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APPLIED ISSUES

Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.)

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SUMMARY

1. The effects of catchment urbanisation on water quality were examined for 30 streams (stratified into 15, 50 and 100 km^2 ± 25% catchments) in the Etowah River basin, Georgia, U.S.A. We examined relationships between land cover (implying cover and use) in these catchments (e.g. urban, forest and agriculture) and macroinvertebrate assemblage attributes using several previously published indices to summarise macroinvertebrate response. Based on *a priori* predictions as to mechanisms of biotic impairment under changing land cover, additional measurements were made to assess geomorphology, hydrology and chemistry in each stream.
2. We found strong relationships between catchment land cover and stream biota. Taxon richness and other biotic indices that reflected good water quality were negatively related to urban land cover and positively related to forest land cover. Urban land cover alone explained 29–38% of the variation in some macroinvertebrate indices. Reduced water quality was detectable at *c.* >15% urban land cover.
3. Urban land cover correlated with a number of geomorphic variables such as stream bed sediment size (–) and total suspended solids (+) as well as a number of water chemistry variables including nitrogen and phosphorus concentrations (+), specific conductance (+) and turbidity (+). Biotic indices were better predicted by these reach scale variables than single, catchment scale land cover variables. Multiple regression models explained 69% of variation in total taxon richness and 78% of the variation in the Invertebrate Community Index (ICI) using *phi* variability, specific conductance and depth, and riffle *phi*, specific conductance and *phi* variability, respectively.
4. Indirect ordination analysis was used to describe assemblage and functional group changes among sites and corroborate which environmental variables were most important in driving differences in macroinvertebrate assemblages. The first axis in a non-metric multidimensional scaling ordination was highly related to environmental variables (slope, specific conductance, *phi* variability; adj. $R^2 = 0.83$) that were also important in our multiple regression models.
5. Catchment urbanisation resulted in less diverse and more tolerant stream macroinvertebrate assemblages via increased sediment transport, reduced stream bed sediment size and increased solutes. The biotic indices that were most sensitive to environmental variation were taxon richness, EPT richness and the ICI. Our results were largely consistent over the range in basin size we tested.

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Introduction

Land cover change is impacting stream ecosystems worldwide (Allan & Johnson, 1997; Rosenberg, McCully & Pringle, 2000; Paul & Meyer, 2001). Much of this change in developed countries is characterised by loss of agricultural and forested land and conversion to residential and commercial uses (i.e. urbanisation) (Grimm *et al*., 2000). Our study was conducted in the Piedmont region of the U.S., where rural land is undergoing rapid conversion due to expansion of nearby metropolitan areas (e.g. Atlanta; Lo & Yang, 2000). This conversion results in a mosaic of agricultural, forested and urban land cover.

The effects of such changing land cover on streams may occur largely via land disturbance, increased impervious surface area and resultant altered hydrology and transport of non-point source pollutants (e.g. sediment, nutrients) to streams. Increased impervious surface in catchments associated with urbanisation causes increased surface runoff (Hollis, 1975), leading to increased channel erosion (Trimble, 1997), altered channel morphology (Pizzuto, Hession & McBride, 2000) and increased concentrations of sediment, nutrients, particulate organics and potentially, toxins in streams (Wilber & Hunter, 1977; Klein, 1979; Herlihy, Stoddard & Johnson, 1998; Ometo *et al*., 2000). Sedimentation of streams caused by high erosion rates from construction activities and tilling of soils degrades physical habitat for stream biota (Waters, 1995; Trimble, 1997). Agricultural land uses can result in contributions of pesticides, fertilisers and animal wastes to streams (see review Cooper, 1993), potentially altering food or water quality for stream biota. Further, forest loss results in decreased assimilation of non-point source pollutants in the landscape (Lowrance *et al*., 1984; Peterjohn & Correll, 1984). Ultimately, this degradation of physical habitat and water quality can result in a reduction in the richness or number of intolerant or endemic species of fish (Karr & Schlosser, 1978; Schlosser, 1991; Wang *et al*., 1997; Klauda *et al*., 1998) or macroinvertebrates (Benke *et al*., 1981; Garie & McIntosh, 1986; Jones & Clark, 1987; Lenat & Crawford, 1994; Kennen, 1999; Thorpe & Lloyd, 1999) that live in receiving streams.

Quantifying and understanding how land cover change affects water quality and stream processes is essential for determining how humans can minimise their impacts on stream ecosystems. Thus, the purpose of this study was to: (1) quantify relationships between macroinvertebrate assemblage attributes and catchment land cover, (2) identify those environmental factors (chemical or geomorphic) that were affected by land cover change and (3) quantify relationships between biotic indicators and environmental factors. We built predictive multiple regression models of biotic indices and variables describing land cover, geomorphic conditions and water quality from 30 stream reaches that varied in catchment land cover. We were specifically interested in determining predictive capabilities of using land cover versus reach-scale (physical/chemical) attributes in determining effects on stream biota. In addition, we used an indirect ordination technique on a matrix of taxa-specific macroinvertebrate densities at each site to determine whether macroinvertebrate assemblage data yielded results similar to the multiple regressions using biotic indices. Finally, we tested whether relationships between land cover and biotic assemblage attributes differed across catchment size (15, 50 and 100 km^2 catchments) to determine whether our results were robust over this size range, relative to management. We also assessed potential breakpoints in urban land cover above which macroinvertebrate community condition indicated rapidly declining stream conditions.

Methods

Study region

Study sites were located in the Etowah River catchment, a 4823 km^2 basin in northcentral Georgia, U.S.A. (Fig. 1). The upper portion of the catchment empties into Lake Allatoona, a reservoir. Downstream of the reservoir, the Etowah River flows into the Coosa River, in the Mobile River Basin, which empties into the Gulf of Mexico in Alabama. The northernmost headwaters of the catchment lie in the Blue Ridge physiographic province and drain steeply sloping forests consisting primarily of secondary-growth

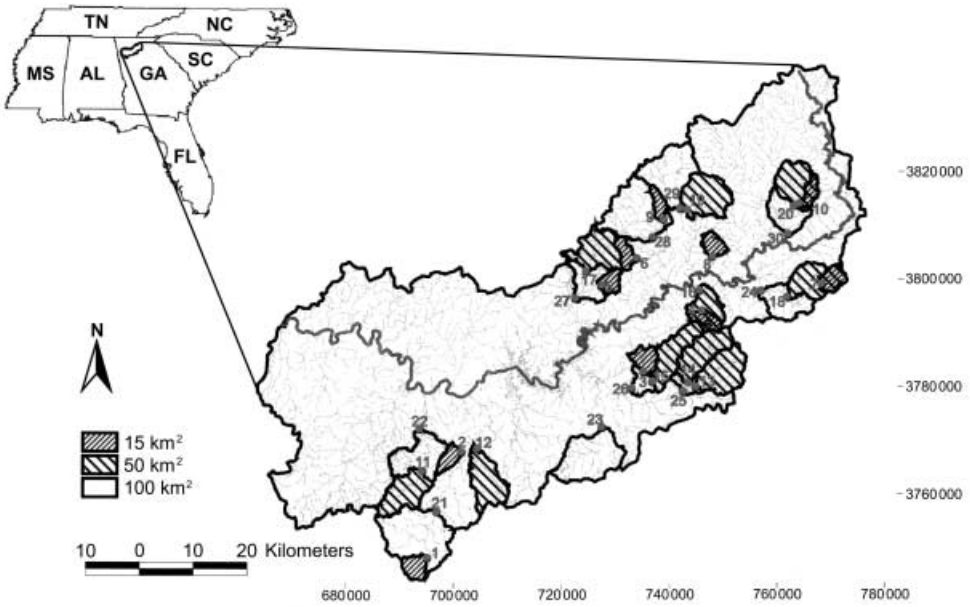

Fig. 1 Map of the 30 study sites within the Etowah River basin, Georgia, U.S.A.

hardwoods. The Piedmont physiographic province, characterised by less steep topography, encompasses the southern portion of the catchment. The 30 stream reaches selected for this study were from the Piedmont, with one catchment having part of its basin in the Blue Ridge. The region has a temperate climate with a mean annual rainfall of 132–163 cm and a mean annual air temperature of 15.6 °C (Georgia Environmental Protection Division (GA EPD), 1998). For a more detailed description of the catchment area, see the Coosa River Basin Management Plan (GA EPD, 1998).

