

## HOW MUCH DO VALLEY FILLS INFLUENCE HEADWATER STREAMS?<sup>1</sup>

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**Abstract.** Valley fill mining has the potential to alter headwater stream habitat in many areas in the eastern United States. In valley fill mining, overburden is removed to expose underlying coal seams. The overburden is then deposited in the adjacent valley. The deposited overburden from mining increases sedimentation, increases stream conductivity, and alters hydrologic regimes downstream of the fill. Changes in downstream communities are not well documented. However, it was suspected the increased sedimentation and conductivity would have deleterious effects upon the downstream macroinvertebrate communities. In southern West Virginia, four pairs of streams, each consisting of a fill and a reference stream, were selected as representative of watersheds experiencing valley fill mining. Stream pairs were selected for similar environmental conditions, with one stream having a valley fill in its headwaters. Each stream was sampled by replicate Surber samples (N = 9 per stream). Water chemistry and sediment measurements also were taken at each location. Valley fill streams had significantly higher specific conductance ( $p < 0.01$ ), but did not have elevated levels of fine sediment. Fills also had significantly elevated levels of Na, K, Mn, Mg, Ca, Ni and Fe relative to reference streams. Additionally, valley fill streams had significantly lower densities of Ephemeroptera, Coleoptera, Odonata, Non-insects, Scrapers, and Shredders ( $p < 0.03$ ) than reference streams. Further, Ephemeroptera richness was negatively related to specific conductivity and many of the richness metrics were negatively related to metals, both of which were generally elevated in fill streams. It appears that at the minimum, valley fills increase specific conductance and metals in streams and this or some other unqualified factors structure the macroinvertebrate community downstream of the valley fill. However, given the level of disturbance in valley fills, it is surprising how little differences existed between fills and reference stream biota.

Additional Key Words: macroinvertebrate, Ephemeroptera, specific conductance.

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## **Introduction**

Valley fills are a by-product of mountaintop mining activities (Peng 2000). In mountain top mining, overburden layers, typically the peaks of mountains are removed to gain access to coal layers (US EPA 1984; White and Barata 1995). The overburden is deposited into the valleys between mountain peaks where it is eventually graded flat and seeded with various species of grasses (Peng 2000). In many cases, this valley fill may bury several kilometers of headwater stream (Peng 2000) and removes the area of the watershed associated with that stream from providing allochthonous inputs of deciduous organic matter from the formerly forested watershed.

Once filled, those sections of headwater streams are permanently removed from production of aquatic invertebrates and fishes. Of considerable interest is the impact and duration of impacts in waters downstream of the fill locations. The magnitude of this type of surface disturbance undoubtedly alters the natural hydrological cycle, opens the canopy, which may permit warming of stream temperatures, and potentially increases sedimentation in streams. Fine sediment is known to be negatively related to stream invertebrate abundance and diversity (Waters 1995). However, there may be mitigating effects of the valley fills upon stream discharge. It is believed that fills may release water more slowly than natural watersheds during rainfall events. This may stabilize flows in response to rainfall and drought events.

Sediment from mining activities increases stream turbidity as well as filling and coating the stream substrate (Nelson et. al. 1991). Fine sediment particles reduce macroinvertebrate populations and community diversity when interstitial spaces between substrate particles are filled and substrate surfaces are coated by fine sediment (Sandine 1974; Richards and Bacon 1994; Vouri and Joensuu 1996). When macroinvertebrate populations are reduced, energy resources available for organisms higher in the trophic cascade are reduced. This limits the biomass of fish and other organisms that are supported by aquatic macroinvertebrates.

The legacy of past land use has been shown to be an influence in aquatic biota long after the event (Harding et. al. 1998). Valley fill mining permits have become common in the eastern U.S. only in the last ten years. Therefore, valley fill mining does not have the depth of research into long-term effects as deep shaft or placer mining (US EPA 1984). The literature is rich with long-term examples of the influences of logging and farming (Harding et. al. 1998). However,

the tremendous changes from filling a valley with overburden are probably different than the consequences of vegetation removal or manipulation. The long-term ramifications of removal of headwater and early order streams in regional stream networks are currently unknown. Therefore the objective of this study was to evaluate the impacts of valley fills upon aquatic biota by examining how fills differ from reference streams with respect to basic water quality parameters, and to examine how these differences may influence abundance and diversity of aquatic macroinvertebrates.

