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Use of diatoms to assess agricultural and coal mining impacts on streams and a multiassemblage case study

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Abstract. We developed and tested a species-level Bray–Curtis (BC) similarity to reference index and a genus-level diatom model affinity (DMA) index to quantify agricultural and acid mine drainage (AMD) impacts on streams in the Western Allegheny Plateau of southeastern Ohio. Decreased similarity to references sites was found in impaired streams, and diatom metrics further indicated how assemblages were impacted. Sites identified by index scores as impaired had significantly greater conductivity, PO_4-P, Ca^{2+}, Mg^{2+}, Na^+, Cl^-, % pasture in the upstream watershed, and forest fragmentation than minimally impaired sites ($p < 0.05$). Percent acidophilic diatoms significantly increased with reduced alkalinity caused by mining impacts ($p < 0.01$). Relative abundances of species indicating low P and N status decreased with increased pasture and row crops in upstream watersheds ($p < 0.01$), whereas abundance of motile diatoms increased ($p < 0.01$). In a case study, diatom, macroinvertebrate, and fish assemblages were compared among 18 sites in a single watershed (6 sites along an AMD impact gradient, 6 NaOH-treated AMD sites, and 6 sites with no mining impacts). All assemblages indicated severe impairment by AMD, but fish were least useful because they were absent from 5 of the 6 sites. Macroinvertebrates indicated unimpaired conditions at treated sites, but fish signaled potential problems (lower than expected biomass and presence of deformities) despite high species richness and index of biotic integrity scores. Diatom indices indicated significant impairment at AMD and treated sites ($p < 0.01$). Diatom metrics and indices very effectively signaled agricultural and mining impacts at the regional scale and were especially useful in the case study because they provided finer resolution of AMD effects and additional information of ecological importance missed by or in conflict between macroinvertebrate and fish assemblages. The DMA and BC indices responded similarly to stressors at the regional scale, but BC more effectively signaled impacts at the watershed scale. However, DMA was an effective assessment tool that might be used more easily than BC by novice phycologists and watershed groups because it requires less taxonomic expertise. If implemented in future watershed or regional studies, diatom indices could greatly benefit policy, management plans, and current monitoring efforts.

Key words: diatom index, biological assessment, nutrients, acid mine drainage (AMD), water quality, metrics, algae, fish, macroinvertebrates, periphyton, forest fragmentation, Bray–Curtis.

Human activities, such as agriculture, urbanization, and coal mining, threaten or impair thousands of stream kilometers throughout Appalachian states (Virginia, West Virginia, Ohio, and Pennsylvania) (USEPA 2000) and around the world (Grimm et al. 2008). Agriculture and coal mining affect streams and biological assemblages in the Western Allegheny Plateau (WAP) Omernik level III ecoregion of southeastern Ohio by contributing to sedimentation, nutrient loading, and acidic pH with high concentrations of metals (Omernik 1987, OEPA 2000). Biological communities damaged by these stressors lose their resiliency, ability to handle anthropogenic pressures, and ultimately, ecosystem functions, and biodiversity (Baron et al. 2002). Assessments of stream biology can characterize and quantify impacts and are needed to develop management plans, policy, or discharge permits.

In the WAP, acid mine drainage (AMD) has acidic pH and high concentrations of metals resulting from oxidation of FeS in coal mining waste products. AMD impairs diversity (Verb and Vis 2000), productivity (Niyogi et al. 2002), and ecosystem functions, such as metabolism and organic matter breakdown (Niyogi et al. 2001), in streams. AMD has devastated >7200 stream kilometers throughout Appalachia (USEPA 2000). In extremely acidic waters, except for a few

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macroinvertebrates (Monteith and Evans 2005), algae might be the only biomonitoring organisms available to complement chemical measurements. Thus, the algal community is a valuable and potentially informative tool for assessing AMD impacts.

Depending on land use and conservation practices, agriculture can increase nutrient and sediment loads to streams (Jones et al. 2001), leading to nuisance algal growths (Dodds et al. 1997, Biggs 2000) and loss of habitat heterogeneity (Roth et al. 1996), diversity, and ecological integrity (Allan 2004). Organic matter processing, stream metabolism, and other ecosystem services also can be negatively affected by agricultural activities (Young et al. 2008). Monitoring methods must provide accurate distinction among and quantification of stressors to be effective tools for restoring, managing, and conserving stream ecological integrity (Karr and Yoder 2004).

Diatoms are especially useful for characterizing impacts because they are predominant primary producers in streams and have a wide range of ecological tolerances (Patrick 1973, Lowe 1974, van Dam et al. 1994). Indices of biotic integrity (IBIs), which assess structural and functional components of assemblages, are effective tools for assessing anthropogenic impacts and are used to inform restoration and conservation policy (Karr 1991, Karr and Yoder 2004). Fish and macroinvertebrate indices are used in the WAP region of Ohio, but diatom indices have not been developed or tested on a regional scale (OEPA 2000). Different assemblages respond to different stressors that operate at multiple scales and, thus, can give conflicting diagnoses. Therefore, multiple lines of evidence from various taxonomic groups are important in stream assessments (Johnson and Hering 2009). Diatoms might be more responsive than other assemblages to certain stressors, and diatom-based monitoring tools that can be used to assess regional stressors are needed to complement or supplement results from other assemblages and help refine management efforts.

Indices and metrics based on biological data from least-impaired reference sites in a region help set realistic restoration and conservation goals and improve our understanding of how pollution and land use affect ecological integrity (Karr and Chu 2000). Least-impaired reference sites reflect how biological communities would exist, function, and persist with minimal anthropogenic impacts and are useful benchmarks from which to judge impairment (Stoddard et al. 2006). Diatom assemblages at least-impaired reference sites converge on a similar structure at the regional scale, and a test site's loss of similarity to reference sites can indicate anthropogenic stressors (Passy and Bode 2004, Wang et al. 2005). Changes in specific metrics, such as representation of taxonomic groups, nutrient optima, and morphologies, can be used to identify specific stressors that might lead to the overall loss of resemblance (Barbour et al. 1999). Metrics and similarity measures are standardized and can be used to communicate stressor effects in streams throughout the country (Barbour et al. 1999).

Our goal was to develop diatom monitoring tools for agricultural and AMD impacts. We created 2 indices that scored sites based on their mean similarity to reference-site diatom assemblages: 1) a species-level index based on mean Bray–Curtis (BC) similarity, and 2) a genus-level index based on % similarity of taxonomic groups. A genus-level index, if effective, might be more useful than a species-level index to novice phycologists and watershed groups wanting to integrate diatoms into their biological monitoring programs. We documented the effects of AMD and agriculture on the ecological similarity of study-site diatom assemblages to reference-site assemblages and correlations of changes with individual metrics. We developed and tested the indices/metrics at the regional scale and further tested them in a watershed-scale case study. We also compared the performances of indices based on diatoms, fish, and macroinvertebrates.

Methods

Study sites

Regional sites.—The geology of the WAP is predominantly sandstone, shale, limestone, and coal (Omernik 1987). We sampled 60 regional sites (10 reference sites, 50 test sites) throughout the WAP during summer 2005 and 2006 (Fig. 1). We chose regional reference and test sites randomly from an Ohio Environmental Protection Agency (OEPA) database of all sites historically sampled for fish, macroinvertebrates, or chemistry. From these 60 sites, we selected 7 reference sites that currently meet OEPA nutrient (PO_4-P $<$ 0.06 mg/L, NO_3-N $<$ 0.47 mg/L), ionic composition ($<$500 μS/cm, $Ca^{2+} < 69$, $Mg^{2+} < 18$, $Na^{+} < 17$, $Cl^{-} < 30$ mg/L), and physical habitat criteria (OEPA 1999). We added the criteria that reference sites have >66% forest in the upstream watershed, no mining activity, and no point sources of pollution. We selected 3 additional reference sites from Omernik level IV subecoregions (Pittsburgh Low Plateau, Unglaciated Upper Muskingum Basin, and Monongahela Transition Zone; Omernik 1987). We included these subecoregions to increase the ability of our indices to represent least-impaired conditions in the

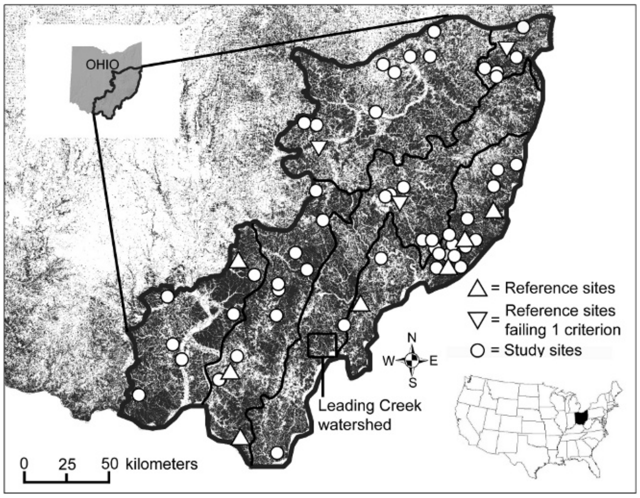

FIG. 1. Distribution of sites in the Western Allegheny Plateau of southeastern Ohio. Gray shading indicates forested land use. Black lines show boundaries of Omernik level IV subecoregions. The rectangle shows the Leading Creek watershed.

WAP. However, these subecoregions have more human disturbance and agriculture and less forested area than other subecoregions in the WAP, and no site in these subecoregions met all reference criteria. In each subecoregion, we chose the site that failed the fewest criteria (each site marginally failed 1 requirement). Fifteen percent (10/60) of the sites sampled were reference sites. Reference sites were on 2nd- to 5th-order wadeable streams with watersheds that ranged from 6.8 to 248 km^2. The 50 test sites were variously affected by agriculture, forest fragmentation, AMD, elevated conductivity, and nutrients.

