#### PRIMARY RESEARCH PAPER

# An assessment of structural attributes and ecosystem function in restored Virginia coalfield streams

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Abstract As human populations continue to grow, expanding energy needs enhance freshwater resource conservation challenges. Mining for coal has significantly altered the landscape in the United States' Appalachian region, with significant negative effects on downstream water quality and ecosystem function. With recent policy changes concerning the impacts of coal mining on aquatic ecosystems, many coal companies choose to restore sections of stream located on older coal mining areas as mandated compensatory mitigation for mining-related stream disturbances. We assessed such mitigation using measures of both structure and function in restored and unrestored streams affected by surface mining operations. Macroinvertebrate assemblages in streams affected by older

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A. J. Timpano · C. Zipper Department of Crop and Soil Environmental Science, Virginia Polytechnic Institute and State University, 363 Smyth Hall, Blacksburg, VA 24061, USA mining and recent restoration practices were rated as "stressed" and "severely stressed," with streams varying from fair to optimal in terms of habitat. All streams were net heterotrophic with varying levels of ammonium uptake. No site differences were found for any measured physicochemical or functional variables, while invertebrate community metric scores were higher in unrestored streams. There were also no significant relationships found between structural and functional measurements in these streams. Principal components analysis implicated the importance of measuring physicochemical, structural, and functional variables in further analyses of restoration success. This study was unable to document pre-disturbance conditions, and as a result, we were unable to find evidence that restoration is currently having a significant effect on ecosystem processes within these systems. Further research is needed to understand the changes in ecosystem structure and function that come with time.

**Keywords** Coal mine streams · Ecosystem function · Ecosystem metabolism · Nutrient cycling · Stream restoration

# Introduction

As human populations grow (United Nations, 2009), anthropogenic impacts to the environment are expanding throughout the world. Coincident with



this increasing human population is the intense need for energy (White, 2007) and freshwater resources (Vörösmarty et al., 2000). One of the major providers of energy for the United States and the world is coal, which is used by homes, industry, and commercial ventures (USDOE, 2011). Coal extraction has taken multiple forms, including underground and surface methods. In recent years, continued development of mining technologies has enabled the expansion of the scale of mining operations, including mountaintop removal and valley fill. Although mountaintop removal coal mining began decades ago, the call to better understand the ecological impairments caused by such practices has been more recent (Peng, 2000).

Streams left unmanaged in a mined landscape can suffer severe changes to hydrology, succession of riparian vegetation, colonization of macroinvertebrates, and ultimately ecological function (Mutz & Schlief, 2005). The highly disturbed nature of a mined landscape will change runoff, infiltration, and stream recharge patterns (Mutz, 1998; Bonta, 2005). More well-known are the chemical impacts of mining on receiving water bodies, especially increases in heavy metals and acidic mine drainage that occur when pyritic minerals are disturbed (Hartman et al., 2005; Merovich et al., 2007; Fritz et al., 2010) unless appropriate reclamation procedures are employed. Mining operations that occur on a large scale, such as those that produce valley fills in Appalachia, also allow leaching of mineral salts from the disturbed rock materials, causing increases in total dissolved solids (TDS) and conductivity of streams draining such areas. Miningrelated conductivity has been associated with decreased numbers of sensitive aquatic taxa (Pond et al., 2008; Pond, 2010). Recent work also suggests decreased success of bacterial and fungal communities, which are essential to ecosystem function in streams, in highly acidic streams on mine lands (Schlief & Mutz, 2005).

At a whole-watershed scale, tributaries draining mined areas will affect downstream water quality (Locke et al., 2006). Impacts on aquatic communities exist even decades after mine abandonment (Lefcort et al., 2010), mainly due to the continual impacts of heavy metal accumulation (Clements et al., 2010; Petty et al., 2010) when appropriate mitigation procedures are not employed. The underlying geology and chemical output of mining practices play an important role in structuring communities (Petty et al., 2010). For example, acid mine drainage, when

mixed with net alkaline waters present in the watershed have led to a precipitation of heavy metals onto the benthic zones of streams, affecting macroinvertebrate colonization (McClurg et al., 2007; Merovich & Petty, 2010; Petty et al., 2010).

Ecosystem function in highly disturbed watersheds has also been assessed recently. Microbial colonization and processing of leaves can be negatively affected by extremes of pH (Simon et al., 2009), thus negatively affecting the palatability for shredding invertebrates (Graca, 2001). In mined watersheds, organic matter decomposition has been severely impacted by the accumulation of iron precipitates, which have been shown to hinder microbial colonization and the initiation of the breakdown process (Schlief & Mutz, 2005). Additionally, toxic metals found in mine drainage can significantly alter the reproduction and function of aquatic hyphomycetes, which are major fungal initiators of organic matter breakdown in streams (Lecerf & Chauvet, 2008).

Given that only a 10% watershed disturbance can reduce the biodiversity of freshwater species by 6% (Weijters et al., 2009), the desire to restore ecosystems has become more predominant. Restoration may take many forms, but typically it involves an alteration to the structure of the channel with additional riparian reconstruction and re-vegetation (KST, 2007). As such, a common measure of the success of restoration has been how the new habitat structure may lead to the successful colonization of new biotic communities (Muotka et al., 2002). Water quality issues in disturbed streams are not simply reflected in biota, but also in water chemistry (e.g., Merovich et al., 2007; Clements et al., 2010), each requiring different forms of restoration (Hobbs & Harris, 2001).

Simply assessing structural responses (physical and/or biological) to restoration provide an incomplete view of success. Hobbs & Harris (2001) point to the importance of also including functional measures of restoration outcomes, and there is a need to monitor these concurrently with structural attributes (Higgs, 1997; Lake et al., 2007). Palmer et al. (2005) suggest standards for successful ecological restoration, including the understanding that aquatic ecosystems are highly dynamic, thus restoration should not only improve their structure and function, but also their resilience to further disturbance.

Under the United States' Clean Water Act of 1972, Section 404, miners are required to reconstruct



affected streams or apply "restoration" to other degraded streams in the watershed as compensatory mitigation for the environmental effects of mining. Thus, coal mining companies often find it convenient to restore streams located on older, previously mined landscapes as a means of satisfying these requirements. Regulatory protocols for evaluation of such efforts commonly rely on assessment of stream structural elements such as channel stability, habitat features, water quality, and benthic macroinvertebrate assemblages. Critics of this process claim that structural replacement provides no assurance that the ecological functions and services provided by headwater streams lost during mining are being restored (Palmer et al., 2010). With the exception of a study by Fritz et al. (2010), few studies incorporating both structural and functional analyses of coal mining-impacted stream restoration have appeared in the literature.