### **Methods**

To evaluate the possible biotic effects of valley fills upon instream biota we conducted a survey of aquatic macroinvertebrates in four pairs of streams in southern West Virginia. Sites were selected based upon several criteria. Streams used in the study were typically two similarly sized, proximal tributaries, or forks of a single stream with similar habitat characteristics. The most important, and limiting, factor in selecting streams was flow. Many streams in the region were intermittent or very small perennial streams. Intermittent streams were excluded due to concerns over different taxonomic composition in perennial versus intermittent streams (Thorp and Covich 1991; Feminella 1996). Additional criteria for fill-stream selection were that streams had passed Phase I reclamation release (fills had been seeded with grasses) and the presence of a proximate reference stream without an excess of other anthropogenic activities such as gas line crossings, industrial dumps, etc.

All study streams are first-order headwater streams located in Boone County, West Virginia. These streams are all within a 21 km radius of the Arch Coal Hobet 21 Facility in Madison, W Va. Stream pairs were selected on the basis of similar geology, stream order and depth. The four pairs of study streams were: Atkins Creek West Branch (reference) and Atkins Creek East Branch (fill), North branch of Sugar Tree Creek (reference) and South branch of Sugar Tree Creek (fill), Big Buck Fork (reference) and Hill Fork (fill), Bend Branch (reference) and Rockhouse Creek (fill), (Fig. 1). Sediment holding ponds on fill streams were typically located near the mouths of streams and all such ponds were downstream of study stream sections and believed to exert no influence on upstream macroinvertebrate fauna.

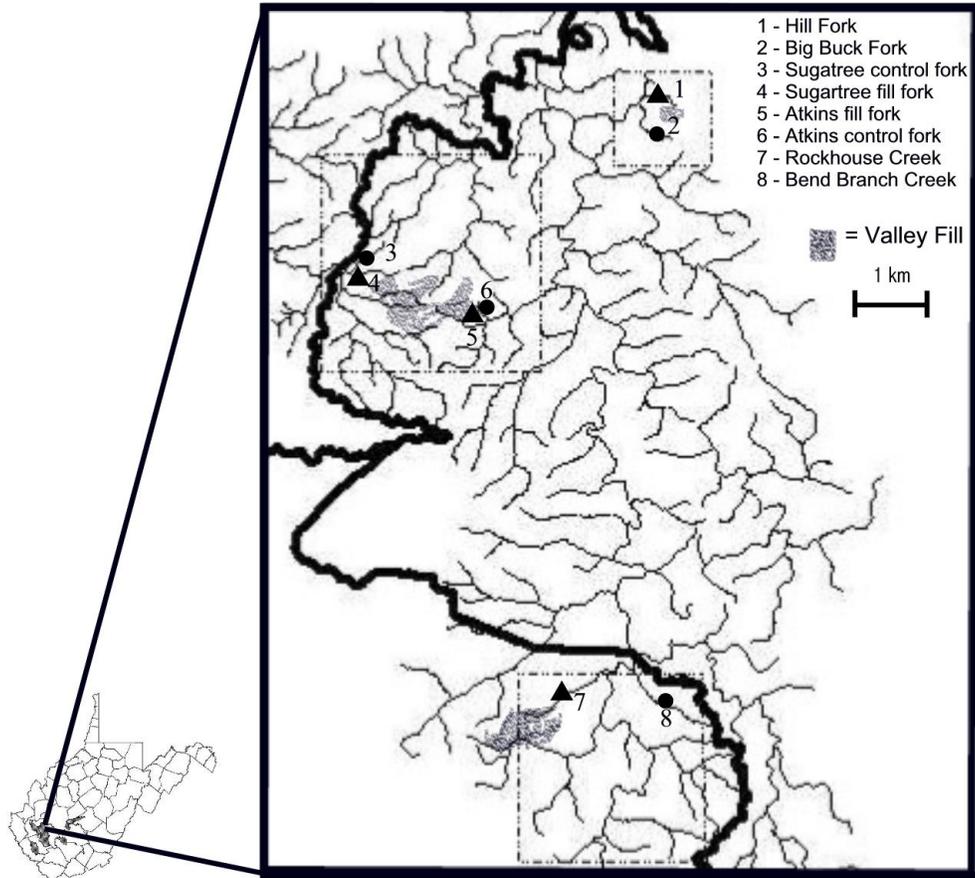


Figure 1. Location of the four pairs of study streams and approximate sampling locations. Reference sites are denoted with the filled circles and fill sites are denoted with the filled triangles.

Streams were sampled for aquatic macroinvertebrates during 26-28 May 1999. Stream samples consisted of three replicate Surber (20 x 25 cm with 0.25 mm mesh) samples taken at three different riffles within each stream (Rabeni 1996). Thus, a total of nine Surber samples were collected from each stream. The exception to this was for the Atkins Creek reference site

where sufficient water to sample was found in only two stations yielding a total of six Surber samples. Selection of riffles was made in fill sites beginning 300 m from the base of the fill and continuing in 300 m increments. Sample sites within the reference streams were sited to be of similar watershed area to the initial upstream fill site based upon the original contours of fill sites. Reference sample sites also continued every 300 m downstream from the uppermost site.

