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SPATIAL CONSIDERATIONS FOR LINKING WATERSHED LAND COVER TO ECOLOGICAL INDICATORS IN STREAMS

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Abstract. Watershed land cover is widely used as a predictor of stream-ecosystem condition. However, numerous spatial factors can confound the interpretation of correlative analyses between land cover and stream indicators, particularly at broad spatial scales. We used a stream-monitoring data set collected from the Coastal Plain of Maryland, USA to address analytical challenges presented by (1) collinearity of land-cover class percentages, (2) spatial autocorrelation of land cover and stream data, (3) intercorrelations among and spatial autocorrelation within abiotic intermediaries that link land cover to stream biota, and (4) spatial arrangement of land cover within watersheds. We focused on two commonly measured stream indicators, nitrate-nitrogen (NO_3–N) and macroinvertebrate assemblages, to evaluate how different spatial considerations may influence results. Partial correlation analysis of land-cover percentages revealed that simple correlations described relationships that could not be separated from the effects of other land-cover classes or relationships that changed substantially when the influences of other land-cover classes were taken into account. Partial Mantel tests showed that all land-cover percentages were spatially autocorrelated, and this spatial phenomenon accounted for much of the variation in macroinvertebrate assemblages that could naively be attributed to certain classes (e.g., percentage cropland). We extended our use of partial Mantel tests into a path-analytical framework and identified several independent pathways between percentage developed land and in-stream measurements after factoring out spatial autocorrelation and other confounding variables; however, under these conditions, percentage cropland was only linked to nitrate-N. Further analyses revealed that spatial arrangement of land cover, as measured by areal buffers and distance weighting, influenced the amount of developed land, resulting in a threshold change in macroinvertebrate-assemblage composition. Moreover, distance-weighted percentage cropland improved predictions of stream nitrate-N concentrations in small watersheds, but not in medium or large ones. Collectively, this series of analyses clarified the magnitude and critical scales of effects of different land-cover classes on Coastal Plain stream ecosystems and may serve as an analytical framework for other studies. Our results suggest that greater emphasis should be placed on these important spatial considerations; otherwise, we risk obscuring the relationships between watershed land cover and the condition of stream ecosystems.

Key words: agriculture; bioassessment; distance weighting; ecological thresholds; land-cover collinearity; land use; macroinvertebrates; nitrate-nitrogen; nutrients; spatial autocorrelation; urbanization; water quality.

INTRODUCTION

As geographic information system (GIS) technology has emerged and become widely available, investigators have relied more heavily on land use and land cover as broad-scale predictors of aquatic conditions (see Hunsaker and Levine 1995, Allan and Johnson 1997). According to O'Neill et al. (1997), linkages between watershed land cover and the biological integrity of stream ecosystems have exciting potential as an inexpensive alternative to ground-based monitoring, particularly at broad geographic scales. Similarly, biological assessments, such as those using an index of biological integrity, have become standard in many regional-scale stream-monitoring programs (e.g., Barbour et al. 1999). It seems natural to extend O'Neill et al.'s ideas to relate watershed land cover to ecological indicators in streams, and many investigators have employed this approach (e.g., Roth et al. 1996, Lammert and Allan 1998, Strayer et al. 2003).

Despite the great potential of land-cover analyses and indicator approaches, they also present particular analytical challenges. First, land cover is most com-

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monly summarized using class percentages, such as percentage cropland in a watershed. Such class percentages are not independent predictors because increasing the percentage of one class necessarily results in a decrease in percentage of one or more other classes (Van Sickle 2003). This lack of independence can confound correlative analyses and yield potentially misleading results (Griffith et al. 2002), yet few watershed investigators have explicitly or implicitly addressed this issue (Osborne and Wiley 1988, Herlihy et al. 1998).

Both among and within regions, land-cover classes also tend to be patchy and spatially autocorrelated. Spatial autocorrelation may be particularly problematic in broad-scale watershed studies because the locations of these land-cover patches often correspond to an underlying pattern in the physical template of the landscape. Consequently, the apparent relationships between land-cover variables and ecological indicators in streams could just as easily be explained by natural, spatial factors that necessarily covary with land cover, such as local or subregional distributional patterns of stream fauna that are dependent upon physical attributes of streams and their watersheds (e.g., Richards et al. 1997). Thus, many apparent linkages between land cover and stream indicators may be spatially confounded (Legendre 1993). This phenomenon is occasionally acknowledged, but rarely addressed quantitatively.

Watershed land cover is also often used as a direct predictor of biological indicators in streams; however it is only indirectly related to stream biota via a dizzying array of near-stream and in-stream abiotic factors. Distinguishing the independent effects of abiotic intermediaries between land cover and stream biota, each of which suffers from its own collinearity (e.g., Norton 2000, Yuan and Norton 2003) and spatial-autocorrelation (e.g., Lichstein et al. 2002) issues, is both challenging and critical for understanding the mechanisms by which land cover interacts with stream ecosystems (Strayer et al. 2003). Moreover, linking land cover to stream condition via specific stressors is critical for diagnosing causes of ecological impairment, currently a major focus of state and federal agencies (e.g., EPA 2000*b*).

Within watersheds, the spatial arrangement of land cover may play an important role in modulating land-cover effects on stream ecosystems (O'Neill et al. 1997). Most investigations of land-cover arrangement utilize land-cover class percentages within a certain distance or series of distances from a sampling location or a stream channel (e.g., Omernik et al. 1981, Schuft et al. 1999, Sponseller et al. 2001). Such fixed-area buffers essentially reduce land-cover arrangement to a step function—characterizing land cover inside the designated area while ignoring land cover outside. Few investigators have employed distance weighting of watershed land cover, an approach whereby patches of a particular land-cover class receive greater emphasis when they occur closer to a feature of interest, such as a sampling station or stream channel. Distance weights have shown some promise for representing the effects of spatial arrangement in watershed studies (e.g., Hunsaker and Levine 1995, Comeleo et al. 1996, Soranno et al. 1996), yet it remains unclear how best to apply this technique.

Watershed size is also an important consideration in land-cover analysis because it not only determines the potential range of land-cover proximity, but also because it influences the range of potential land-cover percentages as measured by the grain size of a particular land-cover map (Turner et al. 1989). Strayer et al. (2003) showed that the effect of land cover on stream ecosystems was dependent upon watershed size, a phenomenon suggested by these authors to be due to differences in the relative importance of spatial arrangement across watershed size classes. However, no previous study has examined the interaction between spatial arrangement of land cover and watershed size. Scale dependency of spatial-arrangement effects has potentially significant implications for land-use planning and watershed management.

In this paper, we use the context of an aquatic-indicator study in the mid-Atlantic region of the United States to explore and address analytical challenges presented by (1) collinearity of land-cover class percentages, (2) spatial autocorrelation of land cover and stream-indicator data, (3) intercorrelations and spatial autocorrelation of abiotic intermediaries between land cover and stream biota, and (4) spatial arrangement of land-cover classes within watersheds. To address these spatial considerations, we employed watershed land cover and stream indicator data from the Coastal Plain physiographic province of Maryland, USA, a large area with distinct spatial patterns of multiple land-cover types. We focused on two commonly used measurement endpoints, nitrate-nitrogen (NO_3-N) and macroinvertebrate assemblages, to evaluate how different ecological indicators can influence our interpretation of land-cover effects on stream ecosystems. The broad objective of this paper is to illustrate several important spatial considerations that have potential to confound the interpretation of similar studies throughout the world.

STUDY AREA

The Coastal Plain of Maryland (USA) encompasses approximately 12 900 km² of the watershed of Chesapeake Bay, one of the largest, most productive estuaries in the world and an ecosystem of high conservation value that faces difficult restoration challenges (Orth and Moore 1983, Officer et al. 1984, Boesch et al. 2001). We focused our analysis on the Coastal Plain because of the potentially important linkage between the condition of coastal streams and the Chesapeake Bay ecosystem. Approximately 9% of the land in the Coastal Plain is residential or commercial develop-