The landscape within the Piedmont of the Etowah River basin has experienced various anthropogenic disturbances, including agriculture and recent urbanisation. Large parts of the basin were cleared in the 19th century when European settlers arrived. Extensive row-crop agriculture, primarily corn and cotton, began in the late 1800s and continued to increase until the boll weevil (*Anthonomus grandis grandis* Boheman) threatened cotton farming in the 1920s (Trimble, 1974). Secondary forests regenerated in many areas, and recently suburban development from the expanding metropolitan Atlanta area has resulted in a rapid increase in residential and commercial development throughout the Etowah River basin. The study sites exhibit a range in forest, agriculture and urban land cover.

Study design

Thirty sites were selected for this study, which included 10 sites in each of three basin size categories (targeted for selection at 15, 50 and 100 km^2 ± 25%). The size categories reflect an approximate doubling of the 2-year flood recurrence interval (~bankfull discharge) between each increase in basin area according to the models of Stamey & Hess (1993). Site selection was intended to exhibit a range of low percentage (<50%) urban land cover. Direct 15 km^2 tributaries (i.e. adventitious streams) of the main Etowah River were not used to avoid potential upstream effects from the main stem. Sites were chosen randomly within the Etowah River basin, independent of whether smaller catchments were nested within larger catchments.

Sampling methods

Macroinvertebrates. Benthic macroinvertebrates were sampled between 6 and 19 March 1999 within a 100-m stream reach. One site (site 11) was re-sampled in April 1999, as the benchmark was changed to a location slightly upstream from the original designation. At each site, macroinvertebrates were collected from three riffle, three pool and three bank habitats within the reach. In riffles, quantitative

samples were taken using a Surber sampler (500 μm mesh net; 0.09 m^2 sampling area, Wildlife Supply Company, Buffalo, NY, USA) and hand-scrubbing rocks for 3 min. In pools, we used a stove-pipe corer (0.04 m^2) and removed the top 10 cm of bottom sediment which was then washed through a 500-μm sieve. Bank habitats were sampled at randomly selected locations along the reach. Each sample was taken by sweeping a rectangular dip net (500 μm mesh; 0.25 m depth from water surface) through a 1-m section of stream bank three consecutive times while vigorously agitating the underwater bank vegetation. All nine samples were separately elutriated in the field using a bucket and a 500-μm sieve. The organic matter and invertebrates were stored in 10% formalin.

In the laboratory, each sample was washed though a 1-mm sieve to separate small macroinvertebrates. All large invertebrates (>1 mm) were hand-picked from debris using a dissecting microscope at 15× magnification and then preserved in 70% ethanol. If necessary, the smallest invertebrates (0.5–1.0 mm) were subsampled using a wheel sample splitter (Waters, 1969) to *c.* 100 invertebrates. Invertebrates were counted, measured and identified to genus, where possible, using standard keys (Merritt & Cummins, 1996; Wiggins, 1996; Brigham, Brigham & Gnilka, 1982). Chironomids were identified as Tanypodinae or non-Tanypodinae, and non-insects were identified to order. The amount of bank habitat was estimated by taking length and depth measurements along the margins of both sides of the stream where submerged snags or live vegetation occurred. This area was added to the amount of riffle and pool area on the bottom of the stream along the thalweg to calculate the proportion of each habitat in the 100 m reach at each site. Macroinvertebrate densities (no. m^{-2}) were multiplied by the proportion of riffle, pool and bank (wetted) habitat present at each site to calculate habitat-weighted density. Taxa were assigned to functional feeding groups (FFGs) based on Merritt & Cummins (1996). For each site, Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, the Benthic Index of Biotic Integrity (B-IBI; Kerans & Karr, 1994) and the Invertebrate Community Index [ICI; Ohio Environmental Protection Agency (Ohio EPA), 1989] were calculated. We modified the original indices to exclude metrics that we could not calculate based on our data (Table 1). To avoid problems using regression on potentially discretely distributed data, we calculated metric scores as continuous variables (0–10). Scores were calculated by dividing the raw metric value for a site (transformed, if necessary) by the 95th percentile (when values decreased with disturbance) and multiplied by 10 (Minns *et al.*, 1994). For metrics that increased with disturbance, the following equation was used:

$$[1 - (x/X_5)(X_{95}/X_5)] \times 10 \quad (1)$$

where x is the raw metric value for a given site, X_5 is the fifth percentile of the range in for that metric, and X_{95} is the 95th percentile. Final metric scores that were >10 were reduced to 10, so the maximum possible score for the B-IBI (11 metrics) was 110 and for the ICI (10 metrics) was 100. High scores in these multimetric indices indicate good water quality. Macroinvertebrates were also assigned tolerance values (where high values indicate poor water quality) using Hilsenhoff's Family Biotic Index (FBI; Hilsenhoff, 1988) and the North Carolina Biotic Index (NCBI; Lenat, 1993). The NCBI species tolerance values were averaged to obtain values for genera. The NCBI scores

Table 1 Metrics for the Benthic Index of Biotic Integrity (B-IBI)* and Invertebrate Community Index (ICI)†

B-IBI Metrics
1. Total taxa richness
2. Ephemeropteran taxa richness‡
3. Trichopteran taxa richness
4. Plecopteran taxa richness
5. Proportion of *corbicula*§
6. Proportion of Oligocheates§
7. Proportion of 2 most abundant taxa§
8. Proportion of Filterers§
9. Proportion of Scrapers§
10. Proportion of Predators excluding chironomids§
11. Total abundance‡

ICI Metrics
1. Total no. taxa
2. No. Mayfly taxa‡
3. No. Caddisfly taxa
4. No. Dipteran taxa
5. Percent Mayfly composition§
6. Percent Caddisfly composition‡
7. Percent predatory Chironomidae composition§
8. Percent other dipteran and non-insects§
9. Percent tolerant organisms§
10. No. EPT taxa

*Modified from Kerans Karr (1994). Metrics omitted from this study include intolerant snail and mussel species and the proportion of individuals as omnivores and scavengers.
†Modified from Ohio EPA (1989).
‡$\log_{10}(x + 1)$ transformed.
§Arcsin square-root transformed.

were adjusted for winter/spring collections by adding 0.2 to all site scores, and water quality ratings were based on the Piedmont ecoregion (Lenat, 1993). Macroinvertebrate or biotic 'integrity' is used throughout the document to refer to scores on multiple biotic assemblage measurements (e.g. richness, biotic indices and multimetric indices) that respond to water quality degradation, with higher integrity reflected by high richness and scores on indices associated with high water quality.

Trophic variables. After invertebrates were removed from the samples, benthic organic matter (BOM) standing crop was quantified. Coarse particulate organic matter (CPOM; >1 mm) and fine POM (FPOM; <1 mm to >500 μm) were dried at 50 °C for 1 week and weighed. The organic material was then subsampled (if necessary), ashed in a muffle furnace at 500 °C and re-weighed to determine the total ash free dry mass (AFDM) of each sample. Algal biomass (as chlorophyll *a*) was determined by scraping algae from the dominant substrate at 10 transects within the 100 m reach at each site between 8 and 19 April 1999 (Rosemond *et al*., 2001). In the laboratory, samples were filtered onto Whatman® GF/F filters (pore size = 0.7 μm, Whatman Inc., Clifton, NJ, USA), rinsed with $MgCO_3$ to prevent chlorophyll degradation and frozen. Chlorophyll *a* was extracted from the filters in the dark in 90% acetone, filters were pulverised and the extract was measured spectrophotometrically as described in Wetzel & Likens (1991). Values used in analyses were mean chlorophyll *a* (mg m^{-2}) of 10 samples per site.

