

Macroinvertebrate Community Response to Spatial Patterns of Water Quality and Habitat within
Mining-influenced Headwater Streams of Central Appalachia

Melanie Ann McMillan

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Daniel L. McLaughlin, Co-chair

Stephen H. Schoenholtz, Co-chair

Sally Entrekin

Greg Pond

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Abstract (Academic)

Benthic macroinvertebrates are heavily relied on to indicate stream condition because of their ease of sampling, broad span of sensitivities to pollution among taxa, and diverse life histories that utilize various habitats and environmental conditions. Surface-coal mining in central Appalachia often results in salinization of headwater streams, with documented responses in macroinvertebrate communities across streams that vary in specific conductance (SC), an index of degree of salinization. Mining-influenced headwater streams can also exhibit within-stream spatial variation in SC, frequently via dilution with downstream distance from mining. However, the extent to which coal-mining alters downstream patterns in water chemistry and macroinvertebrate communities is largely unknown. This study aimed to determine macroinvertebrate community responses to physical and chemical gradients within six Appalachian headwater streams (four mining-impacted, two reference). Streams were sampled for benthic macroinvertebrates, habitat characteristics, and water chemistry in fall 2021 and spring 2022 at six-to-nine locations per stream over a range of 1.5 – 3 km. Mining-impacted streams exhibited greater spatial variation in macroinvertebrate community composition compared to reference streams, particularly in spring. Bray-Curtis Community similarity determined highly-impacted streams experienced the greatest within-stream shifts in community similarity. Metrics of macroinvertebrate communities and community similarity showed some correlation with SC at within-stream scales, particularly in highly impacted streams in spring; however, such trends were much fewer and weaker compared to relationships among streams when collectively examining communities. Redundancy Analysis (RDA) and Variation Partitioning (VP) indicated water quality, habitat, and location do overlap in explanation of community variation although they often additionally explain variance in unique ways. Significant variables identified by RDA within at least two of the six streams include channel slope, streamwater nutrients and hardness, stream channel embeddedness, and percent fines comprising the streambed. Redundancy Analysis also indicated 18 key macroinvertebrate taxa in study streams responding to location within stream, habitat, and water quality. Of those 18 taxa shredders, collectors, and clingers were most frequently impacted. Improved understanding of the spatial scale of coal-mining influences on headwater stream characteristics will help inform bioassessment protocols to most accurately assess stream condition, and inform remediation efforts within the central Appalachian region and in other salinized stream systems.

Macroinvertebrate Community Response to Spatial Patterns of Water Quality and Habitat within Mining-influenced Headwater Streams of Central Appalachia

Melanie Ann McMillan

Abstract (Public)

Small streams (or headwater streams) originating in the central Appalachian Mountains harbor a variety of unique organisms and are essential to the quantity and quality of downstream freshwater for fishing, recreation, and other uses. Coal mining processes, including disturbance of coal-bearing bedrock, often increases the streams salinity by adding pollutants that elevate dissolved minerals, or salts. Salinization of streams can come from a variety of sources in addition to coal mining such as de-icing road salts and crop irrigation and is of growing concern regarding its impacts to the quality of freshwater available for wildlife and human use. A common way to determine stream health is by identifying which aquatic insects (or macroinvertebrates) are present in a stream, because different groups are present based on the type and intensity of a variety of pollutants. Previous studies determined stream health by identifying insects from one location in a stream and comparing it to others. Stream's habitat and water quality naturally change as they join with larger rivers and flow to lower elevations causing different macroinvertebrates to be present at locations within streams. This study aimed to determine how changes along stream distances may be different in streams salinized from coal mining. The objectives of this study were to determine if one sample is adequate to represent the entire condition of a headwater stream. Six streams were sampled for macroinvertebrate, water quality, and habitat at six-to-nine locations within each stream over distances of ca. 2,000 m. Four streams were impacted by mining, of which two were highly impacted and two were impacted to a low-level; the last two streams were unimpacted to represent reference condition. The study found the type and number of macroinvertebrates within streams were changing least within reference streams and most in highly impacted streams. Macroinvertebrate communities in highly-impacted streams changed more within streams because they had high concentrations of dissolved salts upstream near the source of coal-mining pollution and these salts diluted with distance downstream, most likely due to fresh spring water contributions with minimal dissolved salts. Therefore, highly-impacted headwater streams experience greater environmental and macroinvertebrate variability indicating more than one sample location may be helpful in accurately assessing what macroinvertebrates inhabit the stream length of interest. Ensuring accurate sampling technique to determine stream condition is essential to our understanding of stream health and how to remediate and monitor impacts of salinization on our freshwater resources.

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There are bugs in the creek!
Thank god they don't squeak.
Little alien friends,
Sometimes breathe from their hind end;
What a delightful physique.

I am grateful for the privilege to study macroinvertebrates and aquatic systems and to be able to build a career in conserving and restoring freshwater resources. Water flows over every inch of the earth, accumulating our interactions with the land, our homes, and concentrating the consequences of our actions into a source essential for our well-being and survival, water. Support and advice from my friends, family, coworkers and mentors has reinforced the security and determination to pursue my interests and perform this research to the best of my abilities.

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Chapter 1- Introduction

Purpose

Surface coal mining in central Appalachia has altered water quality and benthic macroinvertebrate communities within headwater streams (U.S. EPA 2011), but less is known about the within-stream variability and downstream extent of these impacts. This study seeks to help fill this knowledge gap by documenting within-stream patterns in water chemistry, habitat, macroinvertebrate communities, and their association. Such information will provide the spatial context necessary to improve understanding of downstream effects of surface coal mining and how well previous long-term single-point sampling locations represent longer stream reaches. Improved understanding of the spatial scale of coal-mining influences will help to inform bioassessment protocols and remediation efforts within the central Appalachian region. Furthermore, this study can add to a broader understanding of variable water quality/habitat and associated macroinvertebrate metacommunity dynamics within streams and thus the spatial relevance of single-reach bioassessments. Use of macroinvertebrates in bioassessments has been widely implemented as a primary means of indicating stream condition (U.S. EPA 2003) and is a method under constant scientific scrutiny for refinement (Bonada et al. 2006).

Background

Mountaintop Mining and Valley Fills: Influences on Water Quality

Coal mining has transformed much of the central Appalachian region's economic, ecological, and cultural context. Since the 1970's, surface coal mining has been the dominant land-use change of the central Appalachian region, with roughly 5,900km² (3.5%) of forest land being converted to coal-mining use (Pericak et al. 2018; Sayler 2008). In West Virginia, Virginia, Kentucky, and Tennessee over a 17-year period, 1,165 km of headwater streams were permanently lost due to valley-fill burial (U.S. EPA 2011; 2005). This mining land-use persistently elevates downstream dissolved ion levels, consistently degrades macroinvertebrate and fish communities, and can degrade water quality to acutely toxic levels for some benthic organisms (U.S. EPA 2011).

Surface mining is the process of using explosives to remove up to 300 m of soil and rock, called overburden, and expose coal seams for extraction. The overburden is either used to re-contour the impacted mountain or placed into adjacent valleys as valley fill. This process alters ephemeral, intermittent, and small perennial headwater streamflow patterns and increases sediment loading from mined areas and valley fills (U.S. EPA 2011). During mining, settling ponds are used to store runoff, precipitate trace elements, decrease suspended sediment, and regulate pH before confluence with downstream systems. After mining, the area is graded and planted to control sediment, artificial streams are constructed on the fill, and settling ponds are eventually removed. These changes to the natural system have persistent influences to headwater hydrology, chemistry, and biota in a variety of ways (Hartman et al. 2005; U.S. EPA 2005; 2011; Pond et al. 2008).

Due to the geology of the central Appalachian region, most of the overburden produced from coal-mining consists of sandstone, siltstone, and shale (U.S. EPA 2011). Accelerated weathering of these newly fragmented and exposed substances results in dissolved carbonates rapidly neutralizing any acids,

producing alkaline-mine drainage (U.S. EPA 2011). This drainage consists of elevated levels of dissolved inorganic salts such as sulfate, bicarbonate, calcium, and magnesium (Clark et al. 2018), resulting in increased total dissolved solids (TDS) and associated salinity, often measured as specific conductance (SC). Post-mining elevated SC has been shown to persist in central Appalachian headwater streams, with mean annual SC rates of decline from 1.9% to 3.7% and streams not returning to reference conditions over ca. 40 years (Cianciolo et al. 2020). Critically, such salinity levels have been well-documented to alter freshwater fish and benthic macroinvertebrate communities (Stauffer and Ferreri 2002; Fulk et al. 2003; Pond et al. 2008; Pond 2010; Pond et al. 2014).

Macroinvertebrate Communities Response to Altered Water Chemistry

Since implementation of the Clean Water Act in 1972, benthic macroinvertebrate communities often have been used to assess water quality and stream condition and inform review and assessment of state waters. Benthic macroinvertebrates community structure integrates water and sediment quality and can therefore indicate collective impact of pollution over time. Macroinvertebrates integrate stream condition due to the diversity of life histories creating variable habitat requirements, trophic relations, and water-quality sensitivities among taxa (Huryn and Wallace 1987; Wallace and Webster 1996; Clarke et al. 2008). Furthermore, they are relatively sedentary for aquatic biota and have lifespans of approximately a year, meaning the community composition can reliably represent conditions at that specific time and place. Accordingly, macroinvertebrates have been shown to respond to various sources of anthropogenic disturbances such as urbanization, agriculture, logging, and other various forms of resource extraction and development (Merritt et al. 2019). Macroinvertebrates with greatest response to associated stressors of anthropogenic disturbance, such as habitat degradation, chemical or sediment contamination, and hydrologic alterations, vary depending on scale, i.e. continent, state, region, and stream size (Merritt et al. 2019). As such, it has become common practice to use benthic macroinvertebrate multimetric scoring systems to determine responsive macroinvertebrates and develop stream condition indices capable of assessing the degree of disturbance. As is the case in Virginia, the Virginia Stream Condition Index (VASCI) examined macroinvertebrate collections from 278 non-coastal Virginia sites from 1994-1998 to score streams based on the six metrics that best discriminate between reference and stressed conditions such as those of mining-influence (Burton and Gerritsen 2003).

Stream alterations caused by coal mining can have a variety of consequences on benthic macroinvertebrate community structure. For example, changes in SC impact osmoregulation of aquatic insects by changing the ionic composition and/or concentration of the water (Griffith 2017), but with effects varying among certain aquatic insects (Merritt et al. 2019). That is, some taxa are less sensitive to elevated SC than others depending on physiology (Griffith 2017). In streams impacted by coal mining, elevated SC decreases benthic macroinvertebrate richness, creates changes in specific benthic taxa metrics, and causes shifts in communities to more salt-tolerant taxa (Boehme et al. 2016; Timpano et al. 2018; Vander Vorste et al. 2019). For example, elevated SC has been observed to negatively correlate with Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, Ephemeroptera richness, Plecoptera richness, and clinger richness (Timpano et al. 2018). Conversely, elevated SC has been associated with increases in percent of pollution-tolerant chironomid and oligochaetes, percent of sample that is composed of the two most dominant taxa, percent Trichoptera, and percent of collector-filterer taxa (Timpano et al. 2018; Merricks et al. 2007; U.S. EPA 2011). Although increases in SC are the main influence on biotic condition in Appalachian streams influenced by coal mining, altered

macroinvertebrate communities have also been attributed to other impacts from coal mining such as i) increased selenium concentrations causing declines in scraper richness and predator density (Drover et al. 2020) and ii) a lower proportion of large cobbles-to-fine in streambeds and relative streambed stability increasing clinger richness (Pond et al. 2014).

Changes to benthic macroinvertebrate community composition can have significant impacts on the functionality of headwater streams (Pond et al. 2016). The Appalachian Mountains acted as a refuge during the last glacial period resulting in endemic species and high biodiversity into modern day. The persistence of this biodiversity is in part due to headwater stream spatial and temporal dynamics creating a variety of habitats to support the many different life histories of aquatic organisms (U.S. EPA 2011). Both benthic macroinvertebrate assemblages and their functional roles differ among headwater streams and larger-order streams (Vannote et al. 1980). Compared to larger-order streams, headwater streams consist of relatively more substrate and large woody debris, which slow flow and provide more time for collecting and shredding benthic macroinvertebrates to process organic matter. This can create trophic subsidies that increase downstream biomass and secondary production (Benke and Wallace 1997). Therefore, loss and/or alterations to headwater streams can result in loss of species diversity and functionality, with potential impacts to downstream waters (U.S. EPA 2011). For these reasons, it is important to incorporate spatial context when examining downstream effects and potential benthic macroinvertebrate community recovery with distance from mined areas.

Role of Spatial Gradients in Bioassessments

Bioassessments have become widely used in the US to assess stream condition since implementation of the Clean Water Act (U.S. EPA 2003). These bioassessments commonly utilize collecting benthic macroinvertebrates as they are relatively easily accessible, exhibit limited mobility, and vary in their sensitivity to different stressors, thereby providing insight into local stream condition (Mazor et al. 2016). Since widespread incorporation of bioassessments, their precision and rigor have been consistently investigated, mostly in a sample's capacity to accurately represent the condition of the stream system (Bonada et al. 2006; Pond et al. 2008; Buss et al. 2015). As current protocols delineate sites based on a single rather subjective "representative" stream reach, recent research has further challenged our knowledge of bioassessments in terms of their effectiveness at representing longer stream reaches using one sampling location (Heino 2013; Cai et al. 2019; Barbour et al. 1999).

Spatial studies incorporate concepts, such as the river continuum concept (RCC) (Vannote et al. 1980) and metacommunity theory (Leibold et al. 2004), that are less considered in across-stream studies. Natural shifts in macroinvertebrate communities over a spatial gradient of physical conditions has been defined by the RCC (Vannote et al. 1980). However, physical gradients are not the only drivers of community composition in any single location, where certain taxa prefer specific physical conditions but are not strictly limited to them. A metacommunity is a community linked by dispersal that would otherwise be independent (Leibold et al. 2004). Considering community connectivity can help explain movement outside of preferential conditions through drift and dispersal and thus differences in community similarity/dissimilarity along stream gradients (Leibold et al. 2004). Metacommunity dynamics emphasize the significance of how differences in presence or absence of macroinvertebrates exhibiting specific traits (i.e. high dispersal versus low dispersal) can inform causality of community shifts (or lack thereof) to natural and/or altered habitat gradients. Furthermore, studies on larger-order streams have found metacommunity dynamics may distort the ability of bioassessments to accurately

reflect stream condition at a single location (Heino 2013; Zhengfei et al. 2021), yet less is known about such potential effects in smaller, headwater streams.

Previous research has shown spatial variation in benthic macroinvertebrate communities within streams, and in both undisturbed and disturbed systems. Some within-stream variation (or lack thereof) can be explained by taxa traits. For example, a study of 14 pristine streams in Finland found similarity of macroinvertebrates with poor dispersal ability decreases with increasing distance (Astorga et al. 2012). However, most studies attribute within-stream variability to local environmental factors and have focused on community shifts along larger-order streams (U.S. EPA 2003). For example, study of 14 Wisconsin streams found the distances at which macroinvertebrate assemblages no longer display spatial correlation, known as distance to independence, to range from 1.7-13.5 km (Shupryt and Studinski 2021). Specific to stream salinization, this study and others found distance to independence to be strongly and positively related to SC, most likely due to either homogenization of water chemistry gradients or elimination of sensitive taxa (Voss and Bernhardt 2017; Shupryt and Studinski 2021). Yet, such studies have largely focused on larger-order streams and river systems where pollutants have been observed to accumulate downstream (Thompson and Townsend 2006; Li et al. 2001; Wilson and McTammany 2016; Weigel et al. 2003; Bonada et al. 2012). Due to steeper slopes and higher relative groundwater inputs, headwater streams have been observed to exhibit trends opposite to larger-order streams, diluting pollutants with distance downstream (Cianciolo 2019). This leads to the question: should we expect to see opposite trends in macroinvertebrate communities over such reversed gradients in headwater streams, where if pollutants dilute downstream, do aquatic communities change (and recover) over shorter distances than in larger-order streams?

The extent to which coal mining alters physical gradients, chemical gradients, and metacommunity dynamics of headwater streams remains largely unknown. Headwater streams generally consist of patchily distributed, isolated habitats and endemic species that depend on this heterogeneity, and therefore may have lower dispersal abilities compared to taxa of more commonly studied larger-order streams (U.S. EPA 2005). The RCC and metacommunity theory, combined with unique headwater characteristics, pose the question: If headwater macroinvertebrate communities exhibit lower dispersal along steeper physical and chemical gradients of change, compared to larger-order streams, will communities shift over shorter distances? This leads to the question: how representative of the headwater stream network is a single sampling location in both mined and unmined headwater streams in central Appalachia where coal mining is a dominant land use?

Need for Study

In ongoing work conducted by our research group, 23 headwater streams in the central Appalachians have been studied since 2011 to find a variety of trends relating to the effects of nearby coal mining on stream condition. Most notably, this work has documented a negative benthic macroinvertebrate community response to increased SC, designating SC a primary stressor (Timpano et al. 2018). We have a good understanding of differences in macroinvertebrate communities among streams that vary in SC, but little is known about macroinvertebrate community trends along longitudinal distances within these study streams. Analyzing macroinvertebrate response to within-stream spatial trends in water chemistry and habitat can help inform bioassessment protocols and remediation of coal-mining impacts.

We have also recently observed a spatial gradient of water quality conditions within our study streams. A 2019 survey on a subset of our study streams found within-stream gradients of SC and associated ions, largely exhibiting dilution with downstream distance (Cianciolo, unpublished data). What remains to be assessed is the effect of these within-stream water chemistry gradients on benthic macroinvertebrate community assemblages. Is the within-stream association between water chemistry and macroinvertebrate communities comparable to previously observed associations between water chemistry and macroinvertebrate communities across streams? Further, as typical in most stream-condition studies, we have been utilizing bioassessments at singular 100m sample reaches aimed to represent each headwater stream of interest. A spatial macroinvertebrate study is needed to determine the reliability of a single reach to represent condition of longer stream reaches. A spatial study could further inform assessment of future resilience and recovery trends in mining-influenced streams. We know the water-quality impacts of coal mining persist and thus there is little recovery of macroinvertebrate communities among impacted streams (Cianciolo et al. 2020). Less is known about whether benthic macroinvertebrate communities will have the capacity to re-establish through dispersal if/when water quality conditions within and among streams improve.

Objectives and Predictions

Assess spatial trends in water chemistry and habitat and associated responses by benthic macroinvertebrate communities within reference (unimpacted) and mining-influenced headwater streams.

Objective 1: Determine within-stream variability of benthic macroinvertebrate communities and stream condition.

This objective assesses spatial variation in benthic macroinvertebrate communities and, more broadly, the effect of sample location on stream-condition studies assessing the influence of coal mining. This objective will determine changes of macroinvertebrate communities within streams at different downstream distances from stream origin in both reference and mining-influenced streams.

Prediction 1: There is higher spatial variability of macroinvertebrate communities and stream condition in mining-influenced streams as compared to reference.

Objective 2: Determine if the same changes in macroinvertebrate communities among streams over a gradient of SC are occurring within streams.

Among our 23 long-term study streams, a strong relationship between macroinvertebrate communities and SC gradient has been consistently observed. This objective will assess whether or not this trend occurs within streams that exhibit spatial variation in SC.

Prediction 2: Macroinvertebrate within mining-influenced streams sensitive to SC will prefer locations with lower SC.

Objective 3: Examine influence of water quality and other environmental factors on within-stream benthic macroinvertebrate community variability.

Among mining-influenced streams, SC is a highly influential factor in macroinvertebrate community composition, although macroinvertebrate community assemblages can also be attributed to several other local stream characteristics. This objective will evaluate the extent to which water chemistry (including

SC and other variables) and stream habitat attributes (e.g. stream width, streambed sediment size, and stream channel gradient, etc.) explain within-stream benthic macroinvertebrate variation. Spatial patterns of sample location will also be examined in this objective and provide insight on within-stream dispersal. This knowledge will indicate whether metacommunity dynamics have potential to influence resilience and recovery of benthic macroinvertebrate communities in headwater streams impacted by coal mining.

Prediction 3: Water quality variables, in addition to SC, along with habitat and location variables, will further explain variation in macroinvertebrate communities within streams compared to SC alone.

Chapter 2 – Methods

Site Selection

To assess within-stream variation in water quality and macroinvertebrate communities, a subset of six headwater streams were selected from a set of 23 long-term monitoring stream sites, which include six reference and 17 mining-influenced streams. These 23 streams were selected to have comparable habitat features and each consists of one sampling reach with an *in situ* 30-minute automated SC logger where bi-annual (spring and fall) benthic macroinvertebrate and water-chemistry sampling has occurred since 2011 (see Timpano et al. 2015 for site details and selection criteria). Other than surface coal-mining and natural gas wells, these streams were selected to be in mainly forested areas without other active land uses. Long-term sampling reaches on these 23 streams are typically located on or near the most downstream section practical for sampling as described by the Virginia Department of Environmental Quality Rapid Bioassessment Protocol (VA DEQ RBP) for riffle-run habitat in high-gradient streams (VA DEQ 2008). In order to select six of these streams for my spatial study, nine streams were ruled out due to habitat features, property access, or significant land-use change upstream of the previously established sample location. During summer 2021, the 14 remaining long-term study streams were walked from influential confluence to point of origin, measuring SC, pH, temperature, and dissolved oxygen and multiple habitat characteristics at approximately 200m intervals. Habitat characteristics were selected from the EPA Rapid Bioassessment Protocol for High Gradient Streams and included sediment deposition, available cover, and riffle frequency (Barbour et al. 1999)(Appendix A). Measurements of stream width and depth and ocular estimates of percent canopy cover were also recorded to ensure no major habitat variation among selected streams. Two reference streams (average SC ranging 10-40 uS/cm), two streams experiencing low-levels of impact (average SC ranging 250-600 uS/cm), and two streams experiencing high-levels of impact (average SC ranging 860-1,400 uS/cm) were selected based on the data collected from stream reconnaissance in summer 2021. Thus, stream selection totaled four impacted and two reference study streams (Figure 1, Table 1). All streams were in EPA Level IV Ecoregion 69d (Omernik 1987). Watershed area was previously delineated through Spatial Analyst tools in ArcMap v.10 (ESRI Inc., Redlands, CA, USA) software using USGS digital elevation rasters (United States Geological Survey (USGS); <http://nationalmap.gov/>).

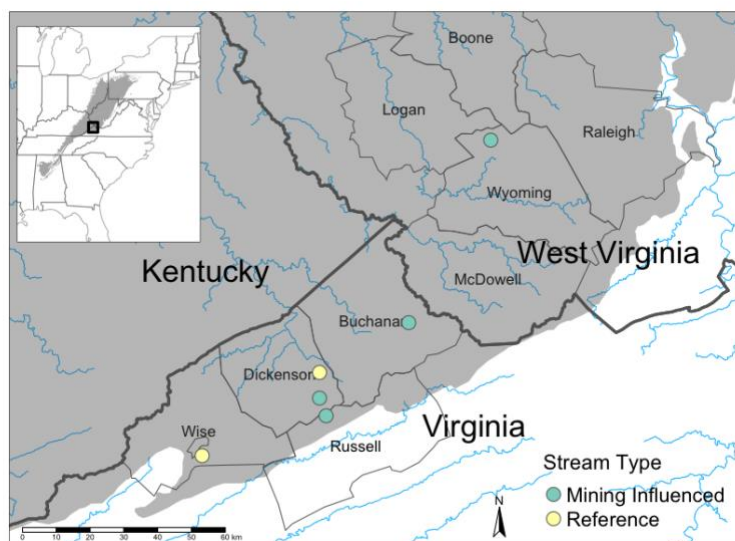


Figure 1. Study stream locations. From South to North: EAS, ROL, FRY, CRO, SPC, LLW.

Table 1. Site attributes for six selected streams in the coalfield of central Appalachia.

Stream	Site ID	Site Type	Approx. Max. (Fall, Spring) SC gradient ($\mu\text{S}/\text{cm}$) ¹	County	State	Watershed Area (km ²)	% Watershed Mined	Max Stream Length (m) ²
Eastland Creek	EAS (R) ³	Reference	10-14	Wise	VA	2.38	0	2,500
Crooked Branch	CRO (R)	Reference	40-10	Dickenson	VA	2.27	1.1	2,050
Fryingpan Creek	FRY (L)	Mining-Influenced (Concentrating)	+ 260, +290	Dickenson	VA	5.73	4.5	2,785
Spruce Pine Creek	SPC (L)	Mining-Influenced (Diluting)	-260, -65	Buchanan	VA	6.71	3.8	2,555
Roll Pone Branch Creek	ROL (H)	Mining-Influenced (Diluting)	-800, -990	Russell	VA	1.30	29.9	1,500
Longlick Branch	LLW (H)	Mining-Influenced (Diluting)	-640, -780	Wyoming	WV	1.98	26.4	1,950

¹ +,- indicate if stream is concentrating (+) or diluting (-) with distance downstream.

² Some stream lengths were shorter in fall due to stream drying, which eliminated some upstream sites.

³ R=reference, L=low-impact mining-influenced, H=high-impact mining-influenced.

Of the six streams selected, the shortest total distance of stream available to sample was ca. 1,500 m, whereas the longest was ca. 2,800 m. At each stream, sampling reaches of 50 m for both macroinvertebrate and water-quality sampling were designated approximately every 300-400 m going from most upstream to downstream, along with additional reaches at the long-term monitoring locations (Timpano et al. 2015) (Figure 2). With this design, the shortest stream had six sampling reaches and the longest had nine sampling reaches. All reaches were flagged and GPS coordinates recorded to replicate sampling locations in different seasons.

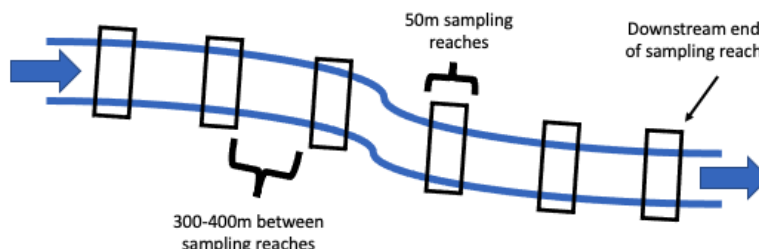


Figure 2. Sampling reach delineation. Arrows indicate direction of flow.