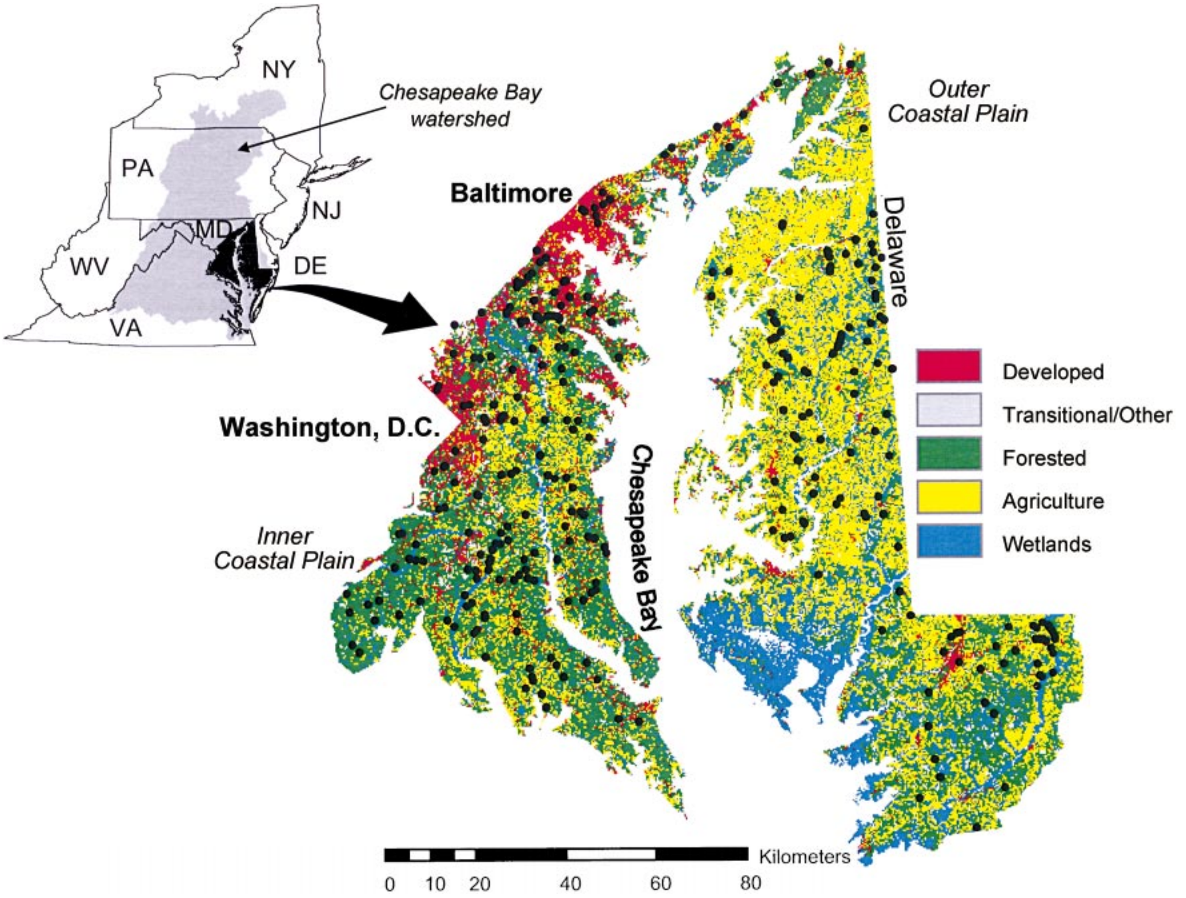

FIG. 1. The study area. (Left) Location of the Coastal Plain physiographic province, Maryland (MD), in relation to the mid-Atlantic region, USA. (Right) Land cover and stream sampling locations ($N = 295$ sites, solid circles) surveyed by the Maryland Biological Stream Survey (MBSS).

ment, as resolved in the National Land Cover Database (EPA 2000*a*). The Baltimore and Washington, D.C., metropolitan areas, located on the western shore of Chesapeake Bay, contribute most of this developed land (Fig. 1). Much of the remaining non-wetland forest cover (39% of total area) is located in the southern half of the Coastal Plain, particularly on the western shore of Chesapeake Bay. The eastern shore is predominantly agricultural. Agricultural land-cover classes are almost exclusively represented by cropland and pasture, each representing a significant percentage (20% and 16%, respectively) of total land cover in the Coastal Plain. Wetlands, including forested and emergent classes, comprise roughly 14% of the total land area, but much of this is restricted to tidal zones in the southeastern part of the province.

The topography of the Coastal Plain varies from the rolling hills of the inner Coastal Plain on the western shore to the extremely flat terrain of the southern portion in the outer Coastal Plain on the eastern shore. Most streams in the province are low gradient, sinuous, and characterized by silt, sand, or gravel substrates (Janicki et al. 1995). Water chemistry is variable, but relatively undisturbed streams are typically characterized by relatively low acid-neutralizing capacity, high dissolved organic carbon, neutral-to-slightly acidic pH, and relatively low conductivity (Janicki et al. 1995).

METHODS

Stream data

We used stream data collected by the Maryland Biological Stream Survey (MBSS; Klauda et al. 1998). The MBSS is a stream-monitoring program based on a probabilistic sampling design stratified by major basins and stream order (1st-to-3rd order on a 1:250 000 stream map). Sampling locations were randomly assigned to non-overlapping, 75-m stream segments across the state of Maryland (USA). Within the randomly selected segments, the survey included measures of stream physical characteristics, water quality, riparian and in-stream habitat, hydrology, and macroinvertebrate assemblage composition. A total of 330 sites were sampled in the Coastal Plain during 1995–1997. For our analysis, we selected 295 of these sites (Fig. 1) that had complete physical, chemical, and biological data, including nitrate-N and genus-level macroinvertebrate assemblage composition. Greater details on the MBSS data set, including information on collection methods, sample analysis, and quality assurance/quality control, are provided in Mercurio et al. (1999). Summaries of variables used in our analysis are provided Appendices A and B.

Although the state of Maryland has developed an index of biological integrity (IBI), a widely used indicator of stream ecosystem health based on attributes of macroinvertebrate assemblages (Stribling et al. 1998), we chose to examine the raw composition data as a multivariate response variable. Preliminary analysis indicated variation in the raw assemblage measurements that was potentially related to land cover might have been lost when the data were reduced to univariate IBI scores. Instead, we converted these data into a distance matrix using Bray-Curtis dissimilarity (BCD; Bray and Curtis 1957). BCD is a robust, ecologically interpretable distance metric that expresses the percentage taxonomic dissimilarity between pairs of samples (Faith et al. 1987, Legendre and Anderson 1999). Increasing percentage dissimilarity (BCD) between pairs of macroinvertebrate samples indicates an increasing loss or replacement of taxa. Thus, BCD served as a relative metric of taxonomic change in response to land cover and other predictors. Prior to calculation of BCD, macroinvertebrate abundance data (323 taxa) were $\log_{10}(x + 1)$ transformed to add greater weight to taxa with low abundance values. In subsequent analyses, BCD was analyzed either as a raw distance matrix or as univariate axis scores obtained from a distance-based ordination technique (nonmetric multidimensional scaling; nMDS) (see *Methods: Data analyses*, below).

Geographic analyses

For each stream sampling point we analyzed land cover in the upstream watershed. Watershed boundaries were delineated from 1:24 000 digital elevation models (DEMs) expressed as a 30-m raster (USGS National Elevation Data Set, *available online*)[4] using a modified version of the method described by Jenson and Domingue (1988). DEMs were modified by lowering the elevation values of mapped stream channels (1:24 000 digital line graphs, DLG; USGS) to force flow-direction maps to match existing stream lines (M. E. Baker, D. E. Weller, and T. E. Jordan, *unpublished manuscript*). Within watersheds, land-cover percentages were summarized from the National Land Cover Database (NLCD), a raster data set developed from 30-m Landsat thematic-mapper images taken during 1992 (EPA 2000*a*). Watershed boundaries were overlaid on the NLCD in a GIS, and land-cover class percentages were calculated for each area.

Percentage developed land in a watershed was defined as the sum of NLCD low- and high-intensity residential and commercial land-cover classes. Pasture was not considered to be a significant source area of nitrate-N (Jordan et al. 1997*a*, 2003, Weller et al. 2003), and preliminary analysis suggested that it was very weakly related to macroinvertebrate assemblages, so percentage cropland was the lone agricultural land-cover class used in subsequent analyses. Percentage forested land was the sum of the NLCD deciduous, coniferous, mixed, and forested wetland classes, whereas percentage wetland was the sum of emergent and forested wetlands.

To characterize land-cover arrangement within watersheds, we used both neighborhood buffers and inverse-distance weights (IDWs). We calculated land-cover percentages within 50-m, 100-m, and 250-m buffers of mapped stream channels, as well as within 250 m, 500 m, and 1000 m of sampling stations. All buffer analyses were further constrained by watershed boundaries. We calculated IDWs by weighting land-cover percentages by their proximity to stream channels or sampling stations (Fig. 2). We used linear (Euclidean) measures (Fig. 2) as well as estimates of flow length to represent distance. Flow-length distance was estimated by tracing the path of steepest descent between sets of points across a DEM using the eight-directional algorithm described by O'Callaghan and Mark (1984) and Jenson and Domingue (1988) and implemented in an ARC/INFO GIS system (ESRI, Redlands, California, USA). Counts of distances were aggregated into unequal-interval distance classes. For distance-to-station IDWs, classes were 0–100 m, 101–250 m, 251–500 m, 501–1000 m, 1001–2000 m, 2001–5000 m, 5001–10 000 m, and >10 000 m. Distance-to-stream IDWs included a 0–30 m class to emphasize land-cover cells immediately adjacent to stream channels and a >1000-m class that replaced 1001–2000, 2001–5000, 5001–10 000, and >10 000 distance-to-station classes because most distances to streams were <1000 m. The ranges were wider for greater distances because inverse-distance functions are less sensitive to distance at large distances than at small ones. The highest distance in each range was used to represent all cells within the range in the IDW calculation. The equation for calculating inverse distance-weighted percentage land cover in a watershed is as follows:

$$\text{IDW \% land cover in watershed} = 100 \times \frac{\sum_{i=1}^{C} n_{\mathrm{X}} W_C}{\sum_{i=1}^{C} n_{\mathrm{T}} W_C} \qquad (1)$$

where C is the number of distance classes, n_{X} is the number of cells of the land-cover class of interest in distance class i, W_C is the inverse-distance weight for distance class i where d^{-1} = the maximum distance between a cell in distance class i and the station or

