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APPLIED ISSUES

Effects of stream restoration and wastewater treatment plant effluent on fish communities in urban streams

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SUMMARY

1. Fish community characteristics, resource availability and resource use were assessed in three headwater urban streams in Piedmont North Carolina, U.S.A. Three site types were examined on each stream; two urban (restored and unrestored) and a forested site downstream of urbanisation, which was impacted by effluent from a wastewater treatment plant (WWTP). Stream basal resources, aquatic macroinvertebrates, terrestrial macroinvertebrates and fish were collected at each site.
2. The WWTPs affected isotope signatures in the biota. Basal resource, aquatic macroinvertebrate and fish δ^{15}N showed significant enrichments in the downstream sites, although δ^{13}C signatures were not greatly influenced by the WWTP. Fish were clearly deriving a significant part of their nutrition from sewage effluent-derived sources. There was a trend towards lower richness and abundance of fish at sewage-influenced sites compared with urban restored sites, although the difference was not significant.
3. Restored stream sites had significantly higher fish richness and a trend towards greater abundance compared with unrestored sites. Although significant differences did not exist between urban restored and unrestored areas for aquatic and terrestrial macroinvertebrate abundances and biotic indices of stream health, there appeared to be a trend towards improvements in restored sites for these parameters. Additional surveys of these sites on a regular basis, along with maintenance of restored features are vital to understanding and maximising restoration effectiveness.
4. A pattern of enriched δ^{13}C in fish in restored and unrestored streams in conjunction with enriched δ^{13}C of terrestrial invertebrates at these sites suggests that these terrestrial subsidies are important to the fish, a conclusion also supported by isotope cross plots. Furthermore, enriched δ^{13}C observed for terrestrial invertebrates is consistent with some utilisation of the invasive C4 plants that occur in the urban riparian areas.

Keywords: fish diets, stable isotope analysis, terrestrial versus aquatic food resources, urban streams, wastewater treatment plant influences

Introduction

Anthropogenic inputs from the urban landscape because of runoff, sedimentation or stormwater discharges can lead to altered stream hydrology, greater discharge volume and poor water quality (Trimble, 1997; Davis *et al*., 2003; Nedeau, Merritt & Kaufman, 2003), which result in decreased in-stream biotic integrity, homogenisation of stream communities and fewer stress-intolerant invertebrate species (Lenat & Crawford, 1994; Davis *et al*., 2003; Roy *et al*., 2003). The lack of natural riparian zones along urban streams allows more anthropogenic inputs into the stream, which can impact biota several

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kilometres away from the source (Ulseth & Hershey, 2005).

Many urban communities have attempted to curtail the negative effects of urbanisation through stream restoration efforts, including bank stabilisation, recurvature of the stream channel, replanting of riparian buffers and introduction of in-stream structure (Charbonneau & Resh, 1992; Purcell, Friedrich & Resh, 2002; Davis *et al*., 2003). Wetlands and riparian parks may also be established to enhance nutrient retention and processing that is disrupted by urban development (Helfield & Diamond, 1997). However, the positive impacts of stream restoration may not be immediately apparent (Charbonneau & Resh, 1992) and noticeable changes may take decades (Kondolf, 1995). Three-year old restored stream reaches in Greensboro, NC, U.S.A., continued to be dominated by pollution tolerant macroinvertebrate species and showed no improvement in metrics of macroinvertebrate quality, although they did show decreased nitrate and increased oxygen concentrations (Lynam, 2004; E. C. Lynam & A. E. Hershey, unpubl. data).

Degraded urban streams typically have low fish diversity. Riparian restoration may impact stream communities by providing important terrestrial invertebrate subsidies (Weaver & Garman, 1994). Cloe & Garman (1996) found that when aquatic macroinvertebrate availability was low, terrestrial inputs of invertebrates provided an important subsidy to stream fishes. Additionally, fish biomass decreased when terrestrial invertebrate inputs were experimentally removed (Kawaguchi, Taniguchi & Nakano, 2003). Riparian invertebrate subsidies appear to vary by season, with the greatest subsidies during the summer leafing period (Garman, 1991; Cloe & Garman, 1996; Nakano & Murakami, 2001).

Streams located in urbanised areas of the North Carolina Piedmont have lost much of their biotic integrity because of inputs of point and non-point pollutants [North Carolina Department of Environment and Natural Resources (NCDENR), 2003a, 2004]. We hypothesised that with low diversity and abundance of aquatic macroinvertebrates, riparian zone subsidies would be important to the diets of urban stream fish. We also hypothesised that stream restoration would result in increased terrestrial invertebrate subsidies, which would enhance fish abundance and diversity. Finally, we hypothesised that point source inputs from wastewater treatment plants (WWTPs) would provide an important basal resource for fishes, but would result in degraded communities because of poor water quality. To evaluate these hypotheses, we studied fish communities and resource use in restored and unrestored urban stream sites and in forested sites below WWTPs. We quantified terrestrial and aquatic invertebrates and basal resource abundances and used stable isotopes to evaluate fish diets and to trace basal resources to fish.

Methods

Study sites

North and South Buffalo Creeks are low order streams in the North Carolina Piedmont with their headwaters originating in Greensboro, NC, a city of 223,891 (United States Census Bureau, 2000; Fig. 1a). Both streams lie within the Haw River catchment of the Cape Fear River Basin and are directly impacted by both point and non-point source pollution along their lengths. Point-source effluent from WWTPs on both streams has resulted in poor water quality downstream of these plants (NCDENR, 2004). The United States Environmental Protection Agency (US EPA) has also identified these streams as impaired because of high levels of faecal coliform contamination (>200 colonies L^{-1}; NCDENR, 2004). In response to these problems, the city has made efforts over the past decade to restore sections of both streams in an effort to improve the biological health and recreational value of the streams.

A third stream, Little Sugar Creek, is a mid-order stream with headwaters based in Charlotte, NC, which has a population of 540 828 (United States Census Bureau, 2000; Fig. 1b). Little Sugar Creek, located in the Catawba River catchment, has a history of poor water quality, although the stream's ratings have improved slightly for macroinvertebrates (NCDENR, 2003a). Some restoration efforts (regrowth/replanting of riparian zones, bank stabilisation and in-channel structures) have been attempted on the stream in recent years, but most of the stream remains unrestored.

