributing 50 to 90% of their annual nutrient loading (Haggard et al. 2001, 2005). WWTP effluent has a long-lasting effect on urban-stream ecosystems by altering their natural ability to process N effectively. Increased anthropogenic NO_3^- transported from streams and rivers to downstream coastal waters accelerates coastal eutrophication, reduces biodiversity, and causes the formation of hypoxic zones and algal blooms (Rosenzweig et al. 2008, Weijters et al. 2009). As N input increases, N-processing efficiency and proportional denitrification decreases, resulting in greater N export to downstream reaches (Peterson et al. 2001, Mulholland et al. 2008). For example, denitrification rate was high in an urban stream receiving WWTP effluent, but denitrification was unimportant compared to total N loads, and therefore, large quantities of N were exported downstream (Lofton et al. 2007). If a stream is unable to retain N, then the downstream system will receive higher N loads (Grimm et al. 2005). N loading from upstream areas is a particularly serious problem in North Carolina estuaries where increased N is causing water-quality problems (Burkholder et al. 2006).

Stable isotopes of N are useful for detecting anthropogenic N in aquatic systems (Ulseth and Hershey 2005). Stable-isotope ratios can be used to trace organic matter through food webs because consumers typically are enriched 3 to 5‰ in ^{15}N and 0.5 to 1‰ in ^{13}C relative to their food sources (Peterson and Fry 1987). Sewage-derived N below WWTPs is readily traced through aquatic food webs because it has a higher $\delta^{15}N$ value than most natural N sources (Wayland and Hobson 2001, DeBruyn and

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Rasmussen 2002, Ulseth and Hershey 2005, Nishikawa et al. 2009).

Corbicula fluminea (Asian clam) is an invasive species in many freshwater ecosystems. It has a high potential to alter seston quality and concentration through filter feeding. Stable-isotope analysis has shown that *C. fluminea* diet is very similar to the diet of the invasive zebra mussel *Dreissena polymorpha* (Delong 2010), which reduces phytoplankton abundance in lakes (Mackie 1991). In lakes and large rivers, *C. fluminea* also decreases phytoplankton abundance (Cohen et al. 1984, Newell et al. 2005) and zooplankton density through filter feeding, thereby increasing water clarity (Sousa et al. 2009). The filtering rate of *C. fluminea* has been estimated to be sufficient to clear the water column in the Chowan River, North Carolina, every 1.6 d (Lauritsen 1986); a reach of the Potomac River, Virginia, in 3 to 4 d (Cohen et al. 1984); and Meyers Branch, South Carolina, in 21 h (Leff et al. 1990).

Corbicula fluminea has the potential to have direct and indirect effects on its sediment habitat. Reduction of phytoplankton abundance by filter feeders can lead to nutrient burial through biodeposition (Newell et al. 2005). Biodeposits of *C. fluminea* have a high N content relative to those of native mussels (Atkinson et al. 2011). Several investigators have suggested that *C. fluminea* uses both filter feeding and deposit feeding to meet nutritional needs (Yeager and Cherry 1994, Hakenkamp and Palmer 1999, Hakenkamp et al. 2001, Vaughn and Hakenkamp 2001, Cummings and Graf 2010). Pedal feeding can result in an estimated sediment organic matter consumption of 50 mg individual^{-1} d^{-1} (Hakenkamp and Palmer 1999).

We evaluated the potential for *C. fluminea* to reduce particulate anthropogenic N in transport via filter feeding in North Buffalo Creek, an urban stream in Greensboro, North Carolina (USA). We hypothesized that *C. fluminea* would be able to filter at a rate sufficient to substantially reduce anthropogenic particulate N in the water column and, therefore, to provide an ecosystem service. We used field campaigns and laboratory experiments: 1) to assess whether suspended particulate organic matter (seston) appeared to be the dominant food source for *C. fluminea* in North Buffalo Creek, and 2) to estimate the effect of *C. fluminea* filtering activity on quality and quantity of seston.

Methods

Site description

North Buffalo Creek originates in Greensboro, North Carolina, a city with a population of 260,083 (Greensboro Convention and Visitors Bureau 2012), and receives both point and nonpoint pollution from the city. The stream is the headwater of the Cape Fear River Basin and is 4th order in the section we studied. The major N point source is the North Buffalo WWTP (Ulseth and Hershey 2005, Lofton et al. 2007). The WWTP primarily treats residential sewage from the northern ½ of the city and contributes ~50% of the baseflow discharge to the stream. Since 1980, the capacity of the North Buffalo WWTP has been 60,567 m^3/d (City of Greensboro Water Resource Department 2011). North Buffalo WWTP uses a process ending with sand filters and the addition of sodium hypochlorite to mitigate pathogenic bacteria. However, nutrient removal is not part of the wastewater treatment process (City of Greensboro Water Resource Department 2011). North Buffalo WWTP is permitted to discharge 8 mg/L NH_4^+ to the river in winter and 4 mg/L in summer (as cited by Lofton et al. 2007). Previous investigators (Ulseth and Hershey 2005, Lofton et al. 2007) found elevated levels of dissolved inorganic N (DIN) throughout the study reach. Elevated levels of DIN are a consistent occurrence in streams receiving urban runoff and wastewater (Grimm et al. 2005, Lofton et al. 2007). Average nutrient concentrations in water at site B, Rankin Mill Road (Fig. 1), were ~0.13 mg/L NH_4^+ and ~5.75 mg/L NO_3^- and ~0.2 mg/L NH_4^+ and ~9.5 mg/L NO_3^- in previous studies (Lofton et al. 2007, Ulseth and Hershey 2005, respectively). A site upstream of the WWTP appeared to serve as an N sink where ~46% of NO_3-N was removed via denitrification, whereas at downstream sites only ~2.3% of NO_3-N was removed through denitrification (Lofton et al. 2007). $\delta^{15}N$ of seston at site B was ~8.3‰ (Ulseth and Hershey 2005), a value consistent with values from other streams receiving WWTP input (Mayer et al. 2002).

North Buffalo Creek has no permitted discharge points upstream of the WWTP (as cited in Ulseth and Hershey 2005). Upstream of site A (Fig. 1), land use is virtually all urban except in forested areas in urban parks and in narrow buffer zones. Runoff is diverted directly to the stream. Between sites A and B, land uses include residential development, forest, a landfill, other municipal and county facilities, and the WWTP. Below the WWTP, land use is primarily forested with some rural pastureland that has forested buffer zones (Lofton et al. 2007). North Buffalo Creek has been studied for a number of years as a part of a US Environmental Protection Agency (EPA) mandated Total Maximum Daily Load (TMDL) project targeting fecal coliform bacteria (NCDENR 2004). North Buffalo Creek has been used as a research site