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[4] ⟨www.usgs.gov⟩

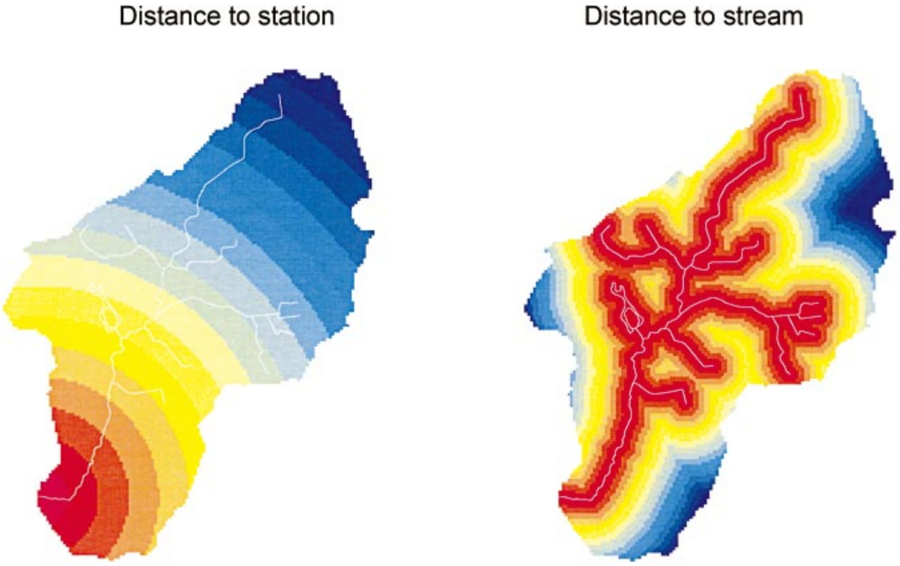

FIG. 2. An example of two Euclidean-distance descriptions used in the analysis of spatial arrangement of land cover. Colors represent a gradient of distance values from near (red) to far (blue) relative to the sampling station or stream.

stream (e.g., 101–250 m distance class was assigned a distance of 250 m), and n_T is the total number of land-cover cells in distance class *i.* With all IDWs we assumed that the effect of a particular land-cover patch would increase with proximity to sampling stations or stream channels.

Data analyses

Land-cover class percentages are not independent.—To evaluate the effect of collinearity among land-cover classes, we explored the relationships between watershed land cover and stream nitrate-N concentration. We focused on the effects of developed, agricultural, forested, and wetland land-cover classes. We expected agricultural land, particularly cropland, to be the primary source area for nitrate-N in streams (Jordan et al. 1997*a, b*) and developed land to be a secondary source (Liu et al. 2000, Jordan et al. 2003, Weller et al. 2003). Increasing amounts of forest and wetland cover were expected to reduce nitrate-N concentrations through removal of source area, and through the processes of uptake, transformation, and dilution (e.g., Peterjohn and Correll 1984, Weller et al. 1998, Jones et al. 2001). We calculated a Pearson product-moment correlation matrix of the land-cover variables to assess the independence of land-cover classes in our data set. We then employed partial correlation analyses to evaluate the independent effect of cropland and developed land on nitrate-N concentrations. Prior to analysis, nitrate-N data were $\log_{10}$–transformed to reduce heteroscedasticity (Sokal and Rohlf 1995).

Land cover is spatially contagious and covaries with the physical template.—We assessed the effect of spatial autocorrelation on apparent linkages between land cover and stream nitrate-N and macroinvertebrate assemblages by using the partial Mantel test, a multivariate extension of partial correlation that uses distance or difference matrices as variables (Mantel 1967, Smouse et al. 1986). Partial Mantel tests estimate the strength of the correlation (Mantel *r*) between two distance matrices after the effect of one or more matrices has been eliminated. Distance matrices can be univariate (e.g., an individual land-cover variable) or multivariate (e.g., geographical *x*-, *y*-coordinates of stream sampling locations). Mantel tests allow the user to remove the spatial component of variation between a predictor and response variable to yield a spatially independent estimate of the relationship. Mantel *r* coefficients are scaled from −1 to 1, although negative coefficients are rare and usually indicate a noisy, heteroscedastic relationship (Dutilleul et al. 2000). Mantel *r* coefficients are typically much smaller than conventional correlation coefficients: coefficients >0.1 are often highly significant statistically (and possibly ecologically), and coefficients >0.5 indicate a very strong relationship (Legendre and Fortin 1989, Dutilleul et al. 2000). Significance of Mantel *r* coefficients is evaluated using random permutations of observations in the distance matrices (e.g., 10 000 permutations; Manly 1997). Greater details on the theory and application of Mantel tests are presented in Legendre and Legendre (1998) and Urban et al. (2002). Mantel tests were performed using the ECODIST library of functions (*unpublished*; D. L. Urban and S. Goslee, Duke University, Durham, North Carolina, USA) in S-Plus 2000 (Mathsoft, Seattle, Washington, USA).

Land cover (percentage) and geographic coordinates (Universal Trans-Mercator *x*- *y*-coordinates, in meters) of the sampling locations were converted to individual distance matrices using Euclidean distance between sample pairs for comparison with the BCD (Bray-Curtis dissimilarity) matrix of macroinvertebrate compositional dissimilarity. Given results from previous studies in other areas (e.g., Roth et al. 1996, Harding et al. 1998, Weigel 2003) we expected both percentage developed land and percentage cropland to negatively affect stream biological condition so that each class would account for unique variation in macroinvertebrate assemblage composition, even after removing the effect of competing land-cover classes and spatial autocorrelation (expressed as the separation distances among sampling locations in the geographical distance matrix; hereafter, ''Space''). We did not include forest cover as a partial predictor because it was not mutually exclusive of the wetland variable. Also, accounting for forest cover in the partial Mantel tests would eliminate much of the variation potentially explained by developed or cropland cover because both of these anthropogenic land-cover types replace forest cover on the landscape. In other words, a significant effect of percentage developed land after accounting for percentage cropland and percentage wetland would also imply a significant percentage forest effect.

Land cover is indirectly related to stream biota via abiotic pathways.—To relate land cover to stream biota via abiotic pathways, we extended the partial Mantel test into a path-analytical framework (e.g., Leduc et al. 1992, King et al. 2004). Spatial, land-cover, and abiotic variables are arranged in a hierarchical manner so that variation explained by confounding variables is factored out, and the remaining variation in the response variable can be evaluated using the predictor variable of interest. This approach yields a pure-partial correlation—variation that cannot be explained by all of the other confounding variables in the analysis. To visualize the results, significant pure-partial correlations are synthesized in a diagram that depicts pathways of significant relationships among variables (Fig. 5). Although this approach is conservative, a significant pure-partial relationship can afford compelling evidence for a potential causal pathway between land cover and biota (King et al. 2004).

We selected a suite of physical and chemical variables measured by the MBSS as reach-scale abiotic predictors of macroinvertebrate assemblage composition. We evaluated the simple relationship between each variable and macroinvertebrate assemblage composition using nonmetric multidimensional scaling (nMDS) ordination (Minchin 1987) and simple Mantel tests. The ordination helped us visualize the direction and magnitude of abiotic correlations with composition, whereas the Mantel tests helped confirm which variables were most strongly related to composition using the raw distance matrices. We selected variables that were most strongly correlated with macroinvertebrate composition and removed redundant metrics of a particular stream attribute (e.g., stream habitat) that were necessarily collinear, unless they appeared to account for a unique component of variation. This analysis helped us identify the following groups of variables to be used as individual distance matrices in the partial Mantel path model: Watershed area (in hectares); Riparian (shading, remoteness, aesthetic rating, minimum width of riparian forest buffer); Gradient (slope, average velocity, velocity–depth diversity); Size (maximum depth, average width, average thalweg depth); Woody (number of pieces of woody debris and number of root wads); SubQual. (in-stream habitat, epifaunal substrate, pool quality, riffle quality, embeddedness); NO_3-N (nitrate-nitrogen), DOC (dissolved organic carbon); Cond. (specific conductivity); Acidity (pH and acid-neutralizing capacity); DO (dissolved oxygen); and Temp. (temperature) (see Appendix A). These groups of variables were converted into individual distance matrices using Euclidean distance. Individual variables in multi-variable distance matrices were first standardized to z-scores (Legendre and Legendre 1998) so that each variable was weighted equally in the matrix. Prior to standardization and conversion to distance matrices, some individual variables were transformed to improve linearity and reduce heteroscedasticity (arcsine square root for percentage variables; $\log_{10}$ for continuous variables; see Appendix A).