Macroinvertebrate-based water quality indices are highly responsive to the synergistic effects of chemical constituents of the streams in mined landscapes (Freund & Petty, 2007), and are thus used as metrics of success for stream restorations. On the other hand, metrics of ecosystem function (e.g., gross primary production (GPP), respiration, leaf breakdown; Young et al., 2008; Young & Collier, 2009) may provide a useful compliment to structural ones in order to determine how resilient a restored ecosystem will be to change (Choi et al., 2008; Palmer et al., 2005). Leaf breakdown has been shown to be an effective indicator of the functionality of disturbed stream ecosystems in Virginia (Simon et al., 2009). As headwater streams are important sources of N and P to downstream water bodies (Alexander et al., 2007), measures of ecosystem metabolism and nutrient uptake should help gauge the effectiveness of restored systems at removal and processing of these nutrients (Young & Collier, 2009). In fact, Bukaveckas (2007) demonstrated increased nutrient uptake in response to the reduction in water velocities accompanying stream restoration.

The Appalachian coalfield, including southwest Virginia, is a primary coal-producing region in the US, and is projected by the US Department of Energy to remain as such over the coming decades (USDOE, 2011). Coal production to date has disturbed >600,000 ha and impacted >1,900 km of headwater and low-order streams in Appalachia (USEPA, 2005).

It is clear that surface coal mining in Appalachia affects the region's dense headwater stream network. The objective of our study was to compare ecosystem structural and functional measures among restored and unrestored stream reaches in coal-mined landscapes. Our primary measure of structure was a multimetric index of benthic macroinvertebrate assemblage structure, the Virginia Stream Condition Index (VSCI; VDEQ, 2006, 2007), which is commonly used in water quality assessments under the Clean Water Act within the Commonwealth of Virginia. Functional measures, such as ecosystem metabolism (GPPecosystem respiration (ER)) and nutrient uptake, address a streams' ability to both support life and process nutrient loads for delivery to downstream reaches (Allan & Castillo, 2007). Metabolism and uptake are also relatively inexpensive, simple measurements that are sensitive to both temporal and anthropogenic changes to the system (Young et al., 2008). A secondary objective was to investigate the potential relationships among the chosen stream structural and functional measures, and identify any variables that may be strong indicators of restoration success. A lack of relationship between the structural and functional measures would indicate that structural metrics alone may not be adequate assessments of stream restoration in mined landscapes.

#### Materials and methods

Site selection

We sampled six restored and three unrestored streams in the coal mining region of southwest Virginia (Fig. 1; Table 1). The restored streams were reconstructed on previously coal-mined areas. Restored streams here generally refer to those that have undergone channel reconstruction using Natural Stream Channel Design (NSCD) methods (KST, 2007), with the exception of Critical Fork. NCSD reconstructions supplement in-stream structures (e.g., cross-vanes, j-hooks, etc.) with riparian re-vegetation in order to restore both habitat and stream function (KST, 2007). In contrast, Critical Fork, the oldest restored stream, was constructed by the coal mining operation as a purposeful effort to create natural stream channel conditions: it travels some 671 m downstream from a sediment retention pond. Portions



Fig. 1 A map of the nine study streams, encompassing three counties (Co.) within the Appalachian coalfield region of southwest Virginia, USA. The shaded area of the map indicates the extent of coal geology in the region. Darkened stream lines represent our study streams, and numbers correspond to those reaches outlined in Table 1

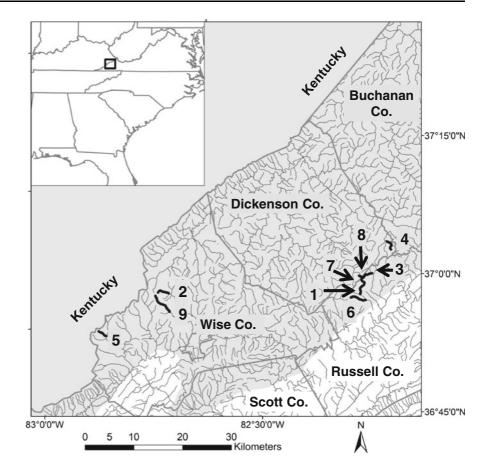


Table 1 Habitat features and metrics of macroinvertebrate community structure for the nine study streams used in this study

| Site                | Year restored | Habitat score   | # Taxa         | # EPT          | VSCI score      | ALU tier      |
|---------------------|---------------|-----------------|----------------|----------------|-----------------|---------------|
| Restored            |               |                 |                |                |                 |               |
| 1. Chaney Creek     | 2006          | 160             | 12             | 5              | 58.2            | Stress        |
| 2. Critical Fork    | 1988-1989     | 145             | 7              | 3              | 45.4            | Stress        |
| 3. Laurel Branch    | 2005          | 171             | 10             | 7              | 56.7            | Stress        |
| 4. Left Fork        | 2007-2008     | 133             | 9              | 4              | 54              | Stress        |
| 5. Lick Branch      | 2003          | 136             | 3              | 1              | 38.8            | Severe stress |
| 6. Stonecoal Creek  | 2008          | 162             | 13             | 6              | 59.2            | Stress        |
| Mean $\pm$ SE       |               | $151.2 \pm 6.3$ | $9.0 \pm 1.5$  | $4.3 \pm 0.9*$ | $52.1 \pm 3.3*$ |               |
| Unrestored          |               |                 |                |                |                 |               |
| 7. N. Chaney Creek  | n/a           | 154             | 14             | 7              | 58.8            | Stress        |
| 8. N. Laurel Branch | n/a           | 170             | 16             | 7              | 62.9            | Good          |
| 9. Powell River     | n/a           | 170             | 10             | 6              | 66.2            | Good          |
| Mean $\pm$ SE       |               | $164.7 \pm 5.3$ | $13.3 \pm 1.8$ | $6.7 \pm 0.3*$ | $62.6 \pm 2.1*$ |               |

Number of taxa (# taxa), Ephemeroptera–Plecoptera–Tricoptera (# EPT) taxa richness, Virginia Stream Condition Index Scores (VSCI, range: 0–100), and aquatic life use (ALU) tiers (VDEQ, 2006, 2008). Habitat assessment scores (range: 0–200) were based on habitat metrics outlined by Barbour et al. (1999) and adapted for VDEQ (2008). Comparisons of stream types (restored vs. unrestored) were made for all variables here, with significant differences (P < 0.05) indicated by asterisks (\*)



of Chaney Creek, Stonecoal Creek, and Laurel Branch were created from drained sediment retention ponds constructed in coal mining spoils, but these streams also receive inputs from larger watershed areas; Left Fork was created as a new channel (Clinton Steele, D.R. Allen and Associates, P.C., Abingdon, VA, personal communication). Lick Branch is the only site that is currently fed by an active deep mine effluent discharge. Riparian zones were rebuilt and planted with vegetation inconsistent from site to site, leading to unique drainage and infiltration patterns across the six restored streams. Most of the stream restorations were completed within the past 5 years. Based on EPA (Barbour et al., 1999) and VDEQ (2006) ratings, half of the restored streams were optimal in terms of habitat while the other three were suboptimal (Table 1).