Immediately after collection all aquatic insects and other matter retained in the Surber sampler were placed in plastic containers and field preserved in 70% ethanol. Water quality information (temperature, dissolved oxygen, specific conductance) was also recorded (N = 3 per stream) simultaneously with each macroinvertebrate collection location and each site was scored for habitat following the Rapid Bioassessment Protocol (USEPA 1989). We felt discharge and hydrology may be important in limiting macroinvertebrates based on concurrent (Kaller 2001) and previous studies (Thorp and Covich 1991; Boulton et. al. 1992; Feminella 1996) and also felt it may be a mitigating factor in evaluating the impact of valley fills upon streams. Discharge and water chemistry measurements were not made during the original sampling therefore, during May 2000, we returned to each stream to measure flow rates and estimate relative discharge among the streams and on 06 June 2001 we collected storm water samples for water chemistry analysis. Although it would have been desirable to collect these measures concurrently with benthic macroinvertebrate collections, water quality samples were taken during the same time of year and likely reflect conditions similar to those during macroinvertebrate sampling. Discharge was calculated from flow rates measured at five positions along an across-stream transect at each study section using standard methods (Gallagher and Stevenson 1999). Water sampled from each station (3 per stream) were chilled with ice and transported (within 24 h of collection) to the water chemistry laboratory at the Appalachian Environmental Laboratory in Frostburg, Maryland where they were analyzed for alkalinity (method 310.1, US EPA 1999a), pH (method 19, US EPA 1987), and metals (method 6020A, 7000B, US EPA 1999b).

We also revisited the streams during December 1999 to gather substrate samples for measurement of sediment composition in each stream. Measurements were taken late in the year due to a belief that reduced transport of fine particles during the fall and winter would give a more representative picture of the fill effect (Murphy and Meehan 1991; Swanston 1991). Two replicate scoop samples were taken at all three macroinvertebrate collection stations in all streams (N = 6 samples per stream). Scoop samples (approximately 2700 cm<sup>2</sup>) have been shown

to provide comparable results to the shovel method for substrate sampling (Grost 1991; Hakala 2000). All substrate samples were oven dried at 60 C for 7 d and then were shaken through a set of modified Wentworth sieves (McMahon et. al. 1996). Sieves sizes included: 32, 16, 8, 4, 2, 1, 0.5, 0.125, 0.063 mm and pan (< 0.063 mm) (McMahon et. al. 1996).

Surber samples were returned to the laboratory for analysis. All samples were stained with rose Bengal solution to facilitate detection of organisms when picking through samples (Rabeni 1996). Samples were processed through two sieves, 1 mm and 0.25 mm. All aquatic invertebrates were counted and identified to the Family level except for organisms of 0.25 – 1.00 mm (mostly Chironomidae) which were subsampled from the small fraction and identified to Family (Angradi 1999). Subsampling employed volumetric dilution to 500 ml with ten 10 ml aliquots removed after thorough mixing from agitation from introduced air (Angradi 1999; Kaller 2001).

Following enumeration and identification the macroinvertebrates were analyzed by calculating several density-related metrics. Density metrics were evaluated at the level of taxonomic order (Ephemeroptera, Plecoptera, Trichoptera, Diptera, Odonata , Coleoptera) as well as by total, Non-insect, and Family Chironomidae density. Functional metrics of collector density, scraper density, and shredder density were also evaluated. We also calculated percent compositional metrics and richness metrics for Ephemeroptera, Plecoptera, and Trichoptera using pooled samples for each stream.

After these initial comparisons the metrics were correlated with water quality, chemistry, and sediment in an effort to determine possible factors explaining differences between treatments. Simple descriptive statistics (mean, standard deviation, minimum, maximum) were calculated for metrics for each stream for comparison. Comparison of the invertebrate metrics in fills versus reference streams was done using Wilcoxon two-sample t-test because the data were not normally distributed (Shapiro-Wilk test). Analysis of water quality and chemistry data was done using a randomized complete block ANOVA. Analysis of sediment data was done using a randomized complete block ANOVA following arcsin-squareroot transformation of the data blocking on stream pair to detect differences between fills and reference streams. Correlation of the metrics with water quality/chemistry and sediment was done using Pearson's Correlation coefficients after arcsin-squareroot transformation of any percent data. In statistical tests involving water chemistry some samples fell below the detection limits for a given element. In

those instances values were set to the detection limit, which should serve as a conservative measure in comparisons between fill and reference streams. We also conducted principle component analysis (PCA) on water quality variables and used stepwise regression (SWR) on the principal components to develop models relating macroinvertebrate density metrics to water quality parameters. Principle components were included in stepwise regression models if  $p < 0.15$ . All statistical procedures were done using SAS 8.0.

