Select carbon dynamics as functional indicators of restoration success: a research approach

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Executive Summary

Permitting of coal mining in the central Appalachian region is dependent on maintaining or restoring hydrologic and ecological function in streams affected by coal extraction. As mandated by the Clean Water Act (CWA) [section 404; compensatory mitigation rule], mining operations permitted by the U.S. Army Corps of Engineers (USACE) must mitigate "unavoidable" stream impacts attributable to valley fill and other mining activities (Federal Register April 10, 2008). Assessment of stream ecosystem structure and function is fundamental to determining ecological condition and success of mitigation techniques employed on these streams. Traditional bioassessment techniques for freshwater streams are cost-effective, efficient, and are often conceptually linked to ecosystem processes through the characterization of functional feeding groups (FFGs) within the benthic macroinvertebrate assemblage. Although common, reliance on measures of biotic assemblage structure may limit the utility of these indices as reliable surrogates for stream function. In contrast, direct measures of ecosystem processes are rarely implemented in the context of restoration assessment.

Gessner and Chauvet (2002) identified lack of expertise, as well as temporal and monetary constraints as primary arguments against using direct, process-based measures as indicators of functional condition. Objections to direct functional assessment persist because monitoring entities require that measures of ecological condition be: (1) easily implemented in space and time, (2) objective, (3) causally linked to stream processes or assemblage structure, (4) sensitive to changes in environmental quality, and (5) able to separate anthropogenic disturbance signals from natural environmental stochasticity. Carbon (C) as organic matter serves as an essential habitat and energy compartment within headwater and downstream environments. Thus, assessment of carbon dynamics is integral to determining levels of ecosystem function for streams receiving restoration treatments.

This project will assess the functional status of eight low-order streams affected by coal mining that have received restoration practices and four reference streams using measures that are relatively simple to implement and relate directly to stream carbon dynamics. Background data collection and physical characterization of these streams are already underway, and a research plan has been developed to assess the functional condition of these streams. Based on the selected measures of C dynamics, this research will:

1. assess the condition of coalfield streams receiving restoration practices relative to forested reference streams,
2. examine relationships between functional and structural assessments of reconstructed streams and reference streams,
3. determine factors affecting these processes in streams receiving restoration practices,
4. evaluate these measures as indicators of stream condition.

Background and Justification:

Stream Restoration Assessment. In the context of ecological restoration of streams, Kauffman et al. (1997) identified the need for reestablishment of biotic and physiochemical factors associated with stream ecosystem function. Though river and stream restoration practices are increasingly common, follow-up assessment of restoration success is generally lacking (Bernhardt et al. 2005; Bernhardt et al. 2007). Post-restoration monitoring is required in mined-land streams as a condition of CWA [section 404] permits. Until recently, assessment of these streams has focused on evaluation of ecosystem structure via measures of water chemistry, channel stability, and biotic assemblages. These practices are likely to change, however, as recent guidance from USEPA (2010) states that quantitative, functional stream impact assessment should be used to determine environmental effects of mining and the capacity of mitigation efforts to restore functions.

Important Carbon Functions: Stream ecology studies have shown headwater streams to be predominantly open ecosystems, governed by allochthonous inputs of organic matter (e.g., Fisher and Likens 1973; Cummins 1974; Vannote et al. 1980; Hall et al. 2000). Organic matter from upstream and riparian zones serves as both energy source and habitat substrate for stream biota and, as such, is an essential component of ecosystem function. Though studies of C budgets provide a holistic view of ecosystem energy flow via measurement of storage, inputs, and outputs, these studies require high-intensity sampling efforts and are costly, thus limiting their utility within management and assessment frameworks. With regard to assessment of C dynamics, measures of organic matter transport, production, and detrital processing within stream corridors provide manageable alternatives to assessment of comprehensive C budgets. These parameters provide insight into the energetic function of stream ecosystems, while restricting sampling requirements to manageable levels. In conjunction with structural assessment and routine physiochemical monitoring, assessment of C dynamics within restored streams not only provides a sound basis for determining levels of ecosystem production and biotic activity, but also allows for insight into factors that control these processes. The C functions to be assessed in this study are:

- *Riparian organic matter subsidies.* The quality and quantity of organic matter input to and transported through the stream corridor greatly influence biotic assemblage structure which, in turn, influences the quality and quantity of organic matter available to downstream environments. Riparian inputs of coarse particulate organic matter (CPOM) as leaves or twigs and branches, for instance, may undergo leaching or fractionation to produce dissolved organic matter (DOM) or fine particulate organic matter (FPOM), respectively. The magnitude and rate of these processes depends on spatiotemporally heterogeneous factors, such as season and stream type, as well as the quality and quantity of CPOM entering the stream across ecosystem boundaries. As such, characterization of terrestrial subsidies to the aquatic system as leaves and other forms of litter is an essential component to determining the availability of resources to the biotic assemblage with clear implications for downstream environments. Ideally, comprehensive understanding of CPOM inputs would include contributions from upstream of research sites as well as the riparian zone. However, in-stream sample nets require frequent retrieval to keep from filling and may interfere with concurrent experiments. In contrast direct-fall inputs of litter can be readily characterized over relatively long periods, while still providing information about resources available to *in situ* and downstream assemblages.