Unrestored streams had coal mining within their catchments, but had undergone no in-stream or riparian restoration. Although all unrestored streams would be considered optimal in terms of habitat score (Barbour et al., 1999; VDEQ, 2006), it is important to note that these streams were not un-mined references. but instead streams with similar watershed disturbance histories due to coal mining. Essentially, the unrestored streams serve as a baseline to which any changes due to restoration may be measured against, with the hope that measures of structure and function in restored streams would be improved compared to those in unrestored streams. Two of the three unrestored streams (N. Laurel and N. Chaney) were upstream segments of streams with downstream restored sections. Riparian areas of unrestored streams contained mature forest vegetation. A 100-m reach was delineated for habitat, biological, and functional measurements for the nine study streams.

## Habitat assessment

In June 2009, stream habitat and riparian structure were assessed using protocols outlined in Barbour et al. (1999) and the Virginia Department of Environmental Quality (VDEQ, 2008). For each 100-m study reach of stream (along with an additional 100-m section upstream), substrate cover, embeddedness, velocity regime, sediment deposition, channel flow status, channel alteration, and riffle/bend frequency were visually scored with up to 20 points each. Riparian features for each bank, including bank

stability, vegetative protection, and riparian zone width were also scored. Streams were classified as Poor, Marginal, Suboptimal, or Optimal, which represent <25, 25–50, 50–75, or 75–100%, respectively, of the total score of 200 points possible.

## Abiotic parameters

Abiotic parameters were measured for each stream, in association with habitat assessment and stream functional parameters. Discharge was estimated using dilution gauging of a NaCl slug (Gordon et al., 2004). Spot measurements of dissolved oxygen (DO) and pH were taken using a portable sonde (Hydrolab Quanta, Hach Instruments, Loveland, CO), and specific conductance (SC) and temperature were taken with a hand-held conductivity meter (Model 30, YSI, Inc., Yellow Springs, OH). TDS was measured in the field via filtration of known volumes (0.45  $\mu$  cellulose ester filter) followed by drying at 180°C (APHA, 1998). Samples were transported and stored at 4°C prior to filtration and dried at 180°C in the laboratory. Additional 60-ml water samples were collected and filtered in the field for background concentrations of chloride and nutrients. Chloride, nitrate, and phosphate concentrations in stream water were analyzed using ion chromatography (DX 500 IC, Dionex Corp., Sunnyvale, CA). Ammonium concentration was measured by flow injection analysis (Lachat QuickChem 8500, Lachat Instruments, Loveland, CO).

## Macroinvertebrate sampling

In June 2009, 6–0.3 m<sup>2</sup> kicks (2 m<sup>2</sup> composite total) were taken with a 500-µm d-frame net for 30 s at each site (Barbour et al., 1999; VDEQ, 2008). All material collected was placed in containers of 95% ethanol for transport back to the lab for identification. Organisms were separated from debris using a randomized subsorting procedure to obtain 110 ( $\pm 10\%$ ) organisms for identification (VDEQ, 2008). Taxonomic keys (Merritt et al., 2008) were used to identify organisms to the family/lowest practicable level. These data were then used to calculate VSCI scores, which were calculated using eight metrics: 1. EPT, 2. total taxa, 3. % Ephemeroptera, 4. % Ephemeroptera + Trichoptera - Hydropsychidae, 5. % Chironomidae, 6. % Top 2 dominant taxa, 7. family level index, and 8. % scrapers (Burton & Gerritsen, 2003). Data for VSCI,



taxa richness, and EPT richness are presented here, but with the caveat that they are not truly independent measures of macroinvertebrate assemblage structure. Aquatic Life Use (ALU) tiers were determined based on each site's VSCI score (VDEQ, 2006, 2008) on the following scale: excellent ( $\geq$ 73), good (60–72), stress (43–59), and severely stressed ( $\leq$ 42).

## Nutrient uptake

We measured ammonium uptake lengths (Webster & Valett, 2006) during the growing season (Summer 2009) in each stream. A co-injection of ammonium (as NH<sub>4</sub>Cl) and a conservative tracer (NaCl) were added to the streams at a constant rate using a metering pump. After the conservative tracer had reached plateau (measured using a conductivity meter), samples were collected at seven transects within the study reach. Triplicates of stream water were collected and filtered through a 0.7 µm glass fiber filter at each transect. Water samples were then placed on ice until laboratory analysis for chloride and ammonium as previously described. Uptake length (S<sub>w</sub>) was calculated as the negative inverse of the regression slope of flow corrected ammonium concentration versus distance. We also calculated uptake velocity  $(v_f)$  and areal uptake (U):

$$v_{\rm f} = \frac{uz}{S_{\rm W}}$$

where u is stream velocity and z is depth, and

$$U = v_{\rm f}C$$

where C is ambient ammonium concentration (Webster & Valett, 2006).

## Ecosystem metabolism

During June 2009 we also measured stream metabolism (GPP and ER) at each stream using whole-stream/open channel methods with conservative gas (SF<sub>6</sub>) injections for estimating site-specific reaeration coefficients (Bott, 2006). DO, water temperature, and conductivity were measured at 2-min intervals over a 36-h period at each site using Hydrolab mini-sondes (Hach Environmental, Loveland, CO). Metabolism was calculated using the single-station method (Bott, 2006, Grace & Imberger, 2006). Net ecosystem metabolism (NEP) was calculated as GPP–ER for each site (Allan & Castillo, 2007).



We considered "structure" to not only be the physical and abiotic parameters of the streams, but also the macroinvertebrate community structure as estimated through EPT and VSCI data. Our "functional" data were measures of GPP, ER, NEP, and ammonium uptake. Basic descriptive statistics, including means, medians, and standard error were generated for all response variables. Differences due to site type (restored vs. unrestored) were assessed using an unequal variance t test ( $\alpha = 0.05$ ) for all functional and structural variables.