Field Methods

Macroinvertebrates

In each 50 m sampling reach across all six study streams, macroinvertebrates were sampled in October 2021 and April 2022 in accordance with the long-term biannual protocol that has been in place in these study streams since fall 2011, which follows the VA DEQ high-gradient, single-habitat, riffle-run procedures (VA DEQ 2008). However, for this study, I used multiple 50-m sampling reaches versus a single 100-m sampling reach to better identify spatial trends. Using a 0.3 m 500 μ m D-frame kick-net, moving downstream to upstream within a 50 m reach, six 1 m kicks were composited as one macroinvertebrate collection reflecting approximately 2 m² of streambed. All macroinvertebrate collections were conducted by the same individual to reduce technique bias. Net placement was secure to stream bottom to prevent flow beneath the net, in areas with enough water to pass through the net to ensure dislodged insects enter the net, but not too high of flows where water passed above the net. Macroinvertebrate collections were preserved in 95% ethanol in the field to be stored in the lab until processing.

Water Quality

At every sampling reach, water-quality measurements were taken for both the October 2021 and April 2022 sampling events. Grab samples were collected from the main thalweg in each sampling reach and analyzed as approved by the EPA for CWA chemical test methods (section 304(h) *Code of Federal Regulations*, title 40, part 136). Streamwater temperature, pH, dissolved oxygen, and SC were recorded using a calibrated YSI Professional Plus (YSI Inc. Yellow Springs, OH). Seven grab samples for each sampling reach were collected for analysis of the following water chemistry parameters: alkalinity, major cations (Ca²⁺, Mg²⁺, K⁺, Na⁺), dissolved trace elements (B, Al, Cr, Cu, Co, Fe, Ni, Mo, Cd, Ba,

Pb, Mn, Se, Zn), major anions (Cl^- , SO_4^{2-}), nutrients (PO_4 , total P, NO_3^- , NO_2^- , NH_3 , total N; 1 sample filtered and 1 unfiltered for totals measurements), and non-purgeable organic carbon (NPOC). Vertically mixed water was collected upon arrival at each reach before any disturbance from macroinvertebrate sampling. Nutrient samples were collected in Nalgene bottles with head space, NPOC in glass amber vials with minimal head space, and the remaining samples were collected in polyethylene sample bags. Water was drawn into a 60 mL syringe before adding it to the total P and N samples, and the remaining samples were filtered through a 0.45 μm pore filter. Fifty ml of stream water was collected for NPOC, and 100 mL was collected for all other samples. For appropriate preparation, nitric acid was added to the trace elements sample and HCL was added to the NPOC sample (U.S. EPA 1996). All samples were stored in a cooler of ice in the field and, upon return to the lab, transferred to a cold room at 4C except for nutrients samples, which were frozen in the transitional time before analysis.

Habitat

In June-July of 2022 each sampling reach was assessed for the following habitat features influential to benthic macroinvertebrate community structure: stream gradient, streambed sediment composition, canopy cover, epifaunal substrate, stream depth at thalweg, stream width, stream wetted-width, bank stability, and riparian vegetative zone width. Epifaunal substrate, bank stability, and riparian vegetative zone width were recorded as described by the EPA for habitat assessment of high-gradient streams (Barbour et al. 1999). Stream gradient, depth at thalweg, stream width, and stream wetted width were collected as outlined by VA Department of Forestry (Austin 1997). Canopy cover was estimated from an average of three measurements from the app “Canopeo” (Patrignani and Ochsner 2015) taken at the most downstream, middle, and upstream point of the 50m reaches. Streambed sediment composition data were collected using a modified Wolman pebble count method (Wolman 1954) with an average of 120 pebbles collected for each 50m reach. Within each reach, 5-6 pebbles from the wetted width were chosen randomly along 21 transects set 2.5 m apart. Pebbles were measured along the B-axis using a gravelometer.

Laboratory Methods

Biological Collections

Field collections for macroinvertebrates were processed according to semi-quantitative VDEQ protocol (VA DEQ 2008). Collections were washed and decanted to remove debris and the remaining fauna and organic matter were spread evenly over a grid. This grid was randomly sub-sampled to a 200 count and selected macroinvertebrates were identified to genus using standard keys (Merritt et al. 2019; Morse et al. 2017). All specimens were identified by the same individual to insure consistency.

Water Chemistry

Alkalinity was measured through titration of the field sample with a potentiometric auto-titrator (TitraLab 865, Radiometer Analytical) and a prepared standard acid (APHA, 2005). Total alkalinity and pH measurements were used to calculate HCO_3^- (APHA, 2005). Major cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+) and dissolved trace elements (B, Al, Cr, Cu, Co, Fe, Ni, Mo, Cd, Ba, Pb, Mn, Se, Zn) were analyzed using ICP-MS with a Thermo iCAP-RQ (APHA, 2005). Chloride and SO_4^{2-} were analyzed using ion chromatography with a Dionex ICS-3000 Ion Chromatography System (APHA 2005). Nutrients (total

N, total P, PO₄, NH₃, NO₂⁻ & NO₃⁻) were determined through colorimetric analysis using a SEAL AutoAnalyzer3, Multitest MT7A/MT8A analyzer (American Public Health Association (APHA 2005). NPOC was analyzed using a Shimadzu TOC-V cph Total Organic Carbon Analyzer (APHA 2005). Together this analysis yielded three main components: YSI (SC, temp, pH, and DO) field measurements, trace elements, and nutrient resulting in 46 water quality metrics to be analyzed (Table 2).

Table 2. Water quality metrics for selected study streams in the coalfield region of central Appalachia.

Category	Metric	Abbreviaton
Trace Elements	Sodium	na.mgl
	Chloride	cl.mgl
	Lithium	li.ugl
	Strontium	sr.ugl
	Calcium	ca.mgl
	Magnesium	mg.mgl
	Nickel	ni.ugl
	Potassium	k.mgl
	Uranium	u.ugl
	Calcium to Magnesium Ratio	ca.mg
	Barium	ba.ugl
	Selenium	se.ugl
	Copper	cu.ugl
	Manganese	mn.ugl
	Vanadium	v.ugl
	Titanium	ti.ugl
	Cobalt	co.ugl
	Cadmium	cd.ugl
	Arsenic	as.ugl
	Zinc	zn.ugl
	Iron	fe.ugl
	Aluminum	al.ugl
	Chromium	cr.ugl
	Lead	pb.ugl
	Silver	ag.ugl
	Molybdenum	mo.ugl
Nutrients	Sulfate	so4.mgl
	Bicarbonate	hco3.mgl
	Ratio of Sulfate to Bicarbonate	so4.hco3
	nitrate + nitrite	no2no3.n.mgl
	Non-Purgeable Organic Carbon	npoc.mgl
	Ortho-Phosphate	ortho.po4.p.mgl
	Total Nitrogen	tn.mgl
	Total Phosphorus	tp.mgl
	Ammonia	nh3.n.mgl
Field Measurements	Specific Conductance	sc.uScm
	Actual Conductance	ac.uScm
	Alkalinity	alkalinity.mgl
	Hardness	hardness
	Dissolved Oxygen	do.mgl
	Temperature Celcius	temp.C
	Potential Hydrogen (pH)	ph

Data Analysis

Using collected data, I first determined metrics describing macroinvertebrate communities and habitat conditions for each sampling location within each study stream, which were then used along with water quality data to assess Objectives 1-3. All data analysis was executed in RStudio version 1.4.1106.

Selected Macroinvertebrate Groups

Taxa (117 taxa identified) were grouped in four ways (tolerance, habit, feeding, and taxonomic) based on differing physiology and behaviors within the macroinvertebrate assemblages to better distinguish the nature of impairments. These groupings are based on tolerance values and movement and feeding methods that have been documented regionally in the Virginia DEQ BIOMON database and broadly in the Invertebrate Community Index (ICI) (DeShon 1995), RBP (Barbour and Gerritsen 1996), and the benthic Index of Biological Integrity (IBI) (Kerans and Karr 1994). Tolerance values represent the sensitivity to perturbation (mainly weighted for organic pollution) as a scale of 1-10, with 10 being the most tolerant. Taxa with tolerance values < 3 were grouped as intolerant and those with scores > 7 were grouped as tolerant. Each taxon was assigned to a habit of clingers (CN), sprawlers (SP), burrowers (BU), climber (CB), or swimmers (SW). A taxon's habit indicates the primary mode of movement, associating it to specific types of habitat, substrate, and/or stream velocities. Taxa were further assigned to one of five functional feeding groups (FFGs): collector-filterers (CF), collector-gatherers (CG), predators (PR), scrapers (SC), and shredders (SH). These FFGs provide information on the balance of feeding strategies, trophic dynamics, nutrient spiraling, and organic matter processing (Barbour et al. 1999). Lastly, taxonomic groups were included for specific orders known to be sensitive to SC and other pollution stressors such as EPT or even further excluding families of sensitive orders known to be more-tolerant such as Baetidae (B), Leuctridae (L or Leuc), and Hydropsychidae (H). Expected responses to disturbance for the following taxa metrics were mainly adapted from the EPA Report (U.S. EPA 2011) summarizing research that examined downstream impacts of valley fills on macroinvertebrates (Table 3).

Taxa Metric Calculations

Community Composition (%)

Benthic macroinvertebrate community composition was determined by proportioning individuals of certain groupings to the total identified community of that sample. Percentage of total composition was calculated for select taxa, FFGs, habits, and tolerances (Table 3).

Taxonomic Richness

Benthic macroinvertebrate taxonomic richness was calculated as the number of taxa summed for the sub-sample to 200 of each sampling reach. Taxonomic richness values did not include damaged or immature individuals that were unable to be identified to genus. Richness was calculated for select taxa, FFGs, habits and tolerances (Table 3).

Diversity and Evenness

Shannon and Simpson Diversity were calculated in R using the *diversity* function in *vegan* (Oksanen et al. 2016).

Evenness (J) (McCune and Grace 2002) was calculated as:

Shannon Diversity/ (log(taxonomic richness))

Virginia Stream Condition Index (VASCI)

Virginia Stream Condition Index was calculated using the following family-level metrics: total richness, EPT richness, percent Ephemeroptera, percent Plecoptera and Trichoptera less Hydropsychidae, percent chironomids, percent scrapers and Hilsenhoff biotic index (Burton and Gerritsen 2003). These metrics were compared to a standard value representative of reference condition for VA streams (Appendix B) to create a score and averaged to quantify stream condition for that macroinvertebrate collection (Burton and Gerritsen 2003). This calculation was performed using data from subsampling to 200 +/- 10 % specimens rather than VDEQ standard 100 +/- 10% specimens subsample. Consequently, VASCI scores calculated with 200 +/- 10% specimens are more likely to include rare species and inflate total and EPT taxa metrics. Therefore, VASCI scores used in this study do not imply impairment, or lack of impairment, which is based on the standard Virginia DEQ VASCI score, but rather are used as a comprehensive metric for additional comparison within and among the six study streams.

Hilsenhoff biotic index is calculated as:

Σh / total abundance

where h equals count of individual multiplied by that individual's tolerance value.

The above macroinvertebrate groupings and taxa resulted in 60 metrics to be analyzed (Table 3). Fifteen metrics with most notable responses based on previous research are reported throughout analyses in this thesis (bolded and underlined in Table 3), whereas Appendix C presents results for all 60 metrics. For the 15 selected metrics, total richness, EPT richness, percent Ephemeroptera, percent Plecoptera and Trichoptera less Hydropsychidae, percent chironomids, and percent scrapers were chosen as indicated by VADEQ as most significant metrics for VA streams (Burton and Gerritsen 2003). Additionally, Ephemeroptera less Baetidae richness and percentage, Ephemeroptera richness and percentage, EPT richness, scraper Richness, total richness, Shannon diversity, and percentage composed of the 5 dominant taxa were selected as indicated to be most responsive to SC in our study streams (Timpano et al. 2018). Lastly, Ephemeroptera richness and percentage, clinger richness and predator richness and percentage were chosen as they have been determined responsive to multiple stressors in our study streams (Drover et al. 2020). Based on strength of documented previous relationships to mining influence (Burton and Gerritsen 2003; Timpano et al. 2018; Drover et al. 2020) and diversity of life history representation, six of these metrics (starred in Table 3) were further selected to be graphically represented throughout this thesis: percent Ephemeroptera, Ephemeroptera less Baetidae richness, EPT richness, scraper richness, clinger richness, and percent 5 dominant taxa.

Table 3. Benthic macroinvertebrate metrics calculated for selected study streams in the coalfield region of central Appalachia.

Category	Metric ¹	Abbreviation	Expected Response to Disturbance ²
Community Composition	% Chironomidae	pChi	+
	% Chironomidae and Oligochaeta	pChiO	+
	% Diptera	pD	+
	*% Ephemeroptera	pE	-
	% Ephemeroptera less Baetidae	pE.less.B	-
	% Ephemeropter, Plecoptera, and Trichoptera	pEPT	-
	% Ephemeropter, Plecoptera, and Trichoptera less Cheumatopsyche	pEPT.less.Cheum	-
	% Ephemeropter, Plecoptera, and Trichoptera less Hydropsychidae, Baetidae, and Leuctridae	pEPT.less.H	-
	% Ephemeropter, Plecoptera, and Trichoptera less Hydropsychidae	pEPT.less.HBL	-
	% Oligochaeta	pOligo	+
	% Plecoptera	pP	-
	% Plecoptera less Allocapnia	pP.less.Allo	-
	% Plecoptera less Amphinemura	pP.less.Amph	-
	% Plecoptera less Leuctra, Allocapnia, and Amphinemura	pP.less.LAA	-
	% Plecoptera less Leuctra	pP.less.Leuc	-
	% Plecoptera and Trichoptera less Hydropsychidae	pPT.H	-
	% Trichoptera	pT	-
	% Trichoptera less Hydropsychidae	pT.less.H	-
Taxonomic Richness	Total Taxa	rich	-
	Coleoptera Taxa	rich.C	-
	Diptera Taxa	rich.D	-
	Ephemeroptera Taxa	rich.E	-
	*Ephemeroptera Taxa less Baetidae	rich.E.less.B	-
	*Ephemeroptera, Plecoptera, and Trichoptera Taxa	rich.EPT	-
	Ephemeropter, Plecoptera, and Trichoptera Taxa less Hydropsychidae	rich.EPT.less.H	-
	All Taxa Except Ephemeroptera	rich.less.E	-
	Plecoptera Taxa	rich.P	-
	Plecoptera and Trichoptera Taxa	rich.PT	-
	Trichoptera Taxa	rich.T	-
	Trichoptera Taxa less Hydropsychidae	rich.T.less.H	-
Functional Feeding Group	% Collector-Filterers	pCF	+
	% Collector-Gatherers	pCG	-
	% Predators	pPR	-
	% Scrapers	pSC	-
	% Scrapers less Ephemeroptera	pSC.less.E	-
	% Shredders	pSH	+
	Collector-Filterer Taxa	rich.CF	+
	Collector-Gatherer Taxa	rich.CG	-
	Predator Taxa	rich.PR	-
	*Scraper Taxa	rich.SC	-
	Scraper Taxa less Ephemeroptera	rich.SC.less.E	-
	Shredder Taxa	rich.SH	+
Habit	% Burrowers	pBurrow	+
	% Clingers	pCling	-
	% Sprawlers	pSprawl	+
	% Swimmers	pSwimm	+
	Burrower Taxa	rich.Burrow	+
	*Clinger Taxa	rich.Cling	-
	Sprawler Taxa	rich.Sprawl	+
	Swimmer Taxa	rich.Swimm	+
Dominance	Pielou's Evenness	J	-
	% 1 Dominant Taxon	p1dom	+
	% 2 Dominant Taxa	p2dom	+
	*% 5 Dominant Taxa	p5dom	+
Tolerance	% Tolerant Taxa	pTOL	+
	Intolerant Taxa	rich.INT	-
	Tolerant Taxa	rich.TOL	+
Diversity	Shannon Diversity	Hshannon	-
	Simpson Diversity	Hsimpson	-
	VA Stream Condition Index Score	VASCI	-

¹ Key metrics for analyses bolded and underlined are reported in tables throughout this thesis; the remaining metrics are reported in the appendices; six starred metrics were selected for graphical representation.

² Responses to disturbance adapted from (U.S. EPA 2011; Burton and Gerritsen 2003; Simpson 1949; Kelso and Entekin 2018; Hamilton et al. 2020; Shannon and Weaver 1971).

Similarity Metric Calculation

Community similarity matrices composed of similarity indices of all collection sites for each stream were created using both the Jaccard (J) and the Bray-Curtis (BC) methods (McCune and Grace 2002; Brown and Swan 2010). Bray-Curtis indices are created using occurrence and abundance data, whereas J utilizes presence/absence data, giving equal weight to rare and abundant taxa. Indices were calculated in R using the *vegdist* function from the *vegan* package (Oksanen et al. 2016)

The Bray-Curtis Similarity (BC) is calculated for abundance as:

$$1-(d[jk]) = (\text{sum abs}(x[ij]-x[ik]))/(\text{sum } (x[ij]+x[ik]))$$

$$\text{binary: } 1- ((A+B-2*J)/(A+B))$$

where $x[ij]$ $x[ik]$ refer to the quantity on taxa (column) i and sites (rows) j and k . In binary (presence/absence standardization performed before analysis) versions A and B are the numbers of species on compared sites, and J is the number of taxa that occur on both compared sites. Scores of 0 indicate no similarity and 1 being the same.

Jaccard Similarity (J) is calculated for incidence (i.e. presence/absence) data as:

$$2BC/(1+BC)$$

where BC is Bray-Curtis similarity

Habitat Metric Calculations

The data manipulations detailed below produced 27 habitat metrics to be analyzed (Table 4). To create one score for each site to be analyzed, means of all three transect values were calculated including channel slope, average depth, wetted width, bank stability, canopy cover, embeddedness, riparian width, and vegetative protection. Streambed sediments collected from the Wolman pebble count were converted into quantiles of the sediment distributions (10, 16, 25, 50, 75, 84, and 90; expressed as D10, D16, etc.), proportion of sediments that occur in each size class (e.g. % fines, % gravel, % cobble) based on the Udden-Wentworth grain-size classification system (Wentworth 1922), ratios of large cobbles to fine (LCF) and small cobbles to fine (SCF), interquartile range, Simpson's diversity of size class proportions, and ratio of D84 to D50. Size classes are as follows: fines (≤ 2.8 mm), pebbles (2-45 mm), small cobbles (64-90 mm), large cobbles (128-180 mm).

Table 4. Habitat metrics calculated for selected study streams in the coalfield region of central Appalachia.

Category	Metric	Abbreviation
EPA Rapid Assessment Sampling Reach Averages	Slope	avg.slope
	Bank Stability Left Bank	avgbankstabL
	Bank Stability Right Bank	avgbankstaR
	Canopy Cover	avgcancov
	Wetted Depth	avgdepth
	Embeddedness	avgembedd
	Riparian Width Left Bank	avgriparianwidL
	Riparian Width Right Bank	avgriparianwidR
	Vegetative Protection Left Bank	avgvegprotecL
	Vegetative Protection Right Bank	avgvegprotecR
	Wetted Width	avgwetwidth
	Large Woody Debris (square meters)	LWD.m2
Sediment Size Distribution	Size at Which 10% of all Particles are Found	D10
	Size at Which 16% of all Particles are Found	D16
	Size at Which 25% of all Particles are Found	D25
	Size at Which 50% of all Particles are Found	D50
	Size at Which 75% of all Particles are Found	D75
	Size at Which 84% of all Particles are Found	D84
	Size at Which 50-84% of all Particles are Found	D84to50
	Size at Which 90% of all Particles are Found	D90
	Sediment Diversity	Hsimpson
	Inner Quartile Range of Sediment Sizes	IQR
	Large Cobbles to Fine Ratio	LCF
	% Fines	pfines
	% Large Cobbles	plargecobble
	% Pebbles	ppebbles
	% Small Cobbles	psmallcobble
	Small Cobbles to Fine Ratio	SCF

Objective 1: Determine within-stream variability of benthic macroinvertebrate communities and stream condition.

Comparisons of Taxa Metrics by Stream

Comparisons of selected taxa metrics and VASCI scores were used to evaluate differences in macroinvertebrate composition among and within streams. Data were tested for normality using *shapiro.test()* function of the *stats* package and homoskedasticity using *leveneTest()* function of the package *car* (Fox and Weisberg 2019). Analysis of variance was performed on all parametric data and Kruskal-Wallis was performed on non-parametric data. Analysis of variance was followed by a Tukeys pairwise post-hoc test with the *aov()* and *TukeyHSD()* function of *stats* package (R Core Team 2022).

Letters denoting significant pair-wise differences were assigned using *multcompLetters()* function from *multcompView* package (Graves et al. 2019). Kruskal-Wallis was followed by a dunn-bonferroni post-hoc test with the *kruskal.test()* function of *stats* package and *dunnTest()* function of *FSA* package (R Core Team 2022; Ogle et al. 2022). Letters were assigned using *cldList()* function of *rcompanion* package (Mangiafico 2023).

Spearman Correlations

Correlations were used to assess responses of taxa metrics and community similarity to distance downstream. Spearman rank correlation were utilized throughout this analysis because distributions of the analyzed variables varied widely and were often not normally distributed. Correlations were done using *rcorr()* function from *Hmisc* package (Frank 2022).

Non-Metric Multidimensional Scaling (NMDS)

Non-metric multidimensional scaling was used to visualize differences in taxa among and within streams. *MetaMDS()* function created the ordination using auto transformation and BC distance (matrix of dissimilarities). *Envfit()* was used to fit taxa vectors. Both functions are from the *vegan* package (Oksanen et al. 2016). Vectors to be graphically represented were chosen through identification of significant vectors and/or visual exploration of raw data to select taxa with the most individuals.

Objective 2: Determine if the same changes among streams over a gradient of SC are occurring within streams

Comparisons of SC by Stream

Specific conductance data were non-parametric; consequently, Kruskal-Wallis was performed to determine differences and variability of SC within streams. Dunn-Bonferroni post-hoc test was performed and letters for pairwise comparison were assigned as described for Objective 1.

Spearman Correlations

First, to further understand within-stream gradients and visualize longitudinal SC variability, SC was correlated with distance downstream in each stream. Next, Spearman correlations were used to assess responses of taxa metrics and community similarity to SC. Taxa metrics and VASCI were correlated to SC both in individual streams and globally with all streams pooled together.

Objective 3: Examine influence of water quality and other environmental factors on within-stream benthic macroinvertebrate variability.

As a first step in examining the influence of different environmental variables, I reduced collinearity of water quality and habitat metrics (via Principal Component Analysis (PCA) analysis) and created variables representing spatial patterns (via Principal Coordinates of Neighbourhood Matrix (PCNM) analysis). With water quality, habitat, and location attributes, I used Redundancy Analysis (RDA) to determine which variables best model taxonomic abundance within and among sites. Lastly, Variation Partitioning (VP) was used to assess the power and relatedness of the three attributes separately and collectively.

PCA to Reduce Collinearity in Water Quality and Habitat Attributes

Collinearity is ubiquitous in environmental data (McCune and Grace 2002) such that environmental studies often face the challenge of the dynamism of nature creating a multitude of variables interacting and influencing ecosystem responses. For water quality measures, especially changes in SC, most are likely collinear, or experiencing similar change, to major ions and trace elements, which directly affect SC. Similarly, as a riparian area becomes less disturbed, associated increases in bank stability, vegetation, canopy cover, etc. are expected. For initial examination of this collinearity, the *pairs* function from the graphics package was used for visual analysis of both water quality and habitat raw data and dictated a clear necessity to reduce collinearity.

To reduce dimensionality PCA, executed using *prcomp()* from the *stats* package, was conducted by a singular value decomposition of the data matrix as is the preferred method for numerical accuracy. In other words, PCA compiles variables to create separate axes, with each representing how multiple variables are related to each other. For the water quality attribute, *do.mgl*, *temp.C*, *sc.uScm*, *hardness.mgl*, and *npoc.mgl* were maintained and PCA grouped nutrients (total nitrogen, total phosphorus, *NH3* ammonia, nitrite and nitrate, and orthophosphate) into one axis variable and trace elements (see trace elements category, Table 2) into another. For the habitat attribute, *pfines*, *ppebbles*, *psmallcobbles*, *plargcobbles*, *LCF*, and *avgwetwidth*, *avgdepth*, *avgembedd*, *avgcancov* and *avg.slope* were maintained as independent variables. Principal Component Analysis was used to group all pebble diameter metrics into one axis and all riparian characteristic metrics into another. After dimensionality was reduced, the Variance Inflation Factor (VIF) was used (*vif* function from *car* package) to further assess the influence of collinearity on the RDA results.

VIF equation:

The VIF for predictor *i* is $1 / (1 - R_i^2)$, where R_i^2 is the R^2 from a regression of predictor *i* against the remaining predictors.

Creating Location Attribute: PCNM

Environmental conditions, created by water quality and habitat, and metacommunity dynamics are often spatially structured, therefore the location of the sample could either be a driving force or confounding variable when considering macroinvertebrate responses (Dray et al. 2006). To address this, Principal Coordinates of Neighbourhood Matrix (PCNM) analysis uses site latitude and longitude data to create variables of space encompassing the shape, arrangement, clustering, and dispersal of site locations using the *Pcnm* function of the *vegan* package (Oksanen et al. 2016). Principal Coordinates of Neighbourhood Matrix uses a spatial weighting matrix and maximizes the Moran's autocorrelation index to create orthogonal maps (Dray et al. 2006). Spatial weights are the relationship of the sample locations to one another, and Moran's spatial autocorrelation evaluates the patterns in space. It is important to note the first PCNM show a broad-scale variation and later PCNM show smaller-scale spatial variation. A broad-scale will highlight the spatial differences between the sites farthest apart, whereas a small-scale will emphasize the relationship of sites closer together (Borcard and Legendre 2002). Spatial structure is important in deciphering the relationship of metacommunity dynamics and local environmental factors when interpreting the impact of coal mining on stream biota. For example, local factors may explain more community variation than space if dispersal is low in that stream (Wilson and McTammany 2016).