Leading Creek sites.—The Leading Creek watershed drains 388 km^2 of steep hills with narrow valley floors (Fig. 1). AMD from coal mining and agriculture heavily affect the watershed, which is ~67% forested and 26% pasture and hay (Kennedy et al. 2004, Rankin 2005). We sampled 18 sites in the Leading Creek watershed during August 2006. Six sites represented an AMD impact gradient (AMD sites), 6 were affected by AMD discharge treated with NaOH (treated sites), and 6 had no mining impacts but some agricultural influences (non-AMD sites). Treated sites were 1.12 to 6.92 km from the next nearest site.

Sampling and laboratory techniques

We used the National Land Cover Database (USGS 1992) to determine % forest, % pasture, and % row crops in the watershed upstream of each sampling site. We determined connectivity, a measure of forest fragmentation, from US Forest Service data (USFS 2005) at 5 spatial scales (0.023, 0.073, 0.656, 5.905, and 53.144 km^2). We calculated connectivity as the probability that 2 adjacent 30 × 30 m pixels are forested, given that the first is forested. We report only the values calculated at the largest spatial scale because they were most strongly associated with indices, metrics, and water chemistry.

We collected samples for water chemistry from each site and filtered them on location through 0.45-μm-pore size filters (Millipore®, Billerica, Massachusetts). We measured PO_4-P, NO_3-N, SO_4^{2-}, Cl^-, alkalinity, and acidity with US Environmental Protection Agency (EPA)-approved protocols (APHA 1995). We analyzed Ca^{2+}, Mg^{2+}, K^+, Na^+, Fe^{2+}, Mn^{2+}, and Al^{3+} with an inductively coupled plasma atomic emission spectrometer (Varian Vista, Palo Alta, California). We measured conductivity, pH, and temperature in situ with handheld probes (Waterproof ECTestr® and pHTestr 30®; Oakton, Vernon Hills, Illinois).

At each stream, we proportionately sampled the available benthic habitats (riffles and pools) and substrata (rock, cobble, silt, and sand) in a 50-m reach to produce a composite sample of periphyton from 10 sampling locations (Stevenson and Bahls 1999). We sampled rocks by scraping them with a firm-bristled toothbrush and soft sediments by suction with a pipette and a 7.1-cm^2 O-ring. We preserved the composite samples with 2.5% $CaCO_3$-buffered glutaraldehyde. We cleaned a 10-mL subsample with 30% H_2O_2 and 50% HNO_3 to remove organic matter and allowed the material to settle on round cover slips in a chamber to disperse diatoms randomly and evenly (Battarbee 1973). We mounted cover slips on slides with NAPHRAX™ (The Biology Shop, Hazelbrook, New South Wales, Australia) and counted diatoms at 1000× with a light microscope (Olympus BX40™, Center Valley, Pennsylvania). We used the keys of Krammer and Lange-Bertalot (1986, 1988, 1991a, b) to identify 500 valves per sample along a transect. We calculated relative abundances of species, Shannon index of diversity, and species richness for each sample.

Diatom indices and metrics

Genus level.—We used the predominant genera found at reference sites to create a model diatom assemblage (diatom model affinity [DMA] index; Passy and Bode 2004). We defined predominant genera as those with >1% relative abundance in >1 reference site. Recent taxonomic splitting of genera (e.g., Round et al. 1990, Lange-Bertalot 2001) was not used because congeneric diatoms frequently represent

TABLE 1. Formulation of the diatom model affinity (DMA), which is based on genus-level taxonomic composition from 10 reference sites. DMA_{MAX} is the maximum possible score for each category and the total index. Scores for each taxonomic category are calculated as the lesser value of the % counted in the sample or the % in the model (DMA_{MAX}).

Taxon/DMA	Minimum	Maximum	Mean	SD	75th percentile	25th percentile	DMA_{MAX}
Achnanthidium + *Cymbella*	16.6	69.0	35.9	16.6	45.3	19.9	50
Nitzschia	11.3	29.9	21.1	6.9	27.5	15.3	20
Navicula	5.7	27.4	16.6	5.8	20.4	14.1	15
Amphora + *Cocconeis*	3.8	15.2	7.8	4.0	10.7	4.8	10
Fragilaria + *Gomphonema*	0.4	7.4	3.8	2.6	6.2	1.0	5
DMA	61.2	86.3	75.5	8.7	84.6	66.7	100

similar conditions (van Dam et al. 1994, Hill et al. 2001). For this index, *Achnanthidium* included *Planothidium* species; *Cymbella* included *Encyonema*, *Encyonopsis*, and *Reimeria* species; *Nitzschia* included *Tryblionella* species; *Navicula* included *Geissleria*, *Hippodonta*, *Luticola*, *Placoneis*, and *Sellophora* species; and *Fragilaria* included *Synedra*, *Staurosirella*, and *Staurosira* species. We grouped genera with similar ecological attributes to increase robustness and ease of model use. We grouped *Achnanthidium* and *Cymbella* because they are typical of waters with high dissolved O_2 and low trophic states (van Dam et al. 1994, Wang et al. 2005). We grouped *Amphora* and *Cocconeis* because they are typical of high pH, saprobity, and nutrients, whereas we grouped *Fragilaria* and *Gomphonema* because the species collected represented high nutrient conditions (van Dam et al. 1994).

We used the mean of each taxonomic group to set the maximum attainable score for each category of the index within 1 standard deviation (SD) as: *Achnanthidium* + *Cymbella*: 50%, *Nitzschia*: 20%, *Navicula*: 15%, *Amphora* + *Cocconeis*: 10%, and *Fragilaria* + *Gomphonema*: 5% (Table 1). We set the maximum attainable score for each taxonomic group close to its respective mean, except for *Achnanthidium* + *Cymbella*, which had a large SD because the 3 subecoregion reference sites had lower-than-expected abundances of these genera (17–20%). The mean relative abundance of *Achnanthidium* + *Cymbella* for the 7 reference sites that met all reference criteria was 44%. We set the value for this group at 50%, which was within 1 SD of the mean of all 10 reference sites, because these genera typically represent less-impaired conditions (KYDOW 1993, Wang et al. 2005). Scoring sites in this manner set the index on a scale of 0–100 for ease of interpretation.

We scored sites based on % similarity to the DMA index. High similarity indicated less impaired conditions, and low similarity indicated more impaired conditions. Percent similarity was calculated as (Whittaker and Fairbanks 1958):

$$\% \text{ similarity} = \sum_{i=1}^{5} \min(m,r),$$

where m is the relative abundance of taxonomic group i in the DMA index (e.g., in Table 1, DMA_{MAX} for *Nitzschia* = 20%) and r is the relative abundance of the same taxon at a study site. Scores were determined by summing the smaller of m or r for each taxonomic group. For example, if a study site had 20% *Achnanthidium* + *Cymbella*, 30% *Nitzschia*, 5% *Navicula*, 8% *Amphora* + *Cocconeis*, and 37% *Fragilaria* + *Gomphonema*, it would be scored as 20 + 20 + 5 + 8 + 5 = 58% similarity. Thus, index scores are reduced if test sites have relative abundances greater or less than DMA values.

Species.—BC similarity is a resemblance coefficient that often is used to analyze species composition patterns with abundance data (Bray and Curtis 1957, Legendre and Legendre 1998). We used a species-based BC similarity index to calculate the similarity between a study site and each reference site as:

$$BC = 1 - \frac{2W}{(A+B)},$$

where W is the sum of minimum abundances of the various species at the 2 sites, and A and B are the sums of abundances of all species at each site (Legendre and Legendre 1998). We calculated scores as the mean BC similarity between assemblages at study sites and at the reference sites.

Metrics.—We used correlations of water chemistry and landuse variables associated with human impacts with 15 diatom assemblage metrics to assess how specific components of diatom communities contributed to loss of resemblance between test- and reference-site assemblages in response to stressors. We tested metrics associated with taxonomic composition (species richness, Shannon diversity, % dominant diatom species), morphology (prostrate, erect, stalked, unattached, motile, very motile), and autecology (trophic state, N and P optima, pH optima, % eutraphentic, % acidophilic). Morphology reflects the physical structure of a diatom assemblage (Hill et al. 2000, Fore and Grafe 2002, Wang et al. 2005). In particular, motile diatoms are useful for assessing siltation (Stevenson and Bahls 1999), agricultural, and anthropogenic impacts (Fore and Grafe 2002). Autecology reflects general ecological conditions and specific stressors. We used diatom species indicators of high and low nutrient (P and N) status developed for the Eastern Highlands (synonymous with Appalachian Forests located in the Temperate Eastern Forests, Omernik level II ecoregion), which includes the WAP, because they were developed and tested specifically for the region and were more powerful indicators of trophic state than were indicators developed from European studies (Potapova and Charles 2007). We also tested a % eutraphentic diatom metric (Hill et al. 2000) that uses genus attributes and relative abundance of acidophilic diatoms, because of the frequent occurrence of AMD in the region. We report only metrics that were significantly correlated with anthropogenic stressors.

Statistical analyses

Exploratory statistics.—We used NCSS2004 to conduct exploratory univariate and multivariate statistical tests (Number Cruncher Statistical Systems, Kaysville, Utah). We screened data for normality, and either log(x)- or $\sqrt{(x)}$-transformed data to correct skewness. We used principal components analysis (PCA) of a correlation matrix to summarize the structure of chemical variables in the data set. We used Pearson correlations to indicate relationships among index/metric scores, environmental, and landscape variables.