Sampling design

A total of nine sites, one of each of three types in each of three streams were used in this study. For each stream, sites were classified into three groups

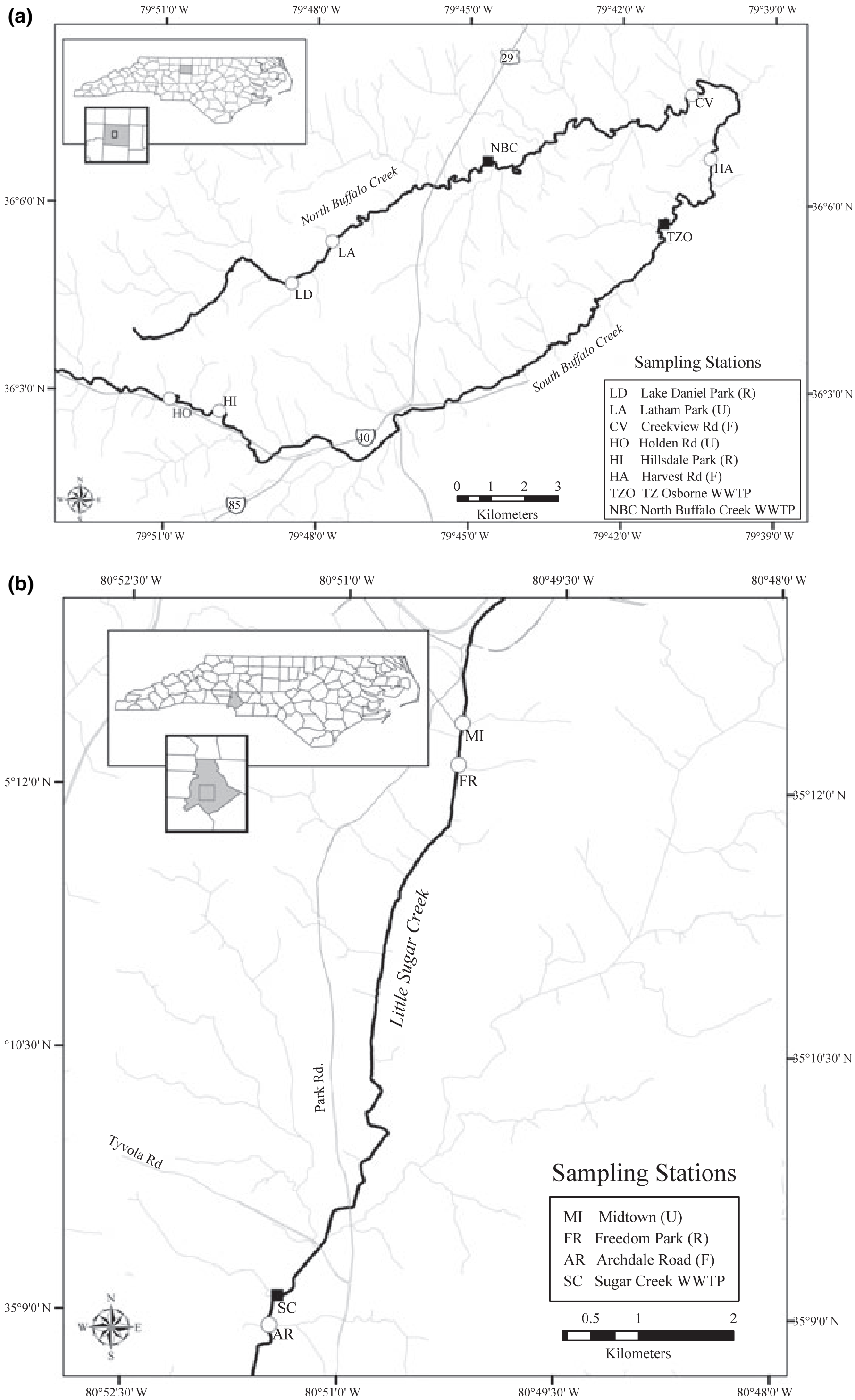

Fig. 1 Maps showing the locations of sampling areas in (a) Greensboro and (b) Charlotte, NC used in this study. Site type designations and sampling station names for North Buffalo Creek, South Buffalo Creek and Little Sugar Creek, respectively, are as follows: urban restored (LD, Lake Daniel Park; HI, Hillsdale Park; FR, Freedom Park), urban unrestored (LA, Latham Park; HO, Holden Road; MI, Midtown) and sewage-influenced forested (CV, Creekview Road; HA, Harvest Road; AR, Archdale Road). WWTPs from North Buffalo, South Buffalo and Little Sugar Creeks are referred to here as NBC, TZO and SC, respectively.

(restored, unrestored or sewage-influenced forested), giving an $n = 3$ for each site type classification. Urban restored sites were classified as those areas of the stream within the city limits (usually within residential areas) that had undergone riparian reconstruction or regrowth, with possible in-stream reconstruction in the last 11 years. Urban unrestored sites were classified as those parts of the stream within a highly urbanised section of the city with no history of stream restoration. Sites located downstream from WWTPs, which were all in forested areas downstream of urban sites, received treated sewage effluent that was known to be isotopically distinct from particulate sources upstream (Ulseth & Hershey, 2005). For all Greensboro urban sites, much of the riparian vegetation surrounding streams was comprised of exotic plants, including three abundant C4 grasses, *Cynodon dactylon* (L.) Pers., *Paspalum dilatatum* Poir. and *Setaria faberi* R.A.W. Herrm. (T. Gilbert Pearson Audubon Society, 1998). Riparian zones of forested sites consisted mainly of mixed native tree and shrub species.

All sampling was completed during the summer of 2004. Each of the sites was visited three times; however, fish and terrestrial invertebrates were only sampled once per site. All sites were sampled at approximately the same hour for each of the visits and in the same order. Stream sampling typically took place at base flow, or at least 3 days after a rain event.

Fish sampling

Fish were sampled along a 100 m transect at each site using back-mounted electrofishing units (BADGER™; Engineering Technical Services, UW-Madison, Madison, WI, U.S.A. and Smith-Root, Inc., Vancouver, WA, U.S.A.). One upstream and one downstream pass was made with the shocker, with the stunned fish being captured using dipnets. Very few fish were lost because several people were netting. All stunned fish were identified to species (Menhinick, 1991), measured for total length and categorised according to functional feeding groups (FFGs; NCDENR, 2001). Three to five individuals of each species were killed by cranial concussion and returned to the laboratory, while the remaining fish were released. Killed fish were dissected in the lab in order to obtain tail muscle and remove intact guts. Tail muscle was prepared for stable isotope analysis and guts were placed in 70% ethanol for later analysis. Gut content analysis was completed by opening the guts and identifying the partially digested contents using a dissecting microscope.