For this study, I used GPS coordinates collected in the field at each sampling location to conduct PCNM analysis for the study streams using *Pcnm* function of the *vegan* package (Oksanen et al. 2016).

Redundancy Analysis (RDA) and Variation Partitioning (VP)

Once the variables for each attribute were finalized, RDA was used to determine the relative influence of environmental variables on taxa abundance within and among streams. Redundancy analysis was performed separately for each attribute (water quality, habitat, and location) and for each stream and all streams pooled together in each season. Before analysis, the dataframe was normalized using the *decostand* function. For each attribute, two RDA models were created using the *rda* function, one null model and one with all variables for that attribute. The *OrdR2step* function performed backwards stepwise model selection using permutation tests on the model of all variables given the scope of the null model. This model selection created a model of best fit to be used in all subsequent analyses. This model of best fit dictated which variables for each attribute were most influential in driving macroinvertebrate community composition. Lastly, *varpart* was used to perform variation partitioning and determine what percent of changes in community composition were due to each attribute and from combinations therein. The best RDA model (representative of selected drivers) for each attribute was used in the function *varpart*, which partitioned the variation in the community matrix due to the three explanatory attributes separately and collectively. All functions utilized were from the *vegan* package (Oksanen et al. 2016).

Chapter 3- Results

Objective 1: Determine within-stream variability of benthic macroinvertebrate communities and stream condition.

Comparing Taxa Metrics by Stream

I evaluated how taxa metrics differed among streams and the extent of variability within them using mean or median comparisons, either ANOVA or Kruskal-Wallis, respectively, for the 15 selected taxa metrics. I found variability of macroinvertebrate community composition and thus these metrics within the study streams, especially in mining-influenced streams in spring (Figures 3-4, Table 5). Generally, reference (R) streams had higher median values for taxa metrics commonly associated with reference conditions and less variability than highly-impacted (H) mining-influenced streams. Percent composed of the five dominant taxa was an exception in that there was no clear separation of this taxa metric among any of the study streams for either season. Although separation of reference and highly-impacted streams was often clearly reflected by the median and within-stream variation of taxa metrics, low-level (L) impacted stream tended to overlap with taxa metrics from either reference or highly-impacted streams. Strongest separation of low-level impacted streams was observed in spring (Figure 3). Long-term sampling locations were often not representative of median stream values and occurred in various positions of within-stream variation. For example, long-term sampling locations were more likely to occur outside the interquartile range (middle 50% of data) in spring for mining-influenced streams (Figure 3).

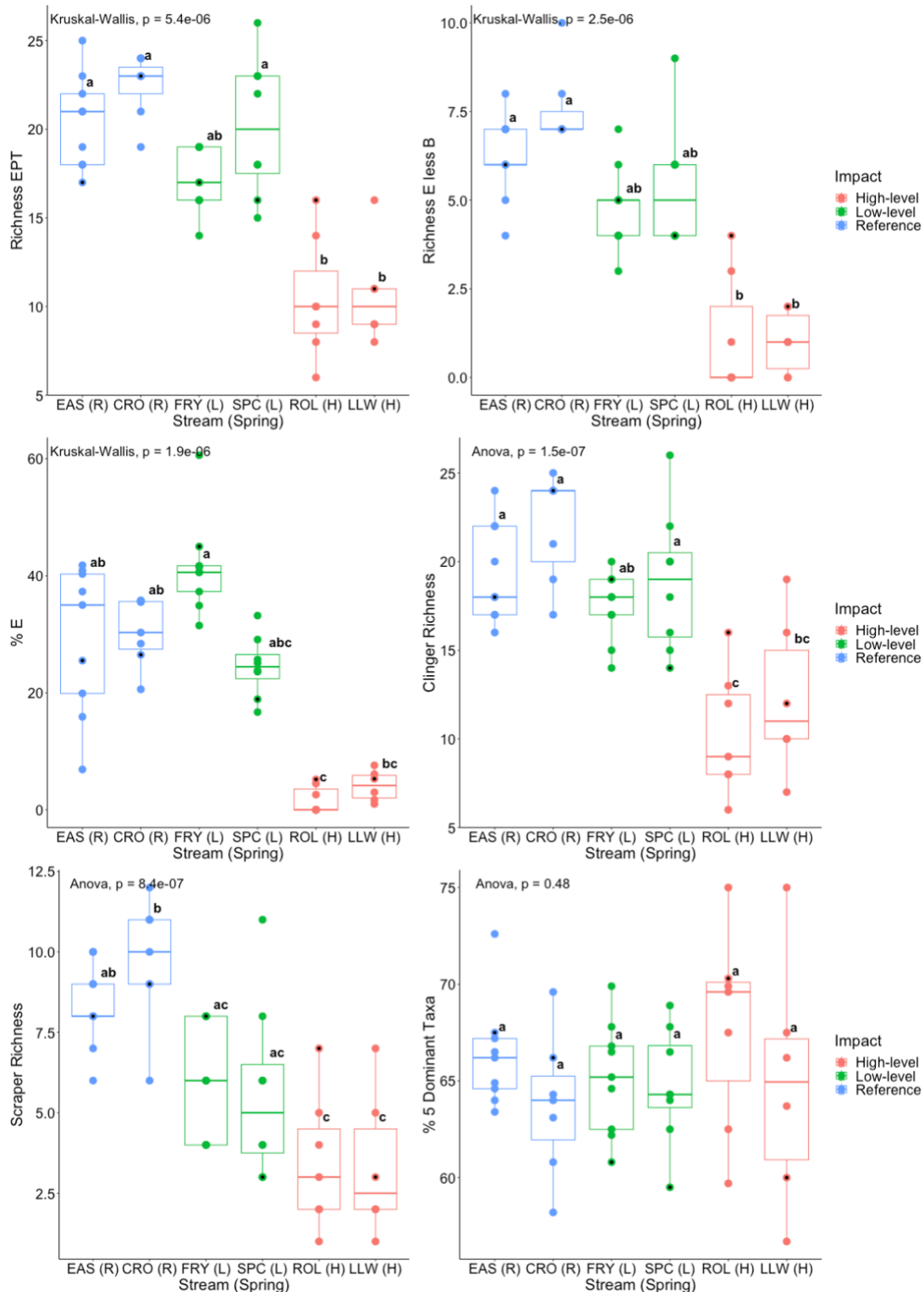


Figure 3. Spring ANOVA and Kruskal-Wallis comparisons among six study streams in the coalfield region of central Appalachia for six taxa metrics of interest. Colors indicate level of impact from mountain top mining valley fill salinization, and black dots represent long-term sampling location. Different letters indicate significant differences at $\alpha=0.05$.

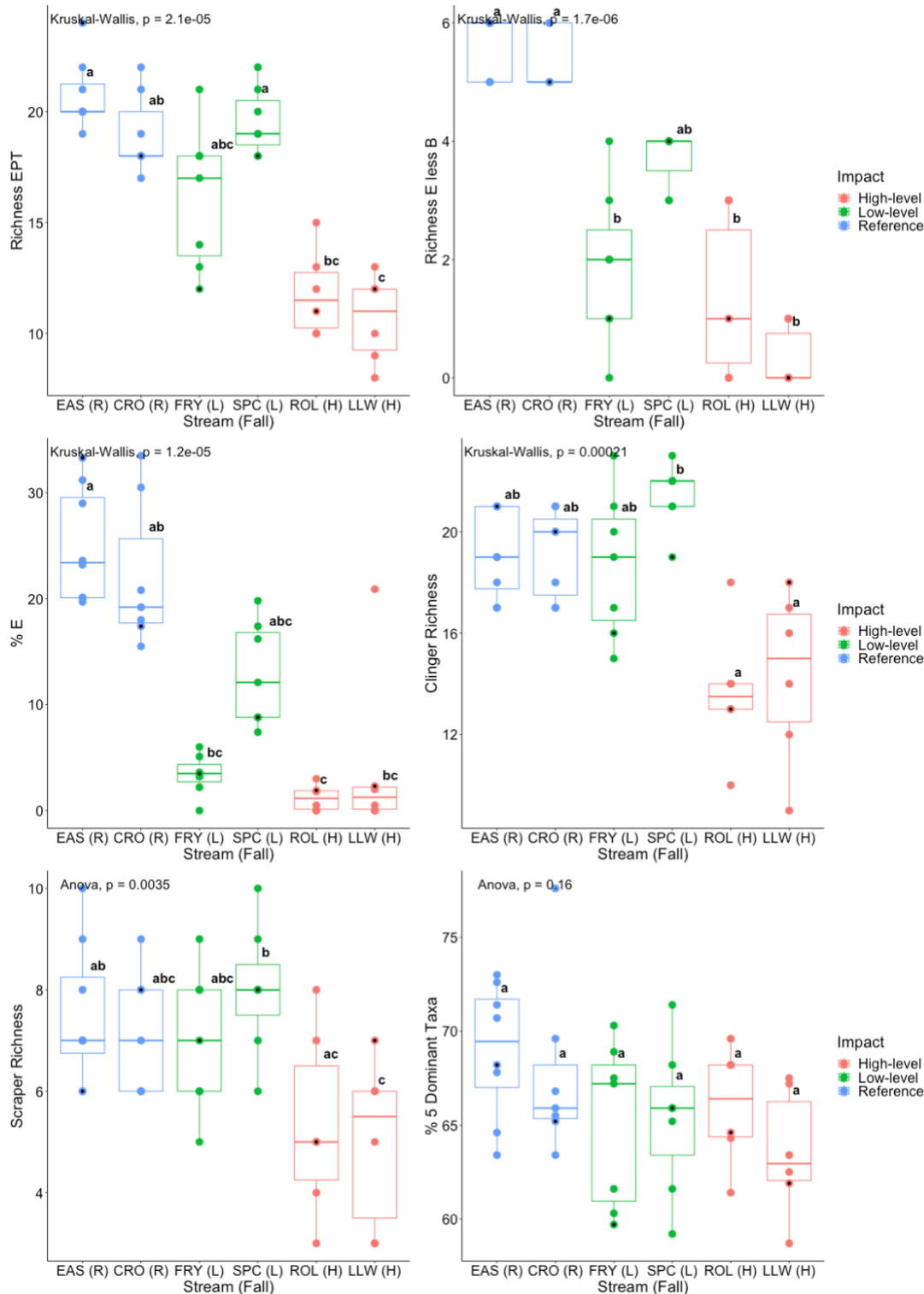


Figure 4. Fall ANOVA and Kruskal-Wallis comparisons among six study streams in the coalfield region of central Appalachia for select taxa metrics of interest. Colors indicate level of impact from mountain top mining valley fill salinization and black dots represent long-term sampling location. Different letters indicate significant differences at $\alpha=0.05$.

Table 5. Kruskal-Wallis and ANOVA chi-squared or F values for 15 selected metrics from six study streams in the coalfield of central Appalachia.

Taxa Metric	Spring			Fall		
	Test	p	X ² or F	Test	p	X ² or F
*p5dom	ANOVA	4.20E-01	0.914	ANOVA	1.55E-01	1.723
*pE	Kruskal-Wallis	1.93E-06	34.453	Kruskal-Wallis	1.22E-05	30.411
*rich.Cling	ANOVA	1.51E-07	13.03	Kruskal-Wallis	2.12E-04	24.057
*rich.E.less.B	Kruskal-Wallis	2.47E-06	33.923	Kruskal-Wallis	1.65E-06	34.796
*rich.EPT	Kruskal-Wallis	5.44E-06	32.192	Kruskal-Wallis	2.14E-05	29.180
*rich.SC	ANOVA	8.35E-07	11.23	ANOVA	3.48E-03	4.352
Hshannon	Kruskal-Wallis	1.43E-06	35.119	ANOVA	2.50E-07	13.410
pChi	ANOVA	6.16E-05	7.295	Kruskal-Wallis	7.62E-05	26.35
pE.less.B	Kruskal-Wallis	6.70E-06	31.737	Kruskal-Wallis	1.07E-06	35.734
pPR	ANOVA	1.00E-06	11.05	ANOVA	9.44E-05	7.253
pPT.H	ANOVA	4.14E-07	11.95	ANOVA	8.97E-03	3.668
pSC	ANOVA	4.05E-08	14.52	KRUSKAL	0.02836	12.516
rich	Anova	8.79E-11	22.91	ANOVA	1.19E-07	14.330
rich.E	Kruskal-Wallis	2.86E-06	33.603	Kruskal-Wallis	1.86E-06	34.544
richPR	KRUSKAL	0.001633	19.379	ANOVA	0.00059	5.714

*Key metrics which are graphically represented throughout this thesis

Kruskal-Wallis of VASCI Scores by Stream

I used median comparison of VASCI scores among streams to indicate variation among and within streams in overall stream condition based on metrics used by VA DEQ (Burton and Gerritsen 2003). Similar to taxa metrics, stream condition, as indicated by VASCI, was highest and least variable in reference and lowest and most variable in highly-impacted streams (Figure 5). However, contrary to taxa metric patterns, however, fall best identified separation of reference streams from low-level impacted streams. In highly-impacted streams, median VASCI scores were lower in spring (Figure 5). Additionally, in multiple streams, VASCI scores at the long-term sites occurred outside of the interquartile range.

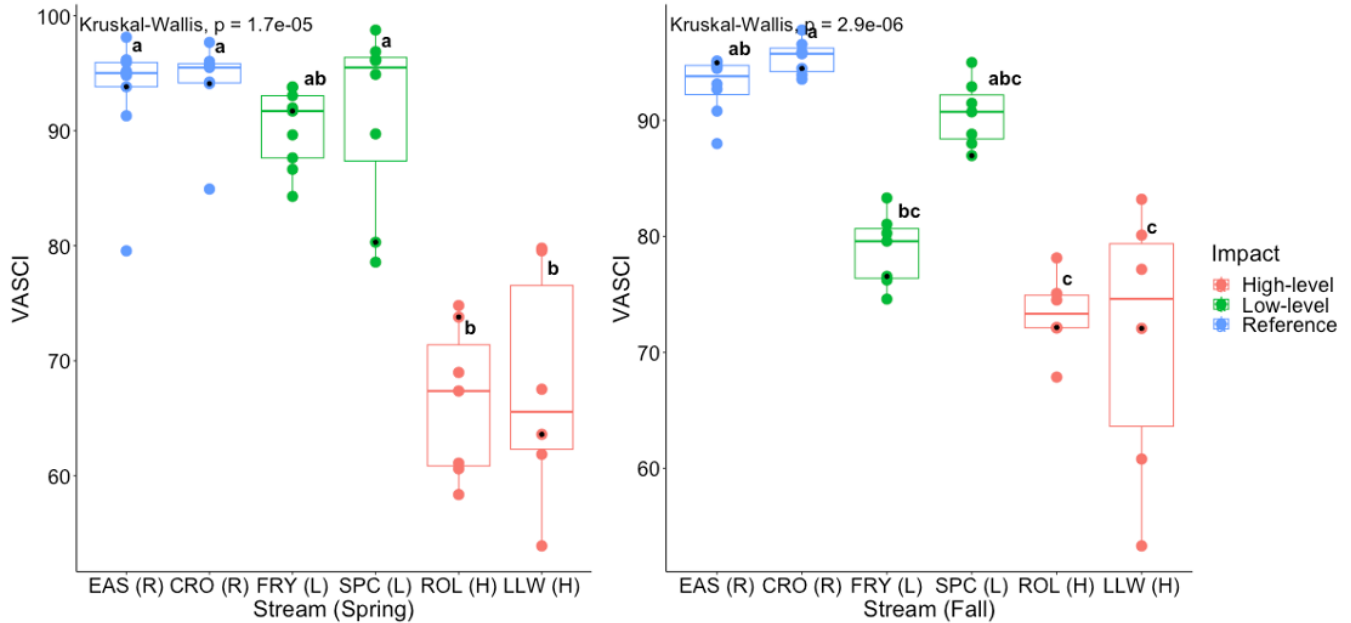


Figure 5. Virginia Stream Condition Index (VASCI) score variability among and within six study streams in the coalfield of central Appalachia by season, with spring results shown left and fall right. Black dots indicate the location of long-term sample collections. Different letters indicate significant differences among medians at $\alpha=0.05$.

Spearman Correlations of Taxa Metrics to Distance Downstream

I used correlations between taxa metrics and distance downstream to identify if longitudinal distance explains within-stream variation in metric values. For this analysis, I illustrate trends for the six selected graphed metrics (Figure 6) and present correlations for the 15 key taxa metrics (Table 6); see Appendix C for all 60 metrics. Generally, more taxa metrics were correlated to distance in spring, except for one reference and one low-level impacted stream (CRO and FRY, respectively). Of the 15 selected metrics, all had correlations to distance downstream in at least one stream in spring but only nine had correlations to distance downstream in fall (Table 6). Of the nine metrics correlated in fall, only two of those metrics were of the six key metrics, percent Ephemeroptera and Ephemeroptera less Baetidae richness. One low-level impacted stream (FRY) did not have any correlations with the six key metrics in either season. One highly-impacted (ROL) and one low-level impacted stream (SPC) had the most taxa metrics (21 and 27, respectively) correlated to distance, both in spring.

Eleven taxa metrics were correlated to distance in at least three of the six streams: percent Ephemeroptera (and less B), clinger richness, EPT richness (and less H), percent scrapers (and less E), scraper richness less Ephemeroptera, CF richness and total richness in spring four of which are not included in the 15 metrics selected to be most responsive in previous studies (Appendix C). In fall, only percent Plecoptera (and less Luectra, Allocapnia, and Amphinemura and less Luectridae) and percent sprawlers were correlated to distance in at least three of the six streams (Appendix C). One highly-impacted stream (ROL) and one low-level impacted stream (SPC) had the most shared taxa metric correlations, although the low-level impacted stream (SPC) had the opposite trend generally with expected response to disturbance downstream instead of upstream. Taxa metrics changing in reference streams that did not change significantly in impacted streams include percent Plecoptera, percent SH and richness of tolerant taxa in spring, and percent CF, percent CG, percent CN, percent SW, percent

Trichoptera, percent tolerant, richness burrowers, richness Coleoptera, richness SC, richness Trichoptera, and richness tolerant in fall (Appendix C). One low-level impacted stream (SPC) had the most unique taxa metric responses in spring, three of which (percent chironomids, percent predator, and richness predator) have been found to respond to disturbance in previous research (U.S. EPA 2011) (Table 6).

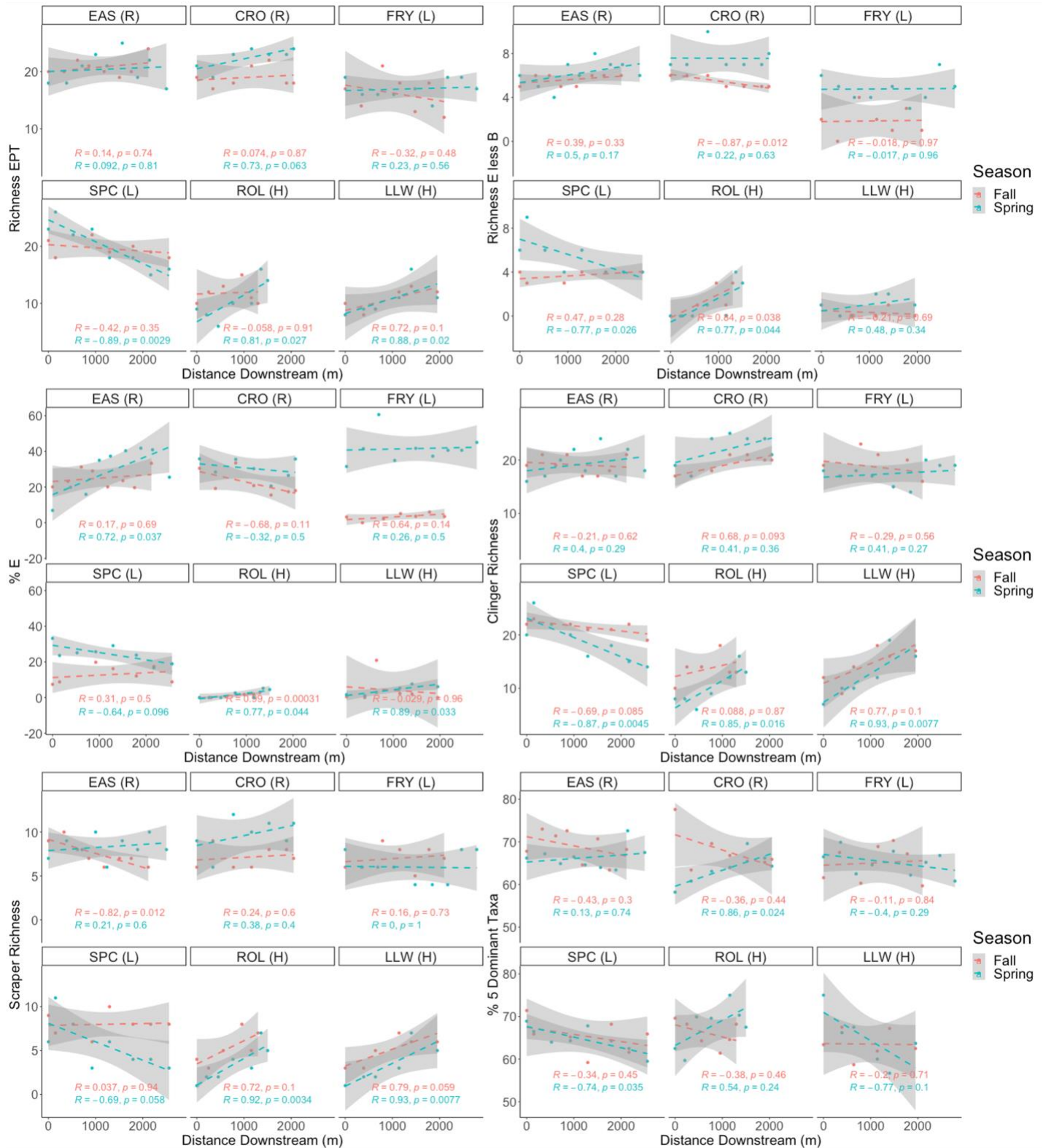


Figure 6. Spearman correlations of six selected taxa metrics to distance downstream for each stream and season for six study streams in the coalfield region of central Appalachia. Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Table 6. Spearman correlation coefficients for selected taxa metrics versus distance downstream with p -value < 0.05 for six study streams in the coalfield of central Appalachia by season.

Taxa Metric	Season	EAS (R)	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)
*p5dom	Spring	NA	8.6E-01	NA	-7.4E-01	NA	NA
*pE	Spring	7.2E-01	NA	NA	NA	7.7E-01	8.9E-01
*rich.Cling	Spring	NA	NA	NA	-8.7E-01	8.5E-01	9.3E-01
*rich.E.less.B	Spring	NA	NA	NA	-7.7E-01	7.7E-01	NA
*rich.EPT	Spring	NA	NA	NA	-8.9E-01	8.1E-01	8.8E-01
*rich.SC	Spring	NA	NA	NA	NA	9.2E-01	9.3E-01
Hshannon	Spring	NA	NA	NA	-7.4E-01	NA	8.3E-01
pChi	Spring	NA	NA	NA	8.6E-01	NA	NA
pE.less.B	Spring	7.3E-01	NA	-8.3E-01	-9.0E-01	8.1E-01	NA
pPR	Spring	NA	NA	NA	-8.1E-01	NA	NA
pPT.H	Spring	NA	-8.2E-01	NA	NA	NA	NA
pSC	Spring	7.5E-01	NA	NA	-9.8E-01	8.9E-01	8.9E-01
rich	Spring	NA	NA	NA	-9.0E-01	9.3E-01	8.4E-01
rich.E	Spring	NA	NA	NA	NA	7.7E-01	NA
richPR	Spring	NA	NA	NA	-8.9E-01	NA	NA
*pE	Fall	NA	NA	NA	NA	9.9E-01	NA
*rich.E.less.B	Fall	NA	-8.7E-01	NA	NA	8.4E-01	NA
Hshannon	Fall	NA	NA	NA	NA	NA	9.4E-01
pE.less.B	Fall	NA	NA	NA	NA	9.9E-01	NA
pPT.H	Fall	NA	NA	-8.2E-01	NA	-8.9E-01	1.0E+00
pSC	Fall	NA	-9.3E-01	8.6E-01	NA	NA	NA
rich	Fall	NA	NA	NA	-9.5E-01	NA	NA
rich.E	Fall	NA	-7.8E-01	NA	NA	8.4E-01	NA
richPR	Fall	7.1E-01	NA	NA	NA	NA	9.3E-01

* Key metrics

Spearman Correlations of VASCI to Distance Downstream

Correlations were tested for VASCI scores with distance to determine if distance downstream can help explain variation in stream condition when considering effects of multiple taxa metrics. In spring, VASCI was significantly correlated to distance downstream positively in both of the highly-impacted streams (ROL and LLW) and negatively in one low-level impacted stream (SPC) (Figure 7). In fall, VASCI was not correlated to distance downstream in any streams. Although some significant correlations of taxa metrics to distance downstream in reference streams were noted, VASCI reflected minimal change, especially when compared to the magnitude of observed changes in highly-impacted streams. In the spring, VASCI in reference streams ranged from ca. 80-98, in low-level impacted from ca. 79-99, and in highly-impacted from ca. 53-79. In the fall, VASCI in reference streams ranged from ca. 88-98, in low-level impacted from ca. 74-95, and in highly-impacted from ca. 53-83. Separation of stream condition due to season was evident in two mining-influenced streams, with higher VASCI score for FRY in spring and ROL in fall (Figure 7).