## **Results**

### **Water quality, Substrate, and Rapid Bioassessment Protocol Scores**

At the time of macroinvertebrate collections stream pairs (fill X reference) were not significantly different with respect to most simple water quality and habitat variables measured. Streams within pairings did not differ in pH, dissolved oxygen, or temperature (Table 1). Significant differences ( $p < 0.01$ ) were noted in all stream pairings for specific conductance with higher specific conductance in fill streams.

Water chemistry and analytical analyses collected during storm water discharge measured in June 2001 detected other differences between fills and reference streams (Table 2). Many elements were found in higher concentrations in the fill streams than in the reference streams (Table 2). Fills had significantly higher pH, alkalinity, calcium, sodium, and potassium than reference streams. In addition, the metals magnesium, copper, nickel, manganese and iron were significantly higher than in the reference streams. Reference streams had higher levels of aluminum than fills.

Substrate analyses failed to detect differences in fine sediment composition (percent  $< 0.5$  mm) between pairs of fill and reference streams (Table 3). Therefore, over the limited temporal scale in which we sampled, valley fills did not appear to significantly increase fine sediment bedloads in the study streams.

Rapid Bioassessment Protocol (RBP) scores generated for each stream indicated that stream pairs were very similar with respect to physical habitat (Table 4). RBP scores ranged from 82 - 211. Most RBP scores from reference and fill streams were indicative of low stream quality. The very similar scores of Hill Fork - Big Buck Fork and Rockhouse Creek - Bend Branch were reflective of the similarity of the streams in the pairs. However, the RBP scores of Rockhouse

Creek - Bend Branch were both much higher than in the other streams and in a range more typical of streams of intermediate quality in the central Appalachians.

#### Macroinvertebrate Abundance and Community Metrics

Six of the macroinvertebrate metrics were significantly different between valley fills and reference streams (Table 5). The densities of ephemeropterans, coleopterans, Odonata, and non-insects were significantly lower ( $p < 0.01$ ) in fills than in reference streams. In addition, the metrics scraper density and shredder density were also lower in fills than in reference streams ( $p < 0.03$ ). There were no differences in total density of aquatic insects or any of the other macroinvertebrate metrics between fills and reference streams.

#### Relationships between taxa and water quality

Many of the macroinvertebrate metrics were negatively correlated with heavy metal concentrations in the storm samples (Table 6). Ephemeroptera density was negatively related to calcium, copper, iron, manganese, and nickel ( $p < 0.04$ ). EPT density was negatively related to calcium, manganese, and nickel ( $p < 0.05$ ). Plecoptera density was negatively related to cadmium ( $p < 0.04$ ). Pearson's correlation coefficients ranged from  $-0.70$  to  $-0.98$  for the significant relationships (Table 6). Percent Ephemeroptera was negatively related to copper and nickel ( $p < 0.04$ ). Ephemeroptera richness was negatively correlated with specific conductivity ( $p < 0.05$ ). None of the metrics were correlated with fine sediment levels in the streams.

Principle component analysis (PCA) is a multivariate statistical method that is very useful for reducing the number of variables in a data set and for obtaining two-dimensional views of a multi-dimensional data. Using PCA we identified three principle components of water quality measures that explained 90.78% of the variation in water quality between streams. Many of the water quality variables, especially specific conductivity and heavy metals were significantly correlated with principle component 1 (PC1, Table 7). Stepwise regression shows strongest relationships between Ephemeroptera-related metrics (EPT Richness and Ephemeroptera Richness) and PC1, suggesting the factors associated with PC1 (metals, specific conductivity and alkalinity) negatively influence these taxa (Table 8).

Table 1. Summary of water quality characteristics in the study streams. Mean values followed by the standard error for each water quality parameter for three samples (one at each site) in each stream are listed.