Macroinvertebrate sampling

Stream macroinvertebrates were collected over a 1.5–2 h period within a 100 m transect at each site using dipnetting (300 μm mesh), kicknetting (500 μm mesh) and substrata (rocks, wood, sediment and leaf packs) picking as described in Barbour *et al.* (1999) and were then preserved in 95% ethanol. Equivalent sampling effort was used at each site to ensure that intersite comparisons of abundance would be as realistic as possible. Samples from each site were separated into their respective orders and identified to the lowest possible taxonomic level (Brigham, Brigham & Gnilka, 1982; Merritt & Cummins, 1996; Epler, 2001). Larval chironomids (Diptera: Chironomidae) were mounted on microscope slides for identification. Stream quality was assessed at each site using the North Carolina Biotic Index (NCBI; Lenat, 1993). This index uses published tolerances (see Lenat, 1993; NCDENR, 2003b) for identified aquatic macroinvertebrates in conjunction with abundance values from each site to obtain a score on a scale from one to five, with five categorised as 'excellent' and one as 'poor.'

Samples of macroinvertebrates were collected from the stream for stable isotope analysis once per visit (for a total of three samples per site, or 27 total samples) and were stored in stream water and placed on ice for transport to the lab. Macroinvertebrates were then placed in deionised water overnight for evacuation of gut contents. Samples were separated into order and family, dried, homogenised and placed in tin capsules for stable isotope analysis.

Flying riparian insects were collected using malaise traps mounted on both banks of each site for approximately 15 h. Each site was sampled once with both traps. Sample jars were not filled with a killing liquid, but following collection, they were placed in a freezer overnight to kill the insects. Insects were then identified to order. Samples from each site were then recombined, dried, homogenised and placed in tin cups for stable isotope analysis.

Basal resource sampling

Three types of detrital organic matter were collected: coarse particulate organic matter (CPOM), fine benthic organic matter (FBOM) and fine suspended particulate organic matter (seston). A 0.093 m^2 Surber sampler (500 μm mesh) was used to collect CPOM, in the form of leaf packs and woody debris. Three samples were taken from each site per sampling day for a total of nine samples per site ($n = 81$ total samples). CPOM from each site was oven dried at 60 °C for 48 h and ashed in a muffle furnace at 550 °C. Ash-free dry mass (AFDM) was calculated (Benfield, 1996) and biomass (g m^{-2}) was determined. Three additional samples of CPOM from each site were also collected for stable isotope analysis.

Three FBOM samples were collected from the stream bottom at each site using a 35 mL baster, for a total of 27 samples. Samples were passed through a 1 mm sieve so that large particles (>1 mm) would be retained (Wallace & Grubaugh, 1996). The sieved samples were collected on pre-ashed 47 mm glass fibre filters using vacuum filtration. Filtered samples were dried and acid rinsed using five to six drops of 1 N HCl to remove any inorganic carbonate contamination that might influence ^{13}C isotope results. Seston was collected in 4-L collapsible plastic containers (Wallace & Grubaugh, 1996) and filtered on pre-ashed 47 mm glass fibre filters using vacuum filtration. Three samples were taken from each site per sampling date for a total of nine samples per site ($n = 81$ total samples). Filters for both FBOM and seston were oven dried for stable isotope analysis.

A wire brush was used to scrape the surface of seven arbitrarily selected rocks for periphyton at each site per sampling day. Three of the rocks were rinsed with deionised water and the remaining liquid was filtered onto glass fibre filters and prepared for stable isotope analysis. The four additional rocks were used for chlorophyll *a* (chl *a*) analysis by scraping an 8.40 cm^2 area of the rock surface with a wire brush using a plastic photographic slide mount as a template. These samples were washed into foil-covered bottles using deionised water, filtered as above and assayed for chl *a* as a surrogate for periphyton biomass at each site. These filters were then placed in foil-covered screw-top 15-mL polystyrene conical tubes with 10 mL of 90% buffered acetone and frozen for 24 h, followed by either fluorometric or spectrophotometric analysis for chl *a*. Results in mass per unit area (μg cm^{-2}) were obtained by following the methods of Steinman & Lamberti (1996) and APHA, AWWA & WEF (1992).

Stable isotope processing

All isotope samples prepared for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis were shipped to the Colorado Plateau Stable Isotope Laboratory (CPSIL) of Northern Arizona University in Flagstaff, AZ, U.S.A., for processing by gas isotope-ratio mass spectroscopy. Lab reported measurement errors (1 SD) were approximately 0.05‰ for $\delta^{13}C$ and approximately 0.11‰ for $\delta^{15}N$ (Ulseth & Hershey, 2005). Each sample collected for isotopes was oven dried at 60 °C for 48 h. Dried filters for FBOM and seston were shipped to CPSIL without further processing. Dried periphyton samples were removed from filters and ground into a fine powder. All of the other samples were homogenised and also ground into a powder. Powdered samples were placed in 4 × 6 mm pressed tin capsules and shipped to CPSIL.

Data analysis

Normality of the data was assessed using the Shapiro-Wilk test. Only AFDM of CPOM was found to be non-normal and these data were natural log transformed. Two-way ANOVAS were performed on fish isotope, abundance and richness data, using site type and FFG designations as factors. Another two-way ANOVA was used on macroinvertebrate isotope data using site type and source (aquatic versus terrestrial) as factors. All other data were compared using one-way ANOVAS to assess site type differences. Tukey's HSD poc-hoc tests were applied to all significant ANOVA results to assess individual site type differences. All data were analysed using SAS v.9.1.1 and JMP v.5.1.2 (SAS Institute, Inc., Cary, NC, U.S.A.).

Results

Fish

Restored sites showed a trend towards greater abundance of fish, although the trend was not significant (Fig. 2a). Fish species richness was significantly higher in restored compared with unrestored sites ($P = 0.04$; Fig. 2b), but was not significantly different from forested sites. $\delta^{13}C$ of fishes in forested sites was significantly lower than fishes at restored sites, which was lower than fishes at unrestored sites ($P < 0.05$; Fig. 2c). $\delta^{15}N$ of fishes