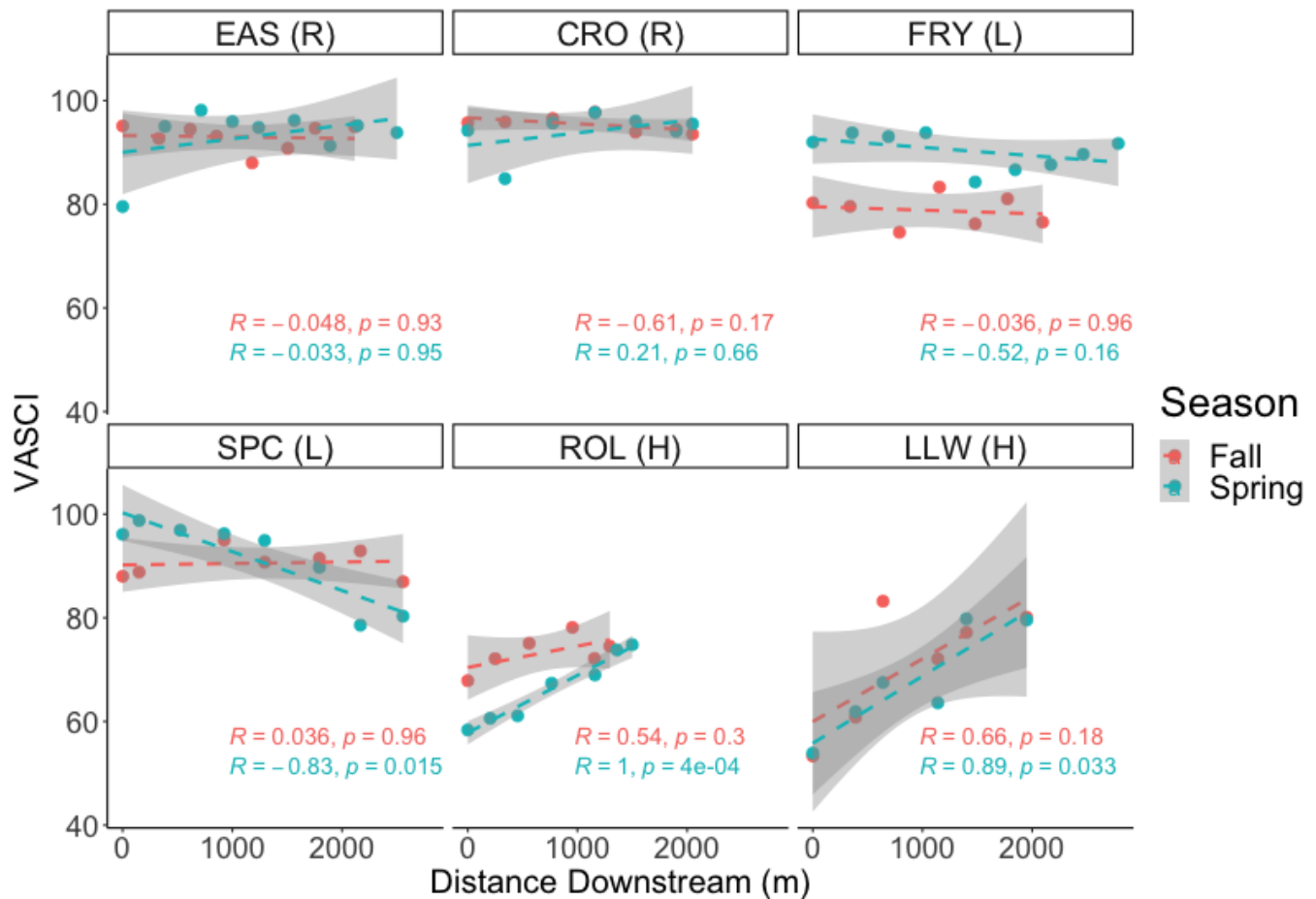


Figure 7. Spearman correlations of Virginia Stream Condition Index (VASCI) to distance downstream by stream and season for six streams in the coalfields of central Appalachia by season. Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Spearman Correlations of Community Similarity to Distance Downstream

To further assess within-stream variation in community composition, community similarities among sampling locations were determined and correlated with distance downstream. Two types of similarity matrices were used to assess the extent to which communities were shifting within streams. Bray-Curtis (BC) emphasizes dominance/abundance of taxa rather than presence/absence. Jaccard (J) standardizes presence/absence data rendering this index sensitive to rare taxa. Before correlations were performed, the two most dissimilar sites in each stream for each season were identified and averages of both reference streams and the remaining four mining-influenced streams were assessed. When considering within-stream BC community similarity, mining-influenced streams most dissimilar sites changed an average 25% in spring and 20.1% in fall (Figure 8). In reference streams, BC similarity indicated an average 20.9% shift in spring and 14.7% in fall. When considering J community similarity, mining-influenced streams most dissimilar sites changed an average 7.8% in spring and 9.98% in fall. Jaccard similarity showed reference streams shifted 3.4% in spring and 5% in fall. This suggests utilizing BC in mining-influenced headwater streams captures the largest shifts in community similarity.

For BC, community similarity related to distance downstream in five streams (all but CRO) in spring, but related to distance downstream in only three streams in fall (CRO, FRY and ROL) (Figure 8). For J, community similarity was related to distance downstream in one reference (EAS) and one highly-impacted stream (ROL) in spring and one reference (CRO) in fall (Figure 8). Although BC captures larger shifts in community similarity, an additional consideration for J is that it may capture unique patterns of change caused by taxa turnover. Considering J, total percent change from upstream to downstream was negligible except in highly-impacted streams, suggesting more change in similarity can be attributed to taxa turnover in these streams.

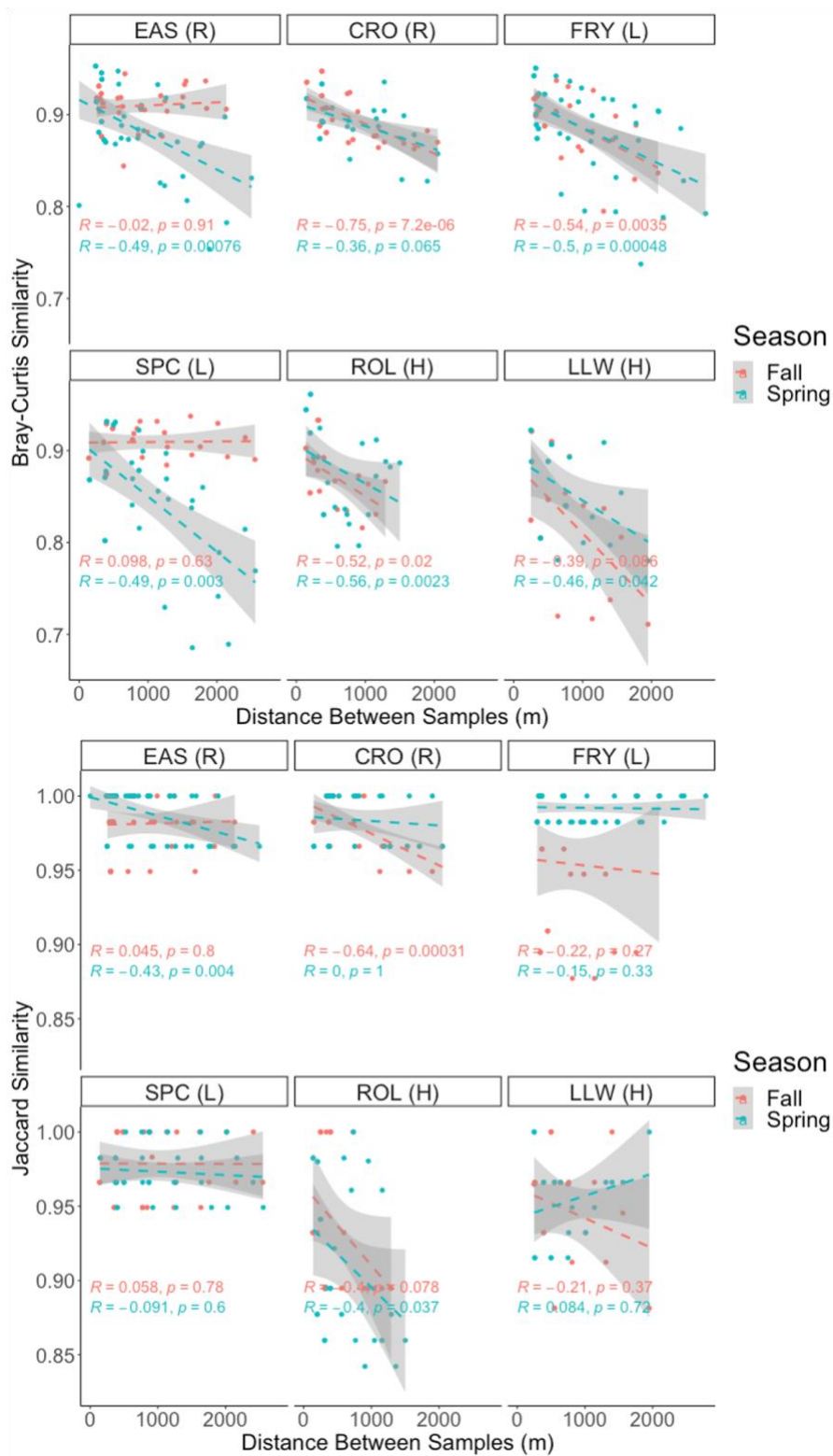


Figure 8. Bray-Curtis (top) and Jaccard Similarity (bottom) versus distance downstream for six streams in the coalfield of central Appalachia by season. Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

NMDS Displaying Site and Species Relationships

To visualize overall relatedness of community composition within and among sites and which taxa drive such variation, NMDS analysis of taxa abundance was utilized. When pooling all streams together, there was some, but not complete, overlap of community composition between levels of impact, although more separation is visible in fall (Figure 9). Taxa that may be weighing most heavily on community similarity and the divide between reference and impacted streams include *Habrophlebioides* and *Stenelmis* (Ephemeroptera and Coleoptera taxa, respectively) in reference and *Tipula* and *Amphinemura* (Diptera and Plecoptera taxa, respectively) in mining-influenced streams. Within streams, there was a general grouping of middle sites and separation of the most upstream and downstream site (Figures 10, 11). In low- and high-level impacted streams, dominant taxa indicate less-sensitive taxa than reference streams (Figures 10,11).

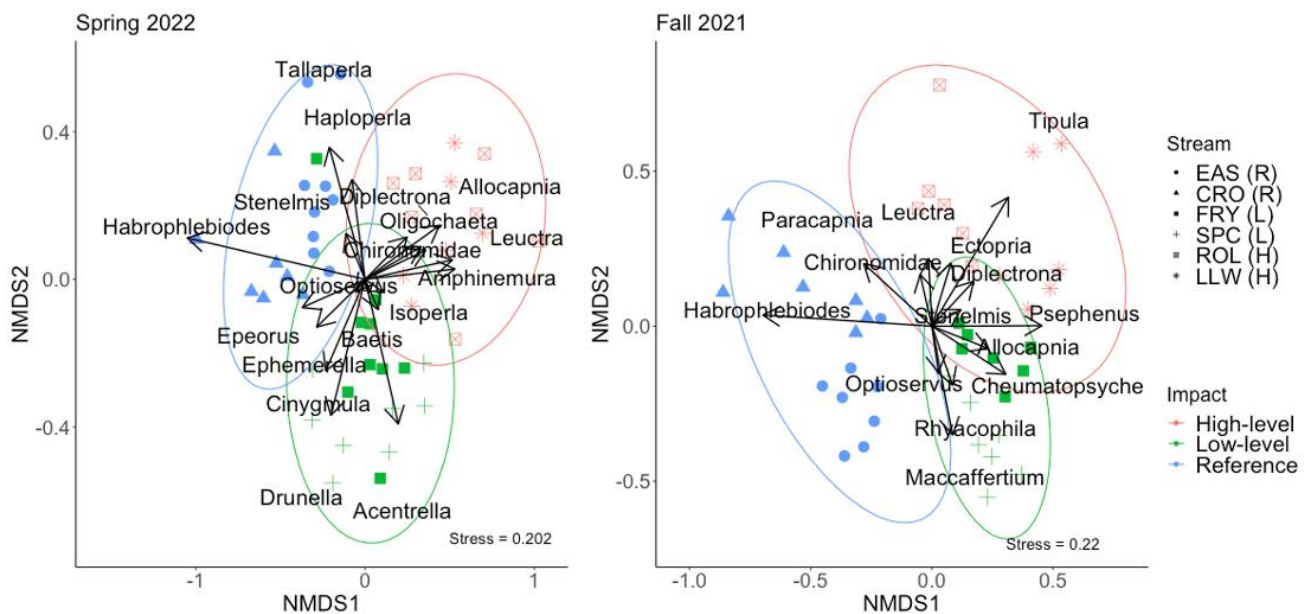


Figure 9. NMDS of six study streams in two seasons from the coalfields of central Appalachia with taxa vectors. Selected taxa vectors represent those with highest proportion of abundance. Ellipses represent level of impact.

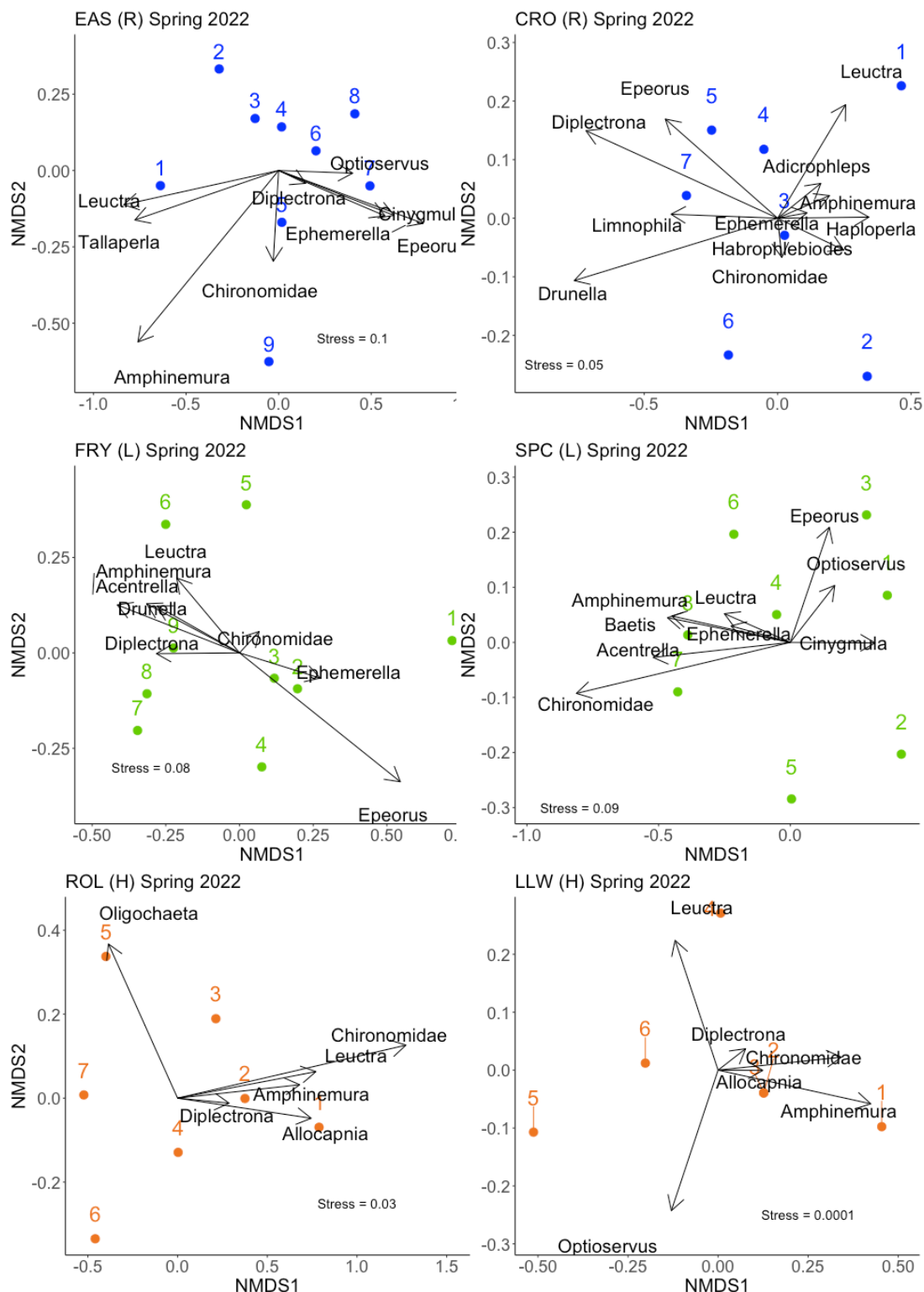


Figure 10. Spring NMDS for six study streams in the coalfield of central Appalachia. Points representing each sampling location within a stream (1 is the most upstream) and arrows indicate taxa with highest proportion of abundance. Stress near zero indicates more samples needed to validate analysis; ideal stress range 0.05-0.2 (McCune and Grace 2002).

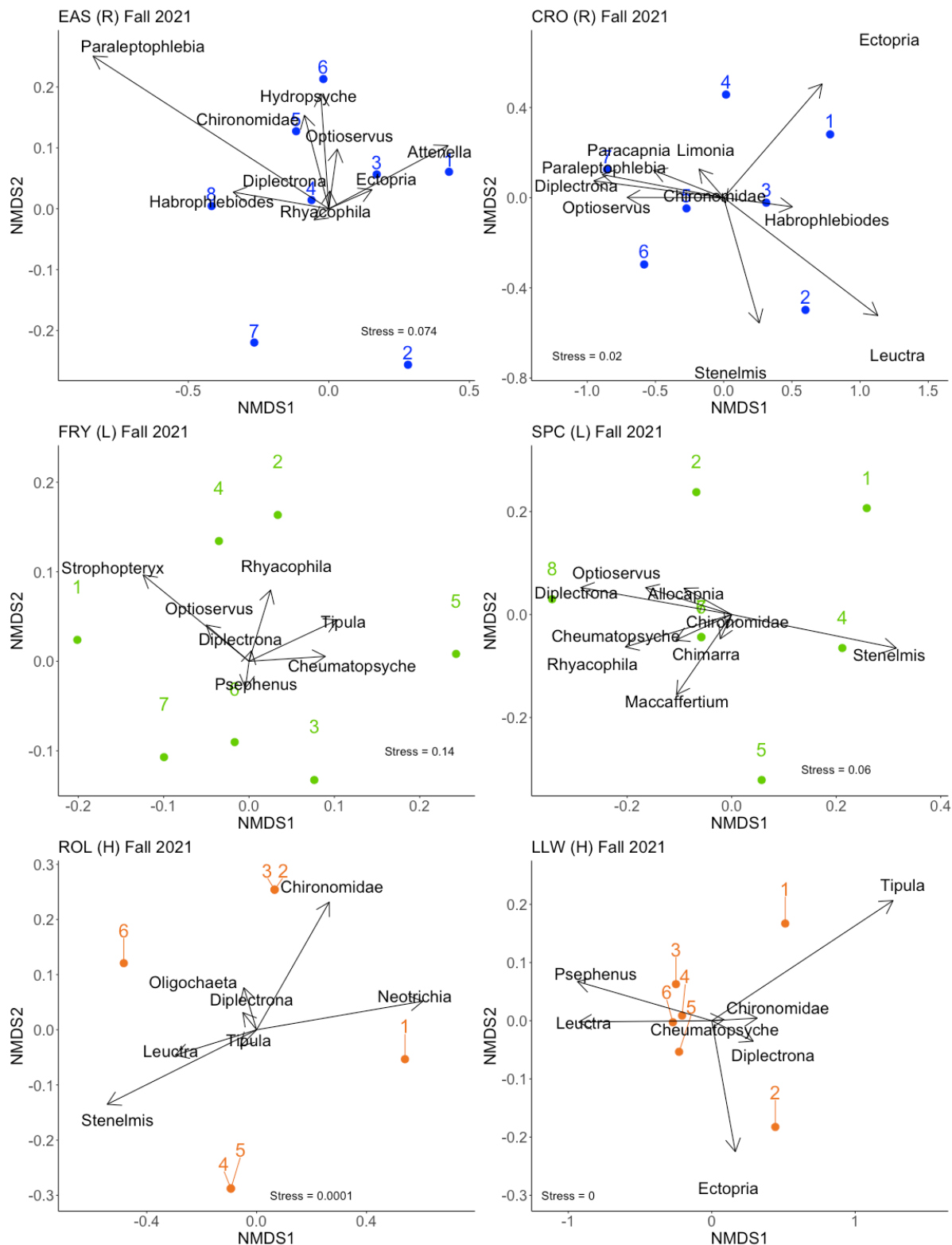


Figure 11. Fall NMDS for six study streams in coalfield of central Appalachia. Points representing each sampling location within a stream (1 is the most upstream) and arrows indicate taxa with highest proportion of abundance. Stress near zero indicates more samples needed to validate analysis; ideal stress range 0.05-0.2 (McCune and Grace 2002).

Objective 2: Determine if the same changes observed among streams over a gradient of SC are occurring within streams.

Determining SC Trends Within Streams

Kruskal-Wallis and correlation of SC to distance were utilized to determine within-stream trends of SC. Reference streams had the lowest SC with the least variability, whereas the two highly-impacted streams (ROL and LLW) had the most within-stream SC variability (Figure 12). In fall, median SC increased, but with reduced within-stream variability in three of the four mining-influenced streams (SPC had low variability in spring). For low-level impacted streams (FRY and SPC) SC ranges did not overlap between seasons.

In both seasons, one reference (EAS) and two highly-impacted streams (ROL and LLW) exhibited significant correlations between SC and distance: EAS had a positive correlation between SC and distance, whereas the highly-impacted streams exhibited negative correlations (Figure 13). Additionally, one low-level impacted stream in fall (SPC) had a significant negative correlation, and one in spring (FRY) had a significant positive correlation (Figure 13). Although the reference stream (EAS) showed a trend in SC, it was over a change of less than 10 uS/cm compared to much larger ranges in low-level impacted (65-290 uS/cm) and highly-impacted (640-990 uS/cm) streams (Table 1, Figure 13). Some streams showed perfect correlation (i.e. $R=-1$) of SC to distance due to identical spearman ranking of the two variables.

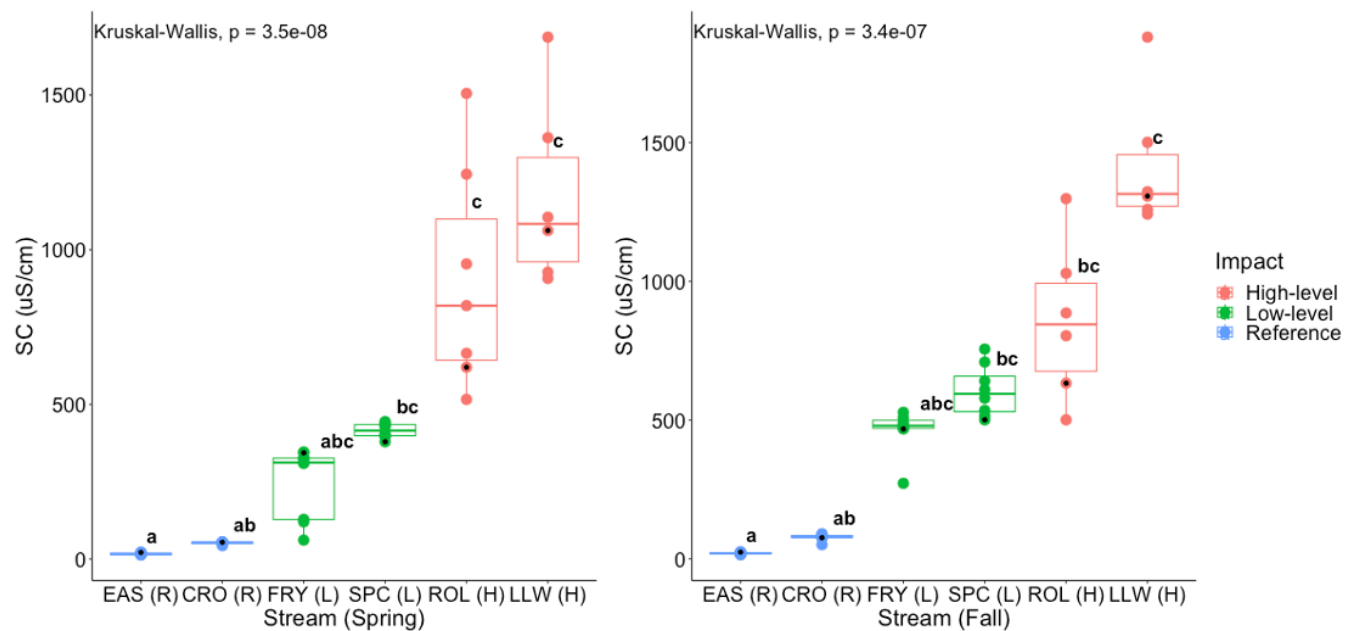


Figure 12. Kruskal-Wallis comparisons of specific conductance (SC) variability among and within six study streams in the coalfield of central Appalachia by season. Black dots indicate the location of long-term sample collections. Different letters indicate significant differences among medians at alpha=0.05. Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

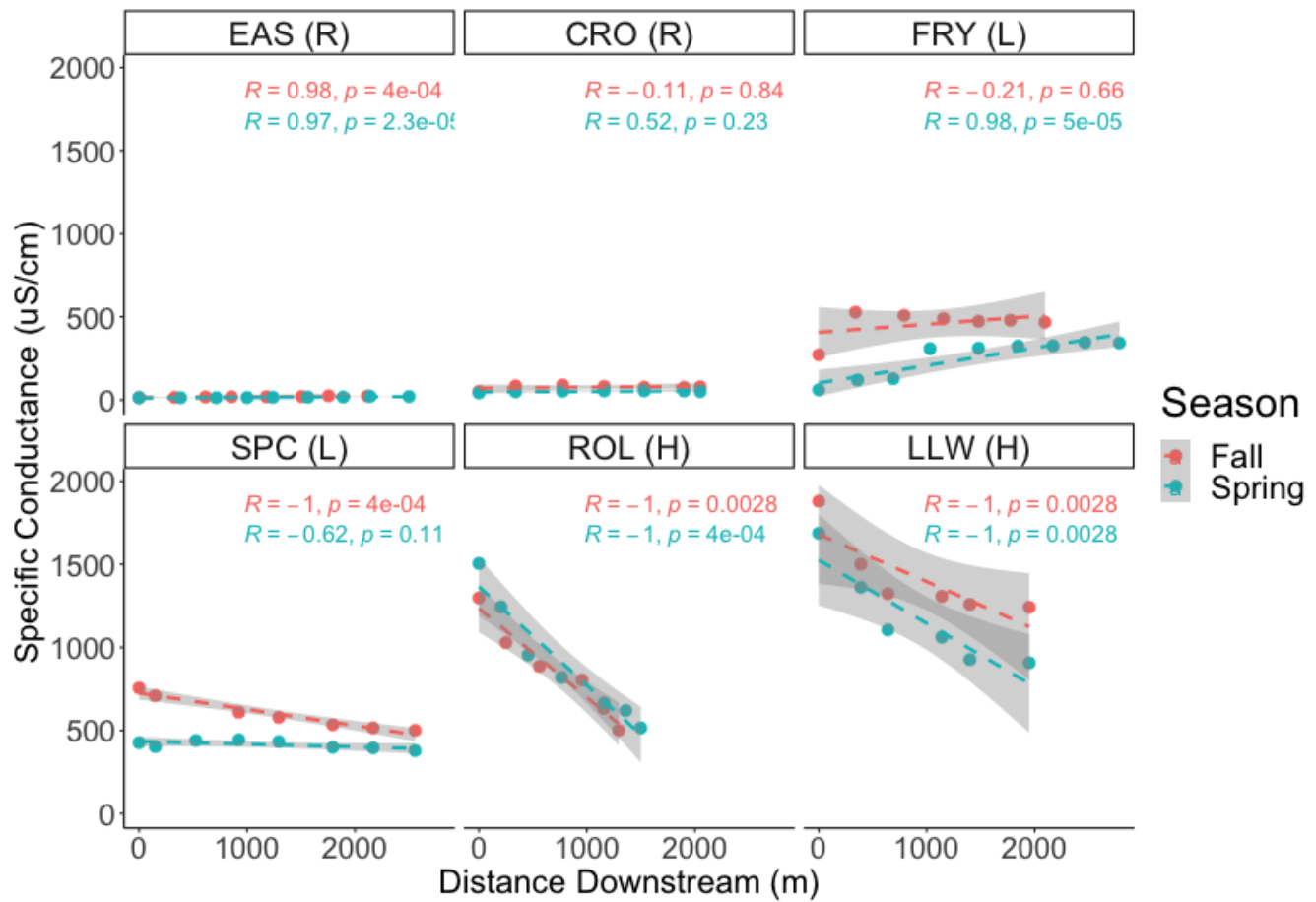


Figure 13. Specific conductance versus distance downstream for six study streams in the coalfield of central Appalachia by season. Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Spearman Correlations of Taxa Metrics to SC by Stream

Correlations between taxa metrics and SC were assessed to determine if taxa responded to SC gradients occurring within the study streams. As spearman correlations ranked distance and SC the same in both seasons in the highly-impacted streams (ROL and LLW) and in fall for one low-level impacted stream (SPC), the same taxa metrics correlated to distance were correlated SC in these streams. The 15 selected metrics were more often correlated to SC in highly-impacted streams in spring (Figure 14, Table 7). All streams had more taxa metrics correlated to SC in the spring with the most correlations occurring in highly-impacted streams (Appendix C). Of the taxa metrics significantly correlated to SC, most exhibited the expected response to disturbance (i.e. sensitive metrics declining and tolerant metrics increasing with SC) except in one low-level impacted stream (SPC) (Table 7, Appendix C). That is, SPC was the only mining- influenced stream that showed a majority of tolerant taxa metrics reflective of disturbance increasing with decreasing SC. In both low-level impacted streams (SPC and FRY), more taxa metrics were correlated to SC in the seasons opposite when the streams experienced the largest SC gradient.