Chemistry. Stream water chemistry was sampled monthly from March 1999 to March 2000 (Paul, Leigh & Lo, 2001). Mean annual values were used in analyses. Samples were collected over two consecutive days each month during baseflow. For ammonium-nitrogen (NH_4-N), nitrate/nitrite-nitrogen (NO_3/NO_2-N) and soluble reactive phosphorus (SRP), water samples were filtered (Gelman® A/E glass fiber, 0.45 μm, Gelman Sciences, Ann Arbor, MI, USA) into acid-washed bottles and transported to the laboratory on ice. Dissolved nutrients were analysed using an Alpkem autoanalyser (Alpkem Corporation, Wilsonville, OR, USA) by the University of Georgia Institute of Ecology Chemical Analysis Laboratory following Standard Methods protocol (American Public Heath Association, 1989). Specific conductance (SC), pH, and dissolved oxygen (DO) were collected with a Hydrolab® Datasonde 4 (Hydrolab Corporation, Austin, TX, USA), which was lab calibrated quarterly for SC and pH and field calibrated daily for DO. Turbidity was measured in the field with a Hach® Turbidimeter (HACH Company, Loveland, CO, USA). Table 2 lists the mean and range of chemistry values across sites.

Geomorphology. Stream geomorphology was measured in summer 1999 (Leigh *et al*., 2001). Stream reach survey lengths were based on stream size (15 km^2: 100 m reach; 50 km^2: 150 m reach; 100 km^2: 200 m reach). Energy grade line slope was determined with a total station survey in the field. Mean water depth and bed sediment size (*phi*) were determined in a zig-zag survey along the length of the study reach. One litre of bed sediment was also collected from riffles, pools and emergent bars and dry sieved in the laboratory to calculate sieved particle size (see Table 2). Details on collection techniques and additional geomorphic variables sampled can be found in Leigh *et al*. (2001).

Land cover. The land cover (implying cover and use) variables were calculated from 1997 *Landsat* TM images (Lo & Yang, 2000). Radiometric normalisation was used to adjust for the seasonal differences in the data. Total urban land cover included high intensity and low intensity urban categories, which were analysed separately in regression analyses. The agriculture category contained cultivated/exposed land and cropland/grassland. Evergreen, deciduous and mixed forests were combined into the forest land cover category. Percent forest in the 100 m riparian buffer for the entire drainage network upstream of sample sites was calculated. Various past land cover (1973 and 1987) and land cover change variables have been calculated for the sites; however, previous analyses indicated that the best predictors for the macroinvertebrate variables were the most recent land cover (Roy *et al*., 2001). Land cover variables that were used in statistical analyses are listed in Table 2.

Data analyses

A subset of geomorphic and chemical variables of those collected were selected to use in statistical analyses. We used correlation analysis (all final variables had $r < 0.70$) to avoid multicollinearity and

Table 2 Variables used in statistical analyses

Acronym	Variable (units)	Min	Mean	Max
Land cover				
Percent urban*	1997 urban land cover (%)	4.9	15.0	60.7
High intensity*	1997 high intensity urban (%)	0.3	2.4	25.5
Low intensity*	1997 low intensity urban (%)	4.4	12.7	35.2
Percent agriculture*	1997 agriculture land cover (%)	6.5	22.3	38.4
Cult/exposed*	1997 cultivated/exposed land (%)	0.0	2.2	5.2
Crop/grassland*	1997 cropland/grassland (%)	5.5	19.9	35.3
Percent forest*	1997 forest land cover (%)	27.2	61.7	87.0
Riparian buffer*	1997 forest land in 100 m buffer (%)	33.7	67.4	94.6
Morphometric and geomorphic variables				
Basin Area	Catchment size (km^2)	11.3	56.9	125.7
Slope†‡	Energy grade line slope	0	0.003	0.009
Local relief‡	Local relief (m)	36.6	67.3	152.4
Entrenchment*‡	Q bankfull/Q 2-year flood	0.09	1.20	3.13
Depth†	Water depth (cm)	16.3	26.3	58.4
Percent riffle*‡	Riffle area (%)	13.2	36.3	60.9
Mean *phi*	Mean bed sediment size (*phi*)	−6.4	−2.9	−0.3
Phi variability†	Bed sediment variability (*phi*)	0.9	2.1	3.7
Bar *phi*	Emergent bar bed sediment size (*phi*)	−4.6	−2.1	0.0
Riffle *phi*†	Riffle bed sediment size (*phi*)	−5.0	−2.8	−0.3
Pool *phi*	Pool bed sediment size (*phi*)	−4.1	−1.4	−0.1
Chemical and other environmental variables				
TSS‡	Total suspended solids (mg L^{-1})	1.25	5.10	13.46
NH_4-N†‡	Ammonium-nitrogen (μg L^{-1})	0.60	1.32	1.97
NO_3/NO_2-N†	Nitrate plus nitrate-nitrogen (μg L^{-1})	34.5	368.2	880.5
SRP	Soluble reactive phosphorus (μg L^{-1})	7.8	77.3	135.2
SC†	Specific conductance (μs cm^{-1})	21.3	69.1	171.6
DO	Dissolved oxygen (mg L^{-1})	7.6	8.6	9.3
Turbidity†‡	Turbidity (NTU)	0.43	0.84	1.25
pH†	pH	6.7	7.1	7.6
BOM	Benthic organic matter (g AFDM m^{-2})	23	256	1043
Chl a‡	Total chlorophyll *a* (mg m^{-2})	0.67	26.54	152.02

*Arcsin square-root transformed.
†Used in multiple regression analysis.
‡$Log_{10}(x)$ transformed.
Q = discharge. *Phi* = negative log of particle size in mm. NTU = nephelometric turbidity units. AFDM = ash free dry mass.

reduce the number of environmental variables used in analyses. Principal components analysis was used to: (1) isolate groups of variables that explained different components of the environmental variation and (2) determine which variable among one or more correlated variables to use in multiple regression analyses by selecting variables that accounted for the greatest proportion of the variance. Although additional variables were used in other analyses, only 11 uncorrelated variables were included in the stepwise multiple regression analyses. To characterise differences among sites, the mean values for selected land cover, geomorphic and chemical variables for the 30 sites are shown in Table 3.