Stream		pH	S. E.	Temperature	S. E.	Dissolved oxygen (mg/L)	S. E.	Specific conductance (muhm/s)	S. E.
Atkins Creek	Fill	7.4	0.3	14.8	0.3	13.0	0.3	1479.0	55.3
	Reference	7.0	0.2	15.0	0.8	12.6	0.3	133.0	42
Hill Fork	Fill	7.5	0.2	10.0	0.7	13.0	0.3	502.0	49.2
Big Buck Fork	Reference	6.7	0.3	13.2	1.2	13.4	0.2	160.1	40.2
Rockhouse Creek	Fill	7.2	0.3	12.8	0.6	9.3	0.3	1024.3	108.3
Bend Branch	Reference	6.5	0.3	15.2	0.3	11.4	0.2	47.6	1.2
Sugar Tree Creek	Fill	7.5	0.5	17.6	0.3	9.1	0.5	1198.6	24.9
	Reference	6.5	0.2	16.2	0.6	8.5	0.4	259.7	15.3

Table 2. Mean storm water quality measures (N = 3 per stream) from the study streams from samples during June 2001. Measures with an asterisk represent those that were significantly higher in fills than in reference streams. Measures of Ag, As, Cd, Cr, Pb, and Sb were generally below detection limits. P-values for comparisons are provided under Significance.

Measurement	Fills		Reference		Significance
	Mean	Range	Mean	Range	
* Alkalinity (mg/l)	163.4	16.2 – 319.3	12.8	0.4 – 46.8	0.001
pH	7.7	6.9 – 8.2	7.2	6.7 – 7.7	0.030
* Na (mg/l)	10.4	3.9 – 22.3	2.9	0.8 – 3.1	0.002
* K (mg/l)	10.2	1.8 – 14.4	3.3	1.5 – 5.1	0.001
* Mg (mg/l)	86.4	4.9 – 125.6	23.0	2.2 – 51.5	0.001
* Ca (mg/l)	126.5	5.9 – 202.2	36.5	2.6 – 67.3	0.003
* Cu (µg/l)	1.2	0.5 – 1.8	0.8	0.2 – 1.9	0.040
* Ni (µg/l)	24.8	<0.3 – 51.2	7.6	<0.3 – 18.4	0.003
Zn (µg/l)	2.8	0.9 – 8.6	2.7	1.4 – 4.7	N.S.
* Mn (µg/l)	62.3	2.0 – 167.8	19.0	1.6 – 45.5	0.002
Al (µg/l)	18.5	0.9 – 74.0	12.3	9.0 – 18.5	0.010
* Fe (µg/l)	47.2	<0.5 – 82.4	15.8	1.4 – 30.2	0.004

\* Detection limits (ppb) were: Al (0.057), Cr (0.805), Ni (0.298), Cu (0.113), Zn (0.045), As (0.180), Cd (0.015), Ag (0.046), Sb (0.015), Pb (0.063), Mn (0.005), Al (0.057), and Fe (0.529).

Table 3. Substrate composition in study streams determined as percent less than the size listed. Means as well as standard errors are reported for 6 sediment samples taken in each stream.

Stream	Type	2 mm	S. E.	1 mm	S. E.	0.5 mm	S. E.	0.25 mm	S. E.	0.125 mm	S. E.	0.063 mm	S. E.
Atkins Creek	Fill	0.46	0.10	0.35	0.05	0.23	0.04	0.08	0.03	0.03	0.02	0.02	0
	Reference	0.35	0	0.28	0	0.18	0	0.06	0	0.02	0	0.01	0
Hill Fork	Fill	0.50	0.06	0.42	0.05	0.25	0.03	0.06	0.01	0.01	<0.01	0.01	<0.01
Big Buck Fork	Reference	0.78	0.03	0.67	0.03	0.43	0.03	0.13	0.03	0.03	<0.01	0.00	<0.01
Rockhouse Creek	Fill	0.23	0.02	0.16	0.02	0.09	0.01	0.02	<0.01	0.01	<0.01	0.01	<0.01
Bend Branch	Reference	0.25	0.07	0.21	0.05	0.11	0.02	0.02	<0.01	0.01	<0.01	0.00	<0.01
Sugar Tree Creek	Fill	0.50	0.04	0.35	0.03	0.18	0.02	0.04	<0.01	0.01	<0.01	0.01	<0.01
	Reference	0.27	0.02	0.22	0.01	0.13	0.01	0.04	<0.01	0.01	<0.01	0	0

Table 4. Summary of Rapid Bioassessment scores for the study streams. Final components listed left bank/right bank.

Component	Atkins Creek		Rockhouse-Bend Branch		Hill Fork-Big Buck		Sugar Tree Creek	
	Fill	Reference	Fill	Reference	Fill	Reference	Fill	Reference
Epifaunal Substrate	18	10	19	19	10	10	4	19
Pool Substrate	9	10	17	17	5	5	9	12
Pool Variability	10	5	19	19	7	9	8	12
Sediment Deposition	10	8	19	20	5	4	16	13
Channel Flow Status	12	6	19	20	9	8	17	15
Channel Alterations	17	20	20	20	7	10	9	18
Frequency of Riffles	15	18	18	19	9	11	12	18
Channel Sinuosity	16	13	19	19	8	10	10	10
Bank Stability	5/5	5/5	8/8	9/9	4/4	2/2	8/8	8/8
Bank Vegetation	5/5	5/5	9/9	10/10	2/2	3/3	8/8	10/10
Riparian Width	10/10	10/10	10/8	10/10	5/5	3/3	2/8	10/10
<b>Total Score</b>	148	130	202	211	82	83	127	173

Table 5. Results of Wilcoxon 2 Sample test for comparisons of invertebrate metrics in fill and reference streams. Sample sizes were 33 and 36 for reference and fills, respectively.