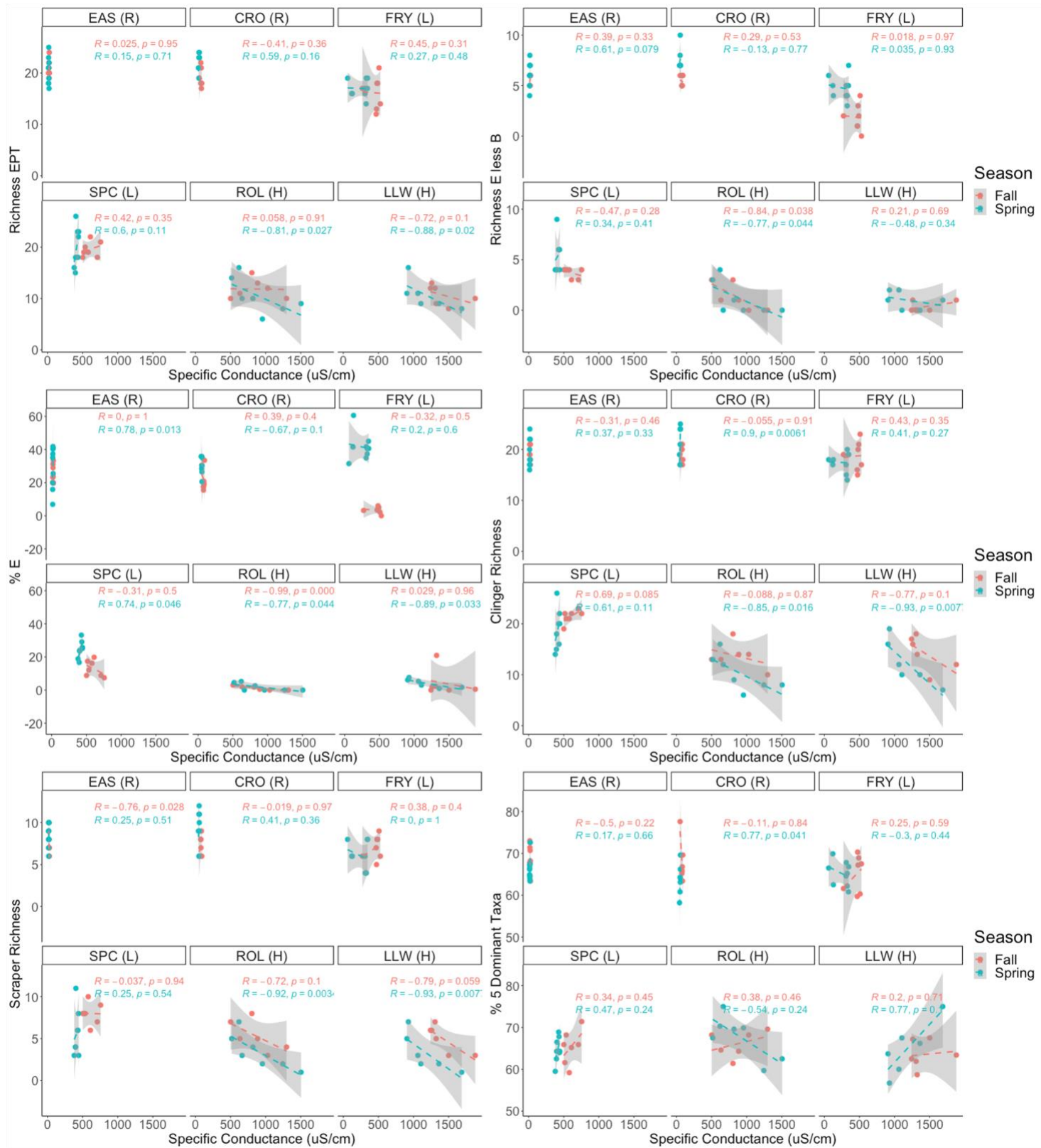


Figure 14. Spearman correlations of six selected taxa metrics to specific conductance by stream and season in six study streams in the coalfield of central Appalachia. Level of impact indicated in () after stream name: R, L, and H indicating Reference, Low-level, and High-level, respectively.

Table 7. Selected taxa metrics Spearman correlation coefficients for 15 selected taxa metrics versus specific conductance with p -value < 0.05 by season for six study streams in the coalfield of central Appalachia.

Taxa Metric	Expected Response	Season	EAS (R) ^{1,2} R	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)	ALL
*p5dom	pos	Spring	NA	7.7E-01	NA	NA	NA	NA	NA
*pE	neg	Spring	7.8E-01	NA	NA	7.4E-01	-7.7E-01	-8.9E-01	-6.2E-01
*rich.Cling	neg	Spring	NA	9.0E-01	NA	NA	-8.5E-01	-9.3E-01	-5.9E-01
*rich.E.less.B	neg	Spring	NA	NA	NA	NA	-7.7E-01	NA	-7.4E-01
*rich.EPT	neg	Spring	NA	NA	NA	NA	-8.1E-01	-8.8E-01	-6.7E-01
*rich.SC	neg	Spring	NA	NA	NA	NA	-9.2E-01	-9.3E-01	-7.2E-01
Hshannon	neg	Spring	NA	7.7E-01	NA	7.1E-01	NA	-8.3E-01	-7.6E-01
pChi	pos	Spring	NA	NA	NA	-8.1E-01	NA	NA	NA
pE.less.B	neg	Spring	8.1E-01	NA	-7.8E-01	NA	-8.1E-01	NA	-7.6E-01
pPR	neg	Spring	NA	NA	NA	NA	NA	NA	-4.4E-01
pPT.H	neg	Spring	-7.1E-01	NA	NA	7.1E-01	NA	NA	6.4E-01
pSC	neg	Spring	7.8E-01	NA	-6.7E-01	NA	-8.9E-01	-8.9E-01	-7.0E-01
rich	neg	Spring	NA	NA	NA	NA	-9.3E-01	-8.4E-01	-7.2E-01
rich.E	neg	Spring	NA	NA	NA	NA	-7.7E-01	NA	-7.1E-01
richPR	neg	Spring	NA	NA	NA	NA	NA	NA	-3.9E-01
*p5dom	pos	Fall	NA	NA	NA	NA	NA	NA	-3.7E-01
*pE	neg	Fall	NA	NA	NA	NA	-9.9E-01	NA	-7.4E-01
*rich.Cling	neg	Fall	NA	NA	NA	NA	NA	NA	-3.5E-01
*rich.E.less.B	neg	Fall	NA	NA	NA	NA	-8.4E-01	NA	-8.3E-01
*rich.EPT	neg	Fall	NA	NA	NA	NA	NA	NA	-6.6E-01
*rich.SC	neg	Fall	-7.6E-01	NA	NA	NA	NA	NA	-4.2E-01
Hshannon	neg	Fall	NA	NA	NA	NA	NA	-9.4E-01	-5.3E-01
pChi	pos	Fall	NA	NA	NA	NA	-9.9E-01	NA	-8.6E-01
pE.less.B	neg	Fall	NA	NA	NA	NA	-9.9E-01	NA	-8.6E-01
pPR	neg	Fall	NA	NA	NA	NA	NA	NA	-5.6E-01
pPT.H	neg	Fall	NA	7.9E-01	NA	NA	8.9E-01	-1.0E+00	NA
pSC	neg	Fall	NA	NA	NA	NA	NA	NA	-3.6E-01
rich	neg	Fall	NA	NA	NA	9.5E-01	NA	NA	-4.1E-01
rich.E	neg	Fall	NA	NA	NA	NA	-8.4E-01	NA	-8.0E-01
richPR	neg	Fall	NA	NA	-8.0E-01	NA	NA	-9.3E-01	NA

¹ Grey cells exhibit opposite of expected response to disturbance.

² Level of impact from mountaintop mining valley fill salinization indicated in () after stream name; R, L, and H indicate Reference, Low-level, and High-level, respectively.

When macroinvertebrate metrics were pooled among all streams, there were more relationships of community composition to SC, with double the number of taxa metrics correlated to SC compared to any one stream in spring and almost triple the correlations compared to any one stream in fall (Table 7). Three of the six key metrics (SC rich, CN rich, and EPT rich) had global correlation that were stronger in spring, compared to fall, which exhibited stronger trends for the other three selected key metrics (% Ephemeroptera, rich Ephemeroptera less Baetidae, % 5 dom) (Figure 15). Reference streams had a within-stream range of ca. 10-40 uS/cm and highly-impacted streams had a within-stream range of ca. 640-990 uS/cm. When all six study streams were pooled together, the SC range substantially increased to ca. 1,670-1,860 uS/cm. Although pooling streams together gives a greater gradient to examine macroinvertebrate response, highly-impacted streams most often exhibited stronger correlations within the individual stream than when all streams were compiled.

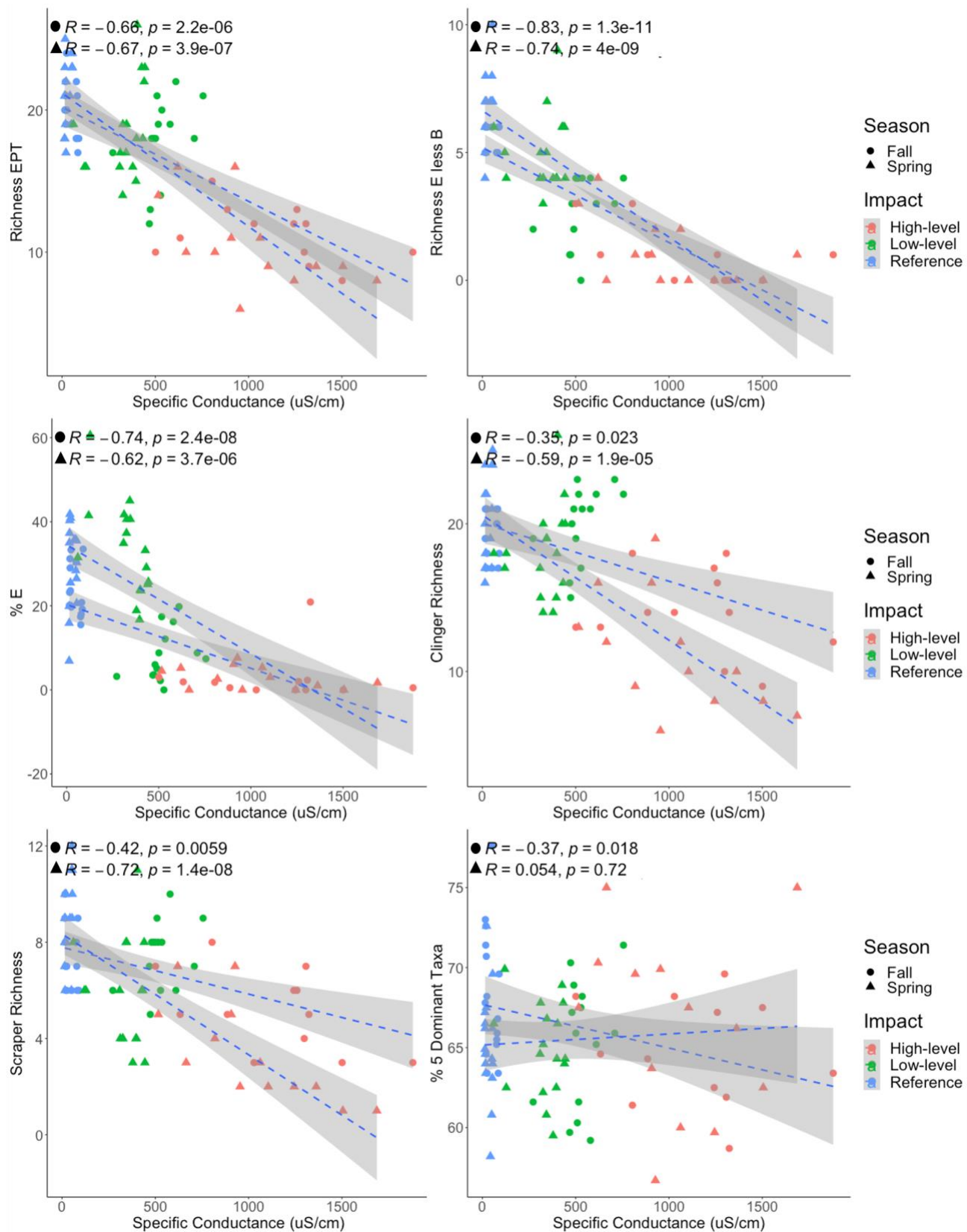


Figure 15. Spearman correlations for six selected taxa metrics versus specific conductance by season pooled among six study streams in the coalfield of central Appalachia.

Spearman Correlations of VASCI to SC

Correlation of VASCI scores to SC were used to further understand how SC variation influences overall stream condition as indicated by multiple combined taxa metrics. VASCI was only significantly negatively correlated to SC in the most highly-impacted streams (ROL and LLW) in spring (Figure 16). Thus, VASCI trends reflect taxa metric trends as highly-impacted streams also had the most significant correlations of key taxa metrics. Change in additional metrics less strongly associated with disturbance, such as were identified in reference and low-level impacted streams, are not reflected in VASCI scores. When VASCI scores for all streams were analyzed collectively, stream condition was more strongly related to SC in fall with clearer separation of low-level impacted streams from reference (Figure 17).

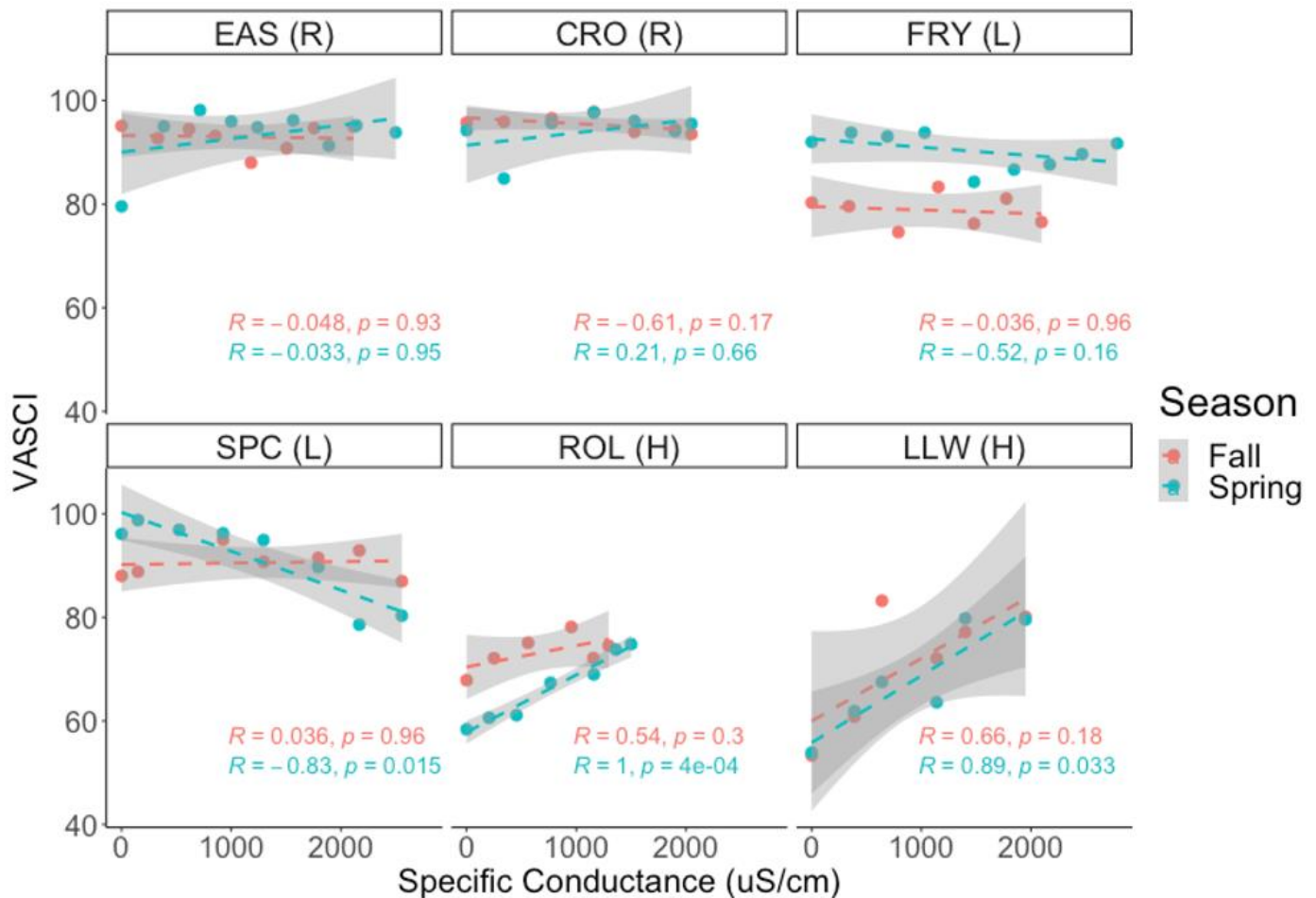


Figure 16. Spearman correlations of Virginia Stream Condition Index (VASCI) to specific conductance by stream and season for six study streams in the coalfield of central Appalachia. Level of impact indicated in () after stream name: R, L, and H indicating Reference, Low-level, and High-level, respectively.

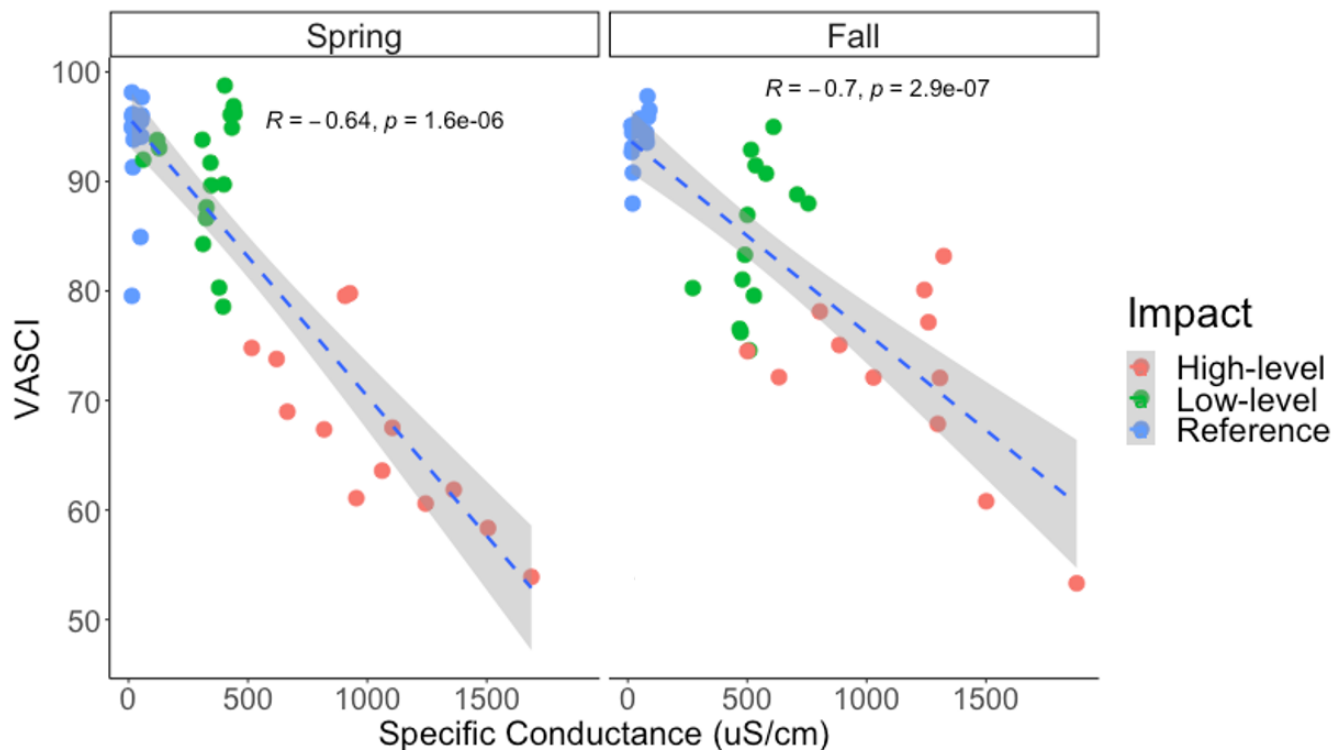


Figure 17. Spearman correlations of Virginia Stream Condition Index (VASCI) versus specific conductance pooled among six study streams in the coalfield of central Appalachia by season.

Spearman Correlations of Community Similarity to SC

Correlations between community similarity and SC were assessed to further evaluate community response to SC gradients. Bray-Curtis explained changes in similarity within streams associated with changes in SC in both highly-impacted streams (ROL and LLW) and one reference (CRO) in both seasons and one low-level impacted stream in spring (FRY) (Figure 18). For J community similarity responses to SC, I observed significant correlations in one reference (EAS) in spring and one low level impacted stream (FRY) in fall (Figure 18). This low-level impacted stream (FRY) had J correlated to SC in fall, but not in spring when BC was correlated to SC in this stream.