The distribution of all dependent and independent variables were checked for normality and appropriately transformed, if necessary. Macroinvertebrate densities were $\log_{10}(x + 1)$ transformed and percentage data were normalised using arcsin square-root. Tranformations used for environmental variables are listed in Table 2. We used least-squares linear regression to determine the relationship between percent urban, agriculture and forest land cover and biotic indices. Bivariate plots were used to look for potential thresholds in percent land cover data, above which change in macroinvertebrate condition indicated rapid decline in water quality. We then analysed potential links between land cover and selected

Table 3 Summary macroinvertebrate assemblage attribute scores for 30 sites. Richness values are totals from nine samples per site. EPT richness is the taxon richness in the macroinvertebrate orders Ephemeroptera, Plecoptera, and Trichoptera. The Invertebrate Community Index (ICI) and Benthic Index of Biotic Integrity (B-IBI) are multimetric indices described in Table 1. The North Carolina Biotic Index (NCBI) and Hilsenhoff's Family Biotic Index (FBI) are biotic indices based on tolerance values for individual taxa. Densities (no. m^{-2}) are habitat-weighted according to the proportion riffle, pool and bank habitat area in the study reach. Thus, reported density values are a linear sum of three riffle, three pool and three bank habitat samples

	Min	Mean	Max
Total richness	21	43	62
EPT richness	3	16	31
ICI	29.3	62.5	88.7
B-IBI	43.7	68.4	91.9
NCBI	4.44	5.60	6.38
FBI	3.89	5.25	5.97
Total density	145.43	2236.50	5893.58
Filterer density	0.00	80.64	399.91
Gatherer density	84.29	1105.52	4280.13
Scraper density	0.71	88.67	336.68
Shredder density	0.00	7.76	36.17
Predator density	2.10	160.66	886.03

geomorphic and chemical variables using simple linear regression. We determined the amount of variation in the macroinvertebrate community that could be explained by these environmental variables using stepwise multiple regression (forward and backward selection; $P = 0.05$ to enter and leave model) for the seven macroinvertebrate indices. All regression analyses were performed using JMP Version 4.0 statistical software (SAS Institute Inc., Cary, NC, U.S.A.).

The effects of basin size on the relationship between land cover and biotic integrity were determined using linear regression, separated by catchment size class. Prior to analyses, we tested whether environmental and macroinvertebrate variables were normally distributed for the 15, 50 and 100-km^2 catchments. The environmental variables that were not normally distributed were eliminated from the stepwise multiple regression analysis for that catchment size (only one variable, pH, for 50 km^2 catchments). We also performed stepwise multiple regression analysis separately for each size category to determine the best environmental predictors of macroinvertebrate integrity based on catchment size.

Variation in biota across sites was also examined using non-metric multidimensional scaling (NMS) with the statistical package PC-ORD Version 4 (MjM Software Design, Glenden Beach, OR, U.S.A.). The purpose of this indirect ordination analysis was to compare the distribution of the entire macroinvertebrate assemblage across sites without including any prior information about how taxa respond to disturbance in an effort to confirm which environmental variables were most important in driving differences among assemblages. Habitat-weighted macroinvertebrate densities [$\log_{10}(x + 1)$ transformed] at each site were used to create a matrix for all taxa. Rare species (present at only one site or abundance <0.01 ind. m^{-2}) were excluded from the analysis. We correlated individual taxa, FFGs and macroinvertebrate metrics from the multimetric indices with ordination axes to determine which taxa best accounted for separation of sites in ordination space. Land cover, chemical and geomorphic variables were regressed against the first two ordination axes to determine which environmental variables best explained the separation of the sites. We then incorporated the ordination information into a stepwise multiple regression analyses by using the NMS axes as dependent variables.

Site 2 was eliminated from all of the analyses, as beaver dams were constructed between sampling days, thus physically and chemically altering the site.

Results

Macroinvertebrate distribution among sites

Over 57 000 macroinvertebrates in 134 distinct taxonomic groups were collected at the 30 sites (Roy, 2000). The most common taxa were Chironomidae, *Corbicula* spp. and oligocheates. Some ephemeropterans (*Ephemerella* spp., *Stenomena* spp. and *Baetis* spp.), Ceratopogoninae, *Simulium* spp., *Cheumatopsyche* spp. and *Optioservus* spp. were also collected in high densities at most sites.

Gatherers (dominated by Chironomidae) were the most abundant functional feeding group, whereas shredders were the least common. Gatherers ranged in density from 84 ind. m^{-2} (Site 23) to 4280 ind. m^{-2} (Site 30), which represented 35.1–93.9% of the total abundance (Table 3). Predators had the second highest mean density and ranged from 22 ind. m^{-2} (Site 23) to 886 ind. m^{-2} (Site 18). Filterers ranged in density from 0 to 400 ind. m^{-2}. Scrapers were found at all sites, with a minimum density of 1 ind. m^{-2} and a maximum of 337 ind. m^{-2}. No shredders were

Table 4 Linear regression models (r^2 reported) with 1997% land cover variables ($n = 29$ sites). Macroinvertebrate variables as described in Table 3. Other variables as defined in Table 2

	% Urban			Agriculture			% Forest	
	Total	High intensity	Low intensity	Total	Cult/exposed	Crop/grassland	Total	Riparian buffer
Macroinvertebrate variables								
Total richness	−0.29**	−0.31**	−0.23**	−0.01	0.00	−0.01	+0.22*	+0.32**
Total density	−0.22*	−0.14*	−0.14*	+0.03	+0.11	+0.04	+0.06	+0.12
EPT richness	−0.31**	−0.27**	−0.27**	−0.04	+0.02	−0.03	+0.28**	+0.36***
B-IBI	−0.19*	−0.24**	−0.14*	−0.03	+0.02	+0.01	+0.20*	−0.24
ICI	−0.38***	−0.30**	−0.36***	−0.03	+0.03	+0.02	−0.31**	−0. 28
NCBI	+0.29**	+0.27**	+0.33**	+0.03	+0.02	−0.03	−0.24**	−0.30**
FBI	+0.28**	+0.27**	+0.30**	+0.02	0.00	−0.02	−0.21*	+0.37***
Morphometric and geomorphic variables								
Basin area	+0.01	+0.00	+0.01	−0.07	−0.02	−0.06	+0.01	0.00
Slope	−0.19*	−0.05	−0.25**	−0.03	0.00	−0.02	+0.14*	+0.18*
Local relief	−0.34***	−0.31**	−0.35***	−0.09	−0.06	−0.05	+0.32**	+0.26**
Entrenchment	0.00	−0.01	−0.01	−0.12	−0.03	−0.11	+0.07	+0.07
Depth	0.00	0.00	−0.01	−0.04	−0.02	−0.03	+0.03	+0.02
Percent riffle	−0.14*	−0.06	−0.18*	−0.01	−0.01	−0.01	+0.01	+0.11
Mean *phi*	+0.22*	+0.05	+0.31**	+0.03	+0.01	+0.02	−0.16*	−0.18*
Phi variability	−0.07	−0.05	+0.16*	−0.04	−0.01	−0.03	−0.10	+0.16*
Bar *phi*	+0.15*	+0.14*	+0.16*	+0.07	+0.01	+0.05	−0.19*	−0.27**
Riffle *phi*	+0.20*	+0.10	+0.21*	+0.04	0.00	+0.04	−0.19*	+0.23*
Pool *phi*	+0.15*	+0.07	+0.19*	+0.01	+0.01	+0.01	−0.10	−0.15*
Chemical and other environmental variables								
TSS	+0.28**	+0.20*	+0.33**	+0.11	−0.01	+0.11	−0.32**	−0.41***
NH_4-N	+0.16*	+0.23**	+0.17*	+0.30**	0.00	+0.29**	−0.39***	−0.55***
NO_3/NO_2-N	+0.24**	+0.47***	+0.16*	+0.34***	−0.01	+0.35***	−0.52***	−0.58***
SRP	+0.24**	+0.13	+0.30**	+0.11	0.00	+0.09	−0.28**	−0.29**
SC	+0.48***	+0.39***	+0.38**	+0.03	−0.09	+0.02	−0.38***	−0.47***
Turbidiity	+0.10	+0.18*	+0.16*	+0.28**	+0.10	+0.24**	−0.27**	−0.37***
pH	+0.01	0.00	0.00	−0.11	−0.03	−0.11	+0.01	0.00
BOM	+0.01	+0.03	0.00	+0.01	0.00	+0.01	−0.01	−0.01
Chl *a*	−0.16*	−0.04	−0.21*	0.00	0.00	+0.01	+0.05	+0.06

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

found at Sites 3, 13 and 23, and the highest density (36 ind. m^{-2}) was found at site 30 (Table 3).