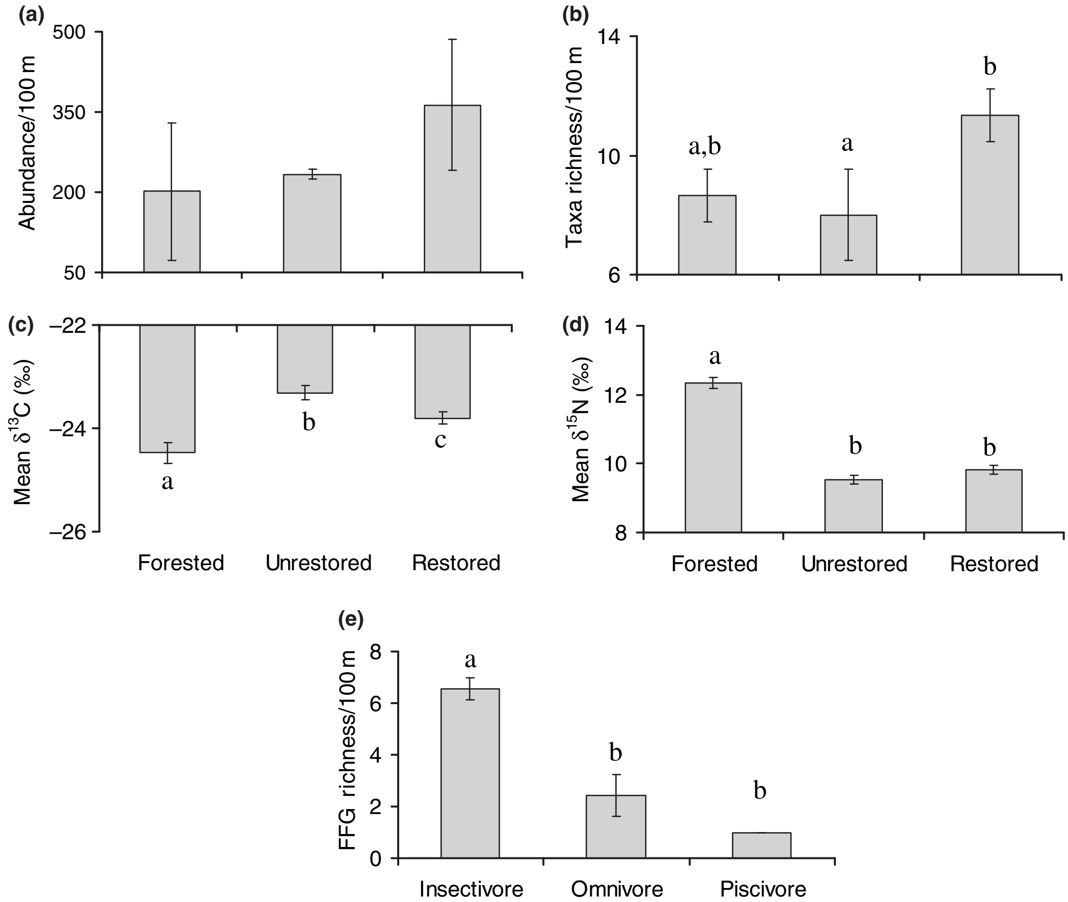

Fig. 2 Differences in fish data for each site type, including (a) total abundance, (b) taxa richness, (c) mean $\delta^{13}C$, (d) mean $\delta^{15}N$ and (e) FFG designations. Error bars represent 1 SE of the mean. All data were pooled by type (forested, unrestored and restored) for analysis ($n = 3$ for each site type). For FFG richness, insectivores and omnivores included pooled data from all sites ($n = 9$ for both), but piscivores were only present in a few of the sites ($n = 3$). Bars with different letters were significantly different at $P < 0.05$.

in forested sites was significantly higher than in restored and unrestored sites ($P < 0.05$; Fig. 2d). There were significantly more insectivorous fishes ($P < 0.05$) compared with both omnivores and piscivores (Fig. 2e).

Redbreast sunfish (*Lepomis auritus* Linnaeus) were the most ubiquitous fish species across sites and showed similar patterns to the pooled fish data. Redbreast sunfish appeared to be more abundant at restored sites compared with unrestored and forested sites, but these differences were not significant (Table 1). Redbreast sunfish also showed a trend (non-significant) toward lower $\delta^{13}C$ in forested sites compared with restored and unrestored sites (Table 1). $\delta^{15}N$ of redbreast sunfish was significantly higher in forested sites compared with restored and unrestored sites ($P < 0.05$; Table 1).

Table 1 Redbreast sunfish (*Lepomis auritus* Linnaeus) differences (mean ± SE) in abundance, $\delta^{13}C$ and $\delta^{15}N$ across site types

	Forested	Unrestored	Restored
Abundance	100.67 ± 93.2	98.67 ± 44.8	161 ± 71.0
$\delta^{13}C$ (‰)	−24.02 ± 0.7	−23.47 ± 0.4	−23.57 ± 0.1
$\delta^{15}N$ (‰)	12.87 ± 0.5*	8.94 ± 0.6	9.17 ± 0.5

*Significantly differ at $P > 0.05$.

Macroinvertebrates

There were no significant effects of site type on either terrestrial or aquatic macroinvertebrate richness or abundance. However, there appeared to be a trend toward greater abundance and richness of both groups at restored sites (Fig. 3a,b). $\delta^{15}N$ of aquatic macroinvertebrates was significantly higher in fores-

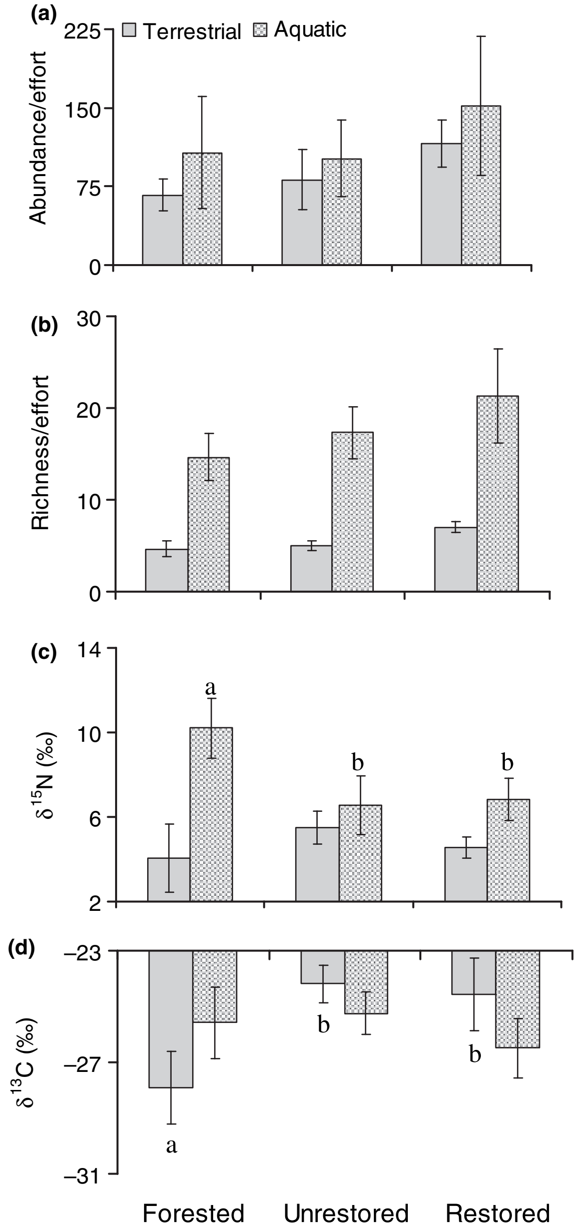

Fig. 3 Differences in terrestrial and aquatic macroinvertebrates across sites for (a) total abundance, (b) taxa richness, (c) δ^{15}N and (d) δ^{13}C. Error bars represent 1 SE of the mean. All data were pooled by type (forested, unrestored and restored) for analysis ($n = 3$ for each site type). Bars with different letters were significantly different at $P < 0.05$.

ted compared with both restored and unrestored urban sites ($P = 0.02$; Fig. 3c). δ^{15}N of terrestrial macroinvertebrates was not significantly different among sites (Fig. 3c). δ^{13}C of terrestrial macroinvertebrates was significantly lower at forested sites compared with both restored and unrestored urban sites ($P = 0.01$; Fig. 3d). δ^{13}C of aquatic macroinvertebrates did not differ among site types (Fig. 3d).