- *Leaf litter processing.* Gessner and Chauvet (2002) have presented a compelling case for use of leaf litter breakdown in functional assessments of stream ecosystems. Moreover, multiple studies have identified leaf litter and organic matter processing as ecosystem functions essential to maintenance of *in situ* and downstream environments (Wallace et al. 1982a; Wallace et al. 1982b; Fisher and Gray 1983; Hutchens and Wallace 2002; Simmons et al. 2008; Aldridge et al. 2009; Benstead et al. 2009). Leaf litter breakdown is a function of both biotic processes, such as microbial and macroinvertebrate activity, and abiotic processes, such as chemical breakdown and leaching of organic compounds. Because biotic activity may be indexed by leaf-litter breakdown rates (Simmons et al. 2008), breakdown coefficients may be regarded as bioindicators of functional condition. Leaf litter breakdown rates integrate changes in environmental quality over time. Comparison of leaf litter breakdown rates of reaches that have received restoration practices to those of reference and/or pre-restoration conditions allows for quantitative and objective assessment of ecosystem function and subsequently can serve as an indicator of ecological restoration success.

- *Primary Production.* *In situ* rates of primary production may be determined by changes in dissolved O_2 or CO_2 concentration, pH, ^{14}C incorporation, or indexed by the change in standing crop over time, given control for losses caused by grazing, scour, and migration (Steinman et al. 2006). Although each method has advantages and limitations, efficient assessment is predicated upon constraints of time and funding. Chlorophyll *a* (chl *a*) is the predominant photopigment common to all primary producers, and concentrations thereof have been used as surrogates for algal standing crop. Measurement of chl *a* is not a demographic measure of population or community (i.e., biotic structure), and as such, calculated differences in this photopigment over time can be used to estimate accrual rates of algal assemblages. Accrual rates incorporate gains in the number and size of individuals, as well as any losses due to herbivory, scour, or sloughing. Conceptually, accrual rates of benthic algae and net primary production (NPP) should be roughly equivalent when controlled for losses due to physical and biotic processes.

Progress to date:

Site Selection. To date, >100 watercourses were evaluated for potential inclusion in this study. A preliminary assessment of ecosystem functions for 6 restored/reconstructed streams and 3 non-restored/reconstructed streams was conducted by previous members of the research team (Northington et al. 2009), and we have continued to collect physiochemical and biotic samples on these streams.

Additional streams have been identified to augment the population of streams used in the previous study. Reconstructed/restored streams affected by active deep mine discharge, as well as those deemed incomparable based on physical characteristics were excluded from the study. Minimally impacted reference streams were identified as those that lack evidence of recent significant watershed disturbance by humans, and with specific conductance $\leq$ 150 µS cm^{-1} and circumneutral pH (6-8), Eight reconstructed streams and four reference streams were finally selected for study (Table 1).

Table 1. Location and general description of streams selected for study.

Stream Name	Location	Stream Type[1]	Stream Order[2]
Sewing Creek	Buchanan County, VA	MRR	1
Shooting Range Creek	Buchanan County, VA	MRR	1
Chaney Creek	Russel County, VA	MRR	2
Laurel Branch	Russel County, VA	MRR	1
Stonecoal Creek	Russel County, VA	MRR	1
Callahan Creek	Wise County, VA	MRR	1
Critical Fork	Wise County, VA	MRR	2
Guest Mountain #3	Wise County, VA	MRR	2
Copperhead Branch	Buchanan County, VA	UFR	1
Big Branch	Dickenson County, VA	UFR	1
Crooked Branch	Dickenson County, VA	UFR	2
Middle Camp Branch	Dickenson County, VA	UFR	1

[1] Two stream type categories have been identified, mined receiving restoration practices (MRR) and un-mined forested reference (UFR).
[2] Stream order was determined using 7.5' USGS quadrangles.