Calculated macroinvertebrate index scores for each site are also listed in Table 3. The EPT richness ranged from three taxa (Site 23) to 31 taxa (Site 30). The ICI ranked Site 23 as the most degraded site, while the B-IBI ranked Site 3 as most degraded. Both multimetric indices ranked Site 30 with the highest water quality. In terms of biotic indices, FBI scores ranged from 3.89 (very good; Site 4) to 5.97 (fairly poor; Site 23). According to the water quality categories for the FBI, no sites were considered excellent, poor or very poor. The NCBI scores ranged from 4.44 (excellent; Site 4) to 6.38 (good-fair; Site 26). Based on the NCBI, nine sites were considered 'excellent', nine were 'good', and 12 had 'good-fair' water quality for Piedmont streams.

Land cover relationships with macroinvertebrates and environmental variables

Increased urban land cover and decreased forested land cover were related to degraded biotic integrity (Table 4). There were significant regressions between both urban and forested land cover and total richness, EPT richness, B-IBI, ICI, NCBI and FBI; regressions with agricultural land cover were not significant. Both high and low intensity urban land cover formed significant correlations with all of the macroinvertebrate variables, indicating negative impacts. Compared with total percent forest/land cover, the percent of the riparian buffer that was forested formed stronger positive correlations with total richness, EPT richness, NCBI and FBI (Table 4).

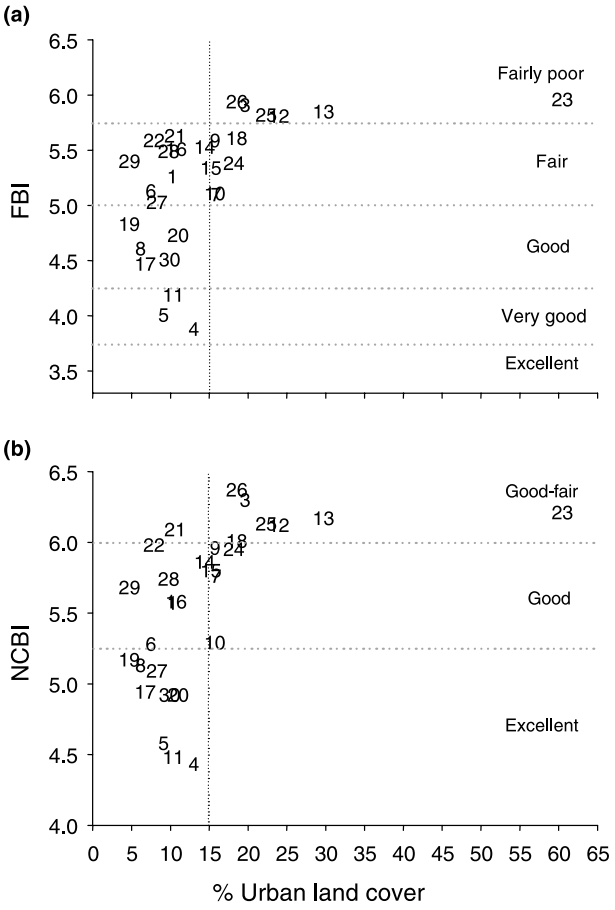

Fig. 2 Relationship between Hilsenhoff's Family Biotic Index (FBI; a) and the North Carolina Biotic Index (NCBI; b) and percentage urban land cover in the catchment. Numbers correspond to sites. Higher biotic index values reflect poorer water quality as indicated by the water quality ratings (horizontal dotted lines). The vertical line at 15% urban land cover represents the suggested approximate urbanisation threshold beyond which sensitive taxa are lost.

Bivariate plots of the NCBI and FBI with urban land cover revealed a breakpoint of about 15–20% urban land cover, above which the indices indicated only fair to fairly poor (FBI) or good to good-fair (NCBI) water quality as opposed to a range of very good to fair (FBI) or excellent to good-fair (NCBI) with less urban land cover (Fig. 2). Mean FBI (5.00 versus 5.63) and NCBI (5.32 versus 6.02) values between sites that had <15% urban land cover ($n = 18$) and >15% urban land cover ($n = 11$), respectively, were significantly different (*t*-test assuming unequal variances; FBI: $t = 3.72$, $P < 0.001$; NCBI: $t = 4.45$, $P < 0.001$).

Land cover was also related to many environmental variables (Table 4). Increased percent urban land cover and decreased percent forest land cover were associated with larger phi and lower slope and local relief. Agricultural land cover was not significantly correlated with any of the morphometric or geomorphic variables. In terms of water chemistry, higher nutrient concentrations (nitrogen and phosphorus) and turbidity concentrations were related to increased urban and agricultural land cover and decreased forest land cover. Increased specific conductance and total suspended solids were correlated with increased urban land cover and decreased forest land cover. An increase in percent urban land cover (specifically, low intensity urban land cover) was negatively related to chlorophyll *a* (Table 4).

Environmental predictors of biotic integrity

Both geomorphic and chemical variables were important in explaining variation in the multimetric and biotic indices and other macroinvertebrate characteristics across sites (Table 5). Specific conductance, which was negatively correlated with biotic integrity, was a significant environmental variable in all seven models. Variation in *phi* was an important explanatory variable in four of the models, with a higher variation being associated with a more diverse macroinvertebrate assemblage. Average depth, turbidity and riffle *phi* were also selected as significant variables in the linear regression models. In the model with the highest variation in the macroinvertebrates explained, ICI was predicted by riffle *phi*, SC and *phi* variability (adj. $R^2 = 0.78$, Fig. 3).

Catchment size

Linear regressions between biotic integrity and land cover variables for the separate catchment size categories revealed a few significant relationships in each of the catchment sizes (Table 6). Total percent urban land cover was the variable most consistently related to richness or biotic indices, but the responding metric or index was not consistent among size groupings (Table 6).

When examining the best linear regression models for each basin size category, the 100 km^2 catchments had the overall highest coefficients of determination, compared with smaller catchments (Table 7). Specific conductance, depth and *phi* variability (the same variables important in the models with all the sites)

	Adjusted R^2	Partial R^2	P	Independent variables
Total richness	0.69	0.40	<0.001	+ *Phi* variability
		0.25	<0.001	– Specific conductance
		0.07	0.020	+ Depth
Total density	0.50	0.40	<0.001	+ *Phi* variability
		0.13	0.012	– Specific conductance
EPT richness	0.66	0.52	<0.001	– Specific conductance
		0.12	0.018	+ *Phi* variability
		0.06	0.042	+ Depth
B-IBI	0.50	0.51	<0.001	– Specific conductance
ICI	0.78	0.60	<0.001	– Riffle *phi*
		0.15	<0.001	– Specific conductance
		0.05	0.015	+ *Phi* variability
NCBI	0.36	0.27	0.008	– Specific conductance
		0.13	0.023	– Turbidity
FBI	0.18	0.13	0.013	– Specific conductance

Table 5 Significant multiple linear regression models using stepwise regression ($n = 11$ environmental variables, forward and backward selection, $P = 0.05$) for selected macroinvertebrate variables, as described in Table 3. Sign (+/–) indicates direction that variable relates to increased biotic integrity. Independent variables as defined in Table 2

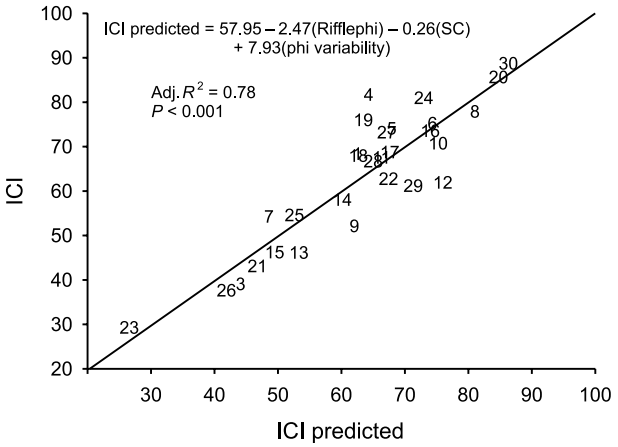

Fig. 3 Fitted multiple linear regression models from stepwise regression analysis for the Invertebrate Community Index (ICI), the macroinvertebrate assemblage attribute that was most predicted by environmental variables. The ICI was predicted by riffle bed sediment size (riffle *phi*), specific conductivity (SC) and bed sediment size variability (*phi* variability).

were important in explaining the variation in macroinvertebrate assemblages for the 100 km^2 catchments. There were no consistently important environmental variables in models for the 50 km^2 sites. For small catchments (15 km^2), NH_4-N was repeatedly important at explaining variation in macroinvertebrate assemblages (Table 7).