Canonical correspondence analysis (CCA).—A detrended correspondence analysis (DCA) of $\sqrt{(x)}$-transformed species relative abundance data had a gradient of 2.3 SD, which indicated a unimodal relationship among most species and environmental variables. Therefore, we conducted CCA with CANOCO version 4.5 (Microcomputer Power, Ithaca, New York; ter Braak and Šmilauer 2002) to explore species–environmental variable relationships. CCA ordinated sites based on relative abundances of diatom species while constraining the solution based on regressions with environmental variables. We eliminated species from CCA if they were not present in >1 site with >1% relative abundance. This step reduced the number of diatom species in the regional data set from 342 to 116. We used a stepwise variable-selection procedure and included only significant (999 Monte Carlo permutations; $p < 0.05$) and noncollinear variables in the CCA. We used 999 Monte Carlo permutations to test significance of axes. We used Pearson correlations of indices, metrics, common genera, water chemistry, and landuse variables with the first 3 CCA axes to indicate their relationships to stressors and diatom assemblages.

Defining levels of impairment

Narrative categories help communicate results to the public and policy makers and can indicate benchmarks of ecological condition (Herlihy et al. 2008). We used DMA scores to define levels of dissimilarity because changes in genera typically are more conservative than changes in species (Hill et al. 2001). We used the 25th percentile score of reference sites to define the boundary between unimpaired and moderately impaired conditions and the 50th percentile of test sites to define the boundary between moderate and severe impairment. We used this arbitrary categorization of impaired sites only for the purpose of determining if diatom similarity measures could indicate different levels of stressors present at the 60 study sites. The 50th percentile probably would change if sites were added to the data set in the future, and therefore, this score should not be used as a level of attainment status in future studies. However, our use of the 25th percentile score to indicate unimpaired conditions was not arbitrary because it was based on the distribution of values at reference sites, and it is a common criterion used in stream biomonitoring (Herlihy et al. 2008).

We tested for differences in environmental variables associated with human activity and correlated with indices/metrics (Ca^{2+}, Mg^{2+}, Na^{+}, PO_4-P, Cl^{-}) among impairment categories (factor variable) with a multivariate analysis of variance (MANOVA; Wilks' Lamda). We used 1-way analyses of variance (ANOVAs) with Tukey–Kramer tests for multiple comparisons to determine significant differences among impairment categories for each variable (Zar 1999). We also used ANOVAs with Tukey–Kramer tests for multiple comparisons to test for differences in metric values among impairment categories.

Leading Creek watershed

We used 18 sites in the Leading Creek watershed as a case study to evaluate the ability of the diatom indices to detect impairment from agriculture and AMD relative to existing fish and macroinvertebrate IBIs. We chose 6 AMD sites, 6 treated sites, and 6 non-AMD sites based on recent studies of water chemistry, macroinvertebrates, and fish (Kennedy et al. 2004, Rankin 2005). We used fish and macroinvertebrate data from Rankin (2005) to calculate species richness, macroinvertebrate impairment scores, and fish IBI scores for each site. Macroinvertebrate scores ranged from 1 to 4, with 4 being the least impaired. Higher fish IBI scores indicated less impaired conditions. We used DCA of $\sqrt{(x)}$-transformed diatom relative abundances to explore patterns among groups of sites along the main axis of variation. We used a MANOVA (Wilks' Lambda) to test for differences in the response variables of pH, SO_4^{2-}, and conductivity (chemical variables indicating mining impairment) among groups of sites. We used 1-way ANOVAs followed by Tukey–Kramer tests to determine significant differences in pH, conductivity, SO_4^{2-}, and assemblage structure (fish, macroinvertebrate, and diatom richness and index scores) among groups of sites. When needed, we transformed variables used in the ANOVAs to meet assumptions of normality and homogeneity of variances.

Results

Regional

BC and DMA.—A total of 342 taxa were identified from the 60 sites. Shannon diversity ranged from 2.0 to 3.8, and species richness ranged from 44 to 80. DMA scores ranged from 30.7 to 86.4, and mean BC similarity ranged from 10.8 to 50.1% for the 60 sites. BC and DMA scores were strongly correlated (r = 0.75, $p < 0.001$).

PCA of chemistry data identified conductivity and nutrients as environmental variables contributing most to the diatom data structure (axis 1: 50% of variation, axis 2: 19% of variation). Factor loadings on axes 1 and 2 were greatest for Ca^{2+} (0.47, 0.46, respectively), Mg^{2+} (0.48, 0.37), Na^+ (0.46, −0.30), Cl^- (−0.26, −0.22), PO_4-P (0.41, −0.20), and K^+ (0.34, −0.69). Percent forested area, forest connectivity, DMA scores, and BC scores were negatively correlated with these water chemistry variables and conductivity (r = −0.29 to −0.65; Table 2). DMA and BC were positively correlated with % forested area (r = 0.42, r = 0.37) and negatively correlated with % pasture (r = −0.34, r = −0.29) and row crops (r = −0.33, r = −0.29) (Table 2). DMA and BC were strongly correlated with forest connectivity at the 53-km^2 scale (r = 0.63 and 0.60, $p < 0.001$; Table 2).

Metrics.—Species richness was correlated with Ca^{2+} (r = −0.46), Mg^{2+} (r = −0.32), conductivity (r = −0.37), and forest connectivity (r = 0.29). Shannon diversity was correlated only with Ca^{2+} (r = −0.35) and pH (r = 0.29). Relative abundances of diatoms indicating low P and low N were positively correlated with forest connectivity (r = 0.48, r = 0.59) and % forest (r = 0.37, r = 0.54) and negatively correlated with % pasture (r = −0.35, r = −0.46, respectively) (Table 2). Relative abundance of diatoms indicating low N also was negatively correlated with % row crops (r = −0.41, $p < 0.01$). Diatoms indicating high P and N were negatively correlated with forest connectivity (r = −0.36, r = −0.34) and % forest (r = −0.36, r = −0.46, respectively) and positively correlated with % pasture (r = 0.32, r = 0.35). Relative abundance of eutraphentic diatoms was not correlated with any land use, but was correlated with Ca^{2+}, conductivity, and alkalinity (Table 2). Acidophilic diatom relative abundances were negatively correlated with pH and alkalinity (r = −0.44, r = −0.68). Percent motile diatoms was the only morphological metric correlated with measures of human disturbance and was negatively correlated with forest connectivity (r = −0.41) and % forest (r = −0.46) and positively correlated with % pasture (r = 0.51) and % row crops (r = 0.35).

Diatom community analysis.—Alkalinity, PO_4-P, Ca^{2+}, Mg^{2+}, Na^+, pH, SO_4^{2-}, and watershed area ($p < 0.05$) were kept in the stepwise CCA with diatom species and habitat data (Fig. 2). These variables explained 31.2% of the total variation in the species data (axis 1: 12%, axis 2: 5.7%, axis 3: 4.4%, all other axes: 9.1%). BC, DMA, % forest, % pasture, and % row crops were used as passive variables to display their relationships with environmental gradients on the CCA biplot (Fig. 2). All 10 reference sites were located in the lower left quadrant of the CCA biplot. Measures of taxonomic composition, genera, index scores, metrics, land use, and water chemistry were significantly correlated with axes 1, 2, and 3 (Table 3).

Axis 1 represented an alkalinity gradient. Sites with positive axis-1 scores were impacted by AMD, indicated by strong correlations of alkalinity, pH, cations (associated with leaching by H^+), and conductivity (Table 3). Percent acidophilic diatoms also was strongly correlated with axis 1 scores (r = 0.77, $p <$ 0.01). Relative abundances of *Cocconeis* and *Amphora* species, which are typical of alkaline conditions, and *Cymbella* species were negatively correlated with axis 1 scores (r = −0.59, r = −0.63, r = −0.37, respectively).

Axis 2 represented an agricultural impact gradient and was significantly correlated with % forest, forest connectivity, % pasture, % row crops, diatom metrics indicating high and low P or N concentrations, and % motile diatoms (Table 3). Sites with greater relative abundances of diatoms indicative of high P and high N conditions had more positive axis 2 scores (r = 0.37, r = 0.36, respectively), whereas sites with greater diatom abundances indicative of low P and low N

TABLE 2. Pearson correlations of diatom index scores, metrics associated with nutrients and acid mine drainage, chemical, and landuse variables. DMA = diatom model affinity, BC = Bray–Curtis similarity to reference sites; low P, high P, low N, high N are relative abundances of diatoms indicating low and high nutrient concentrations (Potapova and Charles 2007) for the Eastern Highlands, which includes the Western Allegheny Plateau; EC = electrical conductivity; eutraphentic = relative abundance of diatoms indicating eutrophic conditions (Hill et al. 2000); acid = relative abundance of acidophilic diatoms; motile = relative abundance of motile diatoms; Pff243 = forest connectivity at the 53-km^2 scale (see Methods for explanation). Values in bold are statistically significant ($p < 0.05$), * = $p < 0.01$, ** = $p < 0.001$.