The majority of eight reconstructed streams selected have received restoration treatments within the past five years. Primarily because of variation in techniques, these stream corridors are characterized by riparian zones in multiple stages of development. Relatively few restoration efforts located are >5 years old, however, and those with developing forest in the watershed generally lack mature riparian canopy. In an effort to expand the study, additional contacts were made to assist in locating mining-impacted streams where restoration or reconstruction efforts have resulted in establishment of more developed riparian canopy. Additionally, “comparably canopied” reference streams, which are relatively unimpacted by other disturbance were sought to discern between scale-dependent (e.g., local riparian vs. watershed forest) controls on selected C dynamics. No such streams were discovered after several scouting trips and contacts, and as such, we are confident that streams of this nature are rare in the region, if they exist.

Experimental Design. The twelve streams selected are within the Central Appalachian ecoregion (69; level III). These first- and second order perennial and intermittent streams are contained within the Appalachian plateau physiographic region of Virginia. Sixty m stream reaches of contiguous channel morphology and riparian structure were delineated and subdivided into two reaches of 30 m for different components of the study. Reaches have been classified into two major categories: un-mined forested riparian (UFR), and mined receiving restoration efforts (MRR). It must be noted that the last class, MRR, is comprised of streams receiving a wide variety of treatments from channel construction alone to those receiving Natural Channel Design (NCD) efforts and diverse riparian plantings. Site history, reconstruction method, temporal estimates of mining and stream restoration occurrence, and physical descriptions of the watercourse are being documented for each stream, and more qualitative information will be collected with the aid of contacts already made.

Materials and Methods.

Habitat and Structure

Physiochemical. Temperature data loggers (HOBO U22-001 Water Temp Pro v2; Onset Computer Corp.) will be deployed in September 2010 and will record stream temperature at 2-h intervals through September 2012. Units will be deployed within 0.5 m of litter bags (see below). Sampling of selected water chemistry variables will begin September 2010, continuing at bi-monthly intervals through July 2012. Dissolved oxygen (mg L^{-1}), temperature (°C), specific conductance (µS cm^{-1}), and pH will be measured in a uniformly mixed portion of the streamwater column immediately below the surface using a Hydrolab water quality meter (Hydrolab Quanta, Hach Instruments, Loveland, CO) at all sites for each sampling date. Additionally, grab samples will be collected at all sites for water chemistry determinations and treated according to Standard Methods (APHA 2005). Following filtration through Millipore brand mixed cellulose ester filters (47 mm diameter, 0.45 µm pore size) samples will be either preserved in 1+1 HNO_3 for determination of heavy metals and major cations, or transported on ice for determination of alkalinity, total dissolved solids (TDS), ammonium (NH_4^+-N), total oxidized nitrogen (NO_3^-N+ NO_2^-N), and soluble reactive phosphate (PO_4^{-3}-P). Flow-injection analysis will be used to determine ammonium (NH_4^+-N), total oxidized nitrogen (NO_3^-N+ NO_2^-N), and ortho-phosphate (PO_4^{-3}-P) concentrations. Concentrations of major dissolved anions, including SO_4^{-2} and Cl^-, will be determined via ion chromatography. Dissolved organic carbon (DOC) will be quantified using an organic carbon analyzer according to Standard Methods (APHA 2005).

Channel and riparian characteristics will be determined as outlined by Fritz et al. (2006). Points will be taken at each end of the reach (0 m; 60 m), and distance between these points will be used as the valley distance. Sinuosity will be calculated as the quotient of thalweg distance (60 m) to valley distance.

The area of each catchment will be quantified from flow direction analysis of the most recent digital elevation model (DEM) available, ensuring that these models reflect post-mining contours. The downstream end of the reach will be identified in the field and used as the pour point for flow direction analysis. This raster will then be used to clip the most recent land-use/land-cover (LULC) data, and catchment level variables will be quantified for each local catchment. These data will be checked against recent aerial photography to ensure their validity. Land-use types will then be calculated as percent of total watershed area and used as explanatory variables.

Benthic macroinvertebrate assemblage. Benthic macroinvertebrate assemblage structure will be evaluated using samples obtained via a flow-independent protocol outlined by Fritz et al. (2006). Specimens preserved in 95% ethanol will be identified to genus/lowest practicable level and assigned to FFGs using Merritt and Cummins (1996) as well as discriminatory methods based on mouth parts. Several assemblage structure statistics will be calculated, including: shredder density, benthic macroinvertebrate density, and Ephemeroptera-Plecoptera-Trichoptera (EPT) richness. All density measures will be reported as number of individuals per g of litter remaining as AFDM. Non-aquatic taxa and life stages will be eliminated from samples and will not be used in further analyses.