Spearman-rank correlations were performed to examine the relationships among a subset of major structural and functional variables. Structural variables assessed here included EPT, VSCI, habitat, and SC. SC was included as the only physicochemical variable due to its significant impact on invertebrates in coal mine impacted streams (Pond et al., 2008). Ammonium uptake and NEP were included as our only functional variables here, as GPP and ER were used in the calculation of NEP.

We then conducted a Principal Components Analysis (PCA) on all structural and functional data to determine which of our variables had a greater impact on the overall variation among streams, and potentially select variables that may indicate restoration success. Principal components with Eigen values above 1 were kept for further analysis (Rencher, 2002). Additional unequal variance *t* tests were performed on the most important principal components to test if these new combinations of variables would show significant difference between restored and unrestored streams. All statistics were performed using JMP v.8 (SAS Institute, Inc., Cary, NC).

#### Results

Habitat and macroinvertebrate community structure

Habitat scores of restored and unrestored streams were not significantly different (P=0.15), although unrestored streams had higher scores, on average (Table 1). Macroinvertebrate taxa richness varied from 7 to 16 among streams, but there were no significant differences between stream types



(Table 1). Both EPT richness and VSCI scores were significantly higher in the unrestored streams; invertebrate community data reflected stress in restored streams (Table 1). The number of EPT taxa was also not different between stream types (Table 1). Seven of the nine study streams showed some degree of stress, as determined by VSCI scores < 60, Lick Branch was the most severely stressed (Table 1). Two of the three unrestored streams, North Laurel and Powell River, had the highest habitat scores and a fully forested canopy, which was reflected by the "Good" macroinvertebrate score (Table 1).

# Abiotic and functional parameters

No significant differences were found between stream types for any physicochemical measures, although restored streams had nominally higher TDS, SC, and nitrate (Table 2). All streams were slightly alkaline, with pH values around 7.9 (Table 2). Background levels of NH<sub>4</sub><sup>+</sup> were below detection (5 μg l<sup>-1</sup>), but these well oxygenated streams had detectable concentrations of NO<sub>3</sub><sup>-</sup> (Table 2). Discharge was quite variable across streams (Table 2), with highest discharges at restored streams occurring at Lick Branch

and Critical Fork (78 and 80 1 s<sup>-1</sup>, respectively) and at Powell River (100 1 s<sup>-1</sup>) for unrestored streams.

GPP was significantly greater in restored streams (P=0.02), while ER was not (Table 2). All streams were net heterotrophic as determined by negative values of NEP (Table 2). Ammonium uptake was also similar between stream types, although with one high value at Lick Branch (124.0  $\mu$ g NH<sub>4</sub><sup>+</sup> m<sup>-2</sup> min<sup>-1</sup>) a restored stream; in the unrestored streams, the relatively low uptake of North Laurel (3.1  $\mu$ g NH<sub>4</sub><sup>+</sup> m<sup>-2</sup> min<sup>-1</sup>) had the most impact (Table 2).

# Relationships between structure and function

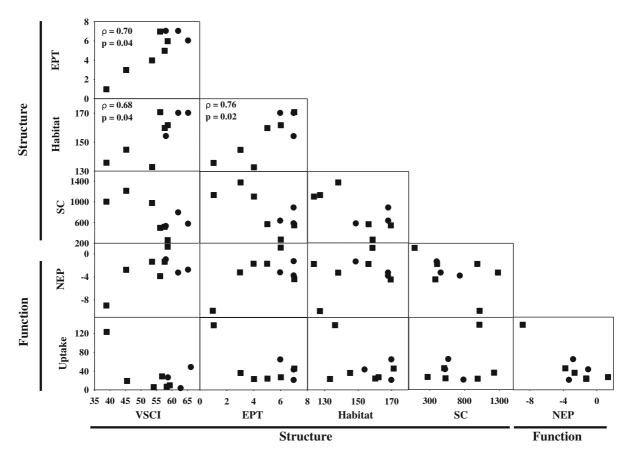
Spearman-rank correlations indicated no relationships between structural and functional measures (Fig. 2). On the other hand, several structural measures were correlated with one another, and habitat was most strongly correlated to metrics of invertebrate community structure ( $\rho = 0.68$ –0.76; Fig. 2). Additionally, both VSCI and EPT showed a negative, but non-significant, trend with SC, suggesting a greater stress on invertebrate communities at higher levels of SC (Fig. 2). More optimal habitats tended to have lower levels of SC (Fig. 2). None of the

Table 2 Physicochemical and functional metrics for restored and unrestored streams

|  | Restored $(n = 6)$ |                   |        | Unrestored $(n = 3)$ |                   |        |
|--|--------------------|-------------------|--------|----------------------|-------------------|--------|
|  | Range              | Mean ± SE         | Median | Range                | Mean ± SE         | Median |
| Physicochemical                                |                    |                   |        |                      |                   |        |
| DO $(mg l^{-1})$                               | 7.5–9.3            | $8.3 \pm 0.3$     | 8.3    | 7.2-9.2              | $8.2 \pm 0.6$     | 8.3    |
| pН   | 7.9-8.1            | $7.9 \pm 0.1$     | 7.9    | 7.7–7.9              | $7.8 \pm 0.1$     | 7.9    |
| TDS $(mg l^{-1})$                              | 324.2-706.8        | $673.8 \pm 169.9$ | 729.2  | 119.2-1223.6         | $529.8 \pm 111.4$ | 558.4  |
| SC ( $\mu$ S cm <sup>-1</sup> )                | 265.6-1220.1       | $750.9 \pm 151.7$ | 753.5  | 525.6-790.0          | $628.9 \pm 81.6$  | 571    |
| $NH_4^+ (\mu g \ l^{-1})$                      |                    | BD                |        |                      | BD                |        |
| $NO_3^- (mg l^{-1})$                           | 0.2-1.1            | $1.0 \pm 0.4$     | 0.8    | 0.1-1.3              | $0.7 \pm 0.4$     | 0.7    |
| $Q (1 s^{-1})$                                 | 3-80               | $41.3 \pm 14.9$   | 39.5   | 7–100                | $41.3 \pm 29.5$   | 17     |
| Temperature (°C)                               | 13.8-19.3          | $16.1 \pm 0.8$    | 16     | 15.5-23.2            | $18.3 \pm 2.4$    | 16.3   |
| Functional                                     |                    |                   |        |                      |                   |        |
| GPP (g $O_2 \text{ m}^{-2} \text{ day}^{-1}$ ) | 0.4-2.6            | $1.6 \pm 0.8*$    | 1.5    | 0.2-0.6              | $0.4 \pm 0.2*$    | 0.5    |
| $R (g O_2 m^{-2} day^{-1})$                    | 1.0-10.6           | $4.3 \pm 1.5$     | 3.2    | 1.2-3.9              | $2.8 \pm 1.4$     | 3.3    |
| NEP (g $O_2 \text{ m}^{-2} \text{ day}^{-1}$ ) | -8.9-1.3           | $-2.7 \pm 1.4$    | -2.0   | -3.3 to $-1$         | $-2.4 \pm 0.7$    | -2.8   |
| $U (\mu g NH_4^+ m^{-2} min^{-1})$             | 6.5-124.0          | $32.9 \pm 18.6$   | 15.1   | 3.1-47.9             | $25.6 \pm 12.9$   | 25.9   |