Table 2 Biomass of chlorophyll *a* (μg cm^{-2}) and ash-free dry mass (AFDM) of CPOM (g m^{-2}) across site types (mean ± SE)

	Forested	Unrestored	Restored
Chlorophyll *a*	3.26 ± 0.61	3.31 ± 0.71	3.50 ± 0.49
AFDM	9.9 ± 7.2	18.3 ± 15.3	10.6 ± 1.6

Scores on the NCBI metric for aquatic macroinvertebrates were not significantly different among site types [$P = 0.34$; mean ± SE, 2.53 ± 0.29 (forested), 3.00 ± 0.58 (unrestored), 2.13 ± 0.13 (restored)], indicating an overall fair to good-fair rating for all streams (see Lenat, 1993; NCDENR, 2003b). All site types, while supporting different aquatic macroinvertebrate assemblages, had the same dominant taxa throughout. *Polypedilum flavum* Johannsen (Diptera: Chironomidae) and *Cheumatopsyche* spp. (Trichoptera: Hydropsychidae) were the most common taxa, with relative abundances ranging from 20.4% to 27.6% of the total samples (Northington, 2005). Both insects also have intermediate tolerance values: 6.6 for *Cheumatopsyche* spp. and 5.3 for *P. flavum* (see Lenat, 1993).

Basal resources

Chlorophyll *a* concentration was similar at all site types ($P = 0.96$; Table 2). AFDM biomass was also similar at all sites ($P = 0.62$; Table 2).

The CPOM samples had very similar δ^{13}C values across site types. FBOM and seston both showed a trend towards lowest δ^{13}C in restored sites and highest δ^{13}C in forested sites, although the difference was not significant. Periphyton appeared to have highest δ^{13}C at restored sites and lowest δ^{13}C at forested sites, but this pattern also was not significant (Fig. 4).

All basal sources showed a similar pattern for δ^{15}N (Fig. 5). FBOM and seston both had significantly higher ($P = 0.03$) δ^{15}N in forested sites compared with restored and unrestored sites. CPOM and periphyton δ^{15}N showed a similar trend as FBOM and seston, but the differences were not significant (Fig. 5).

Isotope biplots

Isotope biplots illustrate that fish were the most ^{15}N-enriched food web component at all sites

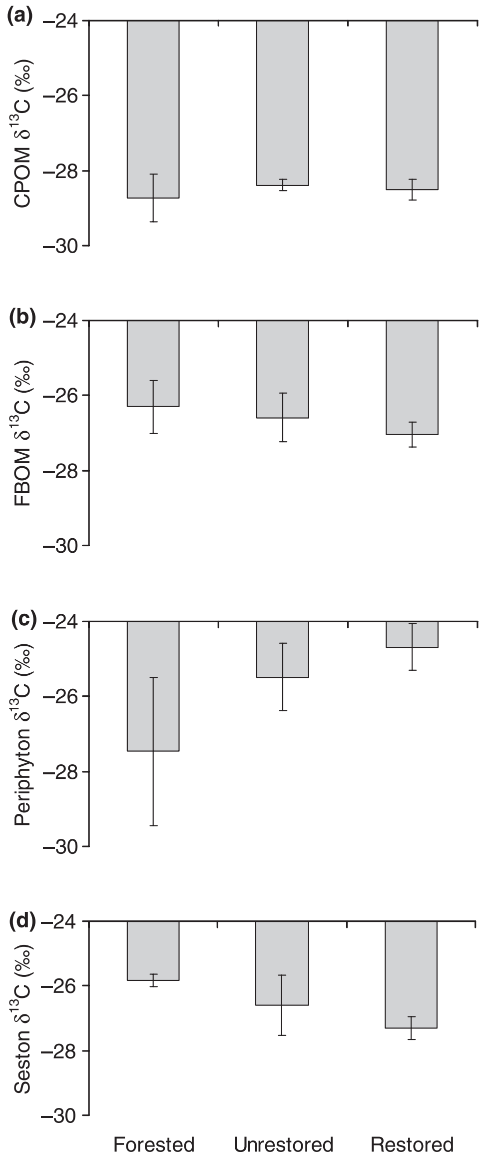

Fig. 4 Mean δ^{13}C signatures for (a) CPOM, (b) FBOM, (c) periphyton and (d) seston across site types. Error bars represent 1 SE of the mean. All data were pooled by type (forested, unrestored and restored) for analysis (n = 3 for each site type).