	DMA	BC	Low P	High P	Low N	High N	Eutraphentic	Acid	Motile	% forest	% pasture	% row crop	Pff243
PO_4-P	−0.42**	−0.44**	−0.08	0.01	−0.38*	0.14	−0.14	0.36*	0.21	−0.47**	**0.32**	0.45**	−0.56**
NO_3-N	−0.15	−0.21	−0.21	**0.32**	−0.24	0.36*	−0.14	0.03	0.15	−0.39*	0.22	**0.26**	**−0.29**
Na^+	−0.48**	−0.46**	−0.16	0.08	−0.40*	−0.08	0.14	0.13	0.21	−0.42**	0.34*	0.21	−0.41*
K^+	−0.34*	−0.45**	0.16	−0.15	−0.19	−0.22	−0.17	0.37*	**0.27**	−0.45**	0.36*	0.52**	−0.40*
Ca^{2+}	−0.62**	−0.50**	**−0.30**	−0.10	−0.51**	−0.08	**0.27**	0.07	0.16	−0.36*	**0.26**	0.19	−0.63**
Mg^{2+}	−0.58**	−0.43**	−0.23	−0.10	−0.51**	−0.07	0.25	0.19	0.19	**−0.29**	0.19	0.25	−0.56**
pH	0.15	0.25	−0.39*	0.66**	−0.16	0.59**	0.22	−0.44**	0.15	−0.38*	0.43**	0.15	−0.19
EC	−0.65**	−0.52**	**−0.28**	0.03	−0.53**	−0.04	**0.27**	0.09	0.17	−0.44**	**0.31**	**0.27**	−0.60**
Alkalinity	0.04	0.14	−0.58**	0.54**	**−0.27**	0.47**	0.52**	−0.68**	0.18	−0.17	0.23	−0.13	−0.24
Pff243	0.63**	0.60**	0.48**	−0.34*	0.59**	−0.36*	−0.23	0.22	−0.41*	0.81**	−0.75**	−0.59**	–
% row crop	−0.33*	**−0.29**	−0.14	0.18	−0.41*	0.19	−0.06	0.11	0.35*	−0.79**	0.61**	–	–
% pasture	−0.34*	**−0.29**	−0.35*	**0.32**	−0.46**	0.35*	0.09	−0.17	0.51**	−0.90**	–	–	–
% forest	0.42**	0.37*	0.37*	−0.36*	0.54**	−0.46**	−0.05	0.07	−0.46**	–	–	–	–

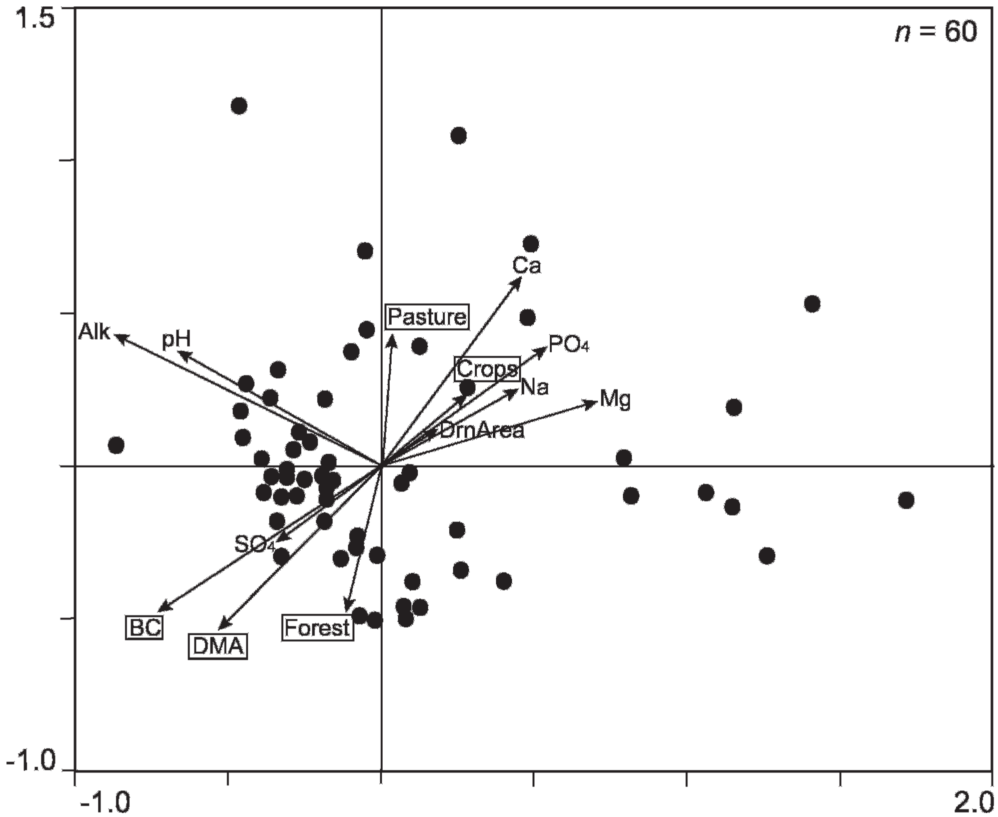

FIG. 2. Canonical correspondence analysis biplot of 60 sites sampled throughout the Western Allegheny Plateau. Variables in boxes were set as passive variables for displaying gradients of land use (% forest, % pasture, % row crop in upstream watersheds), model scores, and their relationships to axis 1 and axis 2. DMA = diatom model affinity, BC = Bray–Curtis similarity to reference sites, DrnArea = watershed area, Alk = alkalinity.

conditions had more negative axis 2 scores ($r = -0.63$, $r = -0.68$). Relative abundances of *Achnanthidium* and *Cymbella* were negatively correlated with axis 2 scores ($r = -0.73$, $r = -0.37$), and were associated with lower % pasture and higher forest connectivity. Relative abundance of eutraphentic diatoms was positively correlated with axis 2 scores ($r = 0.36$), as were relative abundances of *Navicula*, *Nitzschia*, and *Cocconeis* ($r = 0.40$, $r = 0.28$, $r = 0.41$). Percent pasture and % row crops were positively correlated with PO_4-P, Ca^{2+}, Mg^{2+}, and Na^+ (Fig. 2). Sites with high similarity (BC and DMA) to reference sites (i.e., less impairment) had more negative CCA axis 1 and 2 scores (Table 3).

Impairment categories.—Twenty-three sites had scores $\geq 25^{th}$ percentile of DMA reference scores (scores > 67) and were categorized as unimpaired. The remaining 37 sites were categorized as moderately impaired (scores = 55–66) or severely impaired (scores < 55) as set by the 50^{th} percentile of impaired sites in this study. BC scores differed significantly among sites in different impairment categories (ANOVA, $p < 0.01$). BC scores were highest for unimpaired sites, intermediate for moderately impaired sites, and lowest for severely impaired sites ($p < 0.01$). Ca^{2+}, Mg^{2+}, PO_4-P, Na^+, and Cl^-, important indicators of anthropogenic stress, differed significantly among sites in different impairment categories (MANOVA, $F_{10,104} = 4.57$, $p < 0.001$). Conductivity, Ca^{2+}, Mg^{2+}, Na^+, Cl^- and PO_4-P were significantly higher at moderately and severely impaired sites than at unimpaired sites (ANOVA; Fig. 3A–F). Unimpaired sites had higher % forest, lower % pasture (Fig. 3G, H), and higher forest connectivity than did moderately or severely impaired sites ($p < 0.01$). Relative abundances of diatoms indicative of low P and N conditions were highest and relative abundances of motile and eutraphentic diatoms were lowest at unimpaired sites (ANOVA; Fig. 4A–D). Species richness and Shannon diversity tended to be higher at moderately impaired sites than at unimpaired sites, but were significantly lowest at severely impaired sites (ANOVA, $p < 0.01$; Fig. 4E, F).

TABLE 3. Correlations of index scores, taxonomic composition, chemistry, and landuse variables with the first 3 canonical correspondence analysis (CCA) axes. DMA = diatom model affinity, BC = Bray–Curtis similarity to reference sites. Variable names are as in Table 1. Values in bold are statistically significant ($p < 0.05$).

	CCA1	CCA2	CCA3
% variation explained	12.0%	5.7%	4.4%
Variables			
Taxonomic composition			
DMA	**−0.38**	**−0.55**	−0.24
BC	**−0.52**	**−0.50**	0.06
Achnanthidium	−0.06	**−0.73**	−0.17
Cymbella	**−0.39**	**−0.37**	0.18
Navicula	**−0.28**	**0.40**	**−0.42**
Nitzschia	−0.01	**0.28**	0.08
Amphora	**−0.63**	0.02	0.05
Cocconeis	**−0.59**	**0.41**	0.05
Fragilaria	**0.49**	−0.17	−0.08
Gomphonema	0.15	−0.12	**−0.33**
% acidophilic	**0.77**	**−0.26**	−0.11
% motile	**0.41**	**0.48**	−0.18
% eutraphentic	**−0.35**	**0.36**	**0.38**
% low P	**0.42**	**−0.63**	−0.19
% high P	**−0.55**	**0.37**	−0.19
% low N	0.08	**−0.68**	−0.12
% high N	**−0.47**	**0.36**	**−0.26**
Habitat			
PO_4^{3-}-P	**0.41**	**0.41**	**−0.25**
NO_3^--N	0.04	**0.25**	−0.18
Na^+	**0.33**	**0.26**	−0.04
K^+	**0.52**	0.18	**−0.44**
Ca^{2+}	**0.35**	**0.66**	0.07
Mg^{2+}	**0.53**	0.22	**0.32**
SO_4^{2-}	**0.26**	**0.26**	0.16
pH	**−0.50**	**0.40**	−0.07
Alkalinity	**−0.65**	**0.46**	0.14
Conductivity	**0.37**	**0.64**	**0.29**
Watershed area	−0.11	−0.11	**−0.74**
Land use			
% forest	−0.10	**−0.57**	**0.35**
% row crop	0.24	**0.29**	**0.34**
% pasture	0.02	**0.53**	**0.36**
Pff243	−0.22	**−0.73**	−0.14

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A total of 186 diatom taxa were identified from the 18 sites. Shannon diversity ranged from 1.56 to 3.90, and species richness ranged from 12 to 85. The 3 groups of sites differed distinctly in community composition (Fig. 5). Non-AMD sites had scores in the middle of DCA axis 1. Treated sites were on the far left and AMD sites were on the far right of axis 1.