We arranged distance matrices based on our interpretation of their hierarchical structure and causal order. This resulted in five levels of organization: spatial (Space, Watershed area); land cover (Developed, Wetland, Cropland); indirect site-level abiotic variables (Riparian, Gradient, Size); direct site-level abiotic variables (Temp., DO, Acidity, Cond., DOC, NO_3-N, SubQual., Woody); and the biological response variable (Macroinvertebrate assemblage composition). We used this framework to specify appropriate covariates for each partial Mantel test. For all tests, we accounted for Space and Watershed area, as these were spatial factors that could confound apparent relationships at any level in the analysis. Furthermore, we accounted for variation explained by other matrices in the same level of the hierarchy and examined the residual effect of each individual matrix on each matrix in the level immediately below it. These pathways represented pure-partial direct effects. However, we also tested for *residual* effects between indirect predictors and macroinvertebrate composition by factoring out variation explained by matrices on the same level and all levels *below* them (e.g., a significant effect of developed land cover on macroinvertebrate composition after accounting for abiotic intermediaries). Greater details on methods and rationale for partial Mantel path models are presented in King et al. (2004).

Spatial arrangement may be an important modulator of watershed land-cover effects on stream ecosystems.—We explored the effect of within-watershed arrangement of land cover by relating macroinvertebrate assemblages to three ways of representing land cover: (1) percentage developed land in the watershed, (2)

TABLE 1. Pearson product-moment correlation matrix of watershed percentage land-cover summaries from the Coastal Plain physiographic province, Maryland, USA.

	Land cover			
Land cover	Developed	Forested	Cropland	Wetland
Developed	· · ·			
Forested	−0.40****	· · ·		
Cropland	−0.51****	−0.34****	· · ·	
Wetland	−0.43****	0.11^{NS}	0.49****	· · ·

Note: Watersheds correspond to the 295 stream sites sampled by the Maryland Biological Stream Survey (MBSS) in 1995–1997 (see Fig. 1).

**** $P \leq 0.0001$; NS, not significant ($P > 0.05$).

percentage developed land within a 250-m radius buffer around the sampling station, and (3) percentage developed land weighted by its inverse distance to the sampling station. We focused on developed land because our other analyses showed that it was the primary land-cover class linked to changes in macroinvertebrate assemblage composition (see *Results*, below). Preliminary analysis using station and stream buffers of various sizes also indicated that macroinvertebrate composition was more sensitive to development near the station than along the entire stream corridor in the watershed, so we retained the 250-m station buffer for further analysis. We used linear rather than flow-length distance because we did not expect the effect of development to be transmitted solely by hydrologic processes (see *Results,* below).

As a response variable, we used axis scores that corresponded most closely to increasing development in watersheds from an nMDS ordination of genus-level assemblage composition (BCD). The nMDS produced individual site and taxa scores representing a univariate index of taxonomic dissimilarity among sites—increasingly positive nMDS scores represented relatively diverse macroinvertebrate assemblages composed of many pollution-sensitive taxa, while increasingly negative scores corresponded to assemblages dominated by taxa typically associated with impaired streams (Appendix B). These nMDS scores could be evaluated graphically, which improved our ability to contrast sensitivity of responses among the three developed-land metrics. Scatterplots of nMDS scores and developed-land metrics suggested a potential threshold response, so we evaluated the effect of spatial arrangement by contrasting the amount of development that may have resulted in a threshold, as quantified using nonparametric change-point analysis (nCPA; Qian et al. 2003, King and Richardson 2003). Change-point analysis estimates the numerical value of a predictor, x, resulting in a threshold in the response variable, y, represented as the cumulative probability of a threshold. We hypothesized that if developed land near the sampling station had a greater influence on stream biota, then threshold levels of percentage developed land would be lower using the 250-m buffer and the IDW than threshold levels of unweighted developed land in the watershed. Ordination and changepoint analyses were conducted in PC-ORD 4.9 (MjM Software, Gleneden Beach, Oregon, USA) and in S-Plus 2000 using the custom function nonpar.chngp (Qian et al. 2003), respectively.

After previous analyses (see *Results*, below) showed that stream nitrate-N concentrations were strongly linked to percentage cropland in watersheds, we explored this relationship further by contrasting results from three watershed size classes determined by the 33rd and 66th size percentiles in our data set. We then compared these regressions on simple watershed percentages with those using percentage cropland weighted by either the linear or flow-length inverse distance to the sampling station or streams. Analyses reported in *Results* are limited to linear station IDWs because linear and flow-length distance-to-station IDWs captured virtually the same amount of variation in nitrate-N concentrations, but both of these IDWs captured more variation in nitrate-N than IDWs based on distance to stream. Prior to analysis, we $\log_{10}$–transformed nitrate-N concentrations and used partial correlation analyses to remove variation attributable to percentage developed land in watersheds.

RESULTS

Land-cover class percentages are not independent

A Pearson product-moment correlation matrix of the four land-cover variables ($N = 295$ stream sites sampled; Table 1) revealed a negative, triangular relationship among developed, cropland, and forested land covers (i.e., all three dominant classes were negatively correlated with each other). Wetland cover tended to positively covary with cropland and negatively covary with development. However, wetlands were only weakly correlated with forest cover despite sharing the forested wetland class.

Percentage cropland was highly positively correlated with nitrate-N ($r = 0.67$, $P \leq 0.0001$; Fig. 3A) while forest was negatively correlated with nitrate-N ($r = -0.57$, $P \leq 0.0001$). Percentage wetland was positively correlated to nitrate-N ($r = 0.21$, $P = 0.0002$), whereas increasing percentage developed land appeared to reduce nitrate-N ($r = -0.19$, $P = 0.0011$; Fig. 3). However, after the use of partial correlation analysis to factor out the effect of cropland, correlations between both percentage developed land and percentage wetland and nitrate-N residuals were altered. The relatively high concentrations of nitrate-N observed at low percentages of development (Fig. 3B) were markedly reduced when expressed as residuals after the effect of cropland had been removed (Fig. 3C). Consequently, the direction of the correlation reversed, yielding a significant, positive relationship (partial $r = 0.21$, $P = 0.0003$; Fig. 3C). Similarly, factoring out the effect of cropland identified a weak but negative correlation between percentage wetland and nitrate-N (partial $r = -0.15$, $P = 0.021$).

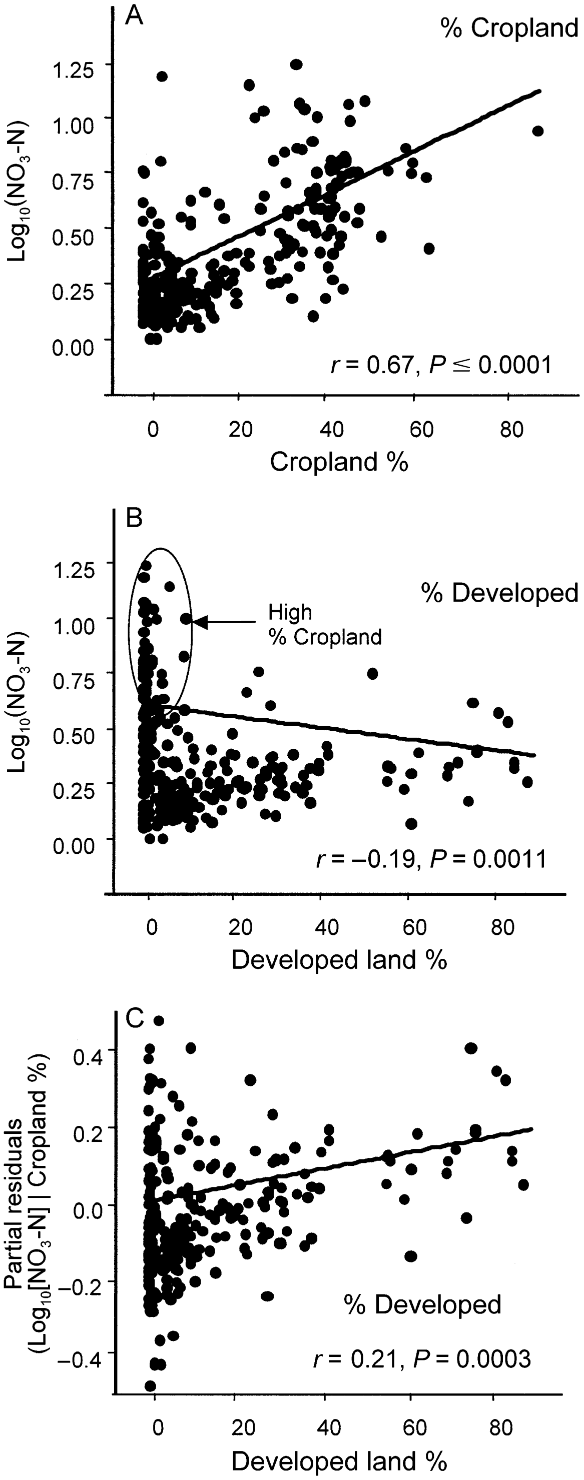

FIG. 3. Simple correlation of (A) percentage cropland and (B) percentage developed land cover in watersheds with stream nitrate-N concentrations in Coastal Plain streams, and (C) the partial correlation between developed land cover and nitrate-N after the effect of cropland was removed.