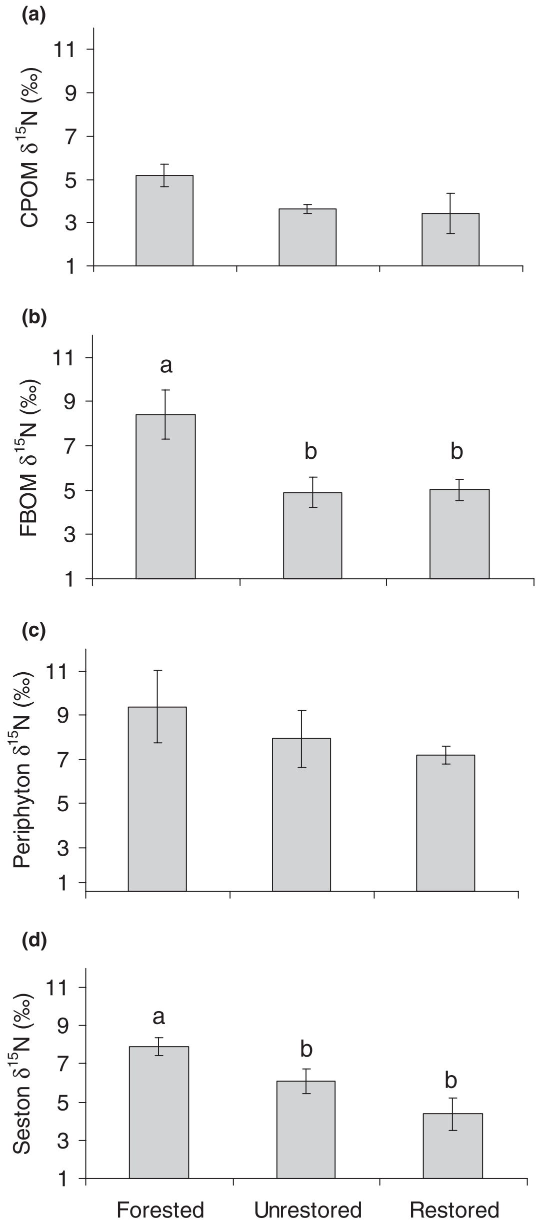

Fig. 5 Mean δ^{15}N signatures for (a) CPOM, (b) FBOM, (c) periphyton and (d) seston across site types. Error bars represent 1 SE of the mean. Bars with different letters were significantly different at $P < 0.05$. All data were pooled by type (forested, unrestored and restored) for analysis (n = 3 for each site type).

(Fig. 6). At each site type, seston and FBOM had very similar values for both δ^{13}C and δ^{15}N, consistent with dynamic exchange between these two types of fine particulate organic matter (FPOM). Henceforth, we consider FBOM and seston collectively as FPOM. However, the isotope signals of FPOM varied among sites.

At forested sites, FPOM was too depleted in ^{15}N and enriched in ^{13}C to have been derived entirely of periphyton and also too enriched in ^{13}C to have been derived from a combination of periphyton and CPOM (Fig. 6a). Discharge from the North Buffalo Creek WWTP has a unique signal (δ^{15}N = 9‰, δ^{13}C = −24‰; Ulseth & Hershey, 2005), therefore, sewage effluent, which is more enriched in ^{13}C than CPOM and periphyton, must have been an important constituent of FPOM. The negative δ^{13}C shift for

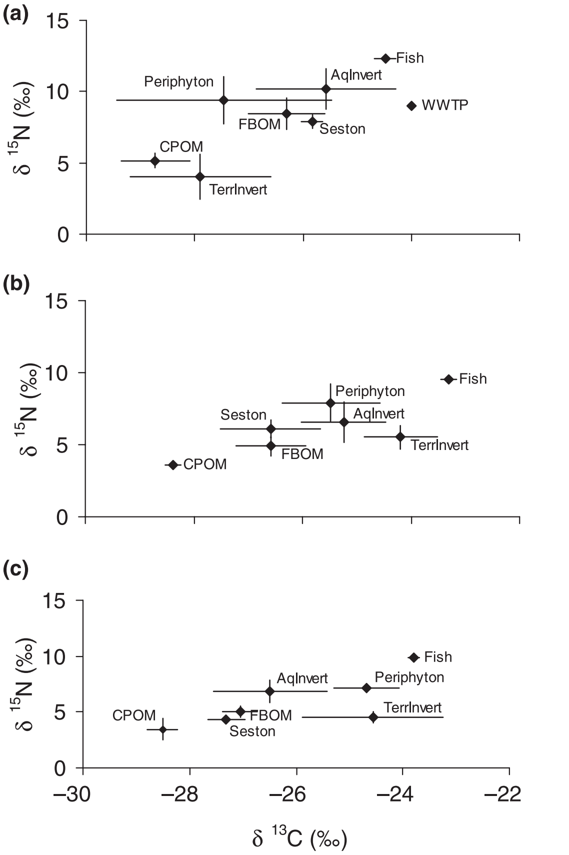

Fig. 6 $^{13}C/^{15}N$ biplot of food web components in (a) sewage-influenced forested, (b) urban unrestored and (c) urban restored sites along the streams used in this study. The isotopic signature of WWTP effluent is taken from Ulseth & Hershey (2005). Error bars represent 1 SE of the mean.

FPOM compared with treated sewage is consistent with a contribution of periphyton to the FPOM pool. CPOM was not a major contributor to the particulate matter pool based on its comparatively low δ^{15}N and δ^{13}C values relative to FPOM.

Aquatic macroinvertebrates at forested sites appeared to be utilising FPOM as their primary food resource, based on their slight positive shift in δ^{13}C and larger positive shift in δ^{15}N. Terrestrial and aquatic macroinvertebrates at forested sites (Fig. 6a) show little overlap in δ^{13}C and no overlap in δ^{15}N, suggesting that the basal resources for aquatic invertebrates is not derived from terrestrial detritus. The isotopic signature of fish at forested sites is consistent with feeding on a diet of aquatic insects. Terrestrial invertebrates at forested sites were depleted in ^{15}N compared with CPOM. However, the CPOM was collected from the stream, where it had been conditioned in WWTP effluent, which should result in ^{15}N enrichment compared with unconditioned terrestrial litter (which we did not collect). The δ^{13}C of terrestrial invertebrates is higher than that of CPOM, consistent with a basal resource with the same δ^{13}C value as CPOM, as would be expected of unconditioned terrestrial litter. Compared with consumers, periphyton at forested sites was too enriched in ^{15}N and depleted in ^{13}C to have been a predominant food source in the food web leading to aquatic invertebrates and fish. Although periphyton δ^{15}N was highly variable at forested sites, mean periphyton δ^{15}N was similar to that of sewage effluent, suggesting that sewage was the primary N source for periphyton at forested sites.

At unrestored urban sites (Fig. 6b), FPOM was intermediate in both δ^{13}C and δ^{15}N compared with periphyton and CPOM, consistent with being derived from these two components. Aquatic invertebrates at unrestored sites were slightly depleted in ^{15}N relative to periphyton, but slightly enriched compared with FPOM. δ^{13}C of aquatic invertebrates also was intermediate between these resources. Thus, basal resources for aquatic invertebrates may have included a combination of periphyton and FPOM. Fish δ^{13}C was too high to be derived entirely from aquatic invertebrates in unrestored sites. However, aquatic and terrestrial invertebrates overlapped in both δ^{13}C and δ^{15}N, and the fish isotope signal reflects a diet of both sources.

At restored urban sites (Fig. 6c), FPOM also appeared to be derived from a combination of CPOM and periphyton. However, the FPOM signal suggested more influence of CPOM compared with periphyton at restored than at unrestored sites (comparison of Fig. 6b,c). The δ^{13}C and δ^{15}N values of aquatic invertebrates indicated a diet of FPOM only, and no direct influence of periphyton. The isotopic signal of fish was consistent with a diet of both terrestrial and aquatic invertebrates. Based on conservative average fractionation values (approximately 3‰ for ^{15}N and approximately 0.5–1‰ for ^{13}C; Peterson & Fry, 1987) fish were too enriched in ^{15}N to be relying entirely on terrestrial insects and too enriched in ^{13}C to be relying entirely on aquatic insects.