Ordination analyses

Two axes, together accounting for 88.7% of the variation in the macroinvertebrate community, were chosen for the NMS ordination which included habitat-weighted densities of all taxa except rare genera. The first NMS axis explained 78.1% of the variation, while the second axis only accounted for 10.6% of the variation (final stress = 14.73).

Percent urban land cover (total, high intensity and low intensity) was negatively related to the first axis, and percent forest land cover and the percentage of the riparian buffer that was forested were positively related to the first axis (Table 8, Figs 4 & 5). Twelve geomorphic, chemical and trophic variables had significant regressions with NMS Axis 1. Larger *phi*, higher chlorophyll *a*, higher DO and greater slope corresponded to sites at the right side of the ordination. Increased SC, SRP, NH_4-N and total suspended solids were negatively correlated with the first NMS axis (Table 8, Figs 4 & 5). No environmental variables used in this study significantly explained the variation in the second axis.

Axis 1 of the NMS ordination was largely influenced by scrapers ($r^2 = 0.78$), filterers ($r^2 = 0.67$) and gatherers ($r^2 = 0.62$) (Fig. 4). Ephemeropterans, trichopterans and dipterans were also significantly correlated to NMS Axis 1 (Table 8, Fig. 5). Percent dipterans and non-insects were highest at sites on the left of the ordination. Individual species analyses indicated that *Ephemera* spp., *Stenomena* spp., *Optioservus* spp., non-Tanypodinae chironomids, *Cheumatopsyche* spp., *Isoperla* spp. and many other taxa densities were highest at the higher quality sites to the right of the ordination. *Corbicula* spp., *Ochrotrichia*

Table 6 Linear regression models (r^2 reported) with 1997% land cover variables for each catchment size category. Macroinvertebrate variables as described in Table 3

	% Urban			Agriculture			% Forest	
	Total	High intensity	Low intensity	Total	Cult/exposed	Crop/grassland	Total	Riparian buffer
15 km^2 ($n = 9$)								
Total richness	−0.60*	−0.78**	−0.49*	−0.28	−0.69**	−0.15	+0.41	+0.39
Total density	−0.04	0.00	−0.09	+0.03	−0.07	+0.01	0.00	0.00
EPT richness	−0.38	−0.56*	−0.28	−0.34	−0.07	−0.29	+0.41	+0.48*
B-IBI	−0.28	−0.44	−0.18	−0.24	−0.15	−0.18	+0.30	+0.28
ICI	−0.58*	−0.55*	−0.51*	−0.24	−0.24	−0.18	+0.39	+0.40
NCBI	+0.33	+0.39	+0.24	+0.05	+0.33	+0.02	−0.14	−0.14
FBI	+0.26	+0.41	+0.17	+0.04	+0.37	+0.01	−0.11	−0.11
50 km^2 ($n = 10$)								
Total richness	−0.09	−0.01	−0.10	−0.07	0.00	−0.06	0.14	0.34
Total density	+0.01	+0.02	+0.01	0.00	+0.22	0.00	0.00	+0.07
EPT richness	−0.21	−0.03	−0.24	−0.02	0.00	−0.01	+0.15	+0.25
B-IBI	−0.06	0.00	−0.07	−0.06	+0.17	−0.08	+0.12	+0.34
ICI	−0.42*	−0.18	−0.45*	−0.17	−0.04	−0.13	−0.45*	−0.46*
NCBI	+0.60**	+0.50*	+0.60**	+0.12	+0.02	+0.09	−0.51*	−0.62**
FBI	+0.59**	+0.50*	+0.59**	+0.12	+0.03	+0.08	−0.50*	−0.57*
100 km^2 ($n = 10$)								
Total richness	−0.41*	−0.38	−0.37	+0.04	+0.26	+0.05	+0.24	+0.38
Total density	−0.45*	−0.38	−0.34	+0.16	+0.52*	+0.18	+0.17	+0.29
EPT richness	−0.46*	−0.37	−0.44*	0.00	+0.23	0.00	+0.37	+0.50*
B-IBI	−0.32	−0.24	−0.31	0.00	0.17	0.00	+0.25	+0.37
ICI	−0.35	−0.26	−0.32	0.01	0.20	0.01	0.25	0.34
NCBI	+0.23	+0.17	+0.30	+0.12	0.00	+0.10	−0.35	−0.40*
FBI	+0.28	+0.21	+0.31	+0.07	−0.04	+0.06	−0.36	−0.44*

*$P < 0.05$, **$P < 0.01$.

spp., *Hydropsyche* spp. and *Corydalus* spp. were negatively correlated with the second axis (Table 8).

When the NMS axes were incorporated into multiple regression models as independent macroinvertebrate variables, Axis 1 was explained by slope, SC and *phi* variability (adj. $R^2 = 0.83$, Table 9). No environmental variables predicted the variation in the macroinvertebrate community encompassed in Axis 2.

Discussion

How well do macroinvertebrate assemblages reflect changes in water quality due to land cover change?

A strength of our study was the diversity of environmental and land cover variables that were quantified, such that both could be related to macroinvertebrate indices. We found significant relationships between biotic indices and single land cover classifications, particularly measurements of urban land cover. Regressions using total urban land cover were roughly as good or better than higher resolution urban classifications. Forest land cover was also highly correlated with macroinvertebrate indices, with the strongest relationships being with percentage forest located in a 100-m wide riparian buffer zone. This result supports previous studies indicating the role of riparian buffers in protecting water quality (Gregory *et al.*, 1991). These relationships were observed over moderate ranges in land cover (5–61% urban land, 34–95% forested riparian buffer).

Relationships between the NCBI and FBI and urban land cover revealed a potential breakpoint of 15–20% urban land cover, above which macroinvertebrate assemblages reflected poorer water quality. Other studies have identified similar thresholds of urban land cover (10–20%, Wang *et al.*, 1997) and impervious surface (8–10%, Booth & Jackson, 1997) within catchments which led to fish impairment (see review Paul & Meyer, 2001). Other macroinvertebrate varia-

Table 7 Multiple linear regression models for separate catchment size categories using stepwise regression (11 environmental variables, forward selection, $P < 0.05$). pH was omitted from the regression for the 50 km^2 sites because it was not normally distributed. Site 23 was excluded from the 100 km^2 models. Models were restricted to two variables. Macroinvertebrate variables as described in Table 3. Model independent variables as defined in Table 2

	Adj. R^2	Model
15 km^2 ($n = 9$)		
Total richness	–	
Total density	0.49	*Phi* variability
EPT richness	0.91	NH_4-N, slope
B-IBI	0.52	NH_4-N
ICI	0.68	NH_4-N
NCBI	–	
FBI	–	
50 km^2 ($n = 10$)		
Total richness	0.72	Slope
Total density	–	
EPT richness	0.87	Riffle *phi*, % riffle
B-IBI	0.46	Specific conductance
ICI	0.81	Slope, NO_2/NO_3-N
NCBI	0.63	Turbidity, entrenchment
FBI	–	
100 km^2 ($n = 9$)		
Total richness	0.69	Depth
Total density	0.69	*Phi* variability
EPT richness	0.81	Depth
B-IBI	0.91	Depth
ICI	0.91	*Phi* variability, specific conductance
NCBI	0.93	Specific conductance, entrenchment
FBI	0.92	Specific conductance, NO_2/NO_3-N

bles (total richness, total density, EPT richness, ICI and B-IBI) had relatively linear relationships with the percent urban land cover. These variables may not be indicating thresholds of disturbance because they give equal credit to sensitive and tolerant taxa, whereas the NCBI and FBI down-weight the importance of tolerant organisms.