Land cover is spatially contagious and covaries with the physical template

We estimated the degree of spatial contagion, or autocorrelation, in each land-cover class using the linear distance between sampling sites, or Space, as a predictor. Results from simple Mantel tests on land-cover percentages indicated that they were spatially autocorrelated among watersheds (Mantel $r = 0.07\text{–}0.48$, $P \leq 0.0001$). The spatial distributions of wetland and cropland were particularly contagious, consistent with large amounts of these land covers observed in the outer Coastal Plain (Fig. 1).

Percentage developed, percentage cropland, and percentage wetland each were significant predictors of macroinvertebrate assemblage composition (simple Mantel test, Mantel $r = 0.193$ (developed), 0.107 (cropland), 0.114 (wetland); all $P \leq 0.0001$). All three land-cover classes remained significant predictors of composition after the effect of each competing land cover was removed; however, the magnitude of the correlations dropped for cropland (partial Mantel $r = 0.053$, $P = 0.0002$) and wetland (partial Mantel $r = 0.054$, $P \leq 0.0001$). In contrast, percentage developed land remained a relatively strong correlate of composition after removing the combined effects of percentage cropland and percentage wetland (partial Mantel $r = 0.172$, $P \leq 0.0001$). Factoring out the spatial component of variation in addition to the competing land-cover percentages reduced the magnitude of the wetland effect to a level of marginal statistical significance (partial Mantel $r = 0.039$, $P = 0.013$) and completely eliminated the correlation between cropland and composition (partial Mantel $r = 0.002$, $P = 0.902$; Fig. 4). In fact, Space alone explained most of the variation in composition that could be attributed to cropland (partial Mantel $r = 0.021$, $P = 0.100$; Fig. 4). Developed land was still a highly significant predictor of composition after factoring out the effects of Space, cropland, and wetland (partial Mantel $r = 0.173$, $P \leq 0.0001$).

Land cover is indirectly related to stream biota via abiotic pathways

The pathways in Fig. 5 represent significant partial correlations between distance matrices, including path-

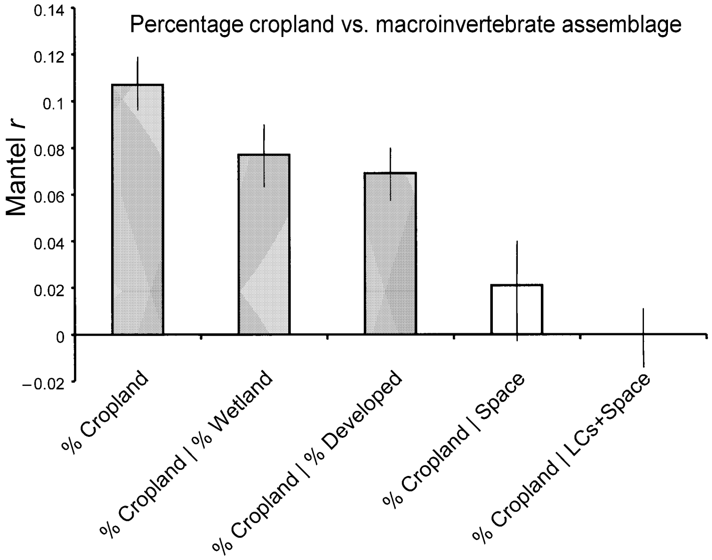

FIG. 4. Results from simple and partial Mantel tests of the percentage cropland as a predictor of macroinvertebrate assemblage composition in Coastal Plain streams. On the *x*-axis, partial tests are indicated by "|", with the covariate(s) listed after the symbol. LCs = land-cover classes (developed + wetland); Space = linear distance between sampling sites. Shaded columns are significantly different from zero ($P \leq 0.05$). Error bars indicate 95% confidence intervals for Mantel *r* coefficients.

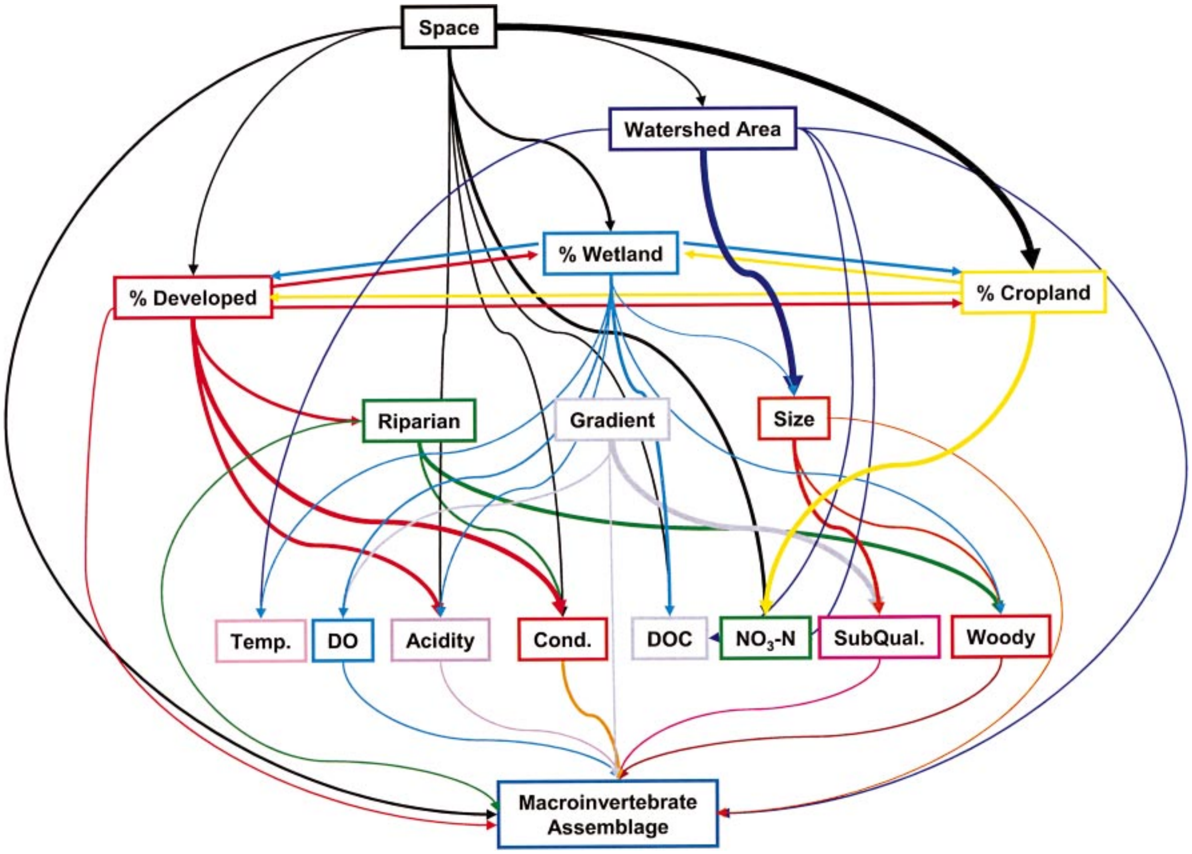

FIG. 5. Partial Mantel path diagram depicting significant linkages among spatial, land-cover, abiotic, and biotic indicators from Coastal Plain streams. The thickness of arrows is proportional to the magnitude of the partial Mantel *r* coefficient. See *Methods: Land cover is indirectly related...* for details on the suite of physical and chemical variables measured.

ways representing residual effects between matrices separated by more than one hierarchical level. For brevity, here we describe only the significant pathways associated with percentage developed land and percentage cropland (see Fig. 5 for all significant partial Mantel results).

Percentage developed land was directly and indirectly linked to several abiotic matrices. Developed land had direct linkages with Riparian (independent of competing land-cover classes and spatial matrices; partial Mantel $r = 0.278$, $P \leq 0.0001$) and Acidity and Cond. (specific conductivity) (independent of intermediate effects of Riparian, Gradient, and Size, plus competing land-cover classes and spatial matrices, partial Mantel $r = 0.081$ and 0.346, respectively; $P \leq 0.0001$). Developed land also had indirect linkages with Cond. (partially due to the direct effect of development on Riparian; partial Mantel $r = 0.167$, $P \leq 0.0001$), and Woody (partially due to the direct effect of development on Riparian, partial Mantel $r = 0.263$, $P \leq 0.0001$). Percentage cropland, on the other hand, was not linked to any abiotic variable, with the exception of nitrate-N.

Acidity, Cond., Woody, and Riparian all had significant direct effects on macroinvertebrate assemblages, and these effects were mostly attributable to percentage developed land (Fig. 5). Percentage developed land also explained variation in macroinvertebrate assemblage composition that could not be explained by the combined effect of all competing land-cover classes, abiotic intermediaries, and spatial factors (partial Mantel $r = 0.101$, $P \leq 0.0001$). There was no effect of percentage cropland on macroinvertebrate assemblage composition.