<b>Metric</b>	<b>Mean Score (fill)</b>	<b>Mean Score (Ref)</b>	<b>Pr &gt;  Z </b>
Coleoptera density	27.1	41.2	0.0027
Diptera density	33.2	33.9	0.8772
Ephemeroptera density	26.6	41.8	0.0009
Odonata density	29.9	37.8	0.0282
Plecoptera density	31.6	35.8	0.3742
Trichoptera density	35.5	31.1	0.3629
Total density	31.7	35.7	0.4062
EPT density	32.3	35.0	0.5579
Chironomidae density	33.0	34.1	0.8317
Non-insect density	28.9	39.0	0.0337
Collector density	32.7	34.5	0.6992
Scraper density	27.5	40.7	0.0034
Shredder density	28.0	40.2	0.0102

Table 6. Summary of correlation between mean aquatic macroinvertebrate metrics and mean water quality measure for each stream. Only those metrics which were significantly correlated with a water quality parameter are presented, all others are  $p > 0.05$ .

METRIC	VARIABLE	PEARSON'S	
		R	p
Ephemeroptera richness	Ca	-0.8224	0.0122
Ephemeroptera richness	Cu	-0.9762	0.0001
Ephemeroptera richness	Fe	-0.8360	0.0097
Ephemeroptera richness	Mn	-0.7367	0.0371
Ephemeroptera richness	Ni	-0.8604	0.0061
EPT richness	Ca	-0.7070	0.0499
EPT richness	Mn	-0.7959	0.0181
EPT richness	Ni	-0.7257	0.0416
Plecoptera richness	Cd	-0.7436	0.0345
Trichoptera richness	Sb	0.8430	0.0086
% Ephemeroptera	Cu	-0.8300	0.0108
% Ephemeroptera	Ni	-0.7458	0.0336
% non-insects	Cr	-0.7475	0.0330
% non-insects	Sb	-0.7316	0.0391
% Trichoptera	Sb	0.8199	0.0127

**Table 7.** Water quality parameters found to be significantly correlated and used for principle component analysis with macroinvertebrate metrics for valley fill and reference streams in West Virginia. Here, PC1, PC2, and PC3 refer to principle components 1, 2, and 3. Variables included in PC1, PC2 and PC3 are listed below each with – indicating negative correlations with the PC. Pearson correlation coefficients and significance levels are given for each parameter. Parameters SPCON, ALK, and FINES refer to specific conductivity, alkalinity, and fine sediment.

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<b>Parameter</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>
ALK	0.936	-0.190	0.113
	0.001	0.652	0.790
pH	0.870	-0.354	0.179
	0.005	0.390	0.671
Na	0.591	-0.708	0.105
	0.123	0.050	0.804
K	0.993	0.013	0.065
	<.0001	0.976	0.879
Mg	0.976	0.021	0.196
	<.0001	0.961	0.642
Ca	0.987	0.148	0.037
	<.0001	0.726	0.932
Cd	0.804	0.201	0.022
	0.016	0.633	0.959

Cu	0.864	0.244	0.157
	0.006	0.561	0.711
Ni	0.965	0.241	-0.044
	0.000	0.566	0.918
Sb	0.282	-0.880	-0.070
	0.498	0.004	0.868
Zn	0.213	0.576	-0.652
	0.612	0.135	0.080
Mn	0.654	0.681	-0.046
	0.079	0.063	0.915
Al	-0.589	0.196	0.669
	0.125	0.642	0.070
Fe	0.989	0.122	-0.008
	<.0001	0.774	0.984
SPCON	0.981	-0.054	0.162
	<.0001	0.899	0.701
FINES	-0.363	0.420	0.811
	0.376	0.300	0.015

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**Table 8.** Results of Stepwise regression relating the principle components (PC1, PC2, PC3) to benthic macroinvertebrate metrics in reference and valley fill streams in West Virginia.