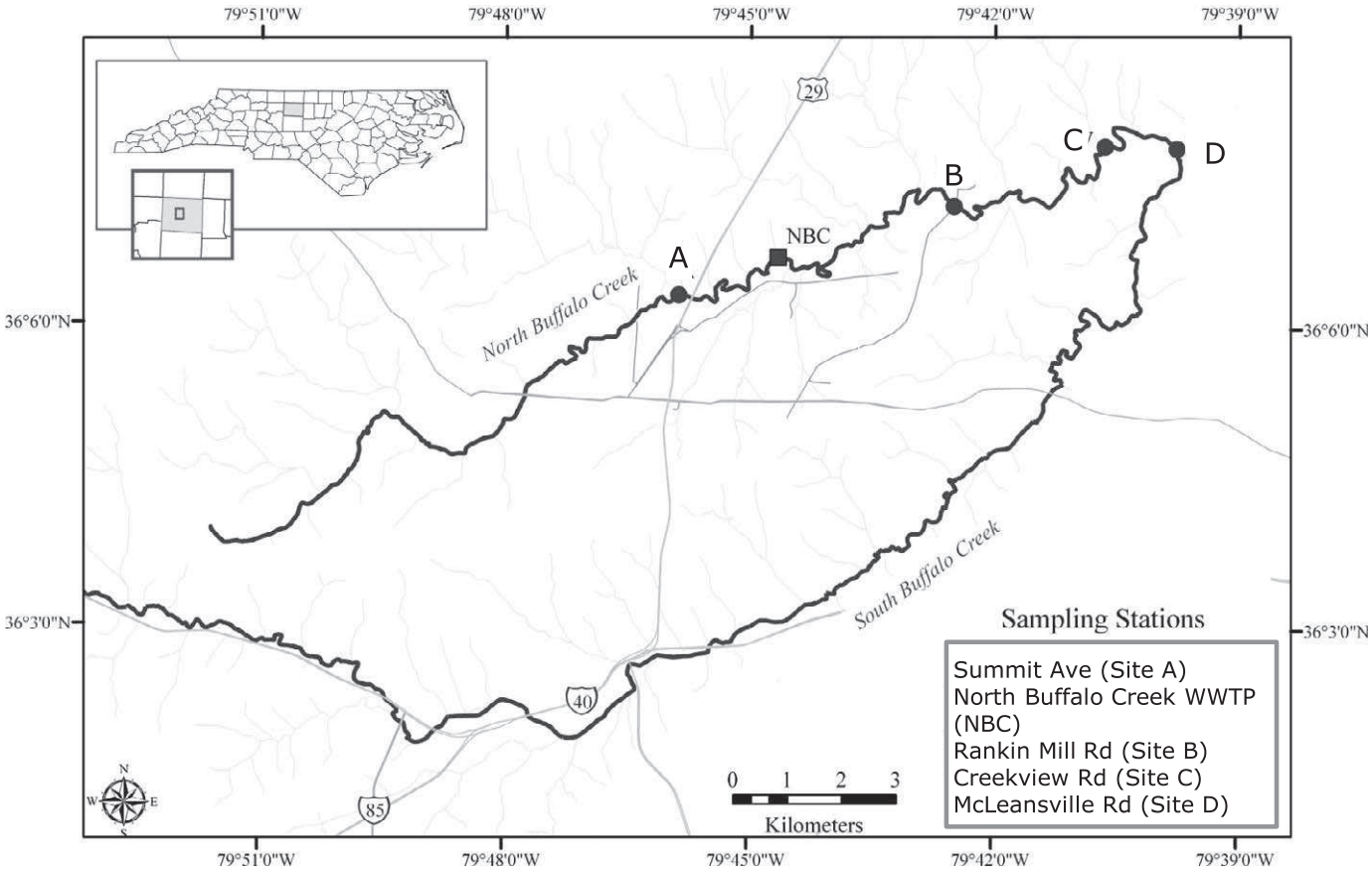

FIG. 1. Map of study sites on North Buffalo Creek, North Carolina, USA. Inset shows the location of North Buffalo Creek in Guilford County, North Carolina.

for studies of urban stream food webs, restoration effects, incorporation of wastewater-derived N into aquatic food webs, and the capacity of the stream to process N (Ulseth and Hershey 2005, Northington and Hershey 2006, Kalcounis-Rüppell et al. 2007, Lofton et al. 2007, Hines and Hershey 2011).

Site A, Summit Avenue has a narrow forested buffer and is the only site upstream of the WWTP that was sampled during our study. Site B is ~8 stream km downstream of the North Buffalo WWTP and is outside Greensboro city limits (Lofton et al. 2007). Site D, McLeansville Road, ~7 stream km downstream of site B is just above the confluence with South Buffalo Creek. Site C, Creekview Road, is at the only road crossing between sites B and D (Fig. 1).

Trophic relationships between seston and C. fluminea *in North Buffalo Creek*

To evaluate objective 1, we sampled *C. fluminea* and seston for stable-isotope analyses in summer and winter. Summer sampling occurred on 23 June 2009 at site B and on 26 June 2009 at sites A, C, and D. Discharge conditions were 0.65 m^3/s and 0.67 m^3/s on those dates (US Geological Survey [USGS] gage 02095500 at site B), respectively. Discharge at site A (not measured) would have been much lower because it is above the WWTP. Winter sampling occurred on 15 December 2009 (discharge = 1.27 m^3/s at site B) at ~500-m intervals along the longitudinal transect from sites B to D, which we accessed by canoeing the reach. We used a global positioning system (GPS) to measure longitudinal stream distances.

We held *C. fluminea* individuals from each site overnight in deionized water and then dried them at 60°C for ≥24 h. We ground dried *C. fluminea* tissue to a powder, which we placed in 4 × 6-mm pressed Sn capsules and sent to the University of California (UC)-Davis Stable Isotope Laboratory for measurement of $\delta^{15}N$ and $\delta^{13}C$.

We collected water for seston analyses in 4-L cubitainers and returned it to the laboratory. We vacuum-filtered subsamples (400 mL) onto 6 pre-combusted glass-fiber filters (0.7-µm pore size) per site. We dried all filters at 60°C for ≥48 h and prepared 3 of the 6 filters/site for stable-isotope

analysis. We put ¼ of each filter for stable-isotope analysis in a Sn capsule and sent the samples to UC-Davis Stable Isotope Laboratory for measurement of $\delta^{15}N$ and $\delta^{13}C$. We calculated seston C:N for each sampling site with the data received from the UC-Davis Stable Isotope Laboratory. We used the 3 remaining filters from each site to estimate seston ash-free dry mass (AFDM) after combustion in a muffle furnace at 500°C.

Evaluation of the effect of C. fluminea *filtering on seston quality and quantity*

To evaluate objective 2, we assessed field density on 2 sampling dates and conducted 2 laboratory experiments. We estimated field density to evaluate whether *C. fluminea* was abundant enough to affect seston. We used the laboratory experiments to measure effects of *C. fluminea* filtering activity on seston quantity and quality with and without sediment present at realistic field densities. We quantified the effect of *C. fluminea* on seston quantity as change over time in seston AFDM. We assessed the effect of *C. fluminea* on seston quality by measuring change in seston $\delta^{15}N$, $\delta^{13}C$, C:N, and chlorophyll *a* (chl *a*) over time.

We estimated *C. fluminea* density with a Surber sampler (0.093 m^2) when we sampled individuals for isotope analyses. *Corbicula fluminea* density is higher in summer than in winter (Cohen et al. 1984). We collected 3 replicate samples in pool, riffle, and run habitats at each sampling site on the summer sampling dates to assess whether *C. fluminea* density was related to habitat. In winter, we collected 3 replicate Surber samples at pools sampled for stable isotopes. We sampled only pools so that we could complete the sampling during daylight hours.

We conducted experiment 1 on 23 September 2009 with *C. fluminea*, sediment, and stream water collected from site B (Fig. 1). This experiment was designed to measure effects of *C. fluminea* on seston quality and quantity when sediments were present. We used 38-L aquaria (0.125 m^2 bottom area) as experimental mesocosms. Three control mesocosms (sediment + water) contained sediment (1.5-cm depth) and unfiltered stream water (34.1 L). Three treatment mesocosms (*C. fluminea* + sediment + water) contained 4 *C. fluminea* (mean length = 21 mm), sediment (1.5-cm depth), and unfiltered stream water (34.1 L). *Corbicula fluminea* density (32 *C. fluminea*/m^2) was at the low end of that observed in North Buffalo Creek (see Results). Based on previous work (Lauritsen 1986), the 4 *C. fluminea* in each mesocosm should have been able to filter the entire water column during the 12-h experiment.