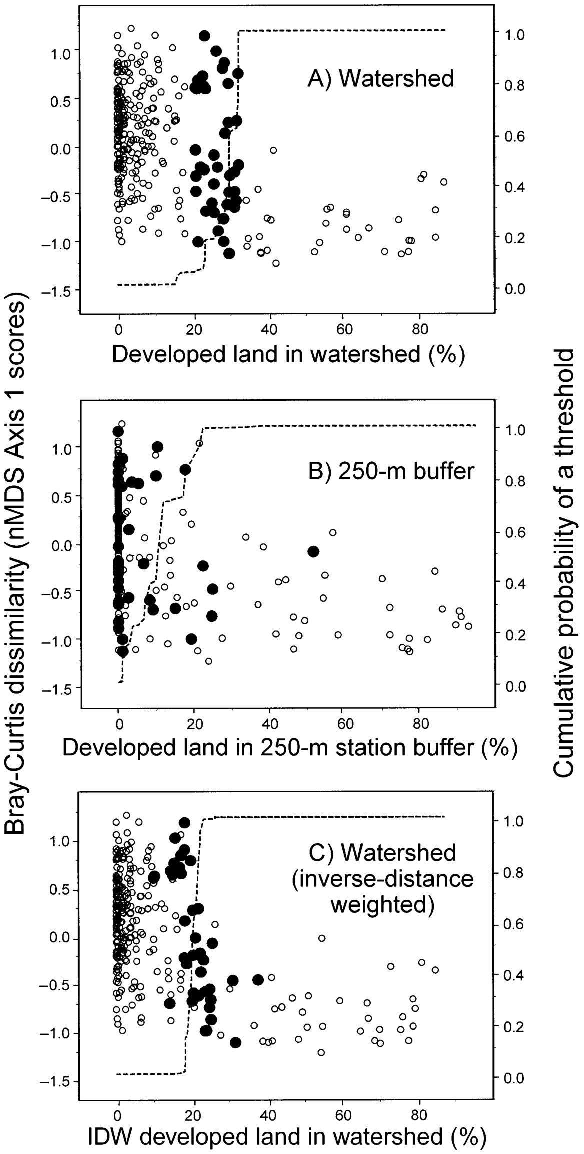

FIG. 6. Scatterplots of the threshold effect of developed land on macroinvertebrate assemblage composition (Bray-Curtis dissimilarity expressed as nonmetric multidimensional scaling [nMDS] Axis 1 scores). (A) Percentage developed land in the watershed. (B) Percentage developed land within a 250-m radius buffer of the sampling station. (C) Percentage developed land in the watershed weighted by its inverse distance (IDW; in meters) to the sampling station. The dotted lines indicate the cumulative probability of an ecological threshold in response to increasing percentage developed land. Samples within the watershed-scale threshold zone of 21–32% developed land in panel (A) are highlighted in black in panels (A)–(C).

Spatial arrangement may be an important modulator of watershed land-cover effects on stream ecosystems

Change-point analysis indicated that as little as 21% developed land in watersheds may result in a threshold in the biotic composition of Coastal Plain streams (Fig. 6A). Macroinvertebrate assemblage composition changed markedly between 21 and 32% developed land, and there was nearly a 100% probability that sharp changes in taxonomic composition would occur beyond 32% developed land. In contrast, there was a 5% probability that as little as 1% developed land and nearly a 100% probability that $>22\%$ developed land in a 250-m buffer around each sampling station would alter stream macroinvertebrate assemblages (Fig. 6B). Here, many of the high-scoring samples that corresponded to watersheds with 21–32% development (the apparent watershed-scale threshold zone) actually had relatively little developed land in the 250-m buffer (Fig. 6B). However, many low-scoring samples within the watershed threshold zone also had little-to-no developed land in the buffer. Contrasting watershed- and buffer-scale results (Fig. 6A, B) with those obtained using inverse distance weighted (IDW) percentage developed land (Fig. 6C) suggested that development near the station had a greater effect on macroinvertebrate assemblages, but developed land elsewhere in the watershed also influenced stream condition. The apparent threshold zone using IDW percentage developed land was 18–23%, much lower than the threshold zone cor-

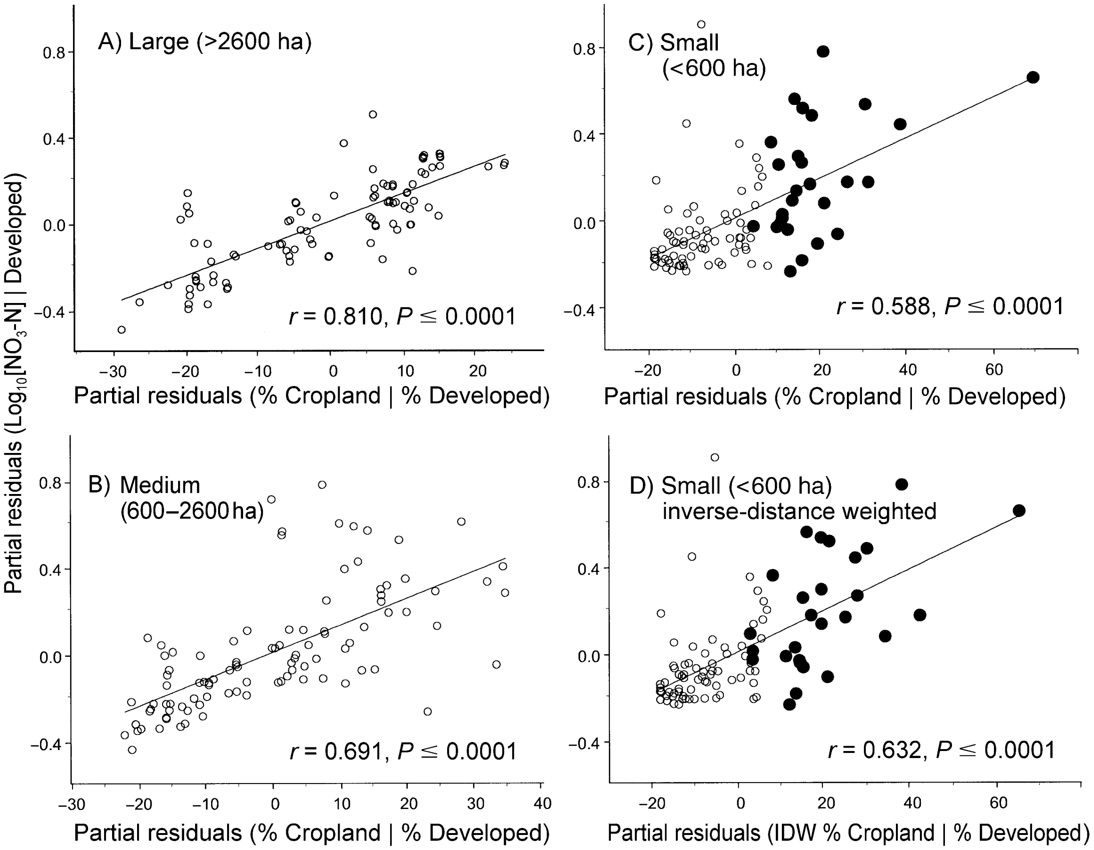

FIG. 7. Regressions of stream nitrate-N concentrations on percentage cropland corrected for percentage developed land in the watershed in (A) large, (B) medium, and (C) small watersheds; and (D) percentage cropland in small watersheds weighted by its inverse distance (IDW) to station. Small watersheds with >25% cropland (unweighted) are indicated by solid symbols. See Fig. 4 legend for explanation of partial-residuals labels.

responding to unweighted percentage developed land in the watershed. Moreover, high-scoring macroinvertebrate samples within the watershed-scale threshold zone (Fig. 6A) shifted to the left of the distance-weighted development threshold, but, contrary to the 250-m buffer results (Fig. 6B), most of the low-scoring macroinvertebrate samples exceeded the threshold (Fig. 6C).

Overall, percentage cropland was a strong predictor of stream nitrate-N in the Coastal Plain, and this relationship was slightly stronger after accounting for percentage development (r = 0.713, P < 0.0001, N = 295 sites). Regression slopes were similar among watershed size classes (0.0092, 0.0124, and 0.0126 for small, medium, and large classes, respectively) but the variance explained by these models varied markedly (Fig. 7). Large watersheds (>2600 ha) exhibited the strongest correlation between percentage cropland and stream nitrate-N (Fig. 7A), but there was a trend toward weaker relationships in medium (600–2600 ha; Fig. 7B) and small (<600 ha; Fig. 7C) watersheds.