Metric	Intercept	PC-1		PC-2		PC-3		Model R-Square
		Slope	p-value	Slope	p-value	Slope	p-value	
EPT Richness	14.375	-0.93891	0.0199	-2.19627	0.0102	*		0.846
Ephemeroptera Richness	4	-0.76955	0.0267	*		*		0.5868
Plecoptera Richness								**
Trichoptera Richness	4.5	*		-1.01294	0.0587	*		0.4748
Odonata Richness	1.625	*		0.51477	0.0359	-0.8196	0.0151	0.8095
Diptera Richness								**
Coleoptera Richness								**
Percent EPT								**
Percent Ephemeroptera	0.17339	-0.02342	0.0903	-0.03822	0.1421	*		0.5975
Percent Plecoptera	0.47355	*		0.04375	0.1433			0.3207
Percent Trichoptera								**
Percent Odonata	0.04224	*		*		-0.02052	0.0689	0.4494
Percent Diptera								**
Percent Coleoptera	0.14159	-0.01369	0.0864	*		*		0.4116
Percent Chironomidae								**
Percent Non-Insect								**

\* Indicates non-significance in stepwise multiple regression at 0.15 level

\*\* Indicates non-significance to any of the principal components at the 0.15 level

### Discussion

The streams (reference and fill) paired for this study appeared to demonstrate great similarity between RBP scores, sediment composition, and macroinvertebrate density. Further, extensive deep mining has occurred in all of the watersheds studied. Thus, the primary difference between the stream pairs was the presence of a valley fill in what was once the headwater of the treatment streams.

Perhaps the most surprising result was that despite the large landscape-level influence of valley fills we were unable to detect as strong of an impact on the biota and habitat as would be expected. It was suspected fills would increase fine sediment accumulation in the substrate and increase turbidity as was found in surface mining studies in Virginia (Matter and Ney 1981). Surprisingly, neither a significant increase in fine sediment accumulation in the substrate nor a significant increase in turbidity was detected between the paired fill and reference streams—this, despite the fact that fill stream study reached were all upstream of sediment ponds. Several possibilities may explain the failure to detect a difference in fine sediment composition or turbidity. By sampling only at one time of year for sediment we may have missed differences that occur over temporal scales (Waters 1995). It is also possible that unlike silviculture or roads that have smaller, chronic inputs of fine sediment (Chamberlin et. al. 1991; Furniss et. al. 1991; Nelson et. al. 1991; Waters 1995) the valley fill causes a massive initial input of fine sediment during the process of dumping the overburden into the stream, which may lessen over time. Natural hydrologic processes easily transport fine sediment leaving only larger rock debris from the valley fill (Leopold et. al. 1964; Swanston 1991). The age of the fills ranged from 5-20 years old, yet there was no significant difference between fine sediment levels or turbidity in fills versus reference streams when we sampled. This suggests that any increases in fine sediment in stream substrate occurred within a short time (< 5 years) of the initial surface disturbance and then subsided to background levels similar to reference streams.

We were also unable to detect a significant difference in total macroinvertebrate density between fill and reference streams. This finding may not be surprising given that fine sediment levels were not significantly different between treatments and previous studies have shown that despite differences in composition of benthic macroinvertebrate communities, macroinvertebrate density did not differ across wide scale differences in fine sediment (Angradi 1999; Kaller 2001).

Sampling at other temporal scales (seasons or years), or more intensive sampling with more stream pairs may also have resulted in more significant findings. Spring is a time known in West Virginia to experience low productivity in streams (Angradi 1999; Kaller 2001). Invertebrates in our samples were not very abundant, or taxonomically diverse even in reference streams.

We did identify several important differences in water quality and taxa densities between fills and reference streams. Specific conductance did significantly differ between reference and fill streams. Specific conductance in fill streams was at least twice as high as reference streams (Table 1). Typical specific conductance levels in low order West Virginia streams measured in previous research ranged from 13 mhos to 253 mhos (Angradi and Vinson 1996; Kaller 2001). Valley fill streams exceed these values (502-1479 mhos). A study of biological indices in Spain found highly negative correlations between specific conductance and EPT metrics (Garcia-Criado et al. 1999). In our study, none of the metrics were significantly correlated with specific conductance. However, PCA and SWR identified EPT and Ephemeroptera richness as being significantly negatively related to PC1. PC1 was largely influenced by correlations with elevated specific conductivity and heavy metals. Although negative relationships between sensitive taxa and heavy metals have been shown in many studies (Clements et al. 1992, 2000; Hickey and Clements 1998; Mebane 2001) it is apparent that in addition to increasing some heavy metals, valley fills also increase specific conductance beyond background levels, and this may affect diversity of Ephemeroptera taxa.