We filled the mesocosms with sediments and water 36 h before initial sampling (t = 0) and addition of *C. fluminea* and housed them in an environmentally controlled room at 23.4°C, the temperature of North Buffalo Creek when water, sediment, and *C. fluminea* were collected. We added 100 mL of a solution of 1 g of 99% ^{13}C-$NaHCO_3$ (^{13}C-dissolved inorganic C [DIC]) in deionized water 24 h before t = 0 to each mesocosm to enrich the seston in ^{13}C via algal uptake of ^{13}C-DIC. Our goal was to increase the likelihood of seeing a change in $\delta^{13}C$ caused by removal of algal components of seston by *C. fluminea* during the 12-h experiment. We aerated the mesocosms via aeration tubes secured halfway up the side walls to minimize settling of seston and to prevent the water from becoming anoxic. We held the mesocosms in full light for 24 h to allow the algae to incorporate the ^{13}C label, then turned the lights off at t = 0 and allowed the experiment to proceed in the dark. We sampled 1.2 L of water from each mesocosm without replacement at t = 0 and at 2-h intervals (in low light during sampling) until t = 12 h. We did not replace water to avoid diluting the experimental water and the isotope tracer. The final volume was 25.7 L.

We filtered samples onto precombusted glass-fiber filters to measure AFDM, stable isotope ratios of C and N, C:N, and chl *a* (n = 3). The procedures for AFDM and stable isotope sample collections were the same as previously described. For the chl *a* analysis, we filtered 400 mL of each sample onto separate glass-fiber filters and froze the filters in a light-tight container until analysis. We used 10 mL of 90% high performance liquid chromatography (HPLC)-grade acetone to extract the chl *a* (APHA 1998). We were unable to obtain chl *a* data for experiment 1 because of spectrophotometer failure. We collected bulk sediment samples from each mesocosm at t = 0 and t = 12 h, dried and weighed them, packed them in Sn capsules, and sent the samples to the UC-Davis laboratory for stable-isotope analysis.

We ran a 2nd laboratory experiment (experiment 2) on 10 March 2010 because we found no evidence of filtering activity by *C. fluminea* in experiment 1 (see Results). We used a different combination of treatments (*C. fluminea* + sediment + water, *C. fluminea* + water, water only) (n = 3 replicates/treatment). The change in treatments was designed to test whether a *C. fluminea* filtering effect would be detected in the absence of sediment resources and whether that filtering activity affected seston quality and quantity. We included the *C. fluminea* + sediment + water treatment because that treatment mimicked the natural environment. In each replicate of the 2 treatments containing *C. fluminea*, we used 5 bivalves

(mean length = 21 mm) per 38-L mesocosm and 32.1 L of unfiltered water with or without sediment (1.5-cm depth) from site B. Mesocosms in the water-only treatment contained 32.1 L of unfiltered water from site B. We expected the increase in *C. fluminea* density and decrease in initial water volume to enhance our ability to assess whether *C. fluminea* was filtering. We conducted experiment 2 in the same manner as experiment 1 except for the following: 1) 100 mL of a solution of 900 mg of 99% ^{13}C-DIC in 900 mL of deionized water was added to each mesocosm, 2) δ^{13}C and δ^{15}N of *C. fluminea* were measured in treatments containing *C. fluminea*, and 3) a fluorometer was used to measure chl *a* concentration (Wetzel and Likens 2000). The final volume in each mesocosm was 23.7 L.

Data analysis

We conducted all statistical analyses in SPSS (version 16.0; IBM, Armonk, New York). We examined all data sets (each transect variable and all response variables from experiments 1 and 2) for normality with Kolmogorov–Smirnov (KS) tests. We ln(x)-transformed transect AFDM data to correct for non-normality, but no other variable required transformation. We evaluated the difference in *C. fluminea* density between different habitats (summer only) and between seasons (pools only) with 1-way analyses of variance (ANOVAs). We pooled replicates within sites to provide a single observation per site. We used independent-sample *t*-tests to compare δ^{15}N and δ^{13}C values of *C. fluminea* and seston at each sampling site (June 2009 sampling) and to compare mean δ^{15}N and δ^{13}C values of *Corbicula* between treatments in experiment 2. We used linear regression with ANOVA to test whether AFDM, δ^{15}N, and δ^{13}C changed with distance along the longitudinal transect (December 2009 sampling). We did not apply isotope-mixing models to *C. fluminea* stable-isotope data because the only potential food source sampled was seston.

We used repeated measures ANOVA with time, treatment, and the time × treatment interaction effect to test for changes in AFDM, δ^{15}N, δ^{13}C, C:N, and chl *a* (experiment 2 only) of seston through time. We compared initial and final measures of seston response variables with *t*-tests for experiment 1 and 1-way ANOVA for experiment 2. In experiment 1, we used a paired *t*-test to evaluate the effect of *C. fluminea* presence on sediment δ^{13}C and a *t*-test to compare sediment δ^{15}N between treatments at t = 12. A paired *t*-test could not be applied to δ^{15}N data because we dropped ½ of the initial samples from the data set because they had highly enriched values indicative of contamination with enriched ^{15}N, which was in use in the laboratory in association with another project. We considered p-values < 0.05 significant.

TABLE 1. Mean ±1 SD (n) *Corbicula fluminea* density (individuals/m^2) in 3 habitats and 2 seasons in North Buffalo Creek. Riffles and runs were not sampled in December.

Month	Pool	Riffle	Run
June	141 ± 146 (4)	32 ± 48 (4)	46 ± 40 (4)
December	41 ± 35 (12)	–	–

Results

In June 2009, *C. fluminea* density in North Buffalo Creek was 141 ± 146 (mean ± SD), 32 ± 48, and 46 ± 40 individuals (ind)/m^2 in pool, riffle, and run habitats, respectively (Table 1). Density did not differ significantly among habitats. *Corbicula fluminea* density in pools was significantly lower in December (41 ± 35 ind/m^2) than in June ($t = -2.34$, df = 14, $p = 0.035$).

Seston δ^{15}N (9.32 ± 0.43‰) was significantly greater than *C. fluminea* δ^{15}N (6.80 ± 0.11‰) at site B, but site B was the only location where the seston was more ^{15}N enriched than *C. fluminea* (Fig. 2A–D, Table 2). At sites C and D, δ^{15}N of seston was significantly lower than δ^{15}N of *C. fluminea* (Fig. 2C, D, Table 2). At all sites except site B, the expected shift of 3 to 5‰ N from resource to consumer was observed, but the comparison was not significant at site A.

Seston δ^{13}C was significantly greater than *C. fluminea* δ^{13}C at all sites in June (Table 2, Fig. 3A–D). The difference in δ^{13}C between seston and *C. fluminea* was greatest at site A (4.6‰) followed by sites D (2.7‰), C (2.5‰), and B (1.5‰). The δ^{13}C and δ^{15}N biplot (Fig. 4) for *C. fluminea* and seston at the 4 sites showed that the isotopic signatures did not correspond to the expected pattern based on a trophic shift from resource to consumer.

Seston AFDM declined exponentially with distance from sites B to D in December ($R^2 = 0.33$, $F_{1,41} = 19.495$, $p < 0.001$; Table 3, Fig. 5A). A decline in seston AFDM was the expected pattern if *C. fluminea* was removing WWTP particulate organic matter from North Buffalo Creek. However, no significant linear regression model was found for changes in seston C:N ($R^2 = 0.01$, $F_{1,42} = 0.434$, $p = 0.514$; Fig. 5B). Seston δ^{13}C decreased exponentially with distance downstream ($R^2 = 0.28$, $F_{1,42} = 16.138$, $p < 0.001$; Table 3, Fig. 5C), but *C. fluminea* δ^{13}C showed no clear longitudinal pattern ($F_{1,42} = 0.688$, $p = 0.412$; Fig. 5C). These results suggest that *C. fluminea* was not using seston as its primary C source. Both seston and *C. fluminea* δ^{15}N increased with distance downstream (Fig. 5D). The regression model for seston δ^{15}N was