Shannon diversity was significantly greater at non-AMD sites (mean ± SD; 3.26 ± 0.48) and treated sites (3.00 ± 0.27) than at AMD sites (2.13 ± 0.44). Diatom assemblages at severely impaired AMD sites (pH < 4) were dominated by *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst and *Frustulia rhomboides* v. *saxonica* (Rabenhorst) De Toni. At pH > 5.8, *Achnanthidium minutissimum* (Kützing) Czarnecki was dominant at AMD sites despite high SO_4^{2-} (>158 mg/L) and conductivity (980 μS/cm). Conductivity at treated sites ranged from 1550 to 9940 μS/cm, and diatom assemblages were dominated by brackish-water species, such as *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Pleurosira laevis* (Ehrenberg) Compère, *Nitzschia reversa* W. Smith, and *Thalassiosira lacustris* (Grunow) Hasle.

Conductivity, pH, and SO_4 (important indicators of AMD) differed among sites in the 3 impairment categories (MANOVA, $F_{6,24} = 15.54$, $p < 0.001$). pH at treated sites was comparable to pH at non-AMD sites (Fig. 6A), but SO_4^{2-} and conductivity were significantly higher at treated than at non-AMD sites (ANOVA, $p < 0.001$; Fig. 6B, C). Conductivity was significantly greater at treated sites than at AMD sites because NaOH treatments increased the already high conductivity associated with AMD. AMD sites had significantly lower pH than did sites in the other 2 groups ($p < 0.001$), higher conductivity and SO_4^{2-} than non-AMD sites ($p < 0.001$), and similar SO_4^{2-} concentrations to treated sites (Fig. 6A–C).

DMA scores and BC % similarity at sites were strongly correlated ($r = 0.89$, $p < 0.001$). With the exception of 1 outlier site (score = 39) with an assemblage dominated by *Navicula perminuta* Grunow, non-AMD sites had DMA scores that ranged from 58 to 79 (Fig. 6D). This site clustered with other non-AMD sites in the DCA, but was separated from the cluster along axis 2 (Fig. 5). Elevated nutrient concentrations (NO_3-N > 1 mg/L, PO_4-P > 0.25 mg/L) at non-AMD sites with low DMA and BC scores indicated that these sites were affected by agriculture. DMA scores reflected the gradient of AMD impairment, but because of the high abundance of *A. minutissimum* in moderately impacted streams (pH > 5.8), scores were higher than expected (>65) (Fig. 6D). DMA scores ranged from 17 to 39 at AMD streams with pH < 5.8 and from 38 to 51 at treated sites. These scores indicated severe impairment based on the regional data set. BC % similarity at non-AMD sites ranged from 35 to 42%, with the exception of the outlier site (18.8%), and were significantly greater than BC % similarity at AMD and treated sites ($p < 0.01$; Fig. 6E). BC % similarity ranged from 1.4 to 27.7% at AMD sites and from 14.6 to 26.2% at treated sites. Diatom richness was significantly higher at non-AMD sites than at AMD sites (Fig. 6F).

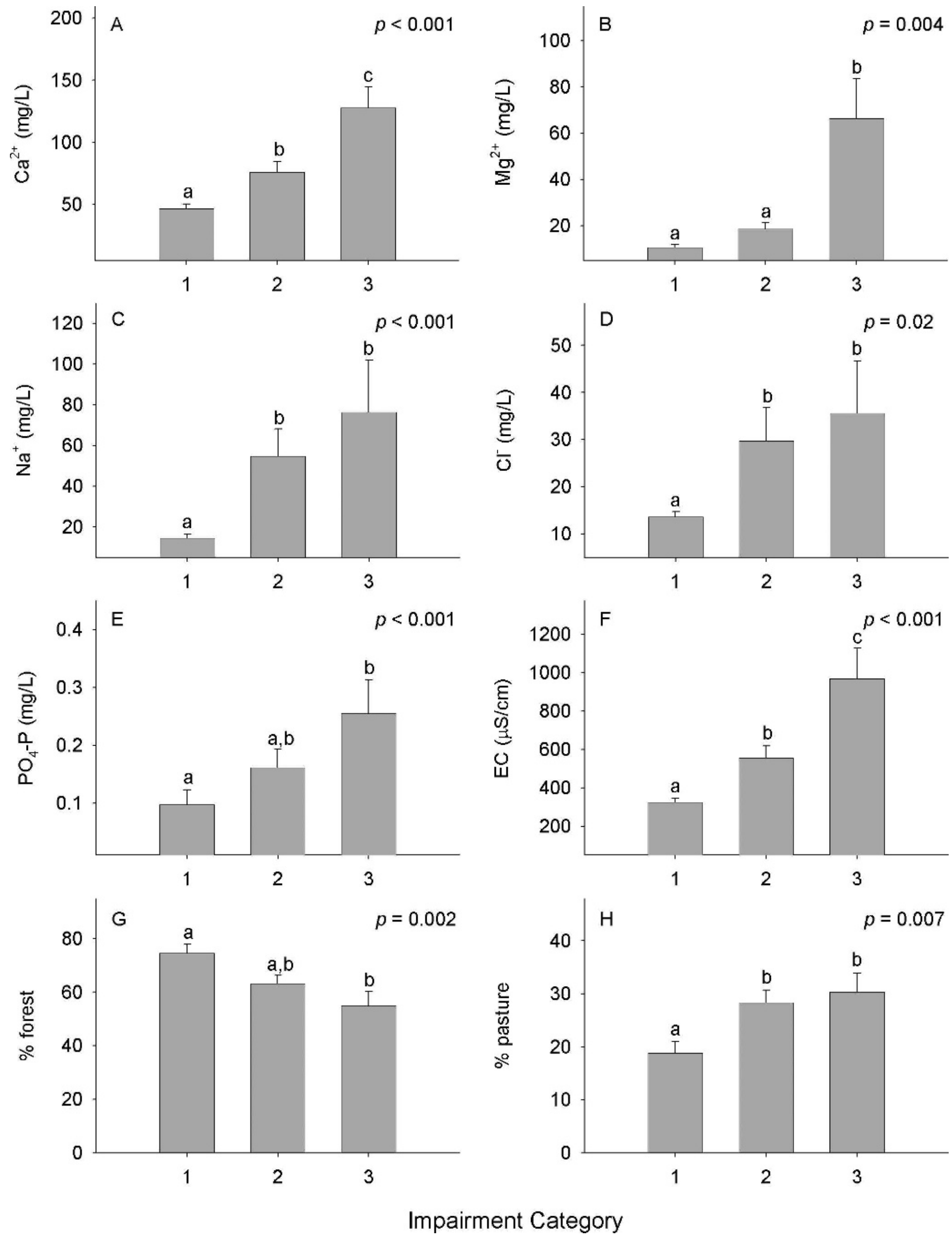

FIG. 3. Mean (+1 SE) Ca^{2+} (A), Mg^{2+} (B), Na^{+} (C), Cl^{-} (D), PO_4-P (E), electrical conductivity (EC) (F), % forest (G), and % pasture (H) at unimpaired (1), moderately impaired (2), and severely impaired (3) sites categorized by diatom model affinity (DMA) scores. Bars with the same letters are not significantly different (analysis of variance followed by Tukey–Kramer multiple comparison tests, $p > 0.05$).

Macroinvertebrate impairment scores and species richness and fish IBI scores were significantly lower at AMD sites than at other sites and indicated impairment, but these metrics were similar between treated and non-AMD sites (Fig. 6G–I). Fish species richness was significantly different among all 3 groups ($p < 0.01$; Fig. 6J). Fish were absent from 5 of the 6 AMD impacted sites, and fish species richness was greatest at treated sites, intermediate at non-AMD sites, and lowest at AMD sites. Fish with deformities were present in 4 of the 6 treated sites, but deformed fish were not observed in any other sites. Fish biomass was lower than expected at treated sites when compared to similar regional reference sites (E. T. Rankin, Environmental Program Manager, Institute for Local Government