Based upon the water chemistry analyses conducted during storm samples, the increased conductivity in fill streams is in part due to higher levels of Ca, Mg, and Mn. Among the elevated metals, Mg, Cu, Ni, Mn, and Fe have been linked to toxicity in aquatic macroinvertebrates (Birge et al. 2000; Clements et al. 1992; Lasier et al. 2000; Wang 1987). Indeed, Ephemeroptera density and EPT density were negatively related to these metals in the study streams. USEPA (1999a) has established aquatic life surface water risk-based exposure limits (RBEL) for 6 of the elements we tested in stream waters. None of the reference streams exceeded any USEPA RBEL criteria. Only the chronic RBEL for manganese exceeded the USEPA limit of 120 ug/l in fill stream water quality ranges. All manganese measurements exceeding the EPA limits were from the Sugar Tree Fill stream (range 125.0 – 167.8 ug/l). The Sugar Tree Fill site had the lowest percent Ephemeroptera and percent EPT taxa metrics of all the other stream pairs. Although most measures of metals in our samples were below the

USEPA RBEL values our samples were taken during storm run-off and levels may be higher during base flow conditions. Further, the USEPA criteria do not consider the potential synergistic effects of a suite of metals elevated above background levels as was the case in the fill stream water chemistry. Therefore, we cannot rule out metals as contributing to the few detected differences in stream biota between our fill and reference streams.

So, why are densities of Ephemeropterans, Coleopterans, Odonata, Non-insects, Scrapers and Shredders lower in fills than in reference streams? Specific conductance is much higher in fill streams than in reference streams, but outside of Ephemeroptera richness, was not related to any of the metrics. Many of the metrics were negatively correlated with levels of metals, which (except for Al) were higher in fills than in reference streams. Perhaps the reason for differences in taxa between fills and reference streams is due to physical changes in the spatial trophic positioning of the fill streams. Many of the taxa with reduced densities in fill streams would be classified as scrapers. Although sites in streams were cited at similar locations relative to original topography and watershed area, fill stream samples began 300 m from the base of the fill. It may be that the apparent reduction in scraper taxa in fill streams may be due to a disruption in the river continuum whereby periphyton and associated trophic guilds are displaced further downstream after valley fill operations causing scrapers to be displaced further downstream in the fill streams.

Alternatively, lower metric levels in the fills could still be related to the elevated specific conductance in fill streams. The Rockhouse stream complicated findings as it had the highest habitat score and perhaps the most diverse aquatic macroinvertebrate community of all eight streams surveyed despite being a fill site. Because this stream was among the best of those we surveyed regardless of treatment, with only four pairs of streams it may have masked the general effect of mountain top mining upon streams and biota. Clearly we detected that metals were higher in fills than in reference streams, and many of these metals were negatively related to macroinvertebrate density. Although the relatively high metric scores at Rockhouse Creek may have clouded our analyses, it also points out that all stream restorations following disturbance (such as a valley fill) are not equal, and some have much better results than others. Further study should examine restoration procedures used at Rockhouse Creek to determine what may have contributed to the relative success of restoration at that site, so it may be applied elsewhere.

Because we did not detect as many significant differences between the fill and reference streams as might be expected from the level of disturbance, some may question the power of tests involving only four pairs of streams. The null hypothesis we tested with the Wilcoxon two-sample test for invertebrate density was  $P(R>F) = P(R<F) = 1/2$  versus the alternative hypothesis of  $P(R>F) \neq P(R<F) \neq 1/2$  where R = reference and F = fill. Under the null hypothesis, if two samples are drawn - one from a fill stream and one from a reference stream - the reference stream density would exceed that of the fill stream 50% of the time. Using methods described in Noether (1987) for sample size requirements of the Wilcoxon two-sample test, our sample size could have detected a  $P(R>F) = 0.69$  with 80% power if it truly existed. Thus, it appears that biologically significant differences in invertebrate communities could have been detected by this study where they existed.

Valley fill mining continues to affect numerous watersheds in West Virginia, Kentucky, Ohio, Virginia, and Pennsylvania (Peng 2000). Thus, with the complications of this study, research into the effects of valley fill mining should not end with a suspected reduction in density of several taxa or metrics. Instead, future research should include additional stream pairs, sampling over several time periods, compensation for varying fill age, and thorough water chemistry assessment. Of particular importance is identifying what characters make the Rockhouse Creek valley fill site so good and attempting to mimic those conditions in other fill sites. This final research topic is, perhaps, most pertinent given the valley fills will persist in West Virginia watersheds long after coal mining ceases in the region. The ultimate effects of valley fill mining upon macroinvertebrate communities downstream of the valley fill and in the watershed as a whole will continue until processes occurring at a geologic time-scale carve new valleys and transport fill materials downstream. Until that time, continued research into this topic is necessary and important in the understanding of the ecologic effects of anthropogenic activities occurring at such a massive scale.

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