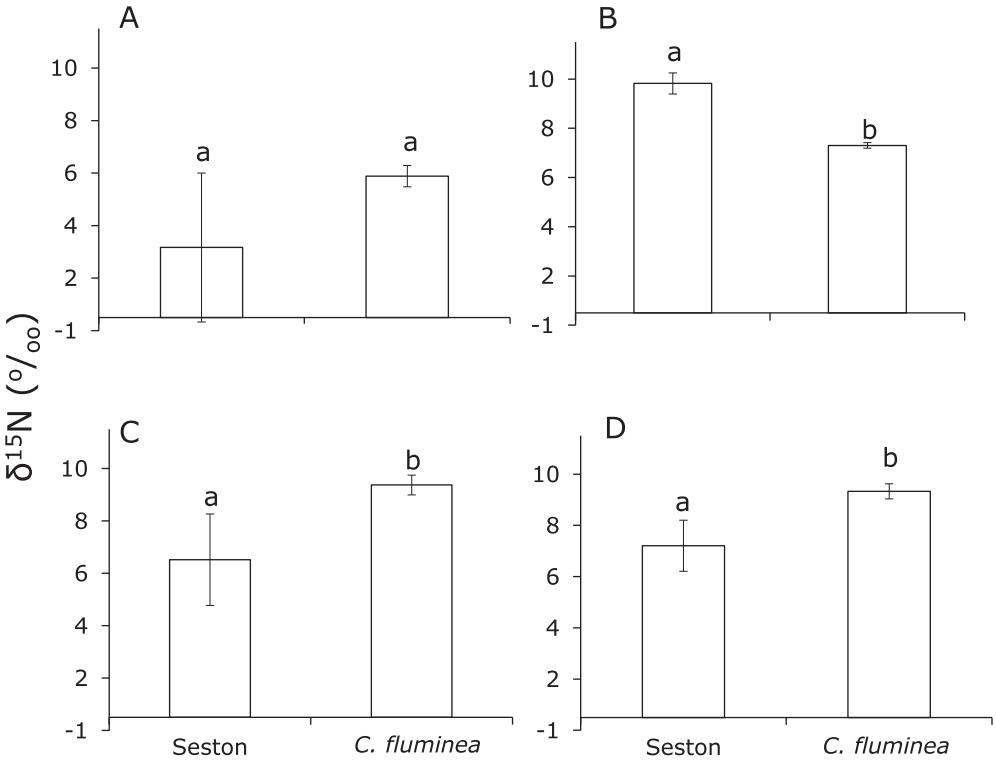

FIG. 2. Mean (±1 SD) $\delta^{15}N$ of seston and *Corbicula fluminea* at sites A (A), B (B), C (C), and D (D) in North Buffalo Creek in late June 2009. Means with the same letters are not significantly different ($p < 0.05$).

significant but weak ($R^2 = 0.14$, $F_{1,42} = 6.632$, $p = 0.014$; Table 3), whereas distance downstream was a stronger predictor of *C. fluminea* $\delta^{15}N$ ($R^2 = 0.29$, $F_{1,44} = 17.804$, $p < 0.001$; Table 3). At each site along the stream transect, *C. fluminea* $\delta^{15}N$ was 4 to 5‰ greater than seston $\delta^{15}N$.

Experiment 1

Seston AFDM did not differ between the sediment + water and *C. fluminea* + sediment + water treatments at t = 0 ($t = -0.405$, $p = 0.706$; Fig. 6A, Table 4) or t = 12 ($t = -1.001$, $p = 0.374$). The repeated measures ANOVA did not indicate a significant effect of time, treatment, or time × treatment over the course of the experiment (Table 5).

TABLE 2. Comparison of stable-isotope signatures between seston and *Corbicula fluminea* samples taken in June 2009 from North Buffalo Creek.

Date	Site	Isotope ratio	*t*	*p*	df
26 June	A	$\delta^{15}N$	−1.640	0.176	4
26 June	A	$\delta^{13}C$	11.313	0.005	4
23 June	B	$\delta^{15}N$	9.802	0.001	4
23 June	B	$\delta^{13}C$	6.663	0.003	4
26 June	C	$\delta^{15}N$	−2.767	0.050	4
26 June	C	$\delta^{13}C$	8.382	0.001	4
26 June	D	$\delta^{15}N$	−3.537	0.024	4
26 June	D	$\delta^{13}C$	6.766	0.002	4

The ^{13}C-DIC enriched the seston by ~13‰ compared to stream seston $\delta^{13}C$. $\delta^{13}C$ of the seston did not differ between the *C. fluminea* + sediment + water treatment and the sediment + water treatment at t = 0 or at t = 12 h (Fig. 6B, Table 4), but the repeated measures ANOVA showed a significant time effect ($F_{1,4} = 28.212$, $p = 0.006$; Table 5), and a significant time × treatment interaction effect ($F_{1,4} = 9.711$, $p = 0.036$). These results showed that seston $\delta^{13}C$ changed differently between treatments over the course of the experiment. The ANOVA results showed that the presence of *C. fluminea* altered the $\delta^{13}C$ of the seston, such that seston $\delta^{13}C$ became more enriched through time in the *C. fluminea* + sediment + water treatment but did not change in the sediment + water treatment (Fig. 6B).

Seston $\delta^{15}N$ did not differ between treatments at t = 0 or t = 12 h ($t = -1.222$, $p = 0.287$, and $t = 1.692$, $p = 0.166$, respectively; Fig. 6C, Tables 4, 5). The repeated measures ANOVA did not indicate a significant time

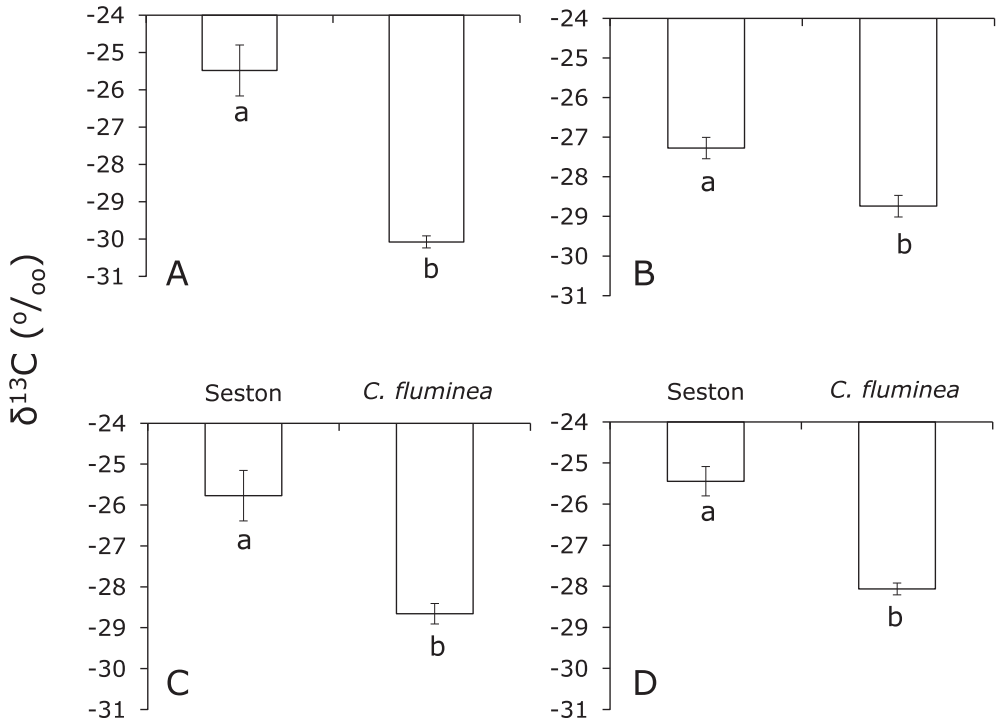

FIG. 3. Mean (±1 SD) $\delta^{13}C$ of seston and *Corbicula fluminea* at sites A (A), B (B), C (C), and D (D) in North Buffalo Creek in late June 2009. Means with the same letters are not significantly different ($p < 0.05$).

($F_{1,4} = 4.18$, $p = 0.117$), treatment ($F_{1,4} = 0.69$, $p = 0.693$), or interaction ($F_{1,4} = 5.28$, $p = 0.083$) effect on seston $\delta^{15}N$.