At both unrestored and restored sites, terrestrial invertebrates were too enriched in ^{13}C compared with CPOM for that resource alone to be representative of their primary food. Invasive C4 grasses were present at all urban sites (T. Gilbert Pearson Audubon Society, 1998). Apparently, these grasses (δ^{13}C −12 to −14 ‰; Peterson & Fry, 1987) were important in the diets of terrestrial invertebrates (primarily comprised of dipterans and homopterans), but did not contribute significantly to the CPOM in the stream, as did leaf litter and twigs from deciduous trees. Thus, CPOM, which was comprised largely of deciduous leaves and twigs, represented only part of the basal resources for terrestrial insects.

Gut content analysis

Gut content analysis confirmed that redbreast sunfish were feeding on both terrestrial and aquatic insects at all site types (Table 3). Mosquitofish (*Gambusia holbrooki* Girard) fed on a diverse diet of terrestrial and aquatic insects at forested sites, but only diptera (mostly Chironomidae) were found in their stomachs at urban restored and unrestored sites. Only chironomids and periphyton were found in stomachs of creek chubsuckers. Stomach contents of flat bullhead at forested sites were dominated by aquatic invertebrates, but also included Hymenoptera. At unrestored urban sites, only aquatic insects and filamentous algae were found in flat bullhead stomachs and these fish were not collected at restored urban sites. Golden shiner (*Notemigonus crysoleucas* Mitchill) stomach contents were dominated by aquatic insects, but also included plant matter at all sites, filamentous algae at

Table 3 Selected fish species and their gut contents across site types. Because of their decomposed state, quantification of individual prey items was not possible, therefore presence/absence data was obtained instead.

	Redbreast sunfish (*Lepomis auritus* Linnaeus)			Mosquitofish (*Gambusia holbrooki* Girard)			Creek chubsucker (*Erimyzon oblongus* Mitchill)			Flat bullhead catfish (*Ameirus platycephalus* Girard)			Golden shiner (*Notemigonus crysoleucus* Mitchill)		
Food Item	F	U	R	F	U	R	F	U	R	F	U	R	F	U	R
Insect Larvae															
Anisoptera	X														
Chironomidae	X	X		X			X	X	X	X		X	X		X
Coleoptera	X		X												
Ephemeroptera		X	X									X	X	X	X
Tipulidae			X												
Trichoptera	X	X		X									X		
Zygoptera		X	X							X					
Insect Adults															
Collembola	X														
Coleoptera		X													
Diptera	X	X	X	X	X	X									
Hymenoptera	X			X						X					X
Orthoptera		X													
Zygoptera	X														
Fish															
Lepomis macrochirus Rafinesque										X					
Gambusia holbrooki Girard										X					
Dipteran pupae		X	X	X	X					X					
Arachnida				X											
Plant matter		X	X		X					X			X	X	X
Algae							X		X			X		X	
Crayfish										X					
Physidae										X					

Site type designations are as follows: F, sewage-impacted forested; U, urban unrestored; R, urban restored.
X, present.

forested and restored sites and Hymenoptera at unrestored sites.

Discussion

Effects of riparian land use and WWTP effluent on fish communities

Fish abundance and richness data suggest that the beneficial effect of the forested riparian zone at the WWTP-influenced sites was not sufficient to overcome the negative effect of degraded water quality associated with the effluent (see Ulseth & Hershey, 2005). Although habitat quality was high below WWTPs, low water quality, especially increased levels of ammonia, higher temperatures and changes in pH, which have been correlated with WWTP effluent (Osborne & Davies, 1987; Nedeau *et al.*, 2003; Roy *et al.*, 2003), are factors that would negatively affect fish (Schlacher *et al.*, 2005). Habitat improvement at restored sites, such as in-stream structure associated with tree roots, woody debris, shade and sinuosity (Bond & Lake, 2003) and slightly improved water quality (Lynam, 2004) are factors that may have contributed to observed improvements in fish communities. Dissolved oxygen (DO) was significantly higher and nitrate was significantly lower in restored compared to unrestored Greensboro sites (Lynam, 2004). At urban sites, despite restoration, stream health was still considered fair to poor based on NCBI scores. However, the beneficial effects of the relatively narrow forested corridors along restored sections combined with within channel improvements and modest water quality improvements apparently was sufficient to benefit fish communities.

Fish richness and abundance may also be influenced by quality and availability of invertebrate food resources (Kawaguchi *et al.*, 2003). Invertebrate abundance and richness have been shown to be influenced by water quality and habitat (Laasonen, Muotka & Kivijarvi, 1998; Nedeau *et al.*, 2003). In addition, fish communities have been shown to benefit (greater abundance and richness) from riparian development that provides in-stream habitat, including overhanging vegetation and woody debris (Gorman & Karr, 1978; Mérigoux, Ponton & de Mérona, 1998; Brazner *et al.*, 2005). Even with significantly higher species richness of fish in restored areas in this study, the roles of habitat structure and resource availability are not clear and require further examination.

Effects of riparian land use and WWTP effluent on stream food webs

Isotope biplots suggest that fish are relying more on terrestrial invertebrates as a source of C at urban (restored and unrestored) sites compared to forested sites. $\delta^{13}C$ is generally considered to be a good tracer of food source, with a 0.5–1.0‰ enrichment per trophic level (Peterson & Fry, 1987), which is very consistent with the relationship between fish and terrestrial invertebrates at unrestored and restored urban sites and between fish and aquatic invertebrates at forested sites (Fig. 6). In addition, terrestrial invertebrates at forested sites were too depleted in ^{13}C to have been important in fish diets. However, as the $\delta^{15}N$ signal of fish at urban sites was approximately 5‰ enriched compared with terrestrial invertebrates, which is higher than the expected range for a ^{15}N trophic shift (approximately 3‰; Minagawa & Wada, 1984), it is likely that there was an additional, more enriched food source that also was important. Aquatic invertebrates and periphyton were both sufficiently enriched in ^{15}N to contribute to the observed fish $\delta^{15}N$, and diet analyses confirm that both sources were consumed. Thus, although fish utilised autochthonous food sources at urban sites, it is clear that the role of riparian macroinvertebrate subsidies was quite important in the urban sites, and of little importance in the forested sites.