Asterisks (\*) indicate significance at P < 0.05. BD below detection, DO dissolved oxygen, TDS total dissolved solids, SC specific conductance, Q discharge, GPP gross primary production, R ecosystem respiration, NEP net ecosystem production, U ammonium uptake





**Fig. 2** Correlation matrix showing the relationships between selected structural and functional variables. All significant (P < 0.05) Spearman-rank correlation coefficients  $(\rho)$  and

associated P values are shown within individual scatterplots. Squares =restored streams, circles =unrestored streams

functional variables were correlated with one another, with trends being driven by one restored site, Lick Branch (Fig. 2). Restored and unrestored streams showed no distinct clustering on the bi-plots, further demonstrating the lack of difference between site types demonstrated in most of the earlier analyses (Fig. 2; Table 2).

Using structure and function to compare streams

The first three principal components explained 87% of the variation in our data set (Table 3). While PC 1 explained the most variation, the similar loadings onto that component (no loadings >0.4, Freund & Petty, 2007) made it difficult to determine the importance of any single variable (Table 3). In contrast, habitat, along with DO and Q to a lesser extent, were the most important factors loading onto PC 2, while nutrient-related factors, such as

ammonium uptake and nitrate concentrations were the most important for PC 3 (Table 3). There were no significant differences between restored and unrestored streams for any of the three principal components (t test, P > 0.05).

## Discussion

The mining influence was evident in all 9 of our streams as indicated by elevated SC. At regional reference sites not impacted by mining, SC concentrations are often <200 µS cm<sup>-1</sup> (Timpano et al., 2010), and all of our measured values were well above this level. SC has been a focus of recent work, implicating it as a major limiting factor in the successful colonization of sensitive invertebrate taxa (Hartman et al., 2005; Timpano et al., 2010), including mayflies (Pond et al., 2008; Pond, 2010).



**Table 3** Principle Components Analysis (PCA) and loadings for variables assessed in this study

| PCA               | PC 1  | PC 2  | PC 3  |
|-------------------|-------|-------|-------|
| Eigen value       | 6.79  | 1.98  | 1.61  |
| % of variation    | 56.6  | 16.5  | 13.4  |
| Cumulative %      | 56.6  | 73.1  | 86.5  |
| Variable loadings |       |       |       |
| VSCI              | -0.31 | 0.34  | 0.07  |
| EPT               | -0.33 | 0.3   | 0.04  |
| Habitat           | -0.22 | 0.52  | -0.16 |
| TDS               | 0.34  | 0.05  | 0.36  |
| SC                | 0.32  | -0.14 | -0.35 |
| DO                | 0.31  | 0.4   | 0.13  |
| pН                | 0.28  | -0.05 | -0.34 |
| $NO_3^-$          | 0.29  | 0.19  | 0.4   |
| Q                 | 0.29  | 0.41  | -0.06 |
| Temp              | -0.18 | -0.36 | 0.13  |
| NEP               | -0.3  | -0.05 | 0.36  |
| U                 | 0.27  | -0.05 | -0.52 |

In this study, there was a non-significant trend suggestive of the negative influence of SC on invertebrate community structure (Fig. 2), a relationship more strongly demonstrated by others (Pond et al., 2008; Pond, 2010). Although we did not directly measure mining-associated ions (e.g., sulfate, bicarbonate, calcium, and magnesium), it is likely that they were the major component driving elevated conductivity present in all streams (Timpano et al., 2010; Orndorff et al., 2010; Table 2). While unrestored sites generally demonstrated much higher habitat quality compared to the poor ratings associated with restored streams, the synergistic effect of high SC and poor habitat may have produced an unsuitable environment for indicator taxa in streams where macroinvertebrate community structure implicated poor water quality (Tables 1, 2; Fig. 2).

Even though many of our invertebrate communities in restored streams were 'stressed' based on VSCI scores (Table 1), our results fall within the range of streams in southwest Virginia (Burton & Gerritsen, 2003). Larger rivers, such as the South Fork of the Pound River had lower scores (27), while others of similar size, such as the South Fork of the Holston River, were higher (74.8; Burton & Gerritsen, 2003). Even within the same stream, VSCI may

vary seasonally, as in Stock Creek (21.7–72.6; Burton & Gerritsen, 2003). This implicates not only the effect of landscape, but season in affecting how results are obtained and interpreted. Therefore, more long-term data from the area must be collected in order to make firmer conclusions on invertebrate community structure.

Clearly, the variability in our stream reaches had a major influence on our ability to differentiate between restored and unrestored streams, and also our ability to find relationships between the structural and functional variables of interest. Bonta (2005) stressed that, in order to have comparable streams (reference), they must be undisturbed and on the same geology (and thus background geochemistry). Timpano et al. (2010) found reference sites within this region, but the size and discharge were much greater, and thus incomparable to, our six restored streams. All nine of our streams were thus indicative of the low-order, mining influenced condition of streams within the region.

Many of the results of this study were found to be either non-significant or opposite to the trends that were predicted. For example, the lack of differences between restored and unrestored streams would likely be due to two reasons. First, the similar watershed disturbance histories (all mined historically) would, by default, place all streams within the similar ranges of physicochemical variables seen here (Table 2). Secondly, all restoration sites were fairly young (Table 1), with 5 of 6 being fewer than 5 years old and the oldest being over 10 years old. The PCA results help clarify these points, as the most significant component (PC 1) had relatively similar loadings for all variables, implicating no one factor in the differentiation of site types here (Table 1). Multiple studies have concluded that restoration is a timedependent process, in which changes between site types may not be noticeable for decades (e.g., Charbonneau & Resh, 1992; Kondolf, 1995). Studies of ecosystem structural and functional restoration should focus on longer-term, repeated measures of both structure and function to track ecosystem resilience and recovery (Bonta, 2005; Palmer et al., 2005).