Seston C:N increased in the sediment + water treatment over time. Initial C:N did not differ between treatments ($t = 0.443$, $p = 0.681$; Fig 6D, Table 4), but the final C:N differed significantly between treatments ($t = 3.767$, $p = 0.020$). The repeated measures ANOVA showed significant time ($F_{1,4} = 8.756$, $p = 0.042$) and treatment ($F_{1,4} = 11.986$, $p = 0.026$; Table 5) effects, but the interaction was not significant ($F_{1,4} = 6.939$, $p = 0.058$). These effects indicate that the C:N of seston changed unless *C. fluminea* was present.

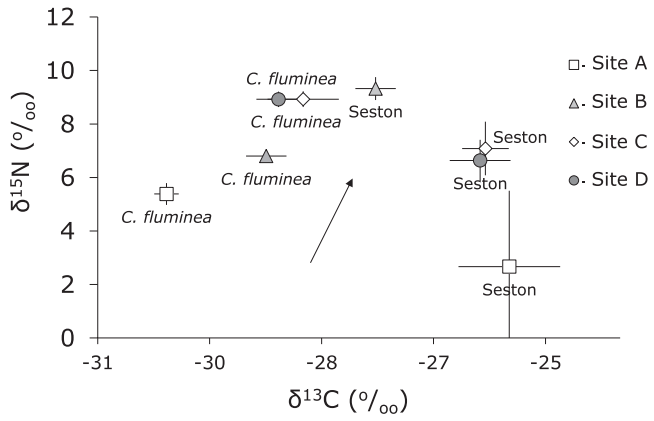

FIG. 4. Mean (±1 SD) $\delta^{13}C$ and $\delta^{15}N$ biplot of seston and *Corbicula fluminea* for sites A, B, C, and D. Arrow shows expected shift between resource and consumer at a given site.

Bulk sediment $\delta^{15}N$ ranged from 17.45 ± 1.21 to 19.32 ± 0.46 and did not differ between treatments ($t = -1.445$, $p < 0.222$; Fig. 7A). Sediment $\delta^{13}C$ ranged from −25.9 ± 0.73 to −23.02 ± 1.05 and did not differ between treatments ($t = -0.309$, $p < 0.772$; Fig. 7B).

Experiment 2

Seston AFDM did not change over time (Fig. 8A, Tables 6, 7), and initial ($F_{2,8} = 2.074$, $p = 0.201$) and final ($F_{2,8} = 0.726$, $p = 0.522$) AFDM did not differ among treatments. The repeated measures ANOVA showed no time ($F_{1,6} = 0.024$, $p = 0.883$), treatment ($F_{2,6} = 1.289$, $p = 0.342$), or interaction ($F_{2,6} = 1.822$, $p = 0.241$) effect. Thus, *C. fluminea* did not filter at a rate sufficient to change seston AFDM regardless of whether sediment was present.

Initial mean seston $\delta^{13}C$ values were −12.28, −11.55, and −9.51‰ in the water only, *C. fluminea* + water, and *C. fluminea* + sediment + water treatments, respectively (Fig. 8B). Initial ($F_{2,8} = 0.734$, $p = 0.519$; Table 6) and final ($F_{2,8} = 0.138$, $p = 0.874$) $\delta^{13}C$ values

TABLE 3. Regression models for longitudinal transect data downstream of a wastewater treatment plant (in m) on North Buffalo Creek (15 December 2009). Seston and *Corbicula fluminea* samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$, and seston was further analyzed for ash-free dry mass (AFDM).

Predicted variable	Model	R^2	df	F	p
AFDM (mg/mL)	= 4.57 − 0.29 ln(distance)	0.33	1,41	19.495	<0.001
Seston $\delta^{13}C$ (‰)	= −26.98 − 0.12 ln(distance)	0.28	1,42	16.138	<0.001
Seston $\delta^{15}N$ (‰)	= 3.27 + ln(distance)	0.14	1,42	6.632	0.014
C. fluminea $\delta^{15}N$ (‰)	= 7.38 + 0.22 ln(distance)	0.29	1,44	17.804	<0.001

did not differ among treatments. $\delta^{13}C$ increased significantly over time in all treatments ($F_{1,6} = 55.82$, $p < 0.001$; Table 7), but the treatment ($F_{2,6} = 0.321$, $p = 0.737$) and interaction ($F_{2,6} = 0.014$, $p = 0.986$) effects were not significant. Thus, seston $\delta^{13}C$ changed in the same way in all treatments over time.

Seston $\delta^{15}N$ increased over the course of the experiment in all treatments (Fig. 8C, Tables 6, 7). Initial $\delta^{15}N$ values differed among treatments ($F_{2,8} = 6.770$, $p = 0.029$). Seston $\delta^{15}N$ differed between the *C. fluminea* + water and *C. fluminea* + sediment + water treatments ($F_{2,8} = 6.770$, $p = 0.026$). Final seston $\delta^{15}N$ values did not differ among treatments ($F_{2,8} = 1.674$, $p = 0.264$). Repeated measures ANOVA showed a significant time effect ($F_{1,6} = 76.52$, $p < 0.001$), but the treatment ($F_{2,6} = 4.595$, $p = 0.062$) and interaction ($F_{2,6} = 4.373$, $p = 0.071$) effects were not significant. Thus, seston $\delta^{15}N$ changed in the same way in all treatments over time.

Seston C:N declined over time (Fig. 8D, Tables 6, 7), but neither initial ($F_{2,8} = 0.283$, $p = 0.763$) nor final ($F_{2,8} = 2.728$, $p = 0.144$) C:N differed among treatments. The decrease in C:N was greatest in the *C. fluminea* + water treatment (2.2), followed by the *C. fluminea* + sediment + water (1.8), and water only (1.1) treatments. Repeated measures ANOVA showed that treatment effect was not significant ($F_{2,6} = 0.540$, $p = 0.609$), but the time ($F_{1,6} = 294.3$, $p < 0.001$) and

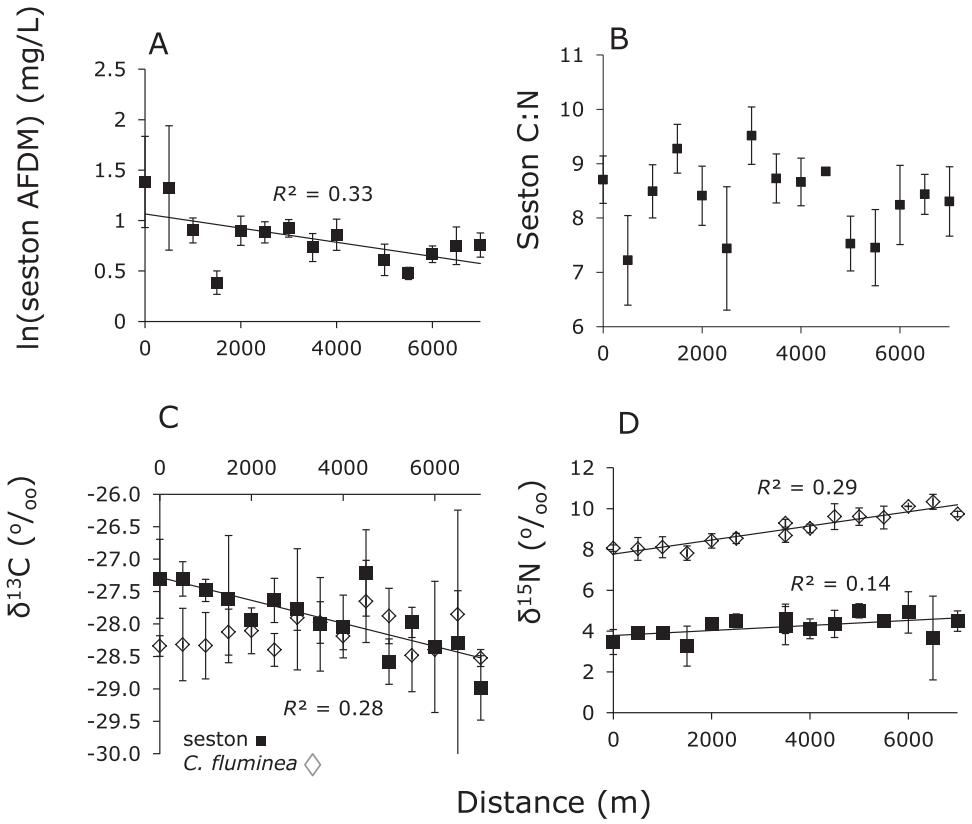

FIG. 5. Mean (±1 SD) seston ash-free dry mass (AFDM) (A) and C:N (B), and seston and *Corbicula fluminea* $\delta^{13}C$ (C) and $\delta^{15}N$ (D) along a longitudinal transect in North Buffalo Creek in December 2009.