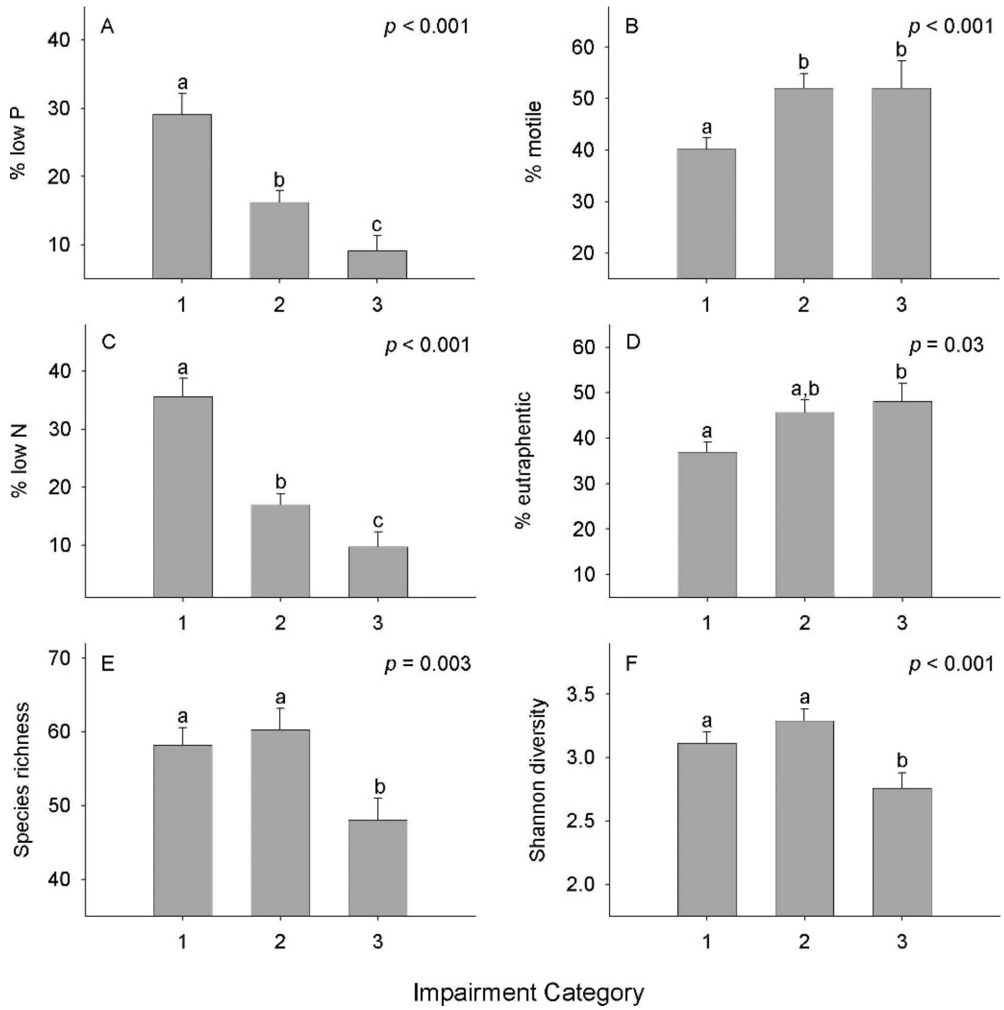

FIG. 4. Means (+1 SE) % low P diatoms (A), % motile diatoms (B), % low N diatoms (C), % eutraphentic diatoms (D), diatom species richness (E), and Shannon diversity (F) at unimpaired (1), moderately impaired (2), and severely impaired (3) sites categorized by diatom model affinity (DMA) scores. Bars with the same letters are not significantly different (analysis of variance followed by Tukey–Kramer multiple comparison tests, $p > 0.05$).

Administration and Rural Development, Ohio University, personal communication).

In general, all 3 assemblages indicated severe impairment at AMD sites, but macroinvertebrates and fish did not detect clear differences between treated and non-AMD sites (Fig. 6G–J). Fish data indicated potential problems (low biomass, deformities) at treated sites, but these results were in conflict with high species richness and IBI scores. The diatom assemblage was the only one to signal impaired conditions clearly via assemblage structure (Fig. 5) and loss of similarity to regional reference streams (Fig. 6D–F). Fish and macroinvertebrates showed clear improvements in treated sites compared to AMD sites, a result that indicated that NaOH treatment improved conditions, even though other stressors associated with the discharge remained ecologically problematic.

Discussion

Regional

BC, DMA, and diatom metrics very effectively represented components of the diatom community that were useful for assessing, interpreting, and communicating how anthropogenic activities impacted streams. Human impacts on landscapes strongly influence habitat conditions and biological communities of streams (Allan 2004). The indices and metrics developed and tested in our study signaled gradients of human impacts, particularly AMD, agriculture, and forest fragmentation. Water chemistry variables, such as conductivity, cation concentrations, and PO_4-P, were associated with landuse patterns and further linked diatom community responses with human impacts. Sites with index and metric scores indicating

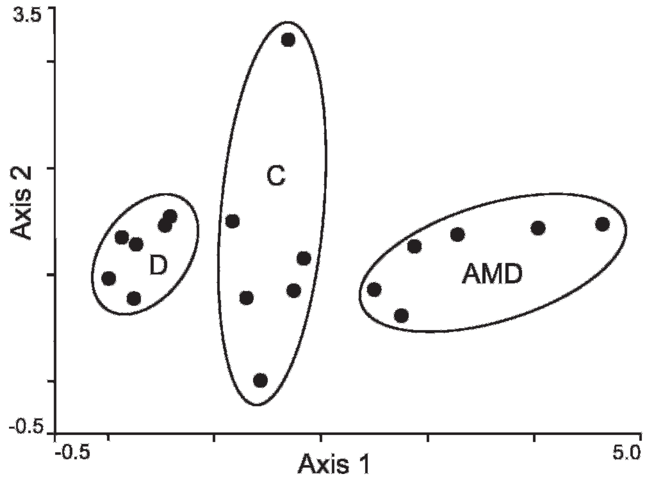

FIG. 5. Detrended correspondence analysis biplot of diatom species relative abundances at acid mine drainage (AMD), NaOH treated AMD (D), and non-AMD (C) sites in the Leading Creek watershed.

moderate or severe impairment were in areas with low forestation, increased agricultural and mining activities, and highly fragmented forests (Figs 2–4). Loss of cations and P from disturbed watersheds, especially as a result of leaching from agricultural (Thornton and Dise 1998, Rhodes et al. 2001) or deforested lands (Swank et al. 2001), can increase stress on aquatic communities.

Ionic composition of stream water is extremely important, and Ca^{2+}, alkalinity, pH, and HCO_3^- strongly influence diatom communities (Biggs 1995, Potapova and Charles 2003, Soininen 2007). Agriculture can elevate conductivity and cation concentrations as a result of fertilizing, liming, animal wastes, and erosion or particulate deposition to streams (Johnson et al. 1997, Carpenter and Waite 2000, Dow and Zampella 2000). AMD also can increase leaching of cations because of elevated H^+ concentrations. Percent acidophilic diatoms was an excellent indicator of AMD with low alkalinity but occasionally high cation concentrations, indicated by strong significant correlations with alkalinity and CCA axis 1 (Fig. 2). Impaired sites had significantly greater Ca^{2+}, Mg^{2+}, and Na^+ concentrations than did unimpaired sites (Fig. 3A–C) because of AMD impacts at some sites (CCA axis 1) and agricultural impacts at others (CCA Axis 2) (Fig. 2). The ratio of the sum of base cations to alkalinity indicates mineral weathering (1:1) or probable anthropogenic sources of cations (>1:1) (Rhodes et al. 2001). Most severely and moderately impacted sites had ratios substantially >1:1, whereas this ratio rarely was exceeded (and then only slightly) at unimpaired sites. The lack of correlation between Ca^{2+} and alkalinity ($r = 0.19$, $p = 0.14$) (Fig. 2) further indicated that both agricultural land use and AMD contribute to increased cations because Ca^{2+} and Mg^{2+} concentrations would be correlated with Ca- or Mg-bicarbonates if natural chemical weathering were the main source of cations (Leland and Porter 2000).

Diatom assemblages were exceptional indicators of AMD and land use in the watershed and adjacent to streams because they were strongly structured by the chemical habitat, which is a result of the water source, percolation through the soils, and runoff in the upstream watershed. Forest fragmentation and deforestation can greatly increase leaching and runoff of PO_4-P and cations into streams and can increase sedimentation, especially if the fragmentation is caused by clearing of land for pasture or crops (Owens et al. 2003, Kayser and Isselstein 2005). In our study, diatom indices were more strongly correlated with forest connectivity than with % forest. Thus, processes mediating nutrient inputs to streams might be affected more by conditions in the area immediately adjacent to sites than by processes at the scale of the watershed, and nutrient input might be high even if the watershed is highly forested in other areas away from the stream. Impaired sites had significantly greater cation, PO_4-P, and Cl^- concentrations, which were more strongly correlated with forest connectivity than % forest in the upstream watershed (Table 2), than did unimpaired sites. Diatoms indicating low P and N status were much more abundant in watersheds with high % forest and connectivity than in watersheds with low % forest or low connectivity (Figs 3, 4, Tables 2, 3). Diatoms indicating low P and N were significantly more abundant at unimpaired than impaired sites (Fig. 4) and were more strongly correlated with chemistry and landuse variables than were high P and N diatoms (Tables 2, 3). Together, these results suggest that sensitive species might be more responsive than tolerant species to increased nutrients. Genus-level % eutraphentic diatoms was less informative than species-level indicators of P or N status as a metric indicating nutrient status (Tables 2, 3). Motile diatoms increased as % pasture increased and % forest decreased, probably because of increased erosion and sedimentation in streams affected by agricultural land use. Motile diatoms also increased along CCA axis 1, which represented an AMD gradient, probably because metal oxide precipitates can smother benthic habitats.