Inverse-distance weighted percent cropland did not improve predictions of nitrate-N over unweighted percentage cropland across all watersheds (r = 0.702), in large (r = 0.762), or in medium watersheds (r = 0.626). Moreover, IDW percentage cropland yielded nearly identical slopes among all watershed size classes (0.0095, 0.0121, and 0.0129 for small, medium, and large, respectively). However, in small watersheds, IDW percentage cropland was a stronger predictor than unweighted percentages and this effect was particularly apparent in watersheds with ≥25% cropland (Fig. 7D). When watersheds with <25% cropland (unweighted) were excluded from the analysis, partial correlations between IDW percentage cropland and nitrate increased from 0.446 to 0.554 in small watersheds, but decreased from 0.362 to 0.267 in large and 0.104 to 0.043 in medium watersheds.

DISCUSSION

Land-cover class percentages are not independent

One of the most common uses of watershed land-cover data is to relate the percentages of specific land-cover classes (e.g., cropland) to stream indicator data using simple linear correlation or regression analysis

(Van Sickle 2003). This is often conducted as an exploratory series of correlations or stepwise multiple regressions in which several land-cover classes are used to predict a response variable. Land-cover variables that yield the largest correlations are often inferred as related in some way, possibly causally, to the response variable. However, few investigators explicitly acknowledge the fundamental limitation that land-cover percentages among watersheds tend to be highly collinear (inter-correlated) so that a significant relationship between a response variable and one land-cover variable may be accompanied by significant relationships with one or more other land-cover classes. It is very difficult to interpret results from such analyses without a clear understanding of the correlation structure among land-cover variables and the ecological processes that yield that structure (MacNally 2000).

We used partial correlation analysis to factor out the effect of cropland and test for independent correlations between the remaining land-cover variables and the nitrate-N residuals. Here, the relatively high concentrations of nitrate-N observed at low percentage developed land were markedly reduced in the residuals after factoring out the effect of cropland. Consequently, the direction of the correlation of nitrate-N and developed land reversed, yielding a significant, positive relationship consistent with our expectations that developed lands are nitrate-N sources (Jordan et al. 2003, Weller et al. 2003). By removing the overwhelming effect of cropland, we were able to isolate the independent effect of developed land. Similarly, factoring out the effect of cropland allowed us to identify a weak but negative correlation between percentage wetland and nitrate-N that matched our understanding of wetlands as N sinks rather than sources (Weller et al. 1996, Baker et al. 2001). Thus, the effects of developed land and wetland on nitrate-N as inferred from simple correlations were very misleading. Only after considering the effect of other, collinear land-cover classes were we able to isolate the individual effect of each class.

Although our initial explorations of the correlation between land-cover percentages and stream nitrate-N were ecologically misleading, they made sense statistically. The highest nitrate-N concentrations were observed in watersheds with a high percentage cropland, and high percentage cropland necessarily resulted in a relatively low percentage developed land. Therefore, developed land would logically be negatively correlated with nitrate-N using simple correlation analysis (Fig. 3B). The positive simple correlation of the percentage wetland with nitrate-N was misleading for a different reason. Percentage cropland and percentage wetland were positively correlated because wetlands spatially covaried with cropland on the outer Coastal Plain (Fig. 1, Table 1), so wetlands superficially appeared to increase nitrate-N concentrations relative to other land-cover classes.

Although these examples may seem somewhat elementary, the analytical problems presented by collinearity among land-cover classes are not trivial and can affect virtually any broad-scale watershed study. Where only two predominant land-cover classes occur, it would not make sense to factor out the effect of one class prior to examining the effect of the other because their mutual effect would be captured when one land-cover type is used as a predictor. However, this scenario is an exception, and competing land-cover classes will need to be accounted for in most studies.

Although we used partial-correlation analysis to illustrate the problem of land-cover collinearity, multiple-regression analysis also isolates the residual effect of each predictor in a similar way. However, our partial-correlation approach forced us to evaluate the interrelationships of all pairs of land-cover predictors and their independent effects on nitrate rather than relying on statistical software to identify the ''best'' suite of predictors, as is commonly done using stepwise multiple regression. We contend that the latter approach may sequentially identify the strongest predictors but obscure the fact that very similar results could have been obtained using collinear, competing land-cover classes excluded by the stepwise selection process. The goal of our analysis was not to simply build the best predictive model, but to try to elucidate how different land-cover classes may be linked to each other and to stream conditions. We suggest partial-correlation analysis may further serve as a fundamental first step for building such predictive models (e.g., Van Sickle 2003, Van Sickle et al. 2004).

Land cover is spatially contagious and covaries with the physical template

We expected that macroinvertebrate assemblage composition would be affected by increasing percentage cropland (e.g., Roth et al. 1996). Cropland was a significant but not a strong correlate of macroinvertebrate composition when we used a simple Mantel test (Fig. 4). The relatively low influence of cropland further decreased when development and wetland effects were removed, suggesting that much of the apparent cropland effect was indicating an absence of developed land and presence of wetland rather than the presence of cropland. This interpretation was reinforced when the magnitude of the effect of developed land scarcely decreased after accounting for cropland and wetland. Thus, the effects of cropland, wetland, and developed land were not ''canceling'' each other out in the partial Mantel analysis. Instead, cropland simply did not explain much unique variation in composition.

The effect of spatial autocorrelation on the relationships between cropland and macroinvertebrates was more interesting (Fig. 4). Given the high degree of spatial contagion in the distribution of cropland in the Coastal Plain (simple Mantel $r = 0.481$), we expected that some of the variation in macroinvertebrates explained by cropland would also be explained by Space (the linear distance between sampling sites). However, the lack of a residual cropland effect after removing the effect of Space suggests that most of correlation between cropland and macroinvertebrate assemblage composition was due to biogeographical patterns or some other spatial covariate. In contrast, cropland was still highly correlated with nitrate-N after accounting for spatial autocorrelation. The coefficient resulting from the Mantel test between cropland and nitrate-N only dropped from 0.401 to 0.312 after removing the effect of Space, and both coefficients were highly significant ($P \leq 0.0001$). Furthermore, removing the combined effects of developed land and wetland in addition to Space actually improved the partial relationship between cropland and nitrate-N (partial Mantel $r = 0.345$, $P \leq 0.0001$). Thus, if percentage cropland in a watershed did indeed affect macroinvertebrate assemblages in a predictable manner, we should have detected this effect regardless of spatial autocorrelation as we did for nitrate-N. Landowner-specific agricultural practices (e.g., removal of riparian vegetation, lack of conservation tillage) may contribute to sharp changes in assemblage structure, but the absence of a partial correlation between cropland and macroinvertebrates casts doubt on the reliability of watershed-scale, *percentage* cropland as a predictor of stream biological integrity in the Coastal Plain of Maryland. It also illustrates the problems spatial autocorrelation will present to other broad-scale stream indicator studies.

In our consideration of spatial autocorrelation, we did not attempt to account for hydrological connectivity. We used simple linear distances as a coarse proxy for ‘‘effective’’ spatial distances among stream samples because our study landscape included dozens of independent drainage basins that emptied directly into Chesapeake Bay. In studies centered on single, large drainage basins, or intensive studies of smaller watersheds with multiple nested sub-basins, it may be important to account for actual separation distances within dendritic stream networks and for the directional component of autocorrelation that arises when samples are taken downstream of other samples (e.g., Fagan 2002). There are a number of other ways of modeling spatial phenomena in ecological data, some of which may be more appropriate depending upon the question and type of response variable (e.g., Koenig 1999, Lichstein et al. 2002).

Land cover is indirectly related to stream biota via abiotic pathways

The partial Mantel path model revealed that the percentage developed land was primarily and directly linked to four abiotic matrices: Riparian, Acidity, Cond., and Woody. The linkage between watershed-scale development and site-scale riparian metrics probably indicated the increased probability of degraded local riparian condition due to *local*, near-station development that was also correlated to watershed-scale percentage development (see *Spatial arrangement...*, below). The pure-partial effects of developed land on stream acidity (increase in pH and acid-neutralizing capacity) and specific conductivity (increase) were likely reflective of nonpoint sources of calcium carbonate, other buffers, and salts associated with human activities in commercial and residential areas (e.g., lawn maintenance, road salts; Liu et al. 2000, Paul and Meyer 2001). Elevated pH also was coincident with supersaturated DO conditions during daytime measurements in some poorly shaded, eutrophic urban streams (although percentage developed land was not a consistent predictor of DO concentrations). The Riparian-mediated effect of developed land cover on Woody suggested a loss of riparian forest buffers, the sources of woody-debris and root-wad habitats.

Acidity, Cond., Woody, and Riparian all had significant direct effects on stream biota, and post hoc comparisons revealed that much of these effects were primarily caused by developed land (Fig. 5). However, percentage developed land also explained residual variation in macroinvertebrate assemblage composition that could not be explained by the combined effects of all competing land-cover classes, intermediaries, and spatial factors (partial Mantel $r = 0.101$, $P \leq 0.0001$). Developed land may have captured the synergistic effect of multiple stressors not accounted for when stressors were considered individually. Alternatively, it may represent other factors (e.g., hydrological variability and its effect on geomorphic attributes of the stream beds; Roy et al. 2003) that were not included in our model and are important pathways between development and stream biota (see *Spatial Arrangement...*, below). Identifying residual, indirect sources of variation between land cover and stream biota could be valuable for designing future studies or monitoring programs because it points out previously unconsidered stressors that may not be effectively measured by existing monitoring protocols.