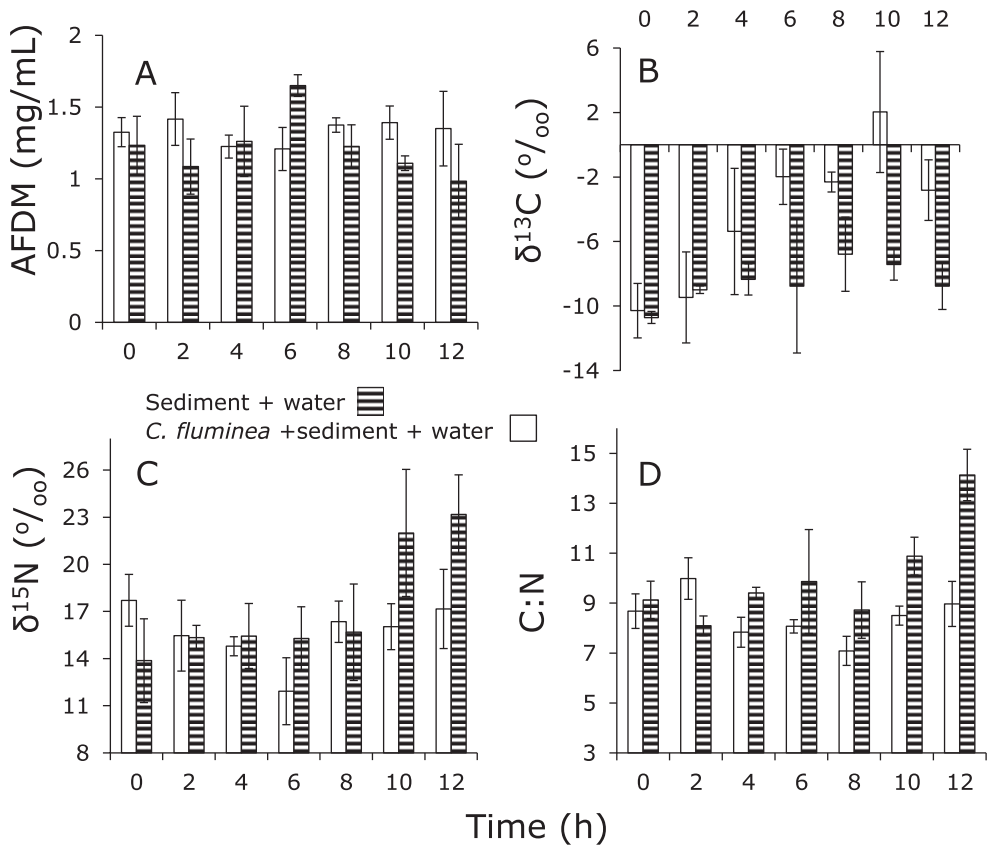

FIG. 6. Mean (±1 SE) seston ash-free dry mass (AFDM) (A), $\delta^{13}C$ (B), $\delta^{15}N$ (C), and C:N (D) in experiment 1.

interaction ($F_{2,6}$ = 10.02, p = 0.012) effects were significant, indicating that *C. fluminea* decreased seston C:N relative to changes in the control treatment.

Seston chl *a* decreased after t = 0 in all treatments (Fig. 8E, Tables 6, 7), probably because the lights were off. Initial chl *a* values were highly variable and did not differ among treatments ($F_{2,7}$ = 2.292, p = 0.197). However, final values differed among treatments ($F_{2,8}$ = 8.864, p = 0.016). Chl *a* was significantly higher in the *C. fluminea* + sediment + water treatment (p = 0.033) than in the water only and *C. fluminea* + water treatments (p = 0.020). Repeated measures ANOVA showed neither a significant time ($F_{1,6}$ = 3.283, p = 0.130), treatment ($F_{2,6}$ = 5.428, p = 0.056), or interaction ($F_{2,6}$ = 0.729, p = 0.527) effect.

Corbicula fluminea $\delta^{15}N$ was significantly lower in the *C. fluminea* + water treatment than in the *C. fluminea* + sediment + water treatment (t = −2.074, df = 4, p = 0.050; Fig. 9A). Mean *C. fluminea* $\delta^{13}C$ (n = 3) did not differ between the 2 treatments with *C. fluminea* (t = −1.306, df = 4, p = 0.13; Fig. 9B). Thus, the presence of sediment had a qualitative effect on *C. fluminea* N composition but not on C composition.

TABLE 4. Comparison of seston variables at the beginning (t = 0) and end (t = 12) of experiment 1. Independent *t*-tests were used to compare seston ash-free dry mass (AFDM), isotopic signatures, and C:N between *Corbicula fluminea* + sediment + water and sediment + water treatments.

	t = 0 h			t = 12 h		
Variable	t	df	p	t	df	p
AFDM	−0.405	4	0.706	−1.001	4	0.374
$\delta^{13}C$	−0.244	4	0.819	−2.504	4	0.066
$\delta^{15}N$	−1.222	4	0.287	1.692	4	0.166
C:N	0.443	4	0.681	3.767	4	0.020

TABLE 5. Repeated measures analysis of variance comparing seston ash-free dry mass (AFDM), isotopic signatures, and C:N between *Corbicula fluminea* + sediment + water and sediment + water treatments.

	Time			Treatment			Interaction		
Variable	*F*	df	*p*	*F*	df	*p*	*F*	df	*p*
AFDM	0.20	1,4	0.667	1.77	1,4	0.255	0.3	1,4	0.613
$\delta^{13}C$	28.2	1,4	0.006	2.87	1,4	0.166	9.71	1,4	0.036
$\delta^{15}N$	4.18	1,4	0.117	0.69	1,4	0.693	5.28	1,4	0.083
C:N	8.76	1,4	0.042	11.99	1,4	0.026	6.94	1,4	0.058

The *C. fluminea* + sediment + water treatment was the only treatment repeated in both experiments. We compared patterns in this treatment qualitatively between experiments. Seston AFDM changed very little and seston ^{13}C increased over time in both experiments. The pattern of change in seston $\delta^{15}N$ differed qualitatively between the experiments. In experiment 1, seston $\delta^{15}N$ remained fairly consistent over time, whereas in experiment 2, seston $\delta^{15}N$ increased over time. Initial seston $\delta^{15}N$ values were lower in experiment 2 than in experiment 1. Values were more typical of a stream receiving WWTP effluent in experiment 1 than in experiment 2. NO_3^- produced from microbially processed wastewater has $\delta^{15}N$ values that range from +7 to +20‰ (Mayer et al. 2002). Seston C:N was affected by *C. fluminea* in both experiments. Proportional N content was greater in the presence than in the absence of *C. fluminea*.