What variables were potentially driving changes in biota in these catchments?

Although the correlative approach used in this study can not be used to infer mechanistic effects of urbanisation on macroinvertebrates, measurements at more than one scale of resolution (catchment and reach scale) allowed us to identify factors that may indicate potential pathways by which changing land cover affected macroinvertebrate assemblages. Biotic indices indicating high water quality were consistently associated with increased bed sediment particle size variability and lower dissolved ion concentration (SC). In some cases, single explanatory variables such as SC, riffle *phi* and *phi* variability explained over half of the variation in the biotic integrity across all sites. Our analyses did not include many other potentially important variables, but we believe that those variables we used reflect the nature of environmental disturbance in these streams. For example, the data set we used in multiple regression analyses was a subset of all potential variables (we chose only those that were uncorrelated). Thus, variables such as mean *phi*, which was not used in multiple regression analyses due to autocorrelation with other variables, also exhibited relationships with macroinvertebrate characteristics among sites (as in our ordination analysis).

The consistently strong relationships we observed between biotic indices and SC indicate that increased SC may lead to biotic impairment of surface waters. Other studies have also found a strong relationship between SC and land cover (Ometo *et al*., 2000) and have determined predictive relationships between SC and changes in macroinvertebrate assemblages (Tate & Heiny, 1995; Imert & Stanford, 1996). Specific conductance might be a good indicator of sediment disturbance as a source of increased ions (in addition to ion input via catchment run off), as it was positively correlated with decreased riffle and emergent bar particle size. Thus, its inclusion in the regression models may partially be due to its relationship with these variables, or as a surrogate 'chemical signal' from increased non-point sources in the catchments (e.g. fertilisers, pesticides, sediment), as suggested by its relationships with forest land cover and ammonium concentration.

Changes in macroinvertebrate assemblage structure were also related to factors indicating variation in physical habitat, particularly bed sediment. Previous studies have also shown positive relationships between macroinvertebrate richness and density and stream bed particle size (see Minshall, 1984). Many macroinvertebrates need large particles and the associated interstitial space for protection from predators and high flows, substrate for periphyton food sources, attachment sites for filter feeding and increased oxygen exchange (Wood & Armitage, 1997). Because large particles are important for biota, sedimentation is a key concern in streams threatened

Table 8 Variables regressed with macroinvertebrate distribution ($r^2 > 0.25$, $P < 0.005$) on axes 1 & 2 of a non-metric multidimensional scaling (NMS) ordination ($P < 0.05$). Ordination used the $\log_{10}(x + 1)$ transformation of the habitat-weighted densities (no. m^{-2}) of all taxa, excluding rare species (present at only one site or abundance <0.01 ind. m^{-2})

	NMS Axis 1 (78.1%)	r^2	NMS Axis 2 (10.6%)	r^2
Environmental variables				
Land cover	% Urban	−0.41		
	Low intensity urban	−0.40		
	Riparian buffer	0.39		
	% Forest	0.29		
	High intensity urban	−0.26		
Geomorphic	Riffle *phi*	−0.69		
	Slope	0.61		
	Mean *phi*	–0.55		
	Phi variability	0.47		
	Bar *phi*	−0.36		
	Pool *phi*	−0.29		
Chemical & tropic	SC	−0.51		
	Chl *a*	0.48		
	DO	0.43		
	SRP	−0.42		
	NH_4-N	−0.31		
	TSS	−0.29		
Macroinvertebrate variables				
FFGs	Scraper density	0.78		
	Filterer density	0.67		
	Gatherer density	0.62		
	% Scrapers	0.33		
	% Filterers	0.25		
Other	% Ephemeroptera	0.65		
	% Dipterans & Non-insects	−0.64		
	Dipteran richness	0.54		
	% Trichoptera	0.47		
	Trichopteran richness	0.46		
	Ephemeropteran richness	0.40		
	Plecopteran richness	0.30		
Taxa	*Ephemera* spp. (E)	0.79	*Corbicula* spp.	−0.55
	Stenomena spp. (E)	0.73	*Ochrotrichia* spp. (T)	−0.28
	Optioservus spp. (C)	0.60	*Hydropsyche* spp. (T)	−0.26
	Non-Tanypodinae Chironomidae (D)	0.53	*Corydalus* spp. (M)	−0.26
	Cheumatopsyche spp. (T)	0.51		
	Isoperla spp. (P)	0.50		
	Antocha spp. (D)	0.47		
	Baetis spp. (E)	0.40		
	Psphenus spp. (C)	0.38		
	Oligocheates	0.36		
	Hemerodromia spp. (D)	0.35		
	Nematoda	0.34		
	Oulimnius spp. (C)	0.33		
	Brachycentrus spp. (T)	0.31		
	Chelifera spp. (D)	0.31		
	Simulium spp. (D)	0.29		

FFGs = functional feeding groups. Letters in parentheses after genus name correspond to insect orders: Ephemeroptera (E), Coleoptera (C), Diptera (D), Trichoptera (T), Plecoptera (P), Megaloptera (M). *Corbicula* spp. is a bivalve introduced from Asia. Variable abbreviations as defined in Table 2.

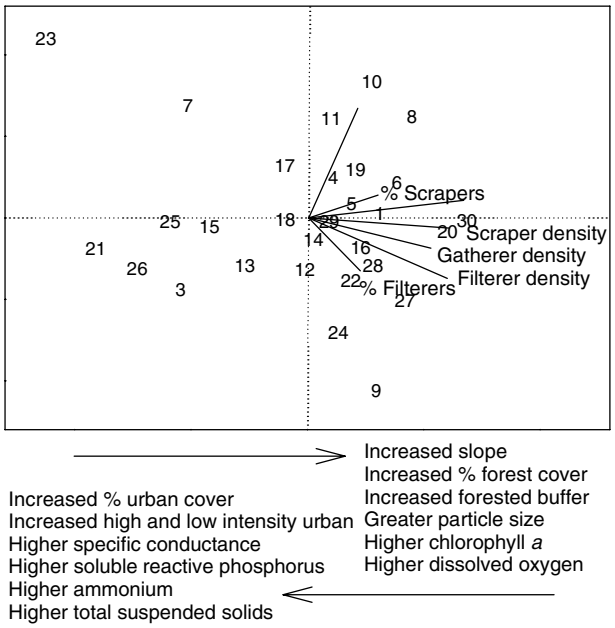

Fig. 4 Non-metric multidimensional scaling (NMS) ordination for all taxa $\log_{10}(x + 1)$ transformed with rare taxa excluded. Densities and proportional densities of all functional feeding groups with regressions of at least $r^2 = 0.25$ ($P < 0.005$) with either axis were plotted as lines on the ordination. Environmental variables with a minimum regression $r^2 = 0.25$ with either axis were indicated on the axes with arrows pointing the direction of increased values.