Leading Creek

Water chemistry, BC, DMA, and diatom assemblages indicated AMD and NaOH treated AMD

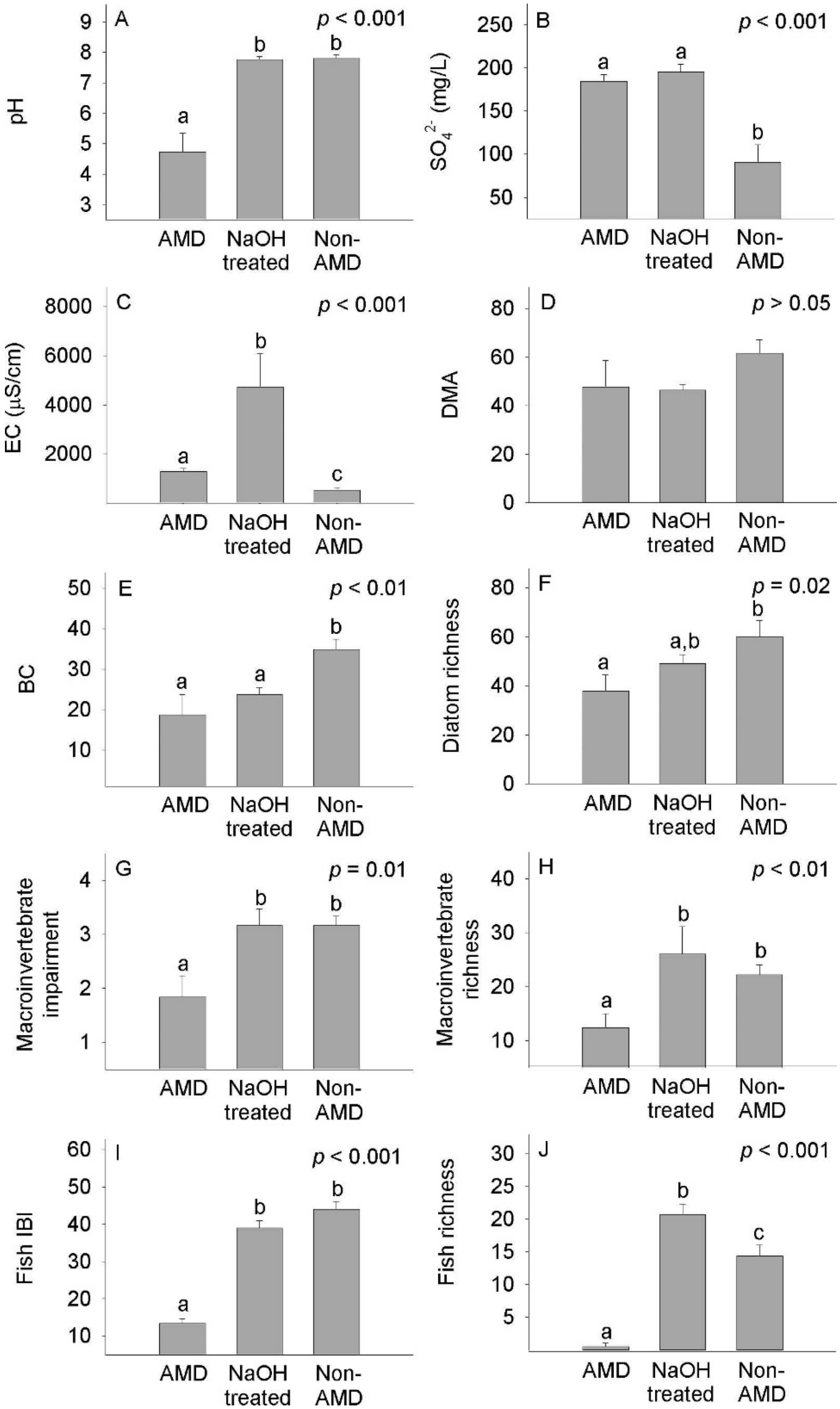

FIG. 6. Means (± 1 SE; $n = 6$) pH (A), SO_4^{2-} (B), electrical conductivity (EC) (C), diatom model affinity (DMA) score (D), Bray–Curtis similarity to reference (BC) score (E), diatom species richness (F), macroinvertebrate impairment score (G), macroinvertebrate species richness (H), fish index of biotic integrity (IBI) scores (I), and fish species richness (J) at acid mine drainage (AMD), NaOH treated AMD, and non-AMD sites in the Leading Creek watershed. Bars with the same letters are not significantly different (analysis of variance followed by Tukey–Kramer multiple comparison tests, $p > 0.05$).

impacts (Figs 5, 6). Severely impaired AMD sites had low BC and DMA scores and low species diversity dominated by acidophilic genera, such as *Eunotia* and *Frustulia*. Fish were less informative of the AMD gradient because they were absent from 5 of the 6 sites. More macroinvertebrate species (6–24 species) than fish species were present along the AMD gradient, but diatoms were most species rich (12–59) and, therefore, have potential to be informative of AMD impact gradients.

NaOH-treated AMD sites had extremely high conductivities, which were reflected in diatom assemblages characterized by high abundances of brackish-water diatoms. Nonnative diatoms also thrive in these sites (Smucker et al. 2008). Assemblages at these sites have experienced chronic Cl^-, Na^+, and SO_4^{2-} toxicity from the discharge (Kennedy et al. 2003). Treated discharge limits fish biomass and growth (E. T. Rankin, personal communication). Deformed fish were found in ⅔ of sites downstream of the discharge. Sensitive aquatic fauna were impaired at sites with >3700 μS/cm in Leading Creek (Kennedy et al. 2003), but the fish IBI and fish richness did not differ between non-AMD and treated AMD sites (Rankin 2005; Fig. 6I, J). Macroinvertebrate data also indicated minimal impairment in these sites (Rankin 2005; Fig. 6D, E). All 6 treated AMD sites were scored as severely impaired by both the DMA and BC indices, but diatom diversity and species richness was not significantly different from non-AMD sites because of the high diversity of brackish-water diatoms. This result underscores the potential problem of using diversity as a metric of human disturbance in some situations.

Diatom communities downstream of NaOH-treated AMD discharge were significantly impaired, and understanding this impairment might indicate mechanisms by which higher trophic levels were impacted. Discharge of NaOH-treated AMD waste is permitted in this watershed, in part because of the conflicting results of macroinvertebrate (no impact) and fish (moderate impact based on the metrics used) monitoring. However, that 3rd-order Leading Creek cannot effectively function and process its effluent is clearly indicated by its diatom assemblage. Including diatoms in the monitoring program might provide the evidence of an ecological problem at these sites that is needed to change the permit.

Species (BC)- vs genus (DMA)-level taxonomic resolution

As with macroinvertebrates, genus-level diatom taxonomy is adequate and informative for some stream assessments (Hill et al. 2001, Wang et al. 2005). Many species within genera have similar growth forms and responses to environmental conditions (Hill et al. 2001). In our study, the DMA and BC indices performed similarly at the regional scale and signaled gradients of AMD, agriculture, and forest fragmentation. Moreover, DMA and BC scores at sites were strongly correlated, probably because numerous genera were represented by a single or few dominant species (e.g., *Achnanthidium* by *A. minutissimum* and *A. deflexum* [C. W. Reimer] J. C. Kingston). These results indicate that DMA could be an effective tool for novice phycologists in biomonitoring programs. However, species-based nutrient metrics more effectively indicated anthropogenic impairments than did the genus-based % eutraphentic diatoms metric.

BC similarity provided finer resolution of environmental impacts than did the DMA index in the watershed case study. Higher-than-expected DMA scores were found in AMD sites because one species of an important DMA genus, *A. minutissimum*, was able to dominate assemblages in slightly acidic (Verb and Vis 2000) and moderately disturbed streams (Stevenson and Bahls 1999). Moderate AMD impairment is difficult to detect and score properly (Verb and Vis 2000, Hamsher et al. 2004). Moderately impaired sites were scored correctly as impaired with the BC index, whereas 2 moderately impaired AMD sites were scored as unimpaired even though conductivity and SO_4^{2-} levels were high at these sites. Thus, BC similarity was a more effective index of impairment than DMA.

In conclusion, the DMA and BC indices, along with diatom metrics, effectively diagnosed anthropogenic impairments (agriculture and AMD) of streams. Diatom assemblages were responsive to landscape level and local environmental conditions and can be used to inform management practices and conservation of streams. Diatom monitoring can improve biomonitoring efforts by contributing information missed by or in conflict between other assemblages.

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Literature Cited

ALLAN, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257–284.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1995. Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

BARBOUR, M. T., J. GERRITSEN, B. D. SNYDER, AND J. B. STRIBLING. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. 2nd edition. EPA 841-B-99-002. Office of Water, US Environmental Protection Agency, Washington, DC.

BARON, J. S., N. L. POFF, P. L. ANGERMEIER, C. N. DAHM, P. H. GLEICK, N. G. HAIRSTON, R. B. JACKSON, C. A. JOHNSTON, B. D. RICHTER, AND A. D. STEINMAN. 2002. Meeting ecological and societal needs for freshwater. Ecological Applications 12:1247–1260.

BATTARBEE, R. W. 1973. A new method for estimating absolute microfossil numbers with special reference to diatoms. Limnology and Oceanography 18:647–653.

BIGGS, B. J. F. 1995. The contribution of flood disturbance, catchment geology, and landuse to the habitat template of periphyton in stream ecosystems. Freshwater Biology 33:419–438.

BIGGS, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient–chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17–31.

BRAY, J. R., AND J. T. CURTIS. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 26:325–349.

CARPENTER, K. D., AND I. R. WAITE. 2000. Relations of habitat-specific algal assemblages to land use and water chemistry in the Willamette Basin, Oregon. Environmental Monitoring and Assessment 64:247–257.

DODDS, W. K., V. H. SMITH, AND B. ZANDER. 1997. Developing nutrient targets to control benthic algal chlorophyll levels in streams: a case study of the Clark Fork River. Water Research 31:1738–1750.

DOW, C. L., AND R. A. ZAMPELLA. 2000. Specific conductance and pH as indicators of watershed disturbance in streams of the New Jersey pinelands, USA. Environmental Management 26:437–445.

FORE, L. S., AND C. GRAFE. 2002. Using diatoms to assess the biological condition of large rivers in Idaho (USA). Freshwater Biology 47:2015–2037.

GRIMM, N. B., S. H. FAETH, N. E. GOLUBIEWSKI, C. L. REDMAN, J. WU, X. BAI, AND J. M. BRIGGS. 2008. Global change and the ecology of cities. Science 319:756–760.

HAMSHER, S. E., R. G. VERB, AND M. L. VIS. 2004. Analysis of acid mine drainage impacted streams using a periphyton index. Journal of Aquatic Ecology 19:313–324.

HERLIHY, A. T., S. G. PAULSEN, J. VAN SICKLE, J. L. STODDARD, AND C. P. HAWKINS. 2008. Striving for consistency in a national assessment: the challenges of applying a reference-condition approach at a continental scale. Journal of the North American Benthological Society 27:860–877.