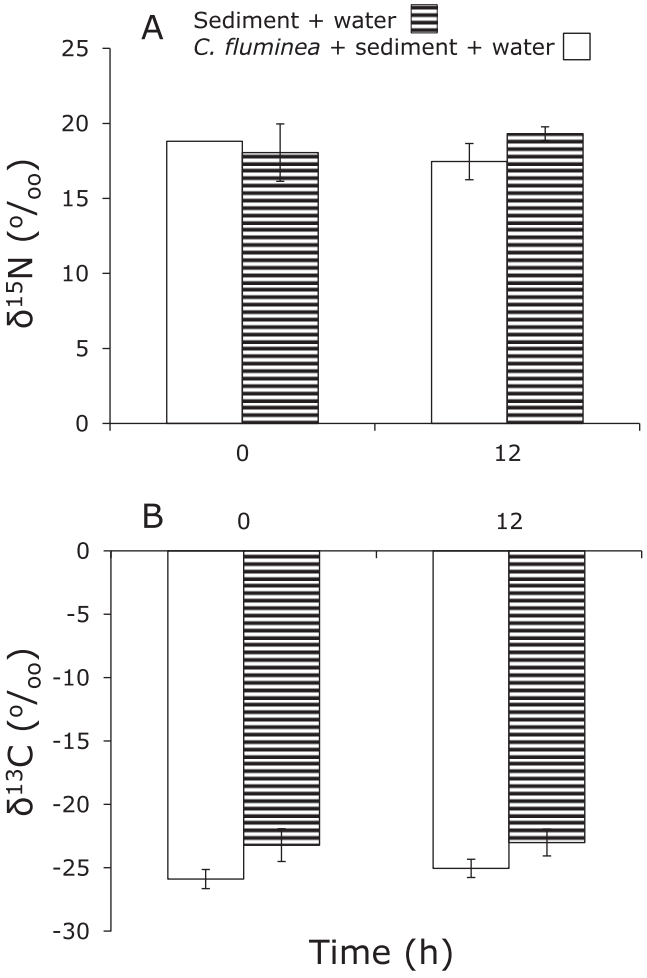

FIG. 7. Mean (±1 SE) sediment $\delta^{15}N$ (A) and $\delta^{13}C$ (B) in experiment 1.

Discussion

Our study was designed to test the hypothesis that *C. fluminea* affects seston quality and quantity in an urban stream through its filtering activity. At densities ranging from 350 to 1400 ind/m^2, *C. fluminea* populations were estimated to clear the water column of streams and large rivers (Lauritsen 1986, Cohen et al. 1984, Leff et al. 1990). However, our laboratory experiments did not support the hypothesis that filter feeding by *C. fluminea* affects seston concentration in North Buffalo Creek, although *C. fluminea* activity significantly affected seston quality. Experimental densities (32–40 ind/m^2) were comparable to those used in previous estimates of filtering rate (Lauritsen 1986), so if filtering occurred, we should have been able to detect it.

Furthermore, our field studies showed that seston was not the primary food source for *C. fluminea* in North Buffalo Creek. Evaluation of the stable-isotope signatures of seston and *C. fluminea* at multiple sites did not support a trophic link between these foodweb components. The ^{15}N enrichment of seston reflected a WWTP point-source input. Treated wastewater has a $\delta^{15}N$ value ranging from +7 to + 20‰ (Mayer et al. 2002), and the $\delta^{15}N$ of seston in the North Buffalo WWTP effluent was within that range (~8.3‰) when measured by Ulseth and Hershey (2005). Greater ^{15}N enrichment of seston than *C. fluminea* at site B during summer 2009 illustrates that seston could not have been an important food source at that site.

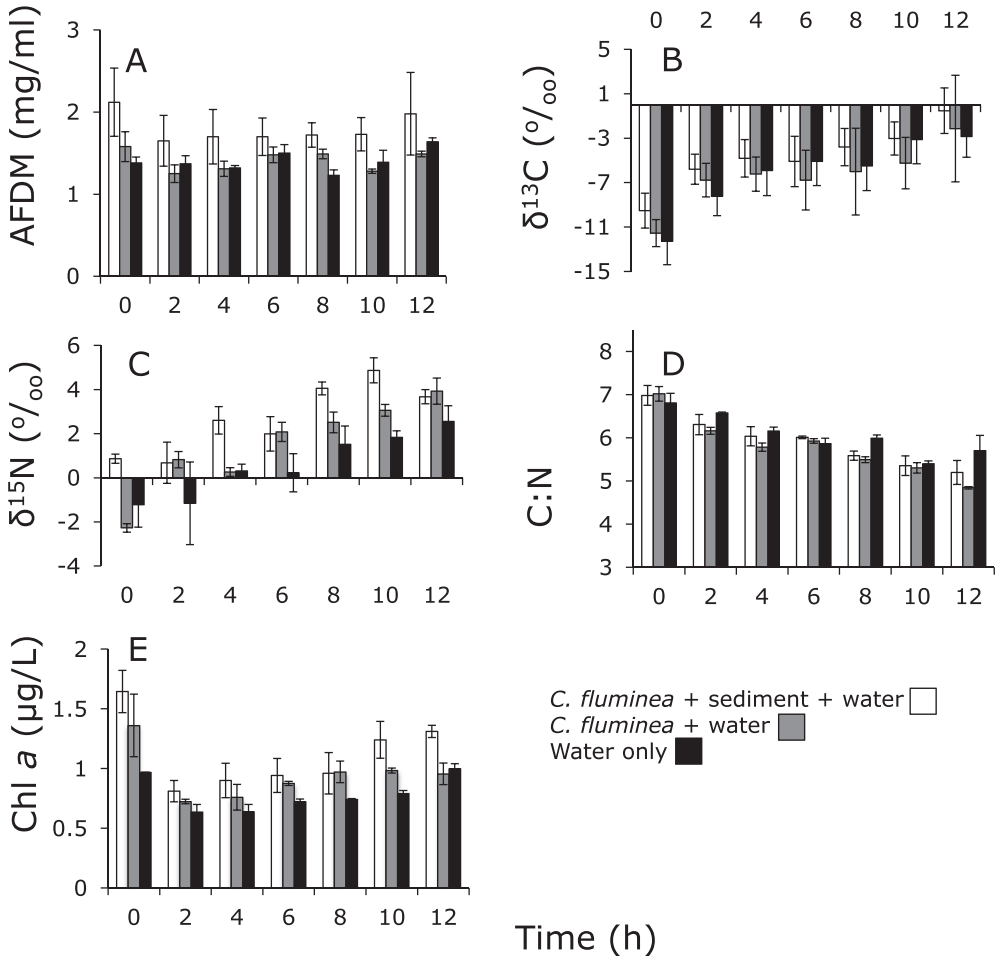

FIG. 8. Mean (±1 SE) seston ash-free dry mass (AFDM) (A), $\delta^{13}C$ (B), $\delta^{15}N$ (C), C:N (D), and chlorophyll *a* (chl *a*) (E) in experiment 2.

At the other 3 sites sampled during the summer of 2009, *C. fluminea* ^{15}N was enriched compared to seston, suggesting that *C. fluminea* could have assimilated primarily seston N. However, *C. fluminea* was ^{13}C depleted rather than enriched compared to seston, indicating that seston was not the primary C source. Data from the longitudinal transect also did not support a trophic link between seston and *C. fluminea*. The progressive decrease in seston $\delta^{13}C$ indicated that processing of sewage-derived C occurred along the longitudinal transect to approach the approximate $\delta^{13}C$ value of terrestrial leaf litter (−28‰). The observed decline in AFDM was consistent with the seston $\delta^{13}C$ results. *Corbicula fluminea*

TABLE 6. Comparison of seston variables at the beginning (t = 0) and end (t = 12) of experiment 2. Analyses of variance were used to compare seston ash-free dry mass (AFDM), isotopic signatures, C:N, and chlorophyll *a* (chl *a*) between water only, sediment + water, and *Corbicula fluminea* + sediment + water treatments.

	t = 0 h			t = 12 h		
Variable	*F*	df	*p*	*F*	df	*p*
AFDM	2.074	2,8	0.201	0.726	2,8	0.522
$\delta^{13}C$	0.734	2,8	0.519	0.138	2,8	0.874
$\delta^{15}N$	6.770	2,8	0.029	1.674	2,8	0.264
C:N	0.283	2,8	0.763	2.728	2,8	0.144
Chl *a*	2.292	2,7	0.197	8.864	2,8	0.016

TABLE 7. Results of a repeated measures analysis of variance comparing seston ash-free dry mass (AFDM), isotopic signatures, C:N, and chlorophyll *a* (chl *a*) for initial and final sampling events among water only, *Corbicula fluminea* + water, and *C. fluminea* + sediment + water treatments.