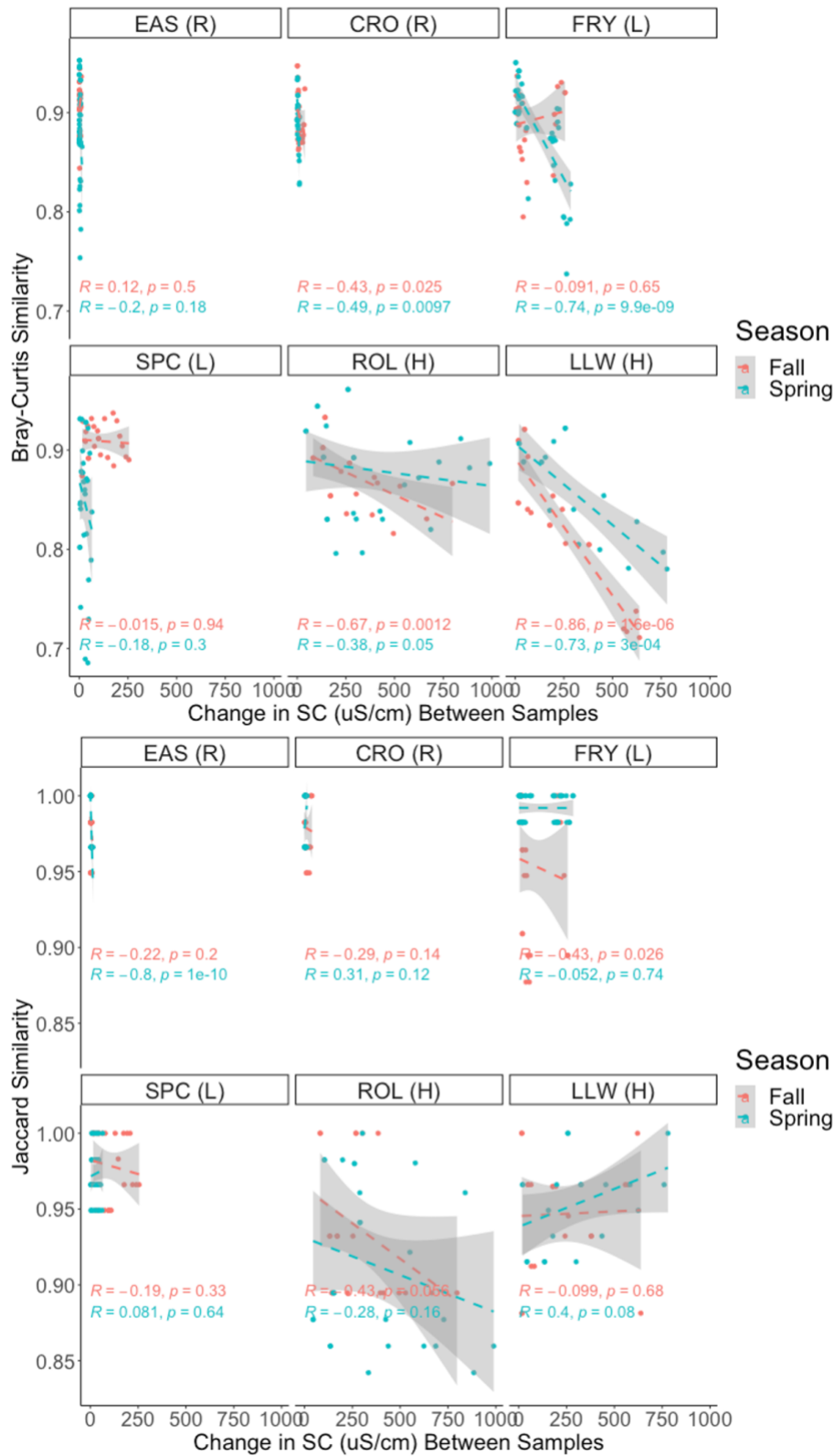


Figure 18. Bray-Curtis (top) and Jaccard Similarity (bottom) versus change in specific conductance (SC) for six study streams in the coalfield of central Appalachia. Level of impact indicated in () after stream name: R, L, and H indicating Reference, Low-level, and High-level, respectively.

Objective 3: Examine influence of water quality and other environmental factors on within-stream benthic macroinvertebrate variability.

RDA Identification of Community Drivers

Redundancy analysis is a popular ordination technique for community ecology capable of determining which explanatory variables are redundant to variation in response variables (Legendre and Legendre 2012). For my research, the explanatory variables were defined collectively by three attributes (water quality, habitat, and location), and the response variables were macroinvertebrate abundances. Some of the collinear variables measured for water quality and habitat attributes were integrated into PCA axes to reduce dimensionality, and location was represented with multiple spatial structures via PCNM analysis (not presented here). Redundancy analysis selected the variables from each attribute that explained the most variation in macroinvertebrate abundance and were thus identified as drivers of community composition (Table 8). It is important to note that one highly-impacted stream (LLW) in both seasons and one highly-impacted and one reference (ROL and CRO, respectively) in fall produced stress near zero, which may indicate insufficient data (Appendix D). Specific conductance was only selected as a driver within one reference stream (EAS) and among streams when analyzed collectively. Water quality drivers that most often attributed to significant influence on community composition in mining-influenced streams include NPOC, hardness, nutrients, elements, and temperature. Reference water quality most influential to macroinvertebrates included nutrients and hardness in addition to SC. Habitat drivers in both mining-influenced and reference streams were oriented around fine sediments, such as % fines and embeddedness in fall, whereas spring was more heavily influenced by stream geometries (e.g. slope) and large cobbles. Within streams, almost all streams indicated importance of the effect of wide-scale variation in location (PCNM1) on community, although among streams, smaller scales were driving community more in spring. Generally, each attribute (water quality, habitat, location) could assign similar proportions of community variance (10-30%) with the exception of LLW, which most strongly attributed community variance to water quality.

Table 8. Redundancy Analysis for selected drivers of macroinvertebrate community structure for six study streams in the coalfield of central Appalachia.

Season	Attribute ¹	EAS (R) ²	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)	ALL
Spring	Water Quality	SC + nutrients	nutrients + hardness	elements	nutrients	NPOC	hardness	SC, hardness, NPOC, temp. DO, nutrients
	adj. R ²	0.31	0.17	0.33	0.21	0.27	0.34	0.37
	Habitat	slope + riparian characteristics	% large cobbles	slope	wetted width	LCF	NA	% fines, slope, pebble dimension, embeddedness, wetted width, and riparian characteristics
	adj. R ²	0.33	0.15	0.31	0.14	0.29		0.23
	Location	PCNM1	PCNM1	PCNM1&2	PCNM1	PCNM1&2	PCNM1	PCNM 4, 2,1 & 5
	adj. R ²	0.22	0.18	0.3	0.27	0.28	0.18	0.32
Fall	Water Quality	hardness	NA	temp.	NA	hardness	hardness	SC, hardness, elements, DO, NPOC
	adj. R ²	0.2		0.15		0.17	0.41	0.32
	Habitat	% fines	LCF	%fines	NA	embeddedness	embeddedness	wetted width, % fines, pebble dimensions, slope, riparian characteristics
	adj. R ²	0.22	0.28	0.1		0.17	0.29	0.22
	Location	PCNM1	PCNM1	PCNM1	NA	PCNM1	PCNM1	PCNM 1,2,4, & 5
	adj. R ²	0.18	0.32	0.15		0.16	0.29	0.34

¹ Adjusted R squared values indicate the proportion of macroinvertebrate abundance variation explained by the selected drivers of that attribute. Drivers were selected using backwards stepwise model selection.

²Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Redundancy Analysis scores the weights of each taxon and collection site based on their ordination position, with the heaviest weighted sites and taxa driving most of the variation in the ordination. Taxa and site score weights from RDA results indicated 18 taxa to be most highly associated with water quality, habitat, and location attributes within each stream and when pooled across the six study streams (Table 9). Of these 18 taxa, there were 7 that were Ephemeroptera, 4 were Plecoptera, 4 were Trichoptera, 1 was Coleoptera, and 2 were Diptera, all of which were most highly associated with measured stream attributes. Overlap in indicator taxa was common for each attribute within streams but often differed among streams. Indicator taxa in reference streams had lower tolerances, were more likely to be shredders, and were all clingers or swimmers (Table 9). Mining-influenced streams had some indicator taxa with higher tolerances and burrowing and sprawling habit. One low-level impacted stream (FRY) in the fall had a predator as an indicator species. Usually, site scores indicative of indicator taxa presence were in upstream locations with the exception of SPC in spring for the habitat parameter.

Table 9. Highest taxa and site score weights from RDA Analysis.

Season	Attribute	EAS (R) ⁴	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)	ALL
Spring	Habitat	Leuctra (0, SH, CN) ²	Habrophlebiodes (0, SC, SW)	Ameletus (1, CG, SW)	Chironomidae (6, CG, BU)	Chironomidae (6, CG, BU)	Amphinemura (2, SH, SP)	Acentrella (4, CG, SW)
		Peltoperla	Baetis	Pteronarcys	Oligochaeta	Haploperla	Prosimulium	Amphinemura
		Tallaperla	Ameletus	Oulimnius	Baetis	Lanthus	Chironomidae	Leuctra
	Sites ¹	1, 3, & 2	2, 1, & 3	1 & 2	7, 8, & 6	2 & 1	1 & 2	FRY 6, 5, & 7
	Water Quality	Peltoperla (2, SH, CN)	Baetis (2, CG, SW)	Ephemerella (2, CG, CN)	Cinygmula (1, SC, CN)	Chironomidae (6, CG, BU)	Amphinemura (2, SH, SP)	Amphinemura (2, SH, SP)
		Leuctra	Haploperla	Epeorus	Isoperla	Haploperla	Chironomidae	Leuctra
		Allocaenia	Eccoptura	Oulimnius	Optioservus	Lanthus	Prosimulium	Acentrella
	Sites	1 & 2	1 & 2	1 & 2	1 & 3	2 & 1	1	ROL 1, 2, & 5
Fall	Habitat	Peltoperla (2, SH, CN)	Habrophlebiodes (0, SC, SW)	Ephemerella (2, CG, CN)	Stenelmis (1, SC, CN)	Chironomidae (6, CG, BU)	NA ³	Leuctra (0, SH, CN)
		Leuctra	Baetis	Epeorus	Isoperla	Haploperla		Amphinemura
		Allocaenia	Haploperla	Oulimnius	Optioservus	Lanthus		Acentrella
	Sites	1, 3, & 2	2 & 1	1 & 2	1, 3, & 2	1 & 2		ROL 1, 5, 6
	Water Quality	Peltoperla (2, SH, CN)	Leuctra (2, SH, CN)	Rhyacophila (0, PR, CN)	NA	Neotrichia (0, SC, CN)	Tipula (4, SH, BU)	Diplectrona (6, CF, CN)
		Maccaffertium	Eurylophella	Paracapnia		Chironomidae	Wormaldia	Tipula
		Attenella	Habrophlebiodes	Neotrichia		Paracapnia	Oligochaeta	Cheumatopsyche
	Sites	1 & 2	2, 1, & 3	3		1 & 2	1 & 2	FRY 4, 7, & 1
Fall	Water Quality	Attenella (1, CG, CN)	NA	Strophopteryx (2, SH, SP)	NA	Neotrichia (0, SC, CN)	Tipula (4, SH, BU)	Cheumatopsyche (6, CF, CN)
		Peltoperla		Rhyacophila		Chironomidae	Isoperla	Tipula
		Maccaffertium		Marilirekus		Allocaenia	Dixa	Diplectrona
	Sites	1 & 2		1 & 3		1	1 & 2	FRY 7, SPC 8, & 6
	Location	Attenella (1, CG, CN)	Leuctra (2, SH, CN)	Rhyacophila (0, PR, CN)	NA	Neotrichia (0, SC, CN)	Tipula (4, SH, BU)	Cheumatopsyche (6, CF, CN)
		Peltoperla	Eurylophella	Strophopteryx		Chironomidae	Dixa	Tipula
		Maccaffertium	Habrophlebiodes	Ectopria		Allocaenia	Peltoperla	Diplectrona
	Sites	2, 1, & 3	1 & 2	3, 2, & 1		1, 2, & 3	1 & 2	FRY 7, SPC 8, & 6

¹ Site 1 being the most upstream. Species with the highest score indicate the taxa whose presence are most representative of how that stream attribute is shifting communities. Sites with highest scores indicate which locations are having the greatest change in these taxa.

² Parentheses indicate tolerance value, FFG, and habit accordingly for the most influential taxa of that attribute. Tolerance values range from 1-10 with one being the most sensitive to disturbance.

³ NA indicates no metrics from that parameter could constrain the site scores in a way that could strengthen the relationship of sites based on macroinvertebrate communities.

⁴ Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

To examine potential for collinearity among variables used in the water quality and habitat attributes and their potential to bias RDA selected drivers, variance inflation factor scores of each selected driver to SC were determined (Table 10). VIF scores were ≤ 10 for all drivers except hardness and elements among all streams pooled together indicating they have notable collinearity to SC.

Table 10. Variable Inflation Factor scores to specific conductance of redundancy-analysis-selected water quality and habitat metrics for six study streams in the coalfield of central Appalachia.

Season	Parameter	Metric	All ^{1,2}	EAS (R)	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)
Spring	Water Quality	do.mgl	3.987	NA	NA	NA	NA	NA	NA
Spring		temp.C	3.12	NA	NA	NA	NA	NA	NA
Spring		hardness.mgl	3.915	NA	2.91	NA	NA	NA	NA
Spring		npoc.mgl	3.276	NA	NA	NA	NA	3.62	NA
Spring		nutrients.pca	2.496	4.69	5.8	NA	1.68	NA	NA
Spring		elements.pca	NA	NA	NA	2.135	NA	NA	NA
Spring		pfines	2.639	NA	NA	NA	NA	NA	NA
Spring		plargecobble	NA	NA	3.405	NA	NA	NA	NA
Spring	Habitat	LCF	NA	NA	NA	NA	NA	3.62	NA
Spring		avgwetwidth	2.2	NA	NA	NA	1.68	NA	NA
Spring		avgembedd	1.72	NA	NA	NA	NA	NA	NA
Spring		avg.slope	2.45	5.08	NA	2.135	NA	NA	NA
Spring		D.pca	1.64	NA	NA	NA	NA	NA	NA
Spring		Rip.pca	1.89	1.63	NA	NA	NA	NA	NA
Fall		do.mgl	2.4	NA	NA	NA	NA	NA	NA
Fall	Water Quality	temp.C	NA	NA	NA	1.293	NA	NA	NA
Fall		hardness.mgl	43.16	4.017	NA	NA	NA	9.15	3.73
Fall		npoc.mgl	2.074	NA	NA	NA	NA	NA	NA
Fall		elements.pca	40.36	NA	NA	NA	NA	NA	NA
Fall		pfines	2.23	4.017	NA	1.292	NA	NA	NA
Fall	Habitat	avgwetwidth	1.645	NA	NA	NA	NA	NA	NA
Fall		avgembedd	NA	NA	NA	NA	NA	9.15	3.73
Fall		avg.slope	2.37	NA	NA	NA	NA	NA	NA
Fall		D.pca	1.73	NA	NA	NA	NA	NA	NA
Fall		Rip.pca	2.17	NA	NA	NA	NA	NA	NA

¹ The higher the score, the greater the collinearity.

² Level of impact from mountaintop mining valley fill salinization indicated in () after stream name; R, L, and H indicate Reference, Low-level, and High-level, respectively.

Variation Partitioning to Determine Influences of Attributes

Variation partitioning was used to determine the relationship of the three attributes (water quality, habitat, and location) to each other in terms of how uniquely and collectively they explained macroinvertebrate community variation in both spring and fall (Table 11). The highest community variation was often explained by the combined variation of all attributes (the spatial structure of water quality and habitat) with the exception of the one highly-impacted stream (LLW) and all streams collectively in spring in which water-quality variation uniquely explained substantial additional community variation. Furthermore, in fall, ROL and LLW (both highly-impacted streams) exhibited unique explanatory power for location and water quality accordingly. Generally, the three attributes could explain ca. 30-50% of community variation, except for one reference (CRO) and one highly-impacted stream (ROL) in spring and one low-level impacted stream (FRY) in fall, all of which could not explain more than 20%. Community variation in one low-level impacted stream (SPC) in fall could not be attributed to any variables associated with any attribute.

Table 11. Variation partitioning individual fractions results for six study streams in the coalfield of central Appalachia.

Stream	Season	Fractions of variation (adj. R^2) ³							Residuals
		WQ ¹	H	L	WQ + H	H + L	WQ + L	All	
EAS (R) ⁴	Spring	0.02	0.10	0.06	0.12	0.00 ²	0.05	0.12	0.54
	Fall	0.11	0.14	0.06	0.00	0.00	0.00	0.17	0.63
CRO (R)	Spring	0.03	0.00	0.00	0.00	0.03	0.00	0.17	0.85
	Fall	NA	0.00	0.03	NA	0.29	NA	NA	0.69
FRY (L)	Spring	0.00	0.10	0.10	0.11	0.00	0.11	0.14	0.52
	Fall	0.00	0.02	0.01	0.01	0.00	0.08	0.07	0.83
SPC (L)	Spring	0.06	0.00	0.07	0.00	0.03	0.03	0.13	0.70
	Fall	NA	NA	NA	NA	NA	NA	NA	NA
ROL (H)	Spring	0.00	0.00	0.00	0.03	0.05	0.00	0.29	0.80
	Fall	0.00	0.02	0.17	0.11	0.00	0.00	0.11	0.72
LLW (H)	Spring	0.25	NA	0.10	NA	NA	0.08	NA	0.57
	Fall	0.31	0.14	0.08	0.00	0.00	0.00	0.35	0.42
ALL	Spring	0.11	0.04	0.07	0.04	0.03	0.10	0.12	0.49
	Fall	0.05	0.03	0.09	0.04	0.02	0.10	0.13	0.54

¹ WQ = Water Quality, H = Habitat, and L = Location.

² Zeros represent R^2 values < 0.01. NA indicates the RDA found no variables from that attribute improved site relationships of macroinvertebrate abundance. LLW in spring had no relationship to any habitat variables, CRO had no relationship to any water quality variables in Fall, and SPC could not be explained by any variables from any attributes.

³ Adjusted R^2 values display the proportion of that attribute that uniquely shares its explanatory power to model macroinvertebrate communities verses the shared explanatory value of the different attributes. ⁴³⁴

⁴ Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Chapter 4- Discussion

In this work, I assessed three objectives to determine macroinvertebrate responses to spatial patterns of water quality and habitat within four streams impacted by mining and two reference streams in central Appalachia. I evaluated macroinvertebrate responses to distance downstream and SC over ca. 1.5 km in the six study streams. I also constrained macroinvertebrate responses within streams to multiple variables comprising three attributes - water quality, habitat, and location - to determine which variables are most influential to community composition within streams. Findings indicated streams with highest mean SC and largest SC gradients within sampling reaches had exaggerated spatial shifts in macroinvertebrate community composition. Reference and low-level impacted streams had smaller community shifts although low-level impacted streams were more variable than reference streams. Specific conductance can be used to reflect community variation among streams; however, within-stream variation in macroinvertebrate community structure is better explained with additional water quality, habitat, and location variables. Furthermore, fall sampling better represented separation of low-level impacted stream condition from reference than spring sampling. Interpretation of previous research focused on between-stream gradients would benefit from improved knowledge on how within-stream dynamics influence representation of stream condition compared to using a single sampling point. Ensuring interpretation of macroinvertebrate communities most accurately reflects biological conditions a stream exhibits is vital for prioritization of monitoring and remediation efforts in central Appalachia streams influenced by mining and in other disturbed streams more broadly.

Objective 1: Determine within-stream variability of benthic macroinvertebrate communities and stream condition.

The RCC defines a continuum of biotic adjustments within stream networks and rivers to accommodate for changing physical systems and to maximize use of energy inputs (Vannote et al. 1980). Since RCC has become widespread, multiple studies have further detailed the relationship of aquatic insects over stream distances (Shupryt and Studinski 2021; Heino 2013; Wilson and McTammany 2016). All six of the study streams here were in accordance with the RCC by exhibiting a significant relationship of community similarity to distance between samples in at least one season. The further the macroinvertebrate collections were apart from each other, the more dissimilar their communities were. The strongest relationships of community similarity to distance downstream were associated with the streams experiencing greatest disturbance from mining. This is in accordance with my hypothesis that in mining-influenced streams, as distance from disturbance increased, local conditions would improve, and therefore macroinvertebrate communities would shift over shorter distances than in reference streams, which remain in good condition throughout headwater stream lengths.

Taxa metrics were utilized throughout the study to integrate understanding of shifts in community function and sensitivity. Specific to taxa metrics, certain changes within streams over distance have been well-documented, such as with mayfly diversity increasing with distance downstream (Pond et al 2016). The RCC outlines how headwaters are strongly influenced by allochthonous (originating outside the stream, i.e. fallen leaves) detritus, which results in communities primarily composed of shredders and collectors. With distance downstream as the stream increases in size, relative contribution of terrestrial input is reduced, which increases the importance of autochthonous (originating within the stream, i.e. algae) primary production and organic transport from upstream. This causes taxa shifts away from shredders towards scrapers (Vannote et al. 1980). Of the taxa metrics examined in this study, ten

corresponded to these aforementioned taxa groups: % and richness of Ephemeroptera, Ephemeroptera less Baetidae, shredders, scraper taxa and scrapers less E. Correlations of these metrics were more responsive in three of the four mining-influenced streams (in spring), further supporting findings that exaggerated gradients in streams caused by mining accelerated natural shifts over shorter distances as compared to reference conditions.

Although large-scale variation (such as at ecoregion scales or among stream studies) often identifies more differences in community composition, it is important to additionally consider mechanisms at play at local scales. It is expected that similarity will decrease with increased distance between macroinvertebrate collections if local conditions are the strongest driver of community composition (Leibold et al. 2004). It has also been determined that headwater streams exhibit less dispersal due to strong niche partitioning (Pond et al 2016). This was reflected in the findings of my research as all significant correlations of community similarity to distance between macroinvertebrate collections were negative, indicating high dependency on local conditions in headwater streams. However, three of the six streams in which community similarity was correlated to distance between collection sites in spring did not have them in fall, which could be indicative of presence of species with higher dispersal abilities or more homogenized local conditions than in spring.

Previous studies found expanding the spatial scale of sampling within streams may improve understanding of factors driving community structures (Li et al. 2001; Wiley et al 1997). Ephemeroptera taxa often have been the focus of explaining level of disturbance among streams (Timpano et al. 2018; Burton and Gerritsen 2003). Within mining-influenced streams, more specific and tolerant taxa (such as percent Plecoptera less Luectra, Allocapnia, and Amphinemura or percent sprawlers) can reveal more about change over distance, and associated functions provided by those taxa, as sensitive taxa may not be present in the first place. Within-stream macroinvertebrate shifts of certain low-level impacted streams may better separate stream condition from reference than would occur using a study among streams. Additionally, richness of CF is an influential metric within the study streams that has not been emphasized in previous studies among streams.

Widespread bioassessment protocols utilize comparison of samples from a single location in one stream to that of a single location in another stream to indicate stream condition (VA DEQ 2008; Timpano et al. 2018; Pond et al. 2008; Hartman et al. 2005). Studies at this scale of comparison among streams have contributed to policy, mitigation and reclamation efforts surrounding coal-mining in central Appalachia. Furthermore, salinization can be attributed to a variety of sources in addition to coal-mining such as urbanization and agricultural impacts, which also benefit from advancing knowledge on impacts of salinized systems to water quality and biota (Cañedo-Argüelles et al. 2013). Best scientific practice considers an issue from multiple angles or scales before forming conclusions to apply best practice and to best mitigate consequences on ecosystem function from anthropogenic sources (Cooper et al. 1997; Wiley et al. 1997; Li et al. 2001). As scale has been influential in understanding of environmental systems, it is essential to be able to support the assumption that a single sample location is representative of headwater streams used in mining research.

This study aimed to evaluate trends at play in salinized systems at a smaller within-stream scale to add a new angle and to benefit understanding of the impacts of coal mining to headwater streams in central Appalachia and potentially more broadly in salinized streams. Analysis of VASCI scores found within-stream sample location has the power to reflect variable stream conditions within mining-influenced

streams, although VASCI scores in this study were inflated and unable to decipher how within stream location impacts impairment status. Highly-impacted site VASCI scores ranged from ca. 55-85, whereas reference streams ranged ca. 90-100. Furthermore, low-level impacted streams exhibited ranges of ca. 75-100 within streams, indicating sampling location could influence how comparable a stream is to reference condition. This study clarified that within-stream scale is influential to interpretation of stream condition in mining-influenced headwaters and potentially in other disturbed systems.

In summary, these results demonstrated that 1) high levels of mining impact prompted macroinvertebrate response over shorter distances, 2) stream sample location is influential to assessing stream condition in mining-influenced streams, and 3) increasing the spatial scale of sampling may reveal finer-scale trends impactful to understanding the influence of mining on stream condition not revealed by larger-scale dynamics.

Objective 2: Determine if the same changes among streams over a gradient of SC are occurring within streams

Natural processes of salinization occur over geologic time frames (100's of thousands of years), but anthropogenic sources, such as coal-mining valley fills, expedite these processes to time frames over decades (U.S. EPA 2011). Anthropogenic salinization has been classified as one of the top stressors posing severe biodiversity loss and insecurity of freshwater resources (Cañedo-Argüelles et al. 2013). Stream salinization is a persistent challenge with documented impacts to many organisms, including benthic macroinvertebrates. For example, EPT taxa are particularly sensitive, with notable influence to Ephemeroptera taxa diversity and ecosystem function such as secondary production (Cianciolo et al. 2020; Tank et al. 2010; Pond et al. 2016; Huryn and Wallace 1987; Merricks et al. 2007; Hartman et al. 2005; Pond 2010; Stauffer and Ferreri 2002). As such, there is a need to understand how salinization impacts differ among stream systems but also, albeit less studied, within streams.

Previous research has reflected the urgency of anthropogenic salinization in headwaters of central Appalachia finding gradients of SC (the measure of salinization) to be the driving variable in community structure among mining-influenced streams in this region (Timpano et al. 2018). Research has emphasized gradients of SC among streams, but less is known about how mining influences within-stream gradients of SC and associated consequences to macroinvertebrates. All four mining-influenced streams exhibited higher variability of within-stream SC when compared to the two reference streams. Highly-impacted streams exhibited strong diluting SC gradients in both seasons: ca. 700-900 $\mu\text{S}/\text{cm}$ as compared to ca. 70-300 $\mu\text{S}/\text{cm}$ in low-level impacted streams and ca. 10-40 $\mu\text{S}/\text{cm}$ in reference streams. In most mining-influenced streams, taxa metrics improved with decreasing SC downstream as expected (U.S. EPA 2011), with the exception of one low-level impacted stream (SPC), where SC was slightly decreased downstream, but community composition deteriorated. One low-level impacted stream (FRY) showed a unique SC concentrating gradient, with lower SC upstream in the fall (below EPA threshold for aquatic life), and presence of high-SC tributaries that escalated SC downstream. Despite these within-stream gradients, results indicated that more macroinvertebrate groups are responding to gradients of SC among headwater streams rather than within them. Contrary to my prediction, low-level impacted streams did not exhibit more (or stronger) correlations of taxa metrics or community similarity with SC even though within-stream gradients of SC were closest to thresholds for biotic life (Pond 2010; U.S. EPA 2011). Although streams with the largest within-stream SC gradients did reflect the more similar macroinvertebrate responses as observed in across-stream studies, correlations of taxa metrics strongest among streams (Timpano et al. 2018) do not fully represent correlations within streams.