The partial Mantel path model also suggested the absence of some linkages that we had expected to be important. Cropland could not account for independent variation in any abiotic variable, except for nitrate-N. Consistent with our previous findings, there was no residual effect of cropland on macroinvertebrate assemblage composition, but, surprisingly, cropland also had no predictable influence on riparian measures. We expected cropland to have a negative effect on riparian measures because other investigations have noted strong negative correlations between watershed agriculture and the amount of near-stream forest (e.g., Baker et al. 2001, Jones et al. 2001). However, in the outer Coastal Plain, this pattern may be limited by the prevalence of very wet riparian floodplains that are more difficult to cultivate or develop than riparian zones on the inner Coastal Plain. Our result may again suggest the problem of spatial covariance among land cover, the physical template, and stream indicators (Richards et al. 1997).

In addition to the absence of a cropland effect, water temperature was not a significant correlate of macroinvertebrates in the path model despite its recognized importance to stream biota (Hawkins et al. 1997). Temperature was only weakly correlated to macroinverebrates in the simple Mantel tests, thus collinearity with other variables in the path model played only a minor role in its insignificance. However, temperature was only measured on one date from each stream location and, consequently, was influenced by temporal variation among dates or times of sampling during the Maryland Biological Stream Survey study (Klauda et al. 1998). Thus, the estimate of temperature used in our analysis was not as robust as a long-term, integrated measure. This may partially explain why Riparian explained residual variation in the macroinvertebrate data after all of the in-stream measures had been factored out of the path model. Riparian measures may have been acting as indicators of a temperature effect not captured by the temperature variable.

Analyzing potential abiotic pathways between land cover and stream biota is clearly complex. All of the variables used in this path model were significant correlates of macroinvertebrate assemblage composition using ordination and simple Mantel tests. However, many of these relationships could not be separated from spatial artifacts or variation shared with other abiotic factors. Our exploratory approach is conservative like a single-edged sword: a significant pure-partial effect makes a strong case for a particular linkage, but the lack of an effect does not necessarily mean a variable is unimportant. We argue that a conservative approach is needed to make strong inference using correlative analyses because observational studies are confounded by the complexity of ecological data and associated spatial dependencies (Legendre 1993, Lichstein et al. 2002). Therefore, our analysis was a series of steps that forced us to think about how to interpret correlative results in the context of several confounding analytical hurdles. We have produced a body of evidence that suggests that development was an important source of stressors to Coastal Plain stream ecosystems, while watershed-scale cropland was not as clearly linked to overall stream condition as we expected. However, cropland was clearly linked to elevated nitrate-N concentrations, and excessive nutrient loading, particularly nitrate-N, is one of the most important stressors to be considered in restoring and managing the Chesapeake Bay ecosystem (Correll 1987, Boesch et al. 2001).

Spatial arrangement may be an important modulator of watershed land-cover effects on stream ecosystems

We found that the threshold of macroinvertebrate-assemblage response was more sensitive to increases in developed land if development was located closer to the sampling station (Fig. 6). When we related developed land near a station to the macroinvertebrate response, we observed a lower percentage developed land threshold than that estimated using development across the entire watershed. This result, together with our path analysis results, suggest that development has its greatest effect when close to the sampling station, where development contributes to riparian degradation and reduced woody-debris recruitment. To explore this question more completely, we highlighted sites with whole-watershed percentage developed land near the threshold levels of effect on macroinvertebrates (Fig. 6A) and tracked the effect of 250-m station buffers and IDWs on the apparent distribution of these sites relative to the threshold. Sites with near-threshold land-cover percentages are of particular interest because greater development across the watershed always results in degradation and there is little evidence that less development predictably degrades streams. Therefore, near-threshold watersheds are where we expect land-cover arrangement to have the greatest influence on macroinvertebrate assemblages. When we quantified developed land for a 250-m buffer (Fig. 6B), all of the high-scoring macroinvertebrate samples (Fig. 6A) moved to the left along the development axis (Fig. 6B). These high-scoring sites—characterized by pollution-sensitive macroinvertebrate taxa (Appendix B)—had moderate percentages of developed land in their watersheds and little or none within 250 m of the sampling station. Distance weighting also moved the high-quality sites further to the left on the development axis (Fig. 6C), but not as much because distant development, excluded from the buffer summary, was incorporated into the development effect. In addition, many low-quality near-threshold sites apparently had substantial percentages of development outside 250 m and the buffer analysis failed to discriminate these from high-quality sites. In contrast, distance weighting shifted the positions of these same sites to the right side of the threshold. Thus, distance weighting accounted for both the whole-watershed and local-scale effects of developed land.

The NLCD (National Land Cover Database) land-cover data used in our analysis did not include impervious surface as a land-cover class. Impervious surface is recognized as an important landscape indicator in watershed studies (e.g., Arnold and Gibbons 1996, Wang et al. 2001). Percentage developed land is likely to be highly correlated to percentage imperviousness, but it is not equivalent (1:1). Thus, the numerical developed-land thresholds reported in this study should not be interpreted as percentage impervious-surface thresholds. However, the clear biotic thresholds observed in response to percentage developed land suggest that a similar analysis using percentage impervious surface is warranted and could be very useful for land-use planning and ecological forecasting in this region (Nilsson et al. 2003, Van Sickle et al. 2004).

We also examined the influence of land-cover arrangement on predictions of stream nitrate-N concentrations, yet these results depended on watershed size. In regressions using percentage cropland corrected for the occurrence of developed land, we observed lower explanatory power in smaller watersheds (Fig. 7C) compared to medium (Fig. 7B) or large (Fig. 7A) watersheds, though the slopes of the responses were similar. Strayer et al. (2003) observed a size effect in the ability of land cover to predict various stream indicators, possibly because land-cover arrangement is more important in small watersheds. Our findings appear to support their observations and hypothesis. Although distance weights did not improve predictions of stream nitrate-N concentrations among large or medium watersheds, they provided a 6% improvement in the amount of variance explained across all small watersheds, and an 11% increase in small watersheds with >25% cropland (Fig. 7D). Incorporating land-cover arrangement in predictions of nitrate-N concentrations is consistent with the idea that landscape sinks can reduce nutrient discharges (Weller et al. 1998). However, our results do not prove conclusively that the spatial arrangement of cropland has a significant effect on nitrate-N concentrations. The distance measures described here only account for surface distance, and other transport pathways, or alternate forms of proximity, may be important, especially for nitrate-N (Jordan et al. 1997*a*, Baker et al. 2001).

Distance weighting can account for land-cover arrangement effects on stream conditions that are missed by land-cover percentages in whole watersheds or neighborhood buffers. Certainly proximal land cover is important, but reliance on buffers alone ignores the potential for simultaneous and synergistic effects of watershed-scale land cover. Further, land-cover percentages in buffers can be so highly correlated with watershed-scale land cover that their unique effect on a response variable is often indistinguishable from watershed-scale effects (Richards et al. 1997, Jones et al. 2001). Arrangement effects may differ among physiographic regions, land-cover classes, response variables, and the specific biophysical processes involved; so it may be prudent to consider different proximity measures (both dependent on and independent of land-cover percentage and watershed size) and different weighting schemes for each combination of land-cover class and stream indicator. The metrics described here are useful exploratory analyses that complement current watershed-scale perspectives and warrant further investigation.

CONCLUSION

As the need for broad-scale assessment of aquatic conditions increases, we will likely see a greater reliance on land cover as an indicator of aquatic condition. We have highlighted several common problems that can arise in the use and relating of land-cover data to ecological indicators in streams. Correctly interpreting land-cover effects in the context of proportional interdependence, spatial autocorrelation, collinearity with intermediaries, and spatial arrangement are nearly universal challenges facing researchers and managers engaged in the conservation and management of aquatic resources. These challenges influenced our interpretation of land-cover patterns and our ability to detect relationships between human activities and stream condition. Simple correlations with land-cover percentages may lead to incorrect interpretations of the magnitude and even the direction of an effect. Previous studies of aquatic condition may well have suffered from these interpretive problems.

The methods we present are certainly not the only solutions available. However, the consideration of land-cover class interdependence, autocorrelation, linkages with abiotic intermediaries, and spatial arrangement should become standards in indicator analyses. Otherwise, we risk obscuring the relationships between land-cover patterns and condition of stream ecosystems.

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APPENDIX A

A table summarizing the spatial, land cover, and environmental variables obtained from the NLCD and MBSS data sets ($N = 295$ stream sites sampled), including distance-matrix assignments and data transformations, is available in ESA's Electronic Data Archive: *Ecological Archives* A015-004-A1.

APPENDIX B

A table summarizing scientific names, nMDS Axis 1 scores, frequencies of occurrence, and tolerance values for all macroinveretebrate taxa with at least 10 occurrences in the MBSS data set ($N = 295$ stream sites sampled) is available in ESA's Electronic Data Archive: *Ecological Archives* A015-004-A2.