The CPOM and FPOM were more important basal resources for aquatic insects in restored sites than in unrestored sites. Furthermore, CPOM made a larger contribution to FPOM than did periphyton at the restored compared with the unrestored sites. A possible explanation for this is that unrestored sites may have less stream bed stability, resulting in greater sloughing of periphyton into the FPOM pool. Aquatic insects at the unrestored sites had an isotopic signature that was so enriched in ^{13}C that it could only be explained by a mixed diet in which periphyton had a very significant role. On the other hand, aquatic insects at the restored sites, which were primarily hydropsychid caddisflies (collectors) had an isotopic signature that would be expected based on a diet of FPOM (approximately 0.6‰ enriched in ^{13}C, approximately 2‰ enriched in ^{15}N). Thus, the greater canopy development associated with the restored urban streams resulted in a significant resource shift for the stream food web away from periphyton and toward an increased role for CPOM.

Invasive grasses were a major component of riparian zone plant community at urban sites, and the dominant among these (*C. dactylon* (L.) Pers., *P. dilatatum* Poir. and *S. faberi* R.A.W. Herrm.; T. Gilbert Pearson Audubon Society, 1998) are C4 plants. Increased prevalence of invasive grasses is well known in riparian zones (D'Antonio & Vitousek, 1992; Tabacchi *et al.*, 1998; Wissmar & Beschta, 1998), although little is known about the consequences of this for urban stream food webs. However, at least during the summer (when our sampling was conducted) the $\delta^{13}C$ data show that these grasses did not contribute quantitatively to CPOM in the stream, to the FPOM food web components or diets of aquatic insects. At the restored sites, the in-stream detrital pathway was clearly derived from C3 plants ($\delta^{13}C$ approximately −28‰), as discussed above. Terrestrial insects, on the other hand, were significantly more enriched in ^{13}C compared with aquatic insects, suggesting a mixed diet of C3 and C4 plants. A more comprehensive study of terrestrial riparian food webs would be needed to evaluate the role of C4 species in the riparian food webs. In addition, study of the stream food webs during the autumn and winter seasons might also show different results regarding use of C4 invasive grasses in the stream detrital pathway. Unrestored sites showed the same general pattern of ^{13}C enrichment for terrestrial invertebrates and fish, although both groups appeared to have slightly higher $\delta^{13}C$ values than at restored sites, indicative of slightly more influence of C4 invasive grasses. This is reasonable because unrestored sites supported fewer trees, which provide a ^{13}C depleted basal resource for terrestrial insects. Thus, our study shows that C4 invasive grasses impact urban stream food webs during the summer via terrestrial insect subsidies, which are important in the diets of fish.

Stable isotope analyses showed that WWTP effluent strongly influenced isotopic signals of food web components. WWTP effluent, reported by Ulseth & Hershey (2005) as 9‰ for North Buffalo Creek WWTP, resulted in a significant downstream ^{15}N enrichment of seston, FBOM, aquatic insects and fish compared with urban sites. A pristine forested stream in this same region had $\delta^{15}N$ values much more depleted than those found downstream of the WWTP in this study (e.g. periphyton = approximately 2‰; Rushforth, 2006), which further illustrates that the observed $\delta^{15}N$ values for food web components at the forested sites could not be explained as a regional background $\delta^{15}N$ signal. There was a trend toward ^{15}N enrichment of periphyton below the WWTPs, although it was not significant. However, the food web leading to fish was clearly derived from FPOM, which was strongly influenced by WWTP effluent. Ulseth & Hershey (2005) also found that WWTP on North Buffalo Creek affected dominant aquatic invertebrates and seston, but that study also showed a significant influence of WWTP effluent on periphyton compared with several upstream sites. Sewage effluent, especially sewage-derived nitrogen (SDN) can be readily traced into aquatic food webs because isotopic fractionation during sewage processing concentrates heavy isotopes into the sewage effluent as light isotopes are preferentially volatilised (Macko & Ostrom, 1994; McClelland, Valiela & Michener, 1997). Most other studies have focused on SDN incorporation into algae or macrophytes in either freshwater or coastal marine ecosystems (Wayland & Hobson, 2001; Cole *et al.*, 2004; Savage & Elmgren, 2004), as opposed through uptake through other routes, including the particulate matter pool. Although not inconsistent with the conclusion that uptake of SDN by primary producers is important in streams receiving sewage effluent, our data show that transfer of SDN from FPOM is a more important route for transferring SDN to aquatic consumers in these streams.

Stream-water nitrogen may be a significant source to riparian ecosystems in arid regions, but may be less so in moist forested catchments because the hydrologic flowpaths are generally from upslope toward the stream (Triska, Duff & Avanzino, 1993; Schade *et al.*, 2005). However, Detweiler (2005) found that sewage was a very significant source of N to riparian C3 plants along a tropical Brazilian urban stream, although her study was conducted during the dry season. In our study, CPOM also showed a trend toward slightly higher $\delta^{15}N$ at forested sites. However, terrestrial insects, which presumably used a basal resource of primarily fresh leaves or unconditioned leaf detritus (these were not sampled), had lower $\delta^{15}N$ than CPOM. These results suggest that any ^{15}N enrichment of CPOM was because of in-stream conditioning of the litter, rather than riparian uptake of SDN.

Conclusions and implications

Fish communities and natural abundance of N and C stable isotopes of food web components are valuable tools for integrating food web responses to riparian changes associated with restoration and with disruptions in the aquatic food web due to sewage effluent. Our data show that transfer of SDN from FPOM to macroinvertebrates is more important than uptake of SDN to periphyton as a route for transferring SDN to aquatic consumers in these Piedmont streams. In addition, stable isotope analysis also showed that SDN did not significantly influence the riparian food web. A management implication of this result is that it is unlikely that riparian vegetation is very important in retaining SND in this ecosystem. We also found that terrestrial insects at both unrestored and restored urban sites were significantly enriched in ^{13}C compared with C3 plants or aquatic insects, which we attribute to a mixed diet of C3 and C4 plants. Furthermore, terrestrial insects were important diet components for fish at urban restored and unrestored sites. Thus, these results demonstrate that invasive C4 plants impact urban stream food webs, at least during the summer, via terrestrial insect subsidies to fish.

Many uncertainties remain regarding water quality and biotic integrity benefits of stream restoration in urban areas. Previous studies have recommended that implementation of porous pavement, stormwater retention ponds or other techniques to reduce the impacts of storm water on the integrity of streams would enhance success of stream restoration efforts (Charbonneau & Resh, 1992). Certainly ameliorating storm water effects is important to more complete stream restoration (Charbonneau & Resh, 1992). However, we have shown some improvements in stream ecosystem parameters in urban stream restoration projects which did not involve reduction of storm water input. Leaf litter was more important in the diets of aquatic invertebrates from restored sites than from unrestored sites, where periphyton was more important in the food web. Fish communities also showed increased richness and a trend toward increased abundance in restored urban sites. Thus, the relatively narrow forested corridors along restored sections do appear to have important beneficial effects on stream ecosystems, especially urban ones.

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