When considering taxa metrics responding to SC within streams it is important to note that reference streams have likely had relatively stable communities throughout time, whereas impacted streams may have been subject to extirpation and recolonization events over time since mining. Therefore, studies assessing differences among streams reveal more about how communities are altered by coal-mining events over time rather than direct taxonomic responses and movement within streams as SC varies. To discern patterns, large-scale among-stream studies emphasize taxa most sensitive to extirpation (VA DEQ 2008). To reveal within-stream patterns, there should be additional emphasis on dominant taxa present and how they are responding, especially in mining-influenced streams due to sensitive taxa scarcity following extirpation events. For example, my study found within-stream changes of Plecoptera and shredders unique to reference streams, compared to within-stream changes of chironomids, clingers, Coleoptera, and Trichoptera in mining-influenced streams. Streams with the largest SC gradients were most likely to exhibit responses in the 15 selected metrics emphasized in my study, indicating sensitive taxa were isolated to locations with the most-favorable environmental conditions within impacted streams.

To further determine if alterations of within-stream SC gradients from coal mining triggered macroinvertebrate response comparable to those among streams, correlations of taxa metrics and community similarity to distance as compared to SC gradients were explored (Table 12). With this comparison, distance was a better descriptor of macroinvertebrate trends than SC in reference and low-level impacted streams. Distance and SC were collinear in both highly-impacted streams with SC declining consistently with distance downstream and therefore both SC and distance were equally correlated to the same taxa metrics in these two streams. When SC and distance were not collinear, the spring had slightly more correlations to SC than distance with the exception of one low-level impacted stream (SPC), and fall had more taxa metrics correlated to distance than SC. Generally, more macroinvertebrates metrics responded to both distance and SC in spring (except FRY), potentially due to the higher diversity of macroinvertebrates inhabiting the stream or higher flows and stream connectivity in spring (Barbour et al. 1999). Furthermore, distance was correlated with stream condition (VASCI) in one reference and one highly-impacted stream in fall and one low-level impacted stream in spring when these streams were not significantly affected by SC. Thus, I determined distance downstream was generally a stronger indicator in fall compared to SC, which was generally the stronger indicator in spring of within-stream macroinvertebrate trends in reference and low-level impacted streams.

Table 12. Summary of number of specific conductance (SC) and distance correlations to all 60 taxa metrics for each season along with stream distances and SC for six study streams in the coalfield of central Appalachia.

	EAS (R) ¹	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)	All Streams
# Metrics Correlated to SC Spring	12	13	7	14	21	17	41
# Metrics Correlated to SC Fall	4	1	1	4	10	13	36
# Metrics Correlated to Distance Spring	11	8	6	27	21	17	8
# Metrics Correlated to Distance Fall	6	14	15	4	10	13	2
SC Range (uS/cm) spring, fall	14, 10	10, 40	290, 260	65, 260	990, 800	780, 640	1673, 1865
Average SC (uS/cm) spring, fall	17, 20	52, 77	252, 460	415, 605	903, 860	1175, 1400	423, 534
Distance sampled (m)	2500	2050	2785	2556	1500	1950	NA

¹ Level of impact from mountaintop mining valley fill salinization indicated in () after stream name: R, L, and H indicate Reference, Low-level, and High-level, respectively.

Examining the relationship of community similarity to SC and comparing it to that of distance helped further assess the effects of both distance and SC gradients, especially in two-highly impacted streams. Correlations to taxa metrics for SC and distance were the same in highly-impacted streams, but similarity was strongly influenced by SC in the one most-highly-impacted stream (LLW) in both seasons and the other highly-impacted stream in fall (ROL). Distance between macroinvertebrate collections explained differences in community similarity for more streams and seasons than SC, although, when change in SC was correlated, the relationship was usually stronger than that of distance. In contrast, a study by Shupyr in 2021 found with greater disturbance (as indicated by increased SC), community structure shifted over longer distances due to homogenization of the system. Yet, this study was conducted over longer distances (10s of km), which may have overpowered finer-scale patterns that were the focus of my study. Macroinvertebrate community dissimilarity in reference streams was not expected due to minimal SC gradients. However, analysis often found stronger relationships of macroinvertebrate communities to SC than distance in spring for reference streams. One possible explanation for this is that SC is collinear to another water quality metric (such as nutrients or hardness) that may have stronger influence on macroinvertebrates over smaller gradients in reference streams. As previously mentioned, the low-impact stream FRY exhibited the only SC concentrating gradient (upstream SC was below EPA threshold) and the only significant J relationship to SC. This suggests that streams with high SC inputs downstream of the stream origin may harbor sensitive taxa above the input that do not inhabit downstream habitat. This is further reinforced by this stream's pattern of high VASCI scores upstream (before source of pollution) and downstream (after pollution dilution) (ca. 92) and lowest in middle sites (most polluted areas) (ca. 82) suggesting macroinvertebrates avoided high SC areas within headwater streams. Furthermore, the streams experiencing the most change in community similarity were highly-impacted, suggesting tolerant taxa in these streams are also structuring themselves to avoid stressful locations.

Objective 3: Examine influence of water quality and other environmental factors on within-stream benthic macroinvertebrate variability.

To further understanding of within-stream drivers of macroinvertebrate communities, relative contributions of three attributes - water quality (in addition to SC), habitat, and location - to macroinvertebrate metrics were further analyzed. Each attribute had multiple variables of which a subset of the most-influential was selected for each individual stream and collectively among streams with multiple sample locations for both seasons. It was determined that SC was not always the strongest indicator of macroinvertebrate communities within my study streams, and that studies at this scale should consider additional water quality, habitat, and location attributes. Further, taxa and sample location that most significantly responded to variables selected for each attribute were identified. Lastly, each attribute was examined to determine whether it explained macroinvertebrate community variation uniquely or in shared ways with other variables.

Water quality drivers and indicator taxa varied by stream. When analyzed collectively, SC, closely followed by hardness, were the primary drivers in both seasons, with additional explanatory power found in NPOC, temp, DO, and nutrients in spring, and trace elements, DO, and NPOC identified in fall. In spring, nutrients were a significant driver in both reference streams and one low-level impacted stream (SPC), which exhibited opposite trends in that the number of sensitive taxa decreased with distance downstream. Trace elements, NPOC, and hardness were indicated as significant drivers of community variation in the remaining three mining-influenced streams (FRY, ROL, and LLW, respectively). This agrees with previous studies over larger scales, which found within-stream community variation attributed to SC, pH, DO, Se, and nutrients (Zhengfei et al. 2021; Shupryt et al. 2021; Cianciolo et al. 2020). Explanatory power of water quality diminished in fall apart from the one highly-impacted stream (LLW) and one reference (EAS). Water quality could not explain any community variation in one reference (CRO) and one low-level impacted stream (SPC) in fall. Macroinvertebrate community variation in the low-level impacted stream (SPC) could not be explained by any attribute in fall. In conclusion, findings on all streams pooled are in agreeance with other studies of this scale finding SC to be the main driver of community composition (Timpano et al. 2015); although in this study, when analyzed on an individual stream basis, intricacies of other water quality variables may overpower effects of SC within streams.

The two strongest drivers of community change attributed to habitat among my study streams collectively were percent fines in the streambed and channel slope in spring, and wetted width and percent fines in fall. These habitat drivers are in accordance with previous research that attributed within-stream community variation to habitat factors such as stream gradient, channel width, and streambed substrate (Zhengfei et al. 2021; Shupryt et al. 2021). Seasonal differences may be attributed to how stream flows are higher in spring and drying is common in headwaters in fall, influencing significance of slope and wetted width accordingly (U.S. EPA 2011). Within individual streams, streambed sediments were a significant driver of macroinvertebrate community in fall. In contrast, spring drivers of macroinvertebrate communities within streams were diverse, including riparian characteristics, percent large cobbles, wetted width, channel slope, and streambed large cobble:fine ratio. Sedimentation is also well-established to negatively impact macroinvertebrate communities and is associated with impacts of coal-mining (Drover et al. 2020; Paybins 2003) and may therefore be causing greater separation of low-level impacted streams from reference conditions in fall when stream flows are typically lower. Habitat did not explain any community variation in the one highly-impacted stream (LLW) in spring and one low-level impacted stream (SPC) in fall. Overall, channel slope in spring and

%fines/embeddedness in fall best explained macroinvertebrate community variation in ways water quality and location variables could not.

Broad-scale location was indicated as a driver in all streams individually and collectively for both seasons (aside from SPC in fall in which no attribute could explain community variance), indicating location within my study streams influenced community variation. Spatial distribution has been used as an indicator of dispersal (Wilson and McTammany 2016; Zhengfei et al. 2021), where the importance of spatial structure decreases with higher dispersal ability and increases with higher importance of local factors. Collectively among streams, finer-scale location patterns explained community variation, which is in accordance with the expectation that the streams acted as clusters with less community variation within streams than between them. As would be expected, only the broadest variables of space (i.e. upstream to downstream) best explained variation within individual streams because communities are better connected (by the water flow) than they are among streams. Consequently, spatial structure is not as strong an indicator within streams as between them, therefore weakening importance of local factors (e.g. within-site SC gradients) in explaining community variation: yet, space does explain some additional community variation within streams.

Most within-stream studies have focused on dispersal of insects and how it influences beta diversity when considering environmental factors and stream distances (Callisto et al. 2021; Astorga et al. 2012; Shupryt et al. 2021). Less is known about derived taxa metrics and how within-stream gradients are changing stream functioning. Compared to larger-order streams, headwaters have lower dispersal and are more dependent on local factors and niche partitioning (U.S. EPA 2011). Macroinvertebrate groupings associated with within-stream variation among all my study streams include mainly shredders, scrapers and collectors in both seasons and additionally predators in fall, clingers and sprawlers in reference streams and low-level impacted streams, and burrowers in highly-impacted streams. Previous studies have found response of predators to selenium in mining-influenced streams indicating the low-level impacted stream (FRY) with predator taxa responding may be experiencing elevated Se in fall (Cianciolo et al. 2020).

The presence of taxa metric groupings was most often associated with upstream location. I determined taxa present at these upstream locations that most differed from downstream communities were often shredders, thus reflecting shifts in stream function of organic matter processing decreasing downstream within headwater streams as is in accordance with the RCC (Vannote et al. 1980). However, upstream sites were not always indicative of indicator taxa presence. The same low-level impacted stream that experienced opposite correlations to SC as would be expected with disturbance and was the only stream to have VASCI degrade with distance downstream (SPC), had habitat site scores indicating most influential taxa were found at downstream locations. This stream's indicator taxa had high tolerance values and the habitat driver included wetted width, which suggests ponding may be a possible influence to macroinvertebrate trends in this stream. Anomalies such as predator taxa and unique habitat-driven taxa may be influential in targeting stream remediation actions.

As was predicted, as the scale at which macroinvertebrate communities are being observed increases (i.e. within streams versus among them versus ecoregion), variation of macroinvertebrate communities can be attributed to different environmental factors at play and is exhibited in different taxa. Using variables for water quality, habitat, and location, this study found compiling six streams with samples at multiple locations could explain similar proportions of variability as within streams individually, with residuals ranging 40-85%. This contradicts a study which found ca. 70% residuals within streams versus ca. 30-60% among streams, although this study was not performed on streams with gradients altered by

coal-mining (Li et al. 2001). The highly-impacted stream (LLW) in both seasons most clearly indicates a dependence on water quality in explaining community variation suggesting macroinvertebrates are responding to exaggerated water quality gradients within mining-influenced streams. Generally, spatial structure and/or habitat added explanatory power to community variation more in fall. The greatest change in within-stream macroinvertebrate BC similarity was 25-30% in low-impact mining-influenced streams (SPC and FRY) in spring and in one highly-impacted stream (LLW) in fall, with the corresponding drivers being PCNM1&2, trace elements, nutrients, wetted width, hardness, embeddedness, and slope. Overall, water quality, habitat, and location attributes in my study accounted for macroinvertebrate community variability in shared ways. However, most explanatory values for streams were supplemented through use of all three attributes.

Chapter 5 – Summary

This study demonstrated the influence of within-stream gradients altered by coal-mining on macroinvertebrate community composition and associated representations of stream condition. Headwater streams compose 70-80% of stream networks in central Appalachia with ca. 724 mi of streams lost to mining (U.S. EPA 2011). Previous monitoring and remediation efforts have centered on representation of a single point sampling location within impacted streams over time. Mining impacts are experienced over multiple scales and have additional consequence within streams that are not reported in among-stream, broader-scale studies based on single-point sampling.

Sampling location within streams matters

Within mining-influenced streams, stream condition and macroinvertebrate community composition vary based on sample location. Low-level impacted streams are especially sensitive to analysis in that some taxa metrics reflected reference conditions, whereas others indicated a clear separation from reference conditions.

Variables to best reflect macroinvertebrate community composition vary based on scale.

I found environmental factors in addition to elevated SC from mountaintop mining were influential to macroinvertebrate community composition within streams. Although previous studies found that mining impacts homogenize downstream systems (i.e. widespread sedimentation or elimination of sensitive taxa) (Shupryt and Studinski 2021), mining-influenced streams are experiencing exaggerated taxonomic shifts near stream origin within headwaters. Furthermore, within-stream shifts may not be fully represented by taxa metrics emphasized in current among-stream protocols and research based on among-stream scales. Documented responses of mayfly taxa to distance downstream have been associated with increased diversity in reference conditions and decreased diversity with distance downstream in salinized streams (Pond 2010; Pond et al. 2008). Small-scale studies should emphasize unique taxa, and for mining-influenced streams, less-tolerant taxa (such as Plecoptera and Collectors) that are more likely to be present and responding negatively to disturbance.

Implications to current protocols

Collaborative implementation of protocols at large scales is important for collective scientific impact, and the goal is to implement widespread sampling protocols that have capacity to adequately focus efforts on streams necessitating greatest concern (Buss et al. 2015). Widely implemented protocols are often limited by their time and resource requirements. Generally, smaller-scale studies require relatively resource-intensive techniques. Finding applicable solutions for consideration of small-scale, within-stream dynamics in mainstream protocols is essential for accurate targeting of remediation/conservation efforts. The main concern with large-scale (i.e. among-stream or ecoregion) studies is often lost emphasis on rare taxa not captured and general accuracy of representation from single-point sampling locations. Previous studies have found rare taxa are added with increased within-stream sampling effort and taxon richness has been thought to adequately represent a system through identification of 100-900 organisms (Barbour and Gerritsen 1996; Zhengfei et al. 2021). Current protocols widely used to assess stream condition in the coalfields of central Appalachia require a subsampling technique with identification of 200 individuals, which this study found to be representative of reference streams, but less so in mining-influenced streams. This study found that sample location can deemphasize or exaggerate impacts of mining, depending on level of disturbance, which is information otherwise lost to single-sample protocols based on among-stream studies. When comparing systems, initial within-stream

observations may be helpful in selecting long-term locations with representation most close to mean stream conditions. Furthermore, broader application of current headwater sampling protocols with emphasis on 2-3 sampling locations above largest confluence, such as a most-upstream, middle, and downstream sample, could refine our knowledge of central Appalachia mining-influenced headwater stream condition.

Future work

This work further justifies within-stream-scale studies of headwater streams impacted by disturbance focused on alterations to environmental gradients. This study consisted of 6-9 sample locations along distances of 1,500-2,800m within six streams and could be strengthened through additional macroinvertebrate collections at these locations over time. Additionally quantitative sampling such as surber-sampling, identification of all individuals (versus sub-sampling to 200), and measurements of biomass would strengthen the accuracy and power of our understanding of within-stream trends. This study did not incorporate a temporal aspect outside of two seasons within one year. Incorporating further temporal considerations, such as locations within streams with the largest or least variation of SC over time (i.e. SC range of 10 fall samples versus one fall sample), could further aid in predicting differences between within-stream sites and understanding the power of within-stream shifts to skew among stream trends. Lastly, to fully understand within-stream dynamics, emphasis on genetic connectivity of downstream waters, and even among watersheds could determine if improvements in water quality and habitat have potential for recolonization of macroinvertebrates.

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Appendix

Appendix A. External Sources

Form A. 1. Best standard values as determined for benthic macroinvertebrate metrics. Sourced from Burton and Gerritsen 2003 “A Stream Condition Index for Virginia Non-Coastal Streams.

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (FRONT)

STREAM NAME	LOCATION	
STATION # _____ RIVERMILE _____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

	Habitat Parameter	Condition Category			
		Optimal	Suboptimal	Marginal	Poor
Parameters to be evaluated in sampling reach	1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime (usually slow-deep).
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (BACK)

Habitat Parameter	Condition Category																				
	Optimal					Suboptimal					Marginal					Poor					
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.					Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.					Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.					Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.					
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.					Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.					Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.					Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.					
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.					Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.					Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.					Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.					
Note: determine left or right side by facing downstream.																					
SCORE ____ (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE ____ (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.					70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.					50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.					Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.					
SCORE ____ (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE ____ (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.					Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.					Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.					Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.					
SCORE ____ (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE ____ (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			

Total Score _____

Table A. 1. Best standard values as determined for benthic macroinvertebrate metrics. Sourced from Burton and Gerritsen 2003 “A Stream Condition Index for Virginia Non-Coastal Streams”.

Benthic Index for Virginia

Table 4-2. Metrics for recommended Draft Virginia non-coastal benthic multimetric index: Standard values and standardization equations.

Metrics that decrease with stress	Standard (best value) X_{95}	$X_{\min}$	Standardization equation (Section 3.6, Equation 1; X =metric value)
Total taxa	22	0	score = $100 \times (X/22)$
EPT taxa	11	0	score = $100 \times (X/11)$
% Ephemeroptera	58.9	0	score = $100 \times (X/58.9)$
% Plec+Tric less Hydropsych.	34.8	0	score = $100 \times (X/34.8)$
% Scrapers	49.1	0	score = $100 \times (X/49.1)$
Metrics that increase with stress	Standard (best value) X_5	$X_{\max}$	Standardization equation (Section 3.6, Equation 2; X =metric value)
% Chironomidae	0	100	score = $100 \times [(100-X)/(100-0)]$
% Top 2 Dominant	29.5	100	score = $100 \times [(100-X)/(100-29.5)]$
HBI (family)	3.2	10	score = $100 \times [(10-X)/(10-3.2)]$
Final index score for a site is determined by averaging the site's 8 unitless standardized metric scores, using a maximum metric score of 100 for any metric whose individual score at a site exceeded 100.			

Appendix B. Supporting Tables

Table B. 1. Spearman correlations ($p \leq 0.05$) for all 60 taxa metrics to distance downstream for six study streams in the coalfields of central Appalachia.

Metrics Correlated to Distance	Season	EAS (R)		CRO (R)		FRY (L)		SPC (L)		ROL (H)		LLW (H)	
		p	R	p	R	p	R	p	R	p	R	p	R
*pSdom	Spring	NA	NA	1.4E-02	8.6E-01	NA	NA	3.5E-02	-7.4E-01	NA	NA	NA	NA
*pE	Spring	3.0E-02	7.2E-01	NA	NA	NA	NA	NA	NA	4.4E-02	7.7E-01	1.9E-02	8.9E-01
*rich.Cling	Spring	NA	NA	NA	NA	NA	NA	4.5E-03	-8.7E-01	1.6E-02	8.5E-01	7.7E-03	9.3E-01
*rich.E.less.B	Spring	NA	NA	NA	NA	NA	NA	2.6E-02	-7.7E-01	4.4E-02	7.7E-01	NA	NA
*rich.EPT	Spring	NA	NA	NA	NA	NA	NA	2.9E-03	-8.9E-01	2.7E-02	8.1E-01	2.0E-02	8.8E-01
*rich.SC	Spring	NA	NA	NA	NA	NA	NA	NA	NA	3.4E-03	9.2E-01	7.7E-03	9.3E-01
Hshannon	Spring	NA	NA	NA	NA	NA	NA	3.7E-02	-7.4E-01	NA	NA	4.2E-02	8.3E-01
Hsimpson	Spring	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.2E-02	8.3E-01
J	Spring	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
p1dom	Spring	NA	NA	2.7E-02	8.1E-01	NA	NA	1.6E-02	-8.1E-01	NA	NA	NA	NA
p2dom	Spring	NA	NA	1.4E-02	8.6E-01	NA	NA	3.5E-02	-7.4E-01	NA	NA	NA	NA
pBurrow	Spring	NA	NA	NA	NA	5.0E-02	-6.7E-01	1.0E-02	8.3E-01	NA	NA	NA	NA
pCF	Spring	NA	NA	3.6E-02	7.9E-01	NA	NA	NA	NA	1.4E-02	8.6E-01	NA	NA
pCG	Spring	NA	NA	NA	NA	NA	NA	1.0E-02	8.3E-01	NA	NA	NA	NA
pChi	Spring	NA	NA	NA	NA	NA	NA	6.5E-03	8.6E-01	NA	NA	NA	NA
pClino	Spring	NA	NA	NA	NA	1.0E-02	8.3E-01	NA	NA	NA	NA	NA	NA
pCling	Spring	NA	NA	NA	NA	1.3E-02	-7.8E-01	2.6E-04	-9.5E-01	NA	NA	NA	NA
pD	Spring	NA	NA	NA	NA	NA	NA	1.0E-02	8.3E-01	NA	NA	NA	NA
pE.less.B	Spring	2.5E-02	7.3E-01	NA	NA	5.3E-03	-8.3E-01	2.0E-03	-9.0E-01	2.8E-02	8.1E-01	NA	NA
pP	Spring	5.0E-02	-6.7E-01	3.6E-02	-7.9E-01	NA	NA	NA	NA	NA	NA	NA	NA
pP.less.Allo	Spring	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
pP.less.Amph	Spring	1.3E-02	-7.8E-01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
pP.less.LAA	Spring	2.5E-02	-7.3E-01	NA	NA	NA	NA	2.0E-03	-9.0E-01	NA	NA	NA	NA
pP.less.Leuc	Spring	3.6E-02	-7.0E-01	NA	NA	5.8E-03	8.3E-01	NA	NA	NA	NA	NA	NA
pPR	Spring	NA	NA	NA	NA	NA	NA	1.5E-02	-8.1E-01	NA	NA	NA	NA
pPT.H	Spring	NA	NA	2.3E-02	-8.2E-01	NA	NA	NA	NA	NA	NA	NA	NA
pSC	Spring	2.0E-02	7.5E-01	NA	NA	NA	NA	3.3E-05	-9.8E-01	6.8E-03	8.9E-01	1.9E-02	8.9E-01
pSC.less.E	Spring	NA	NA	NA	NA	NA	NA	6.8E-04	-9.3E-01	2.5E-03	9.3E-01	4.8E-03	9.4E-01
pSH	Spring	3.0E-02	-7.2E-01	3.6E-02	-7.9E-01	NA	NA	NA	NA	NA	NA	NA	NA
pSprawl	Spring	NA	NA	NA	NA	1.3E-02	7.8E-01	NA	NA	NA	NA	NA	NA
pSwimm	Spring	NA	NA	NA	NA	NA	NA	NA	NA	4.0E-02	7.8E-01	1.9E-02	8.9E-01
pT	Spring	NA	NA	NA	NA	NA	NA	NA	NA	4.5E-04	9.6E-01	NA	NA
pTOL	Spring	2.5E-02	7.3E-01	NA	NA	NA	NA	NA	NA	NA	NA	1.9E-02	-8.9E-01
rich	Spring	NA	NA	NA	NA	NA	NA	2.0E-03	-9.0E-01	2.5E-03	9.3E-01	3.6E-02	8.4E-01
rich.C	Spring	NA	NA	NA	NA	NA	NA	NA	NA	8.4E-04	9.5E-01	3.1E-04	9.9E-01
rich.CF	Spring	2.9E-02	7.2E-01	NA	NA	9.9E-03	8.0E-01	NA	NA	4.0E-02	7.8E-01	3.9E-02	8.3E-01
rich.CG	Spring	NA	NA	NA	NA	NA	NA	NA	NA	5.8E-03	9.0E-01	NA	NA
rich.E	Spring	NA	NA	NA	NA	NA	NA	NA	NA	4.4E-02	7.7E-01	NA	NA
rich.EPT.less.H	Spring	NA	NA	NA	NA	NA	NA	2.6E-03	-9.0E-01	2.7E-02	8.1E-01	7.7E-03	9.3E-01
rich.INT	Spring	NA	NA	NA	NA	NA	NA	2.6E-04	-9.5E-01	1.6E-02	8.5E-01	NA	NA
rich.less.E	Spring	NA	NA	NA	NA	NA	NA	2.5E-02	-7.7E-01	4.1E-02	7.7E-01	NA	NA
rich.P	Spring	NA	NA	NA	NA	NA	NA	3.5E-02	-7.4E-01	NA	NA	NA	NA
rich.PR	Spring	NA	NA	NA	NA	NA	NA	2.9E-03	-8.9E-01	NA	NA	NA	NA
rich.PT	Spring	NA	NA	NA	NA	NA	NA	4.7E-02	-7.1E-01	NA	NA	2.1E-02	8.8E-01
rich.SC.less.E	Spring	NA	NA	1.2E-02	8.7E-01	NA	NA	3.7E-02	-7.4E-01	9.1E-03	8.8E-01	1.2E-03	9.7E-01
rich.Swimm	Spring	NA	NA	NA	NA	NA	NA	3.5E-02	-7.4E-01	2.8E-02	8.1E-01	NA	NA
rich.TOL	Spring	2.5E-02	7.3E-01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
*pE	Fall	NA	NA	2.5E-02	7.3E-01	NA	NA	NA	NA	3.1E-04	9.9E-01	NA	NA
*rich.E.less.B	Fall	NA	NA	1.2E-02	-8.7E-01	NA	NA	NA	NA	3.8E-02	8.4E-01	NA	NA
Hshannon	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.8E-03	9.4E-01
Hsimpson	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.8E-03	9.4E-01
J	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.2E-02	8.3E-01
pBurrow	Fall	NA	NA	NA	NA	1.4E-02	8.6E-01	NA	NA	NA	NA	NA	NA
pCF	Fall	NA	NA	4.5E-04	9.6E-01	NA	NA	NA	NA	NA	NA	NA	NA
pCG	Fall	NA	NA	2.3E-02	8.2E-01	NA	NA	NA	NA	NA	NA	NA	NA
pCling	Fall	3.7E-02	-7.4E-01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
pD	Fall	NA	NA	NA	NA	2.5E-03	9.3E-01	NA	NA	NA	NA	NA	NA
pE.less.B	Fall	NA	NA	NA	NA	NA	NA	NA	NA	3.1E-04	9.9E-01	NA	NA
pEPT	Fall	NA	NA	NA	NA	6.8E-03	-8.9E-01	NA	NA	NA	NA	NA	NA
pEPT.less.Cheum	Fall	NA	NA	NA	NA	1.4E-02	-8.6E-01	NA	NA	NA	NA	NA	NA
pEPT.less.H	Fall	NA	NA	NA	NA	2.3E-02	-8.2E-01	NA	NA	NA	NA	NA	NA
pEPT.less.HBL	Fall	NA	NA	NA	NA	2.3E-02	-8.2E-01	NA	NA	4.8E-03	-9.4E-01	NA	NA
pOligo	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
pP	Fall	NA	NA	NA	NA	2.5E-03	-9.3E-01	NA	NA	NA	NA	0.0E+00	1.0E+00
pP.less.Allo	Fall	NA	NA	NA	NA	2.5E-03	-9.3E-01	NA	NA	NA	NA	4.8E-03	9.4E-01
pP.less.Amph	Fall	NA	NA	NA	NA	2.5E-03	-9.3E-01	NA	NA	NA	NA	0.0E+00	1.0E+00
pP.less.LAA	Fall	NA	NA	2.3E-02	8.2E-01	2.5E-03	-9.3E-01	NA	NA	1.9E-02	-8.9E-01	NA	NA
pP.less.Leuc	Fall	NA	NA	3.6E-02	7.9E-01	2.5E-03	-9.3E-01	NA	NA	1.9E-02	-8.9E-01	NA	NA
pPT.H	Fall	NA	NA	NA	NA	2.3E-02	-8.2E-01	NA	NA	1.9E-02	-8.9E-01	0.0E+00	1.0E+00
pSC	Fall	NA	NA	2.5E-03	-9.3E-01	1.4E-02	8.6E-01	NA	NA	NA	NA	NA	NA
pSC.less.E	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.2E-02	8.3E-01
pSprawl	Fall	2.6E-04	9.5E-01	NA	NA	2.5E-03	-9.3E-01	NA	NA	4.8E-03	-9.4E-01	NA	NA
pSwimm	Fall	3.9E-03	8.8E-01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
pT	Fall	NA	NA	4.5E-04	9.6E-01	NA	NA	NA	NA	NA	NA	NA	NA
pT.less.H	Fall	NA	NA	NA	NA	2.3E-02	-8.2E-01	NA	NA	4.8E-03	-9.4E-01	NA	NA
pTOL	Fall	NA	NA	1.2E-02	-8.7E-01	NA	NA	NA	NA	NA	NA	NA	NA
rich	Fall	NA	NA	NA	NA	NA	NA	8.4E-04	-9.5E-01	NA	NA	NA	NA
rich.Burrow	Fall	NA	NA	3.6E-02	-7.9E-01	NA	NA	NA	NA	NA	NA	NA	NA
rich.C	Fall	NA	NA	2.8E-02	8.1E-01	NA	NA	NA	NA	NA	NA	NA	NA
rich.CF	Fall	NA	NA	3.9E-02	7.8E-01	NA	NA	4.0E-02	-7.8E-01	NA	NA	NA	NA
rich.CG	Fall	NA	NA	NA	NA	NA	NA	4.4E-02	-7.7E-01	NA	NA	NA	NA
rich.E	Fall	NA	NA	4.0E-02	-7.8E-01	NA	NA	NA	NA	3.8E-02	8.4E-01	NA	NA
rich.less.E	Fall	NA	NA	NA	NA	NA	NA	2.7E-03	-9.3E-01	NA	NA	NA	NA
rich.P	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.1E-02	8.8E-01
rich.PR	Fall	5.0E-02	7.1E-01	NA	NA	NA	NA	NA	NA	NA	NA	7.7E-03	9.3E-01
rich.PT	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.0E-02	8.1E-01
rich.SC	Fall	1.2E-02	-8.2E-01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
rich.SC.less.E	Fall	1.9E-02	-7.9E-01	NA	NA	NA	NA	NA	NA	NA	NA	5.1E-03	9.4E-01
rich.Swimm	Fall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.4E-02	-8.5E-01
rich.T	Fall	NA	NA	4.6E-02	7.6E-01	NA	NA	NA	NA	NA	NA	NA	NA
rich.TOL	Fall	NA	NA	1.2E-02	-8.7E-01	NA	NA	NA	NA	NA	NA	NA	NA