We consider it likely that greater differences in structural metrics of restored streams will occur with time (Kondolf, 1995). Woody vegetation has been planted and is growing within riparian areas of most



of the restored reaches; eventual maturation of this vegetation should improve habitat (Orzetti et al., 2010). Additionally, a growing riparian zone would be better able to retain and process N and P from within the watershed to reduce in-stream export of nutrients (Naiman & Decamps, 1997; Diebel et al., 2009), which would otherwise contribute to decreased water quality downstream (Alexander et al., 2007; Robertson et al., 2008; Paerl, 2009). TDS and SC is also likely to decline with further weathering of mining-disturbed materials within the streams' watersheds (Orndorff et al., 2010), although the length of time necessary (possibly centuries) to produce a significant decline remains unknown.

Low rates of nitrogen uptake in all streams would most likely be a result of disturbance to fluvial geomorphology and alterations in organic matter retention, either through past mining or restoration. In streams draining Fort Benning, GA, Roberts et al. (2007) noted extremely low levels of NH<sub>4</sub><sup>+</sup> uptake in streams with major upstream disturbances including major losses of riparian vegetation and sediment. The relatively low nutrient uptake was mainly a result of the lack of organic matter, which when added to the stream, significantly increased uptake rates (Roberts et al., 2007). In addition to biotic controls, discharge plays a major role in the ability of streams to process nutrients (Webster et al., 2001). Variations in discharge were driven, to a great extent, by rainfall patterns in this region. Low rates of autochthonous primary production, along with inconsistent patterns of discharge in our streams, may further explain the low levels of nutrient uptake.

Streams were net heterotrophic (negative NEP; Table 2), indicating high rates of respiration. This was expected in heavily shaded unrestored streams (Vannote et al., 1980), while it was more surprising in open-canopied restored streams. There seemed to be sufficient nitrate (Table 2), while phosphate was below detection (5  $\mu$ g l<sup>-1</sup>) in all streams (Northington, unpublished data), possibly indicating a P-limitation (Webster et al., 1991). Tordoff et al. (2000) has suggested that some mined streams may lose significant amounts of P due to the landscape disturbances of coal extraction. Alkaline waters, as seen in this study (Table 2), may then immobilize phosphate, making it unavailable as a nutrient for aquatic autotrophs (Boström et al., 1988). Alternately, enhanced microbial respiration has been associated with organic matter in mined streams (Schlief & Mutz, 2006), which may explain the altered metabolism. More long-term seasonal data on metabolism, organic matter processing, and land-scape fluxes of nutrients are needed to better establish mechanisms for these findings.

Even with no significant relationships found between structural and functional variables (Fig. 2), we believe that these measures should continue to be an active area of research. Our PCA analysis indicated the importance of both structural and functional measures (Table 3). Although PC1 showed no loading of any particular variable, structural and functional measures heavily loaded onto PC2 and PC3, respectively (Table 3). Although some studies have addressed this relationship in mined, restored streams (Fritz et al., 2010), the continued monitoring of structure (physicochemical, habitat, and invertebrate metrics) and additional forms of function (including leaf breakdown) should be included to give a more dynamic representation of ecosystem recovery (Palmer et al., 2005; Young et al., 2008; Young & Collier, 2009).

Lick Branch was a consistent outlier in all analyses in this study (Table 1; Fig. 2). This stream had fairly rapid discharge ( $\sim 80 \text{ 1 s}^{-1}$ ) due to consistent deep mine water drainage. Additionally, substrata at the site mainly consisted of large, sediment-covered boulders. This poor habitat was reflected in the highly skewed (and severely stressed, Table 1) benthic community, with 96% tolerant Ephemeroptera dominating 99% of the total abundance of macroinvertebrates found in the site (Northington, unpublished data). Sediment is well-known to be an impediment to macroinvertebrate colonization (Larsen & Ormerod, 2010), and paired with the generally degraded nature of the reach, was a major influence on the poor status of the site. The high areal uptake (Fig. 2) would normally be indicative of a stream efficient at cycling and utilizing nutrients due to high biotic demand (Webster et al., 2001). Here, it is more likely that ammonium was being adsorbed to the surfaces of rocks and deposited material (i.e., Richey et al., 1985; Triska et al., 1994) as opposed to being taken up by primary producers, especially since the low NEP (-8.9 g O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>; Fig. 2) indicated a highly heterotrophic system. Even with other physicochemical measures (e.g., SC, DO, pH) at Lick Brach within the range of those seen at the other



streams, the role of deep mine discharges into the channel must not be discounted.

#### **Conclusions**

The major theme of this study was to gather information on the functional and structural attributes of restored stream ecosystems in the coal mine regions of southwest Virginia in order to enhance our understanding of these systems. Although this is a small study with no pre-disturbance data for studied streams, we were able to collect valuable baseline information that will contribute to the knowledge of restoration practices and their effects on stream function in this region, including streams that have been reconstructed on coal mines.

We were unable to find evidence that restoration is currently having a significant positive or negative effect on ecosystem processes within these systems given the short time spans between restoration and this study and the lack of pre-restoration baseline data. Measured structural and functional metrics were highly variable; and most did not differ between streams on older coal mine streams, which had been reconstructed and restored, and unrestored streams in mining-impacted watersheds. Measured structural metrics did not exhibit direct relationships to functional indicators of stream process. Our findings demonstrate the importance of habitat on biotic condition in these mining-affected streams. It is likely that the indicators of restoration success will further change with time, if development of riparian vegetation improves channel habitat and if watershed processes cause an eventual conductivity decline, as would be expected. How much time will be required for such changes to occur, and how they would be likely to affect stream functional indicators are unknown. Further research is needed to capture effects of restoration that may come with time.