HILL, B. H., A. T. HERLIHY, P. R. KAUFMAN, R. J. STEVENSON, F. H. MCCORMICK, AND C. B. JOHNSON. 2000. Use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Benthological Society 14:451–457.

HILL, B. H., R. J. STEVENSON, Y. PAN, A. T. HERLIHY, P. R. KAUFMANN, AND C. B. JOHNSON. 2001. Comparison of correlations between environmental characteristics and stream diatom assemblages characterized at genus and species levels. Journal of the North American Benthological Society 20:299–310.

JOHNSON, L. B., C. RICHARDS, G. E. HOST, AND J. W. ARTHUR. 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshwater Biology 37:193–208.

JOHNSON, R. K., AND D. HERING. 2009. Response of taxonomic groups in streams to gradients in resource and habitat characteristics. Journal of Applied Ecology 46:175–186.

JONES, K. B., A. C. NEALE, M. S. NASH, R. D. VAN REMORTEL, J. D. WICKHAM, K. R. RIITTERS, AND R. V. O'NEILL. 2001. Predicting nutrient and sediment loadings to streams from landscape metrics: a multiple watershed study from the United States Mid-Atlantic Region. Landscape Ecology 16:301–312.

KARR, J. R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66–84.

KARR, J. R., AND E. W. CHU. 2000. Sustaining living rivers. Hydrobiologia 422/423:1–14.

KARR, J. R., AND C. O. YODER. 2004. Biological assessment and criteria improve total maximum daily load decision making. Journal of Environmental Engineering 130: 594–604.

KAYSER, M., AND J. ISSELSTEIN. 2005. Potassium cycling and losses in grassland systems. Grass and Forage Science 60:213–224.

KENNEDY, A. J., D. S. CHERRY, AND R. J. CURRIE. 2003. Field and laboratory assessment of a coal processing effluent in the Leading Creek Watershed, Meigs County, Ohio. Archives of Environmental Contamination and Toxicology 44:324–331.

KENNEDY, A. J., D. S. CHERRY, AND R. J. CURRIE. 2004. Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using *Isonychia*. Environmental Monitoring and Assessment 95: 37–55.

KRAMMER, K., AND H. LANGE-BERTALOT. 1986. Bacillariophyceae, Teil 1: Naviculaceae. Gustav Fischer Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1988. Bacillariophyceae, Teil 2: Bacillariaceae, Epithemiaceae, and Surirellaceae. Gustav Fischer Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1991a. Bacillariophyceae, Teil 3: Centrales, Fragilariaceae, and Eunotiaceae. Gustav Fisher Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1991b. Bacillariophyceae, Teil 4. Achnanthaceae. Gustav Fischer Verlag, Stuttgart, Germany.

KYDOW (KENTUCKY DIVISION OF WATER). 1993. Methods for assessing biological integrity of surface waters. Kentucky Department of Environmental Protection, Frankfort, Kentucky. (Available from: Kentucky Department of Environmental Protection, 14 Reilly Road, Frankfort, Kentucky 40601 USA.)

LANGE-BERTALOT, H. 2001. Diatoms of Europe: *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lato, *Frustulia*. A.R.G. Ganter Verlag K.G., Ruggell, Liechtenstein.

LELAND, H. V., AND S. D. PORTER. 2000. Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. Freshwater Biology 44:279–301.

LEGENDRE, P., AND L. LEGENDRE. 1998. Numerical ecology. 2nd English edition. Developments in environmental monitoring 20. Elsevier Science B.V., Amsterdam, The Netherlands.

LOWE, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. EPA/670/4-74/005. US Environmental Protection Agency, Cincinnati, Ohio.

MONTEITH, D. T., AND C. D. EVANS. 2005. The United Kingdom acid waters monitoring network: a review of the first 15 years and introduction to the special issue. Environmental Pollution 137:3–13.

NIYOGI, D. K., W. M. LEWIS, JR, AND D. M. MCKNIGHT. 2001. Litter breakdown in mountain streams affected by mine drainage: biotic mediation of abiotic controls. Ecological Applications 11:506–516.

NIYOGI, D. K., W. M. LEWIS, JR, AND D. M. MCKNIGHT. 2002. Effects of stress from mine drainage on diversity, biomass, and function of primary produces in mountain streams. Ecosystems 5:554–567.

OEPA (OHIO ENVIRONMENTAL PROTECTION AGENCY). 1999. Appendices to association between nutrients and the aquatic biota of Ohio rivers and streams. Ohio EPA Technical Bulletin MAS//1999-1-1. Ohio Environmental Protection Agency Columbus, Ohio. (Available from: http://www.epa.state.oh.us/dsw/document_index/docindx.html)

OEPA (OHIO ENVIRONMENTAL PROTECTION AGENCY). 2000. Year 2000 Ohio water resource inventory. Ohio Environmental Protection Agency, Columbus, Ohio. (Available from: http://www.epa.state.oh.us/dsw/document_index/305b.html)

OMERNIK, J. M. 1987. Ecoregions of the coterminous United States. Annals of the Association of American Geographers 77:118–125.

OWENS, L. B., R. W. VAN KEUREN, AND W. M. EDWARDS. 2003. Non-nitrogen inputs and outputs for fertilized pastures in silt loam soils in four small Ohio watersheds. Agriculture, Ecosystems and Environment 97:117–130.

PASSY, S. I., AND R. W. BODE. 2004. Diatom model affinity (DMA), a new index for water quality assessment. Hydrobiologia 524:241–251.

PATRICK, R. 1973. Use of algae, especially diatoms, in the assessment of water quality. Pages 76–95 *in* J. Cairns and K. Dickson (editors). Biological methods for the assessment of water quality. ASTM special technical publication 528. American Society for Testing and Materials, Philadelphia, Pennsylvania.

POTAPOVA, M. G., AND D. F. CHARLES. 2003. Distribution of benthic diatoms in US rivers in relation to conductivity and ionic composition. Freshwater Biology 48: 1311–1328.

POTAPOVA, M. G., AND D. F. CHARLES. 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecological Indicators 7:48–70.

RANKIN, E. T. 2005. Fish and macroinvertebrate study of Leading Creek. Midwest Biodiversity Institute technical report MB/2005-3. Midwest Biodiversity Institute, Columbus, Ohio. (Available from: Midwest Biodiversity Institute, 553 Olentangy St, Columbus, Ohio 43202 USA.)

RHODES, A. L., R. M. NEWTON, AND A. PUFALL. 2001. Influences of land use on water quality of a diverse New England watershed. Environmental Science and Technology 35: 3640–3645.

ROTH, N. E., J. D. ALLAN, AND D. L. ERICKSON. 1996. Landscape influences on stream biotic integrity at multiple spatial scales. Landscape Ecology 11:141–156.

ROUND, E. F., R. M. CRAWFORD, AND D. G. MANN. 1990. The diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge, UK.

SMUCKER, N. J., M. B. EDLUND, AND M. L. VIS. 2008. The distribution, morphology, and ecology of a non-native species, *Thalassiosira lacustris* (Bacillariophyceae), from benthic stream habitats in North America. Nova Hedwigia 87:201–220.

SOININEN, J. 2007. Environmental and spatial control of freshwater diatoms—a review. Diatom Research 22: 473–490.

STEVENSON, R. J., AND L. L. BAHLS. 1999. Periphyton protocols. Pages 6-1–6-22 *in* M. T. Barbour, J. Gerritsen, B. D. Snyder, and J. B. Stribling (editors). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. 2nd edition. EPA 841-B-99-002. Office of Water, US Environmental Protection Agency, Washington, DC.

STODDARD, J. L., D. P. LARSEN, C. P. HAWKINS, R. K. JOHNSON, AND R. H. NORRIS. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–1276.

SWANK, W. T., J. M. VOSE, AND K. J. ELLIOTT. 2001. Long-term hydrologic and water quality responses following commercial clearcutting of mixed hardwoods on a southern Appalachian catchment. Forest Ecology and Management 143:163–178.

TER BRAAK, C. J. F., AND P. ŠMILAUER. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, New York.

THORNTON, G. J. P., AND N. B. DISE. 1998. The influence of catchment characteristics, agricultural activities and atmospheric deposition on the chemistry of small streams in the English Lake District. Science of the Total Environment 216:63–75.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2000. Mid-Atlantic Highlands streams assessment. EPA/903/R-00/015. US Environmental Protection Agency Region 3, Philadelphia, Pennsylvania.

USFS (US FOREST SERVICE). 2005. National landscape analysis and assessment. Southern Research Station, US Forest Service, Asheville, North Carolina. (Available from: www.forestthreats.org)

USGS (US GEOLOGICAL SURVEY). 1992. National land cover data. (Available from: seamless.usgs.gov)

VAN DAM, H., A. MERTENS, AND J. SINKELDAM. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28:117–133.

VERB, R. G., AND M. L. VIS. 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and nonimpacted sites. Journal of the North American Benthological Society 19:274–288.

WANG, Y. K., R. J. STEVENSON, AND L. METZMEIER. 2005. Development and evaluation of a diatom-based Index of Biotic Integrity for the Interior Plateau Ecoregion, USA. Journal of the North American Benthological Society 24: 990–1008.

WHITTAKER, R. H., AND C. W. FAIRBANKS. 1958. A study of plankton copepod communities in the Columbia basin, southeastern Washington. Ecology 39:46–65.

YOUNG, R. G., C. D. MATTHAEI, AND C. R. TOWNSEND. 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. Journal of the North American Benthological Society 27:605–625.

ZAR, J. H. 1999. Biostatistical analysis. 4^{th} edition. Prentice Hall, Upper Saddle River, New Jersey.

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