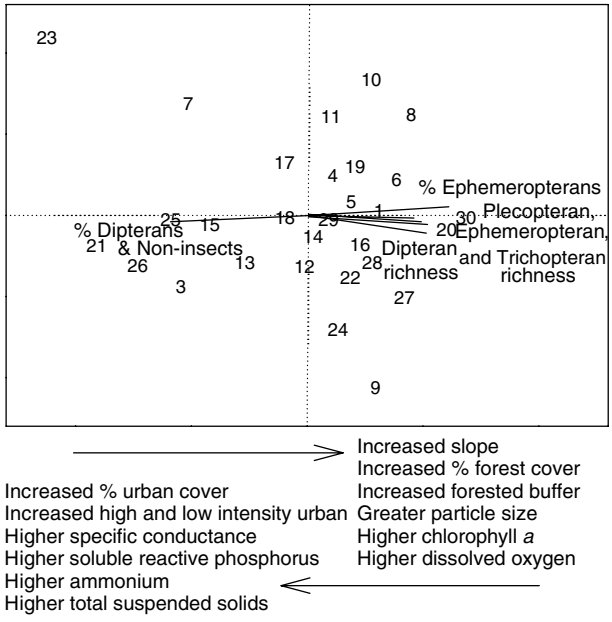

Fig. 5 Non-metric multidimensional scaling (NMS) ordination for all taxa $\log_{10}(x + 1)$ transformed with rare taxa excluded. Macroinvertebrate variables that were included as metrics in the multimetric indices (ICI or B-IBI) that had regressions of at least $r^2 = 0.25$ ($P < 0.005$) with either axis were plotted as lines on the ordination. Environmental variables with a minimum regression $r^2 = 0.25$ with either axis are indicated on the axes with arrows pointing the direction of increased values.

by anthropogenic disturbance in the Piedmont (Waters, 1995; Wood & Armitage, 1997).

Are land cover or reach scale variables better predictors of macroinvertebrate integrity?

Both land cover and reach-scale variables formed significant relationships with biotic indices and macroinvertebrate richness and density across sites. These two scales are not exclusive of each other or independent, as reach-scale geomorphic and chemical variables were related to urban and forested land cover. However, a comparison of the predictive capabilities of the two groups of variables indicates that land cover relationships were weaker than those with environmental variables for predicting variation in biotic indices. These results are consistent with other studies in which reach-scale variables were more important than land cover in explaining macroinvertebrate variability and are therefore potentially more important in building predictive models (Lammert & Allan, 1999). However, because many of our reach-scale variables appear to be related to land cover, the effects of land cover apparently transcended multiple scales to ultimately impact biotic integrity, as seen in other studies (Richards, Johnson & Host, 1996; Roth, Allan & Erickson, 1996; Allan, Erickson & Fay, 1997).

Table 9 Significant multiple linear regression models using stepwise regression ($n = 11$ environmental variables, forward and backward selection, $P = 0.05$) for the NMS ordination axes. Sign (+/–) indicates direction that variable relates to increased biotic integrity. Independent variables as defined in Table 2. Riffle phi was initially included in the model for NMS Axis 1, but was removed at the end by the stepwise selection procedure due to its low *P*-value

	Adjusted R^2	Partial R^2	*P*	Independent variables
NMS Axis 1	0.83	0.61	0.007	+ Slope
		0.15	<0.001	– Specific conductance
		0.09	<0.001	+ *Phi* variability
NMS Axis 2	–			(none)

The ordination analysis was a useful technique for describing macroinvertebrate assemblage changes

across various disturbances and determining the environmental or land cover variables most highly associated with their distribution. As the species composition information was correlated to environmental variables after the ordination distribution was set, this allowed for some corroboration of our multiple regression results. We found that the variables most highly associated with distribution axes, out of all those tested, were slope, SC and *phi* variability. These results are consistent with those obtained via multiple regression and are also consistent with our finding that environmental variables were more highly associated with changes in macroinvertebrate structure than land cover variables.

Which macroinvertebrate variables were most sensitive to environmental change?

Our results indicated that single macroinvertebrate indices (e.g. ICI, total richness, EPT richness, B-IBI) formed good, predictive models. Although the B-IBI and ICI were developed in other areas of the U.S. (Tennessee, B-IBI and Ohio, ICI), these variables formed strong relationships with environmental variables in the Piedmont of Georgia. Rather than developing region-specific indices for studies without the large number of sites required for metric calibration, it may be adequate to use well-defined (i.e. stringently developed and tested), physiographically relevant indices after adjusting scores for regional conditions and removing attributes irrelevant to the study system (Kerans & Karr, 1994). Total richness and EPT richness were also highly correlated with environmental variables. Total richness and EPT richness metrics have the advantage of being easy to calculate and applicable to all systems, adding to their value as biotic measures of degradation. Conversely, macroinvertebrate density was only related to particle size variability and did not appear to be a good indicator of disturbance. The NCBI and Hilsenhoff's FBI responded more to chemistry (turbidity, SC and pH) and land cover (percent urban, percent forest), with a limited ability to detect geomorphic disturbances.

Macroinvertebrate assemblage response to environmental variation was synthesised using ordination analysis. This analysis showed that densities of scrapers, filterers and gatherers were more sensitive to land cover change than other functional feeding groups, and were positively related to increased forest and decreased urban land cover. Shredders were not important in explaining the distribution of macroinvertebrates, presumably because the amount of organic material was not directly related to changes in land cover. Habitat-specialising taxa, such as riffle-dwelling taxa (*Stenomena* spp. and *Optioservus* spp.) and fine sediment-dwelling groups (*Ephemera* spp., Chironomids and *Corbicula* spp.) were best at detecting environmental change compared with habitat generalists, as indicated by their relationships with differences among sites in the ordination.

Did relationships between land cover and biotic indices vary with catchment size?

This study was also designed to test whether catchment size affected relationships between environmental variables and biotic indices. Catchment size was not related to any land cover variables, nor was it important in explaining macroinvertebrate assemblage structure across all sites. When sites were divided into three distinct size categories, our results indicated that relationships between land cover and macroinvertebrate variables were not the same among size categories, although percentage of urban or forested land cover was consistently related to at least two macroinvertebrate variables for each category. The low power of regressions using $n = 10$ versus 30 sites may also limit any definitive conclusions we can make regarding catchment size.

Large catchments were related to the same variables as with all sites because they encompassed the range in SC (21.3–171.6 μs cm^{-1}) and *phi* variability (1.0–2.8 phi) that was included within all sites (21.3–171.6 μs cm^{-1}, and 0.9–3.7 *phi*, respectively). These catchment size effects suggest that large catchments include more of the cumulative effects of disturbance, so detection ability at this size might be better than with smaller catchments. However, all of our catchments may be considered 'small', and comparisons with catchments that are an order of magnitude larger (*c.* 1000 km^2) may exhibit very different patterns. Overall, our results suggest that ecological managers should pick a large and meaningful size range to capture potential variability in land cover and environmental variables.

Conclusions

Our *a priori* predictions concerning effects of land cover change in these catchments were supported by our results and are consistent with other studies indicating negative impacts of urbanisation on macroinvertebrate communities (Benke *et al*., 1981; Jones & Clark, 1987; Kennen, 1999). Current land use coverages showed that as land is deforested and urbanised, increases in nutrients and SC occurred. In addition, sediment-related characteristics such as increased turbidity and decreased *phi* were observed, presumably decreasing habitat and food available to invertebrates. Even across a fairly low percentage of urban land cover (<30%), dramatic changes in invertebrate assemblages were driven by these variables. The FBI showed that above *c.* 15% urban land cover, macroinvertebrate communities shift from being characterized as good or very good, to fair or fairly poor. Likewise, using fish as indicators of water quality led to similar findings indicating that sediment and other non-point pollutant delivery to these streams is impacting fish populations (Walters *et al*., 2001). These results indicate that landscape degradation is seriously impacting these catchments and that management strategies that include reduction of sediment and other urban runoff pollutants are necessary to maintain viable and healthy macroinvertebrate assemblages.

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