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#### References

- Alexander, R. B., E. W. Boyer, R. A. Smith, G. E. Schwarz & R. B. Moore, 2007. The role of headwater streams in downstream water quality. Journal of the American Water Resources Association 43: 41–59.
- Allan, J. D. & M. M. Castillo, 2007. Stream Ecology, 2nd ed. Springer, Dordrecht, The Netherlands: 436.
- American Public Health Association (APHA), 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC.
- Barbour, M. T., J. Gerritsen, B. D. Snyder & J. B. Stribling, 1999. Rapid Bioassessment Protocols for Use in Streams and Rivers; Periphyton, Benthic Macroinvertebrates, and Fish, 2nd ed. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 841-b-99-002.
- Bonta, J. V., 2005. Challenges in conducting hydrologic and water quality research in drastically disturbed watersheds. Journal of Soil and Water Conservation 60: 121–133.
- Boström, B., G. Persson & B. Broberg, 1988. Bioavailability of different phosphorous forms in freshwater systems. Hydrobiologia 170: 133–155.
- Bott, T. L., 2006. Primary productivity and community respiration. In Hauer, F. R. & G. A. Lamberti (eds), Methods in Stream Ecology, 2nd ed. Elsevier, San Diego, CA: 663–690.
- Bukaveckas, P. A., 2007. Effects of channel restoration on water velocity, transient storage, and nutrient uptake in a channelized stream. Environmental Science and Technology 41: 1570–1576.
- Burton, J. & J. Gerritsen, 2003. A Stream Condition Index for Virginia Non-Coastal Streams. Report prepared for Virginia DEQ and US EPA by Tetra-Tech, Inc. Owings Mills, Maryland [available on internet at www.deq. state.va.us/watermonitoring/pdf/vastrmcon.pdf].
- Charbonneau, R. & V. H. Resh, 1992. Strawberry Creek on the University of California, Berkeley Campus: a case history of urban stream restoration. Aquatic Conservation: Marine and Freshwater Ecosystems 2: 293–307.
- Choi, Y. D., V. M. Temperton, E. B. Allen, A. P. Gootjans, M. Halassy, R. J. Hobbs, M. A. Naeth & K. Torok, 2008. Ecological restoration for future sustainability in a changing environment. Ecoscience 15: 53–64.
- Clements, W. H., N. K. M. Vieira & S. E. Church, 2010. Quantifying restoration success and recovery in a metalpolluted stream: a 17-year assessment of physicochemical and biological responses. Journal of Applied Ecology 47: 899–910.



- Diebel, M. W., J. T. Maxted, D. M. Robertson, S. Han & M. J. Vander Zanden, 2009. Landscape planning for agricultural nonpoint source pollution reduction III: assessing phosphorous and sediment reduction potential. Environmental Management 43: 69–83.
- Freund, J. G. & J. T. Petty, 2007. Response of fish and macroinvertebrate bioassessment indices to water chemistry in a mined Appalachian watershed. Environmental Management 39: 707–720.
- Fritz, K. M., S. Fulton, B. R. Johnson, C. D. Barton, J. D. Jack, D. A. Word & R. A. Burke, 2010. Structural and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine. Journal of the North American Benthological Society 29: 673–689.
- Gordon, N. D., T. A. McMahon & B. L. Finlayson, 2004. Stream Hydrology: An Introduction for Ecologists, 2nd ed. Wiley, New York, NY.
- Graca, M. A. S., 2001. The role of invertebrates on leaf litter decomposition in streams—a review. International Review of Hydrobiology 86: 383–393.
- Grace, M. R. & S. J. Imberger, 2006. Stream Metabolism: Performing & Interpreting Measurements. Water Studies Centre Monash University, Murray Darling Basin Commission and New South Wales Department of Environment and Climate Change: 204 [available on internet at http://www.sci.monash.edu.au/wsc/docs/tech-manual-v3. pdf].
- Hartman, K. J., M. D. Kaller, J. W. Howell & J. A. Sweka, 2005. How much do valley fills influence headwater streams? Hydrobiologia 532: 91–102.
- Higgs, E. S., 1997. What is good ecological restoration? Conservation Biology 11: 338–348.
- Hobbs, R. J. & J. A. Harris, 2001. Restoration ecology: repairing the Earth's ecosystems in the New Millennium. Restoration Ecology 9: 239–246.
- Keystone Stream Team (KST), 2007. Guidelines for Natural Stream Channel Design for Pennsylvania waterways [available on internet at http://www.keystonestreamteam.org/docs/NSCD\_Chaps/NSCD\_Guidelines\_03.31.07.pdf].
- Kondolf, G. M., 1995. Five elements for effective evaluation of stream restoration. Restoration Ecology 3: 133–136.
- Lake, P. S., N. Bond & P. Reich, 2007. Linking ecological theory with stream restoration. Freshwater Biology 52: 597–615.
- Larsen, S. & S. J. Ormerod, 2010. Low-level effects of inert sediments on temperate stream invertebrates. Freshwater Biology 55: 476–486.
- Lecerf, A. & E. Chauvet, 2008. Diversity and functions of leafdecaying fungi in human-altered streams. Freshwater Biology 53: 1658–1672.
- Lefcort, H., J. Vancura & E. L. Lider, 2010. 75 years after mining ends stream insect diversity is still affected by heavy metals. Ecotoxicology 19: 1416–1425.
- Locke, B. A., D. S. Cherry, C. E. Zipper & R. J. Currie, 2006. Land use influences and ecotoxicological ratings for upper Clinch River tributaries in Virginia. Archives of Environmental Contamination and Toxicology 51: 197–205.
- McClurg, S. E., J. T. Petty, P. M. Mazik & J. L. Clayton, 2007. Stream ecosystem response to limestone treatment in acid

- impacted watersheds of the Allegheny Plateau. Ecological Applications 17: 1087–1104.
- Merovich, G. T. & J. T. Petty, 2010. Continuous response of benthic macroinvertebrate assemblages to a discrete disturbance gradient: consequences for diagnostic stressors. Journal of the North American Benthological Society 29: 1241–1257.
- Merovich, G. T., J. M. Stiles, J. T. Petty, P. F. Ziemkiewicz & J. B. Fulton, 2007. Water chemistry-based classification of streams and implications for restoring mined watersheds. Environmental Toxicology and Chemistry 26: 1361–1369.
- Merritt, R. W., K. W. Cummins, & M. B. Berg, eds. 2008. An introduction to the aquatic invertebrates of North America, 4th ed. Kendall Hunt Publishing Company, Dubuque, IA, USA.
- Muotka, T., R. Paavola, A. Haapala, M. Novikmec & P. Laasonen, 2002. Long-term recovery of stream habitat structure and benthic invertebrate communities from instream restoration. Biological Conservation 105: 243–253.
- Mutz, M., 1998. Stream system restoration in a strip-mining region, eastern Germany dimension, problems, and first steps. Aquatic Conservation: Marine and Freshwater Ecosystems 8: 159–166.
- Mutz, M. & J. Schlief, 2005. Scenario for undirected progression of extremely acidic streams in the Lusatian postmining landscape, Germany. Ecological Engineering 24: 59–65.
- Naiman, R. J. & H. Decamps, 1997. The ecology of interfaces: riparian zones. Annual Reviews of Ecology and Systematics 28: 621–658.
- Orndorff, Z., W. L. Daniels, M. Beck & M. Eick, 2010. Leaching potentials of coal spoil and refuse: acid-base interactions and electrical conductivity. In ASMR (ed.), Proceedings, 27th National Meeting of the American Society of Mining and Reclamation (ASMR). ASMR, 3134 Montevista Road, Lexington, KY, USA: 736–766.
- Orzetti, L. L., R. C. Jones & R. F. Murphy, 2010. Stream condition in piedmont streams with restored riparian buffers in the Chesapeake Bay watershed. Journal of the American Water Resources Association 46: 473–485.
- Paerl, H. W., 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential. Estuaries and Coasts 32: 593–601.
- Palmer, M. A., E. S. Bernhardt, J. D. Allan, P. S. Lake, G. Alexander, S. Brooks, J. Carr, S. Clayton, C. N. Dahm, J. F. Shah, D. L. Galat, S. G. Loss, P. Goodwin, D. D. Hart, B. Hassett, R. Jenkinson, G. M. Kondolf, R. Lave, J. L. Meyer, T. K. O'Donnell, L. Pagano & E. Sudduth, 2005. Standards for ecologically successful river restoration. Journal of Applied Ecology 42: 208–217.
- Palmer, M. A., E. S. Bernhardt, W. H. Schlesinger, K. N. Eshelman, E. Foufoula-Georgiou, M. S. Hendryx, A. D. Lemly, G. E. Likens, O. L. Loucks, M. E. Power, P. S. White & P. R. Wilcock, 2010. Mountaintop mining consequences. Science 327: 148–149.
- Peng, S. S., 2000. Mountaintop removal controversy slows West Virginia coal mining. Mining Engineering 52: 55–58.