	Time			Treatment			Interaction		
Variable	*F*	df	*p*	*F*	df	*p*	*F*	df	*p*
AFDM	0.024	1,6	0.883	1.289	2,6	0.342	1.822	2,6	0.241
$\delta^{13}C$	55.82	1,6	<0.001	0.321	2,6	0.737	0.014	2,6	0.986
$\delta^{15}N$	76.52	1,6	<0.001	4.595	2,6	0.062	4.373	2,6	0.070
C:N	294.3	1,6	<0.001	0.540	2,6	0.609	10.02	2,6	0.012
Chl *a*	3.283	1,6	0.130	5.428	2,6	0.056	0.729	2,6	0.527

was 3 to 5‰ enriched in ^{15}N compared to seston along the transect, results commensurate with a trophic shift from seston to *C. fluminea* (Peterson and Fry 1987), but *C. fluminea* $\delta^{13}C$ did not show a pattern comparable to that of seston. Thus, the combination of stable-isotope and seston AFDM data suggest that the seston dynamics were controlled by processes other than filter feeding by *C. fluminea*. These results also suggest that an alternate food source or sources that were not evaluated must have been present. *Corbicula fluminea* can pedal feed (Vaughn and Hakenkamp 2001), so the sediments probably served as a major food source.

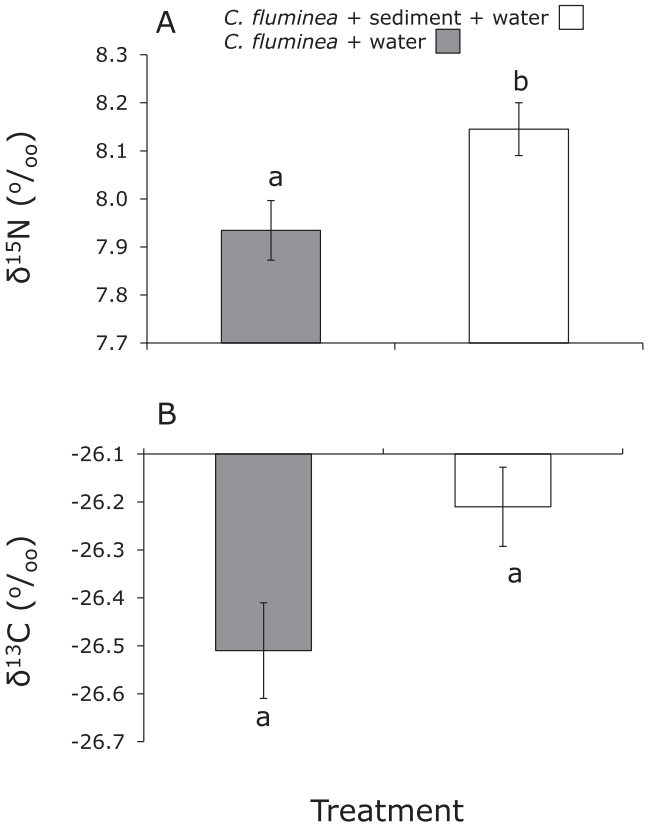

FIG. 9. Mean (±1 SE) $\delta^{15}N$ (A) and $\delta^{13}C$ (B) of *Corbicula fluminea* in experiment 2. Means with the same letters are not significantly different ($p < 0.05$).

Changes in seston C:N and $\delta^{13}C$ in experiment 1 probably were caused by *C. fluminea* activity in the sediments. This activity apparently suspended ^{13}C-enriched particles (resulting from the ^{13}C-DIC enrichment) from the sediment surface into the water column. *Corbicula fluminea* activity also could have resuspended ^{13}C-enriched algae that settled from the water column during the experiment. In the absence of *C. fluminea* (sediment + water treatment), settling algae could have caused the observed increased C:N (Fig. 6D) because algae and other microbes typically have lower C:N than terrestrial detrital particles (Caraco et al. 1998, Frost et al. 2002, Dodds et al. 2004). Resuspension of settled algae by *C. fluminea* could maintain, but not increase, seston $\delta^{13}C$, so this mechanism alone cannot explain the observed differences in $\delta^{13}C$ and C:N. However, a combination of suspension of ^{13}C-enriched benthic algae and resuspension of settling algae could increase seston $\delta^{13}C$ and maintain C:N, consistent with our results.

Experiment 2 provided additional supporting evidence that *C. fluminea* affects seston quality and showed that microbial processing in the water column affects seston quality independently of *C. fluminea* activity. The increase in seston $\delta^{15}N$ and decrease in seston C:N (although uneven across treatments) over time in all treatments probably was caused by microbial processing of N in the water column. The effect of microbial processing of inorganic N on seston $\delta^{15}N$ is not well studied. However, available experimental evidence shows that activity of heterotrophic microbes increases $\delta^{15}N$ and decreases C:N (Caraco et al. 1998, our study).

In experiment 2, *C. fluminea* could have become more ^{15}N-enriched in the *C. fluminea* + sediment + water treatment only by feeding on a sediment N source because seston $\delta^{15}N$ was isotopically ~5‰ lighter than *C. fluminea*. The bulk sediments were slightly enriched in ^{15}N compared to seston in experiment 1 and >10‰ enriched compared seston

in experiment 2 (Fig. 7A compared to Figs 6C and 8C, respectively). Seston $\delta^{15}N$ at site B, where materials for experiments were collected, is temporally highly dynamic, reflecting WWTP input and runoff effects that vary with discharge (Ulseth and Hershey 2005). However, $\delta^{15}N$ of sediment is unlikely to be as temporally variable as seston. The effect of sediment feeding on seston quality was to increase the proportion of seston N relative to C content, as shown by the proportionally greater seston N content in the *C. fluminea* + sediment + water treatment than in the water only treatment in experiment 2 and sediment + water treatment in experiment 1.

Deposit feeding can be an important method of feeding for juvenile bivalves (Cummings and Graf 2010). Several investigators have suggested that *C. fluminea* uses both suspension and deposit feeding (Yeager and Cherry 1994, Hakenkamp and Palmer 1999, Hakenkamp et al. 2001, Vaughn and Hakenkamp 2001, Cummings and Graf 2010), and that pedal feeding affects stream C dynamics via the return of buried organic matter to the water column (Hakenkamp and Palmer 1999). We could find no evidence of significant filter-feeding activity, but we did find that the sediment was an important source of N for *C. fluminea* and that *C. fluminea* activity increases proportional N content of seston at this N-enriched site.

Conclusions

Our study was designed to estimate how much seston N *C. fluminea* removes from North Buffalo Creek via filter feeding. We observed a decline in AFDM along a 7-km transect in North Buffalo Creek downstream of the WWTP, but our laboratory experiments demonstrated that the effect could not be attributed to *C. fluminea*. Furthermore, although *C. fluminea* $\delta^{15}N$ was 4 to 5‰ enriched compared to seston $\delta^{15}N$ along the longitudinal transect, $\delta^{13}C$ values indicated that seston was not the primary food source for *C. fluminea*. Laboratory data showed that *C. fluminea* $\delta^{15}N$ shifts away from that of seston when sediments are present, indicating that sediments provide a food source. *Corbicula fluminea* activity in the sediments also affected seston C:N and $\delta^{13}C$. The most parsimonious mechanism for the observed qualitative changes in seston is that *C. fluminea* activity in sediments redistributes benthic algae and may also resuspend settling algae to the water column. However, further studies are needed to understand the food sources and feeding behavior of *C. fluminea* and the effect of its behavior on stream seston.

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