¹ Level of impact from mountaintop mining valley fill salinization indicated in () after stream name; R, L, and H indicate Reference, Low-level, and High-level, respectively.

² Fifteen selected from relevant research bolded and underlined, six key metrics stared.

Table B. 3. ANOVA and Kruskal-Wallis comparison's pairwise values for 15 selected taxa metrics in six study streams in the coalfields of central Appalachia.

Season	Test	Metric	EAS (R) ¹	CRO (R)	FRY (L)	SPC (L)	ROL (H)	LLW (H)
Spring	ANOVA	*p5dom ²	a ³	a	a	a	a	a
Spring	Kruskal-Wallis	*pE	ab	ab	a	abc	c	bc
Spring	ANOVA	*rich.Cling	a	a	ab	a	c	bc
Spring	Kruskal-Wallis	*rich.E.less.B	a	a	ab	ab	b	b
Spring	Kruskal-Wallis	*rich.EPT	a	a	ab	a	b	b
Spring	ANOVA	*rich.SC	ab	b	ac	ac	c	c
Spring	Kruskal-Wallis	Hshannon	ab	a	bc	abc	c	c
Spring	ANOVA	pChi	a	a	a	a	a	b
Spring	Kruskal-Wallis	pE.less.B	a	a	ab	ab	b	b
Spring	ANOVA	pPR	ab	c	a	a	b	ab
Spring	ANOVA	pPT.H	ab	b	ab	ac	c	ac
Spring	ANOVA	pSC	a	a	bc	ac	b	b
Spring	Anova	rich	a	d	ab	a	c	bc
Spring	Kruskal-Wallis	rich.E	a	a	abc	ac	b	bc
Spring	KRUSKAL	richPR	ab	a	ab	ab	b	b
Fall	ANOVA	*p5dom	a	a	a	a	a	a
Fall	Kruskal-Wallis	*pE	a	ab	bc	abc	c	bc
Fall	Kruskal-Wallis	*rich.Cling	ab	ab	ab	b	a	a
Fall	Kruskal-Wallis	*rich.E.less.B	a	a	b	ab	b	b
Fall	Kruskal-Wallis	*rich.EPT	a	ab	abc	a	bc	c
Fall	ANOVA	*rich.SC	ab	abc	abc	b	ac	c
Fall	ANOVA	Hshannon	a	a	b	a	b	b
Fall	Kruskal-Wallis	pChi	ab	a	c	bc	abc	abc
Fall	Kruskal-Wallis	pE.less.B	a	ab	bc	abc	c	c
Fall	ANOVA	pPR	a	a	a	a	b	b
Fall	ANOVA	pPT.H	ab	ab	ab	b	ab	a
Fall	KRUSKAL	pSC	a	a	a	a	a	a
Fall	ANOVA	rich	a	c	ab	c	b	b
Fall	Kruskal-Wallis	rich.E	a	ab	bc	abc	c	c
Fall	ANOVA	richPR	a	b	ab	ab	a	a

¹ Level of impact from mountaintop mining valley fill salinization indicated in () after stream name; R, L, and H indicate Reference, Low-level, and High-level, respectively.

² Six selected key metrics starred.

³ Different letters indicate significant differences at alpha=0.05.

Appendix C. Supporting Graphs

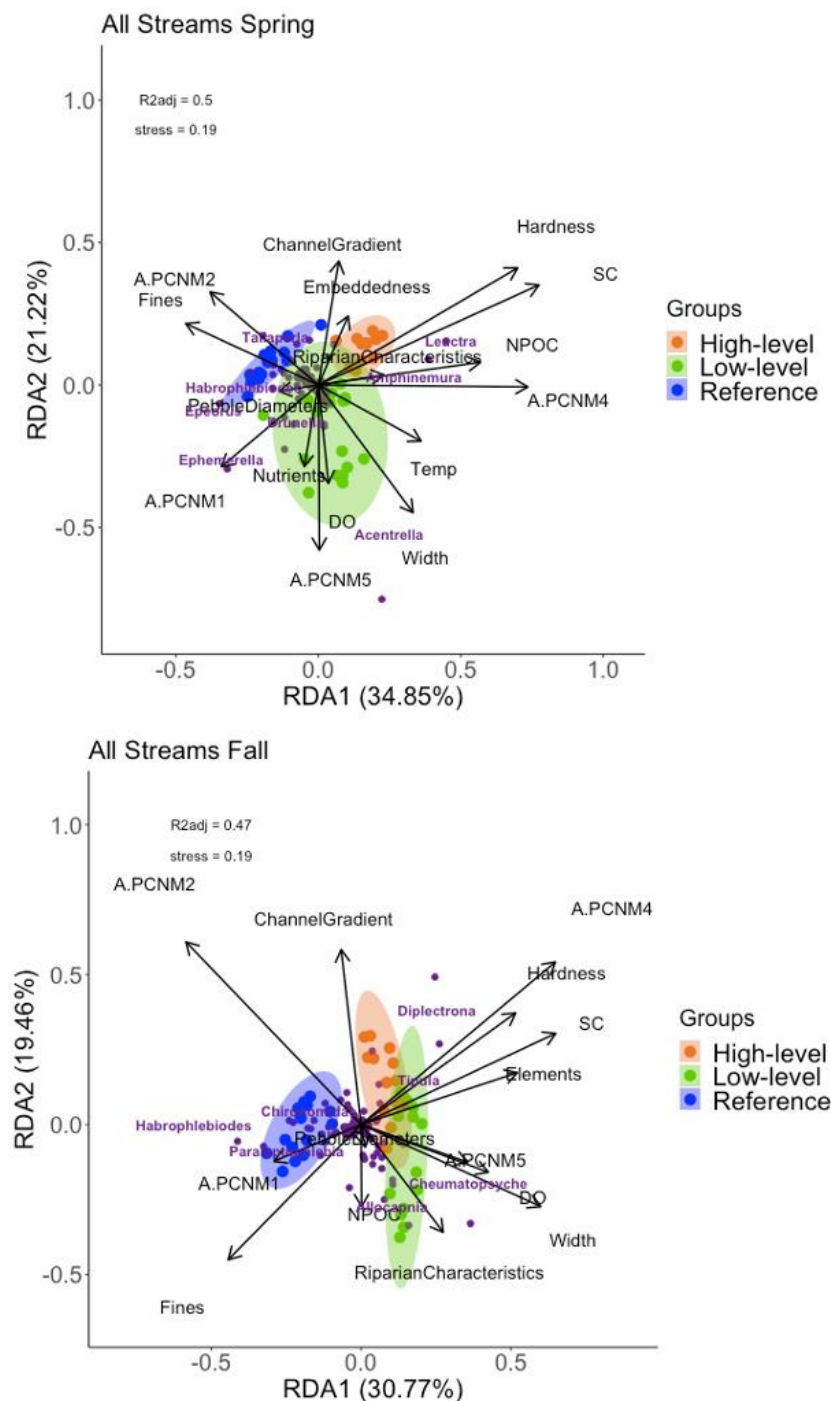


Figure C. 1. RDA of all Streams in Spring (top) and Fall (bottom) with vectors of water quality, habitat, and location. Vectors chosen with ordi2step function using backward selection. Larger points indicate sites and ellipses indicate stream level of impact. Species position indicated by purple dot and most heavily weighted taxa are labeled in purple text.

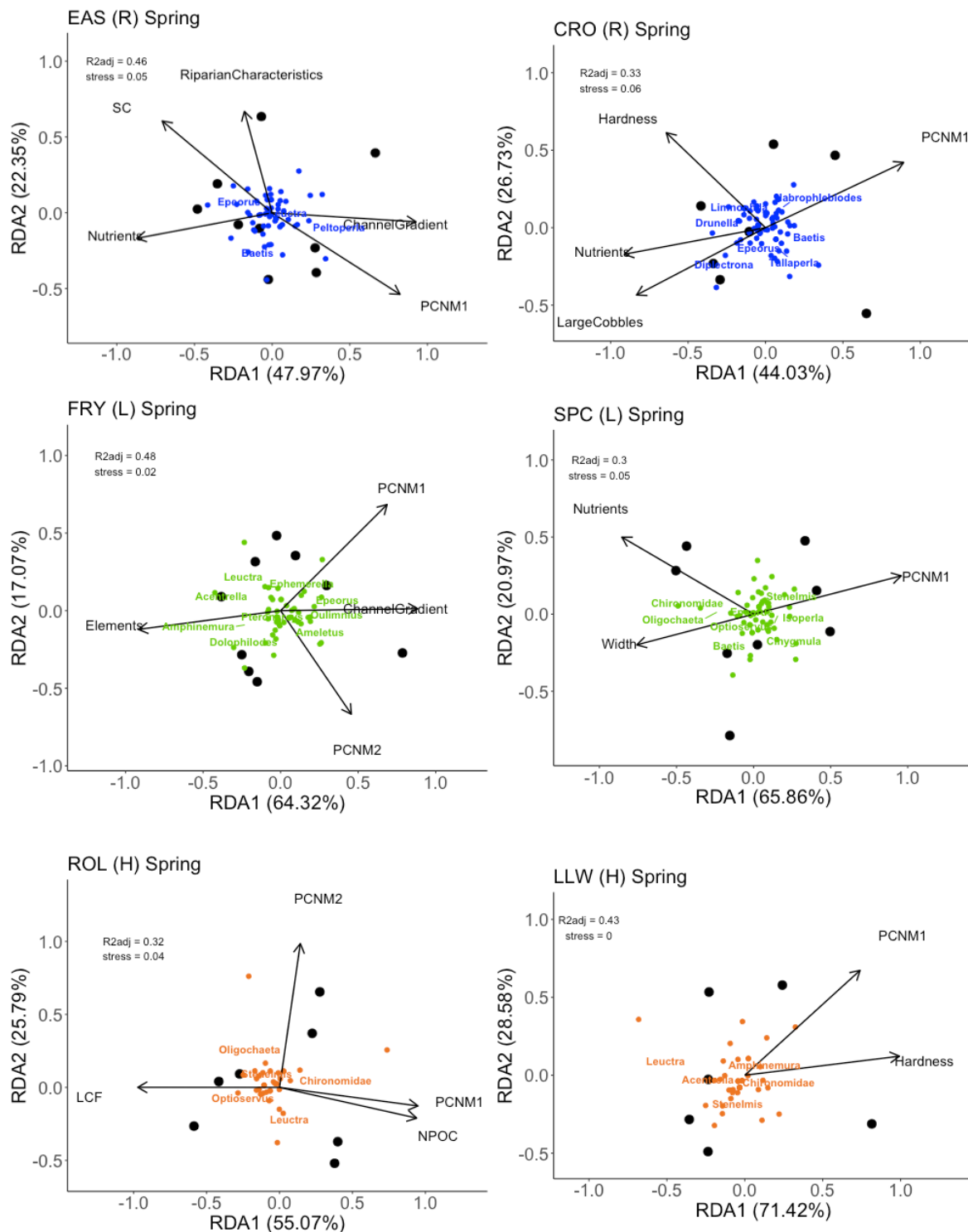


Figure C. 2. Spring RDA results for all selected variables of each attribute (water quality, habitat, and location) by stream. All taxa indicated by colored points, highest scoring taxa indicated by text; color of text and points indicated level of impact (blue = reference, green = low-level, orange = high-level). Vectors for each variable represented by arrows, and black dots indicate sampling locations. Stress = 0 may indicate insufficient data.

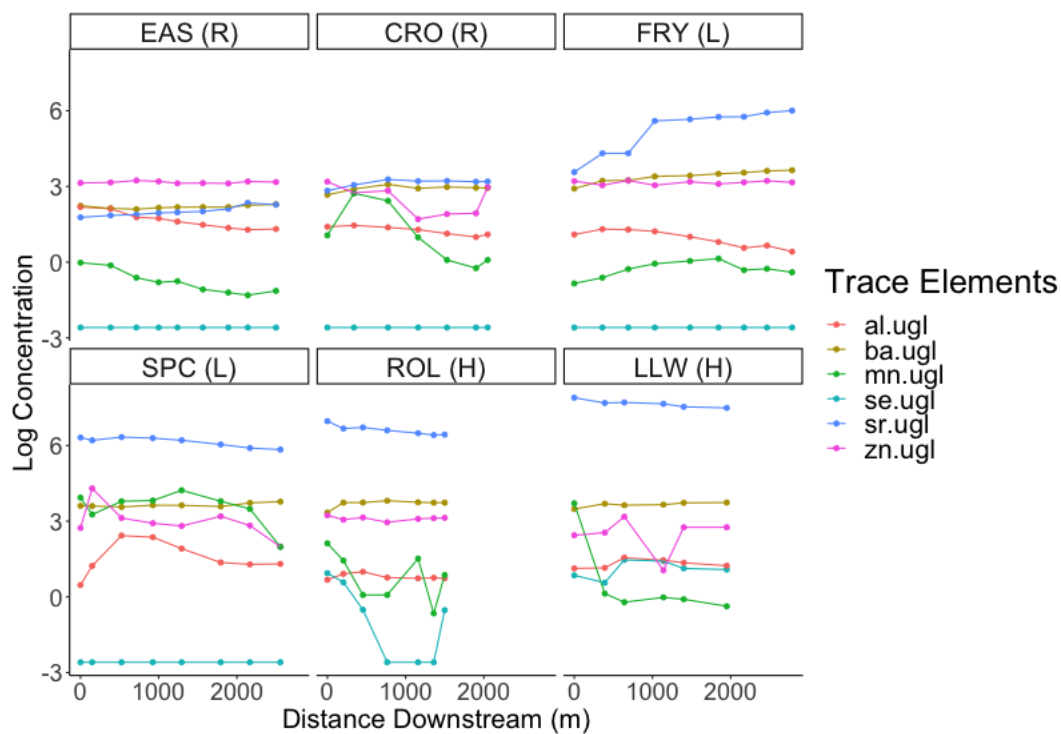
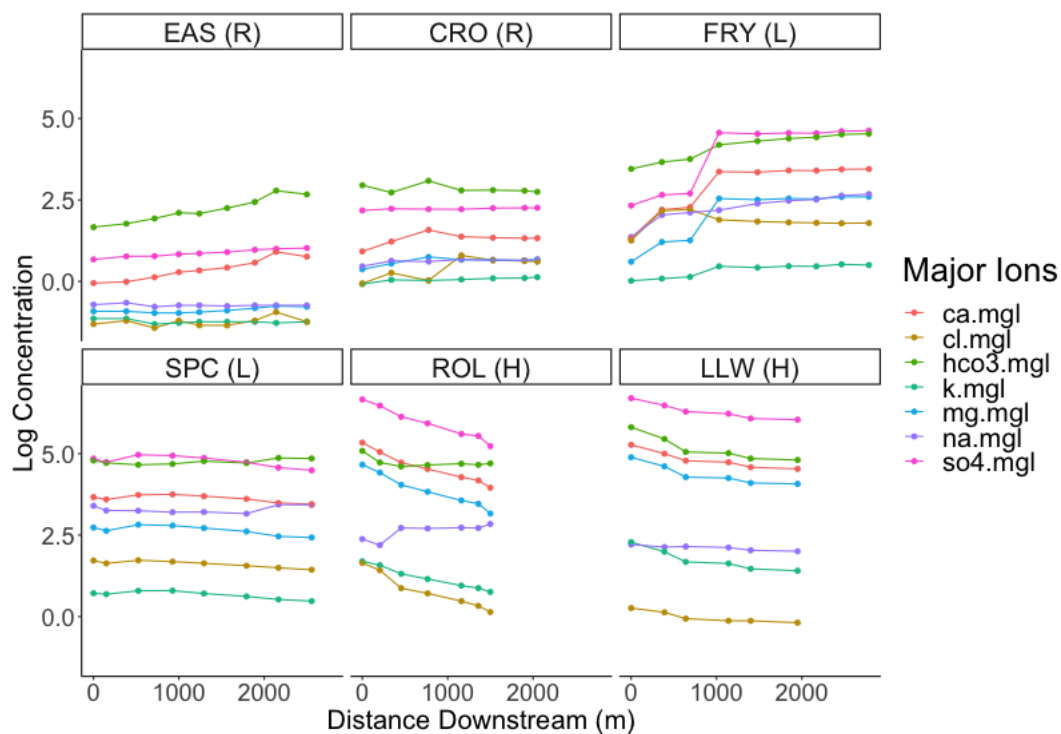


Figure C. 4. Log concentrations of major ions (top) and trace elements (bottom) plotted against distance downstream for spring by stream.

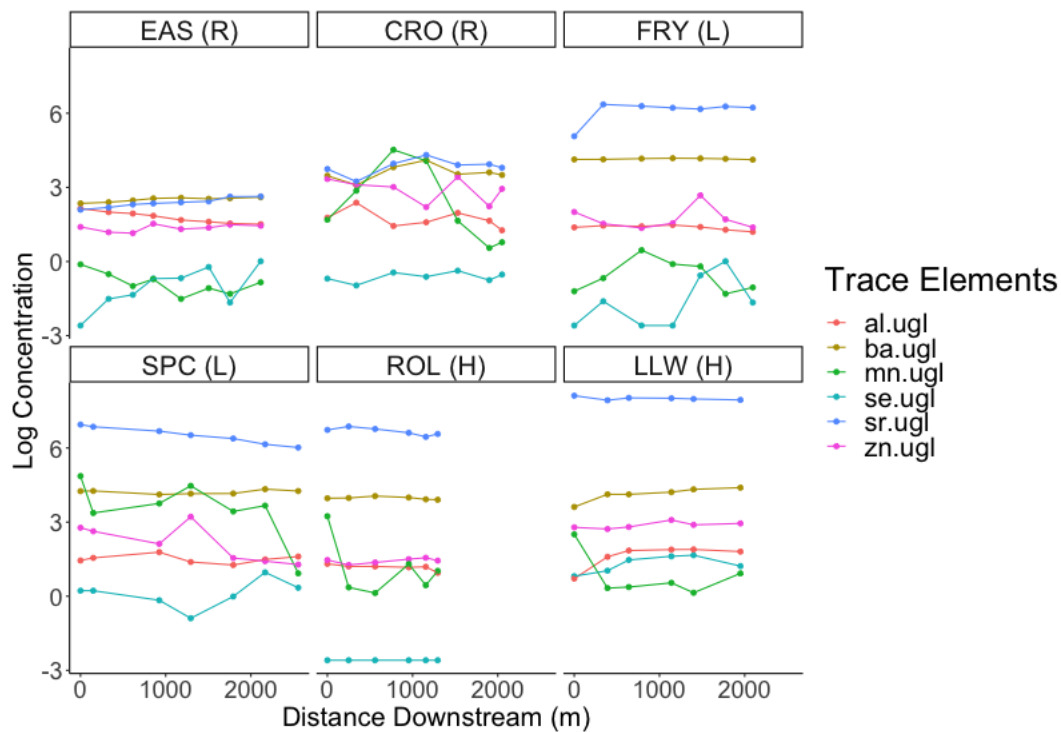
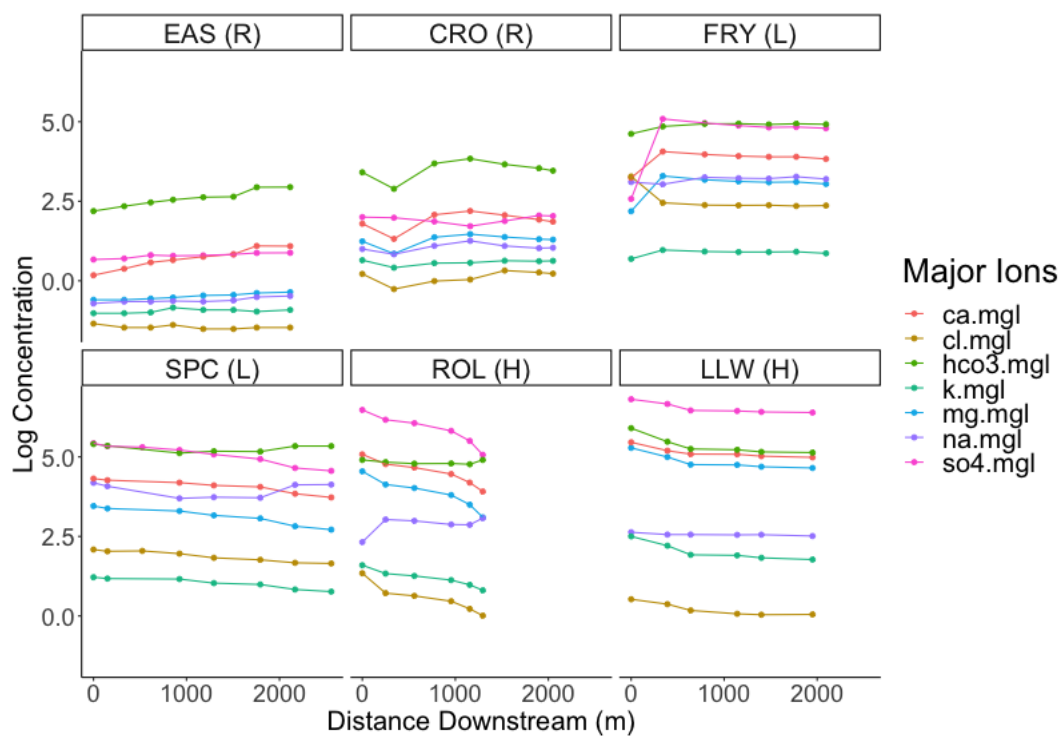


Figure C. 5. Log concentrations of major ions (top) and trace elements (bottom) plotted against distance downstream for fall by stream.