- Petty, J. T., J. B. Fulton, M. P. Strager, G. T. Merovich, J. M. Stiles & P. F. Ziemkiewicz, 2010. Landscape indicators and thresholds of stream ecological impairment in an intensively mined Appalachian watershed. Journal of the North American Benthological Society 29: 1292–1309.
- Pond, G. J., 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). Hydrobiologia 641: 185–201.
- Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds & C. J. Rose, 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society 27: 717–737.
- Rencher, A. C., 2002. Methods of Multivariate Analysis. Wiley, New York, NY.
- Richey, J. S., W. H. McDowell & G. E. Likens, 1985. Nitrogen transformations in a small mountain stream. Hydrobiologia 124: 129–139.
- Roberts, B. J., P. J. Mulholland & J. N. Houser, 2007. Effects of upland disturbance and instream restoration on hydrodynamics and ammonium uptake in headwater streams. Journal of the North American Benthological Society 26: 38–53.
- Robertson, D. M., S. G. Schladow & C. G. Holdren, 2008. Long-term changes in the phosphorous loading to and trophic state of the Salton Sea, California. Hydrobiologia 604: 21–36.
- Schlief, J. & M. Mutz, 2005. Long-term leaf litter decomposition and associated microbial processes in extremely acidic (pH < 3) mining waters. Archiv für Hydrobiologie 164: 53–68.
- Schlief, J. & M. Mutz, 2006. Palatability of leaves conditioned in streams affected by mine drainage: a feeding experiment with *Gammarus pulex* (L.). Hydrobiologia 563: 445–452.
- Simon, K. S., M. A. Simon & E. F. Benfield, 2009. Variation in ecosystem function in Appalachian streams along an acidity gradient. Ecological Applications 19: 1147–1160.
- Timpano, A. J., S. H. Schoenholtz, C. E. Zipper & D. J. Soucek, 2010. Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield streams. Proceedings, National Meeting of the American Society of Mining and Reclamation. ASMR, 3134 Montevista Road, Lexington, KY, USA: 1284–1302.
- Tordoff, G. M., A. J. M. Baker & A. J. Willis, 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41: 219–228.
- Triska, F. J., A. P. Jackman, J. H. Duff & R. J. Avanzino, 1994. Ammonium sorption to channel and riparian sediments: a transient storage pool for dissolved inorganic nitrogen. Biogeochemistry 26: 67–83.
- United Nations, 2009 World Population Prospects, the 2008 Revision [available on internet at http://esa.un.org/unpd/wpp2008/index.htm].
- United State Department of Energy (USDOE), 2011. Energy Information Administration [available on internet at http://www.eia.doe.gov].

- United States Environmental Protection Agency (USEPA), 2005. Mountaintop Mining/Valley Fills in Appalachia: Final Programmatic Environmental Impact Statement [available on internet at http://www.epa.gov/Region3/mtntop/].
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing, 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.
- Virginia Department of Environmental Quality (VDEQ), 2006.

  Using probabilistic monitoring data to validate the noncoastal Virginia Stream Condition Index. Water Quality
  Monitoring, Biological Monitoring and Water Quality
  Assessment Programs. Richmond, Virginia. VDEQ
  Technical Bulletin WQA/2006-001.
- Virginia Department of Environmental Quality (VDEQ), 2007. Water Quality Assessment Guidance Manual for Y2008. Division of Water Quality Programs, Richmond, VA.
- Virginia Department of Environmental Quality (VDEQ), 2008. Biological Monitoring Program Quality Assurance Project Plan for Wadeable Streams and Rivers. Water Quality Monitoring and Assessment Programs, Richmond, VA.
- Vörösmarty, C. J., P. Green, J. Salisbury & R. B. Lammers, 2000. Global water resources: vulnerability from climate change and population growth. Science 289: 284–288.
- Webster, J. R. & H. M. Valett, 2006. Solute dynamics. In Hauer, F. R. & G. A. Lamberti (eds), Methods in Stream Ecology, 2nd ed. Elsevier, San Diego, CA: 169–185.
- Webster, J. R., D. J. D'Angelo & G. T. Peters, 1991. Nitrate and phosphate uptake in streams at Coweeta Hydrologic Laboratory. Verhandlungen des Internationalen Verein Limnologie 24: 1681–1686.
- Webster, J. R., J. L. Tank, J. B. Wallace, J. L. Meyer, S. L. Eggert, T. P. Ehrman, B. R. Ward, B. L. Bennett, P. F. Wagner & M. E. McTammany, 2001. Effects of leaf litter exclusion and wood removal on phosphorous and nitrogen retention in a forest stream. Verhandlungen des Internationalen Verein Limnologie 27: 1337–1340.
- Weijters, M. J., J. H. Janse, R. Alkemade & J. T. A. Verhoeven, 2009. Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 19: 104–112.
- White, T. J., 2007. Sharing resources: the global distribution of the ecological footprint. Ecological Economics 64: 402–410.
- Young, R. G. & K. J. Collier, 2009. Contrasting responses to catchment modification among a range of functional and structural indicators of river ecosystem health. Freshwater Biology 54: 2155–2170.
- Young, R. G., C. D. Matthaei & 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.