Riparian litter inputs. Energy inputs to stream ecosystems occur through one of three vectors: biologic, meteorologic, or geologic (Fisher and Likens 1973). We are concerned primarily with meteorologic inputs of litterfall. Because extensive sampling of riparian inputs within or upstream of the reach may influence the ambient biotic assemblage, litter inputs will be estimated using 10 direct-fall traps (five on each bank) spaced equidistant across a 30 m sub-reach downstream of leaf bag deployment sites. Inputs from these traps will be assumed to accurately represent meteorologic inputs of litter from the riparian assemblage where litter bags will be deployed. Direct-fall traps are being constructed using 5-gal. buckets lined with mesh screen for drainage. Traps will be placed in September 2010, emptied at least monthly for the first two months and bimonthly through July 2012. Total mass of input, species-specific relative contribution of trees to input, and input rate (a function) will be determined.

<u>Functional Measures</u>

Leaf litter breakdown. A standard substrate is necessary to assess relative stream condition based on leaf litter breakdown (Wieder and Lang 1982). *Quercus alba* leaves will be used as the standard substrate in these streams. Q. *alba* leaves will be collected from a single location using aerial litter traps constructed of plastic netting as described in Crowl et al. (2006) during September and October of 2010. *Q. alba* was chosen because it is common to many of the selected catchments for study, readily available, can be processed without undue concern for loss of coarse fragments (unlike *Tsuga canadensis* for example), and has been used in a previous study for assessment of mining effects on ecosystem function (viz., Fritz et al. 2010). In addition, because *Q. alba* is a "slow processor" (Petersen and Cummins 1974; Webster and Benfield 1986; Benfield 2006), periodic retrieval is logistically feasible in comparison with "fast processors". Leaves will be uniformly mixed, air dried (~20° C), and repeatedly "fluffed" until relatively constant dry mass has been achieved to avoid unrealistic degradation of organic matter (Boulton and Boon 1991). This process could take ca. 5-8 d (Benfield 2006) to ca. 30 d (Fritz et al. 2010).

Eight grams of leaves will be weighed and placed in ~28 cm x 30 cm nylon mesh bags. In accordance with recommendations of Young et al. (2008), both coarse (6 mm) and fine (0.5 mm) meshes will be used. Breakdown in coarse mesh is an index of leaf litter processing due to macroinvertebrates, microbial action, and physical processes, whereas fine mesh excludes any processing due to macroinvertebrates. Thus, assuming weight loss due to physical fragmentation and leaching is minimal, breakdown in fine mesh serves as an indicator of microbial activity in leaf packs. Twelve bags of both coarse and fine mesh (24 total) will be deployed in each 30 m upstream sub-reach in late October of 2010, and will be anchored in glides (transitional zones between riffles and pools) using gutter nails or other means where bedrock prohibits. Because some of our study streams are intermittent, care must be taken to ensure that bags are placed in areas with maximum likelihood of remaining inundated for the entirety of incubation. Multiple authors (Webster and Benfield 1986; Boulton and Boon1991; Young et al. 2008; Fritz et al. 2010) recognize the effect leaf pack placement has on leaf litter decomposition. Although reviews of leaf litter breakdown methods often stress the importance of placement of packs where breakdown in packs would approximate natural conditions, Boulton and Boon (1991), Young et al. (2008), and Fritz et al. (2010) opt for consistent deployment of packs in pools as a safeguard against fluctuation in hydrologic permanence (i.e., drying) of ephemeral and intermittent channels. However, breakdown rates may not be representative of typical processing in pools because they are often relatively hypoxic areas of sediment deposition, and shredders may not be well represented. We will use glides as the standard habitat unit as compromise between representative characterizations of leaf litter breakdown processes and safeguarding against drying of the channel. Leaf bags will be deployed in consistent habitat types across all sites.

Bags will be collected at 0 d, ~30 d, 90 d and ~150 d (Benfield 2006). Including the initial sampling instance at 0 d, bags will be collected in triplicate, taking care not to damage or lose fragments, placed into Ziplocs, and stored on ice for transport back to the lab. Leaves will be removed from the Ziplocs and mesh bags, and rinsed over 250 μm sieves to separate litter from mineral deposits and macroinvertebrates. Leaves from triplicate samples will be placed in separate paper bags to air dry (~20°C) to constant dry mass (DM), and subsequently triplicate samples will be aggregated, milled, and ashed at 550°C in previously ashed and weighed pans. Percent organic matter will be calculated and multiplied by DM to obtain ash free dry mass (AFDM), and percent AFDM remaining will be determined as described by Benfield (2006). The processing coefficient (*k*) will be determined based on a first-order decay model of the form, $m_t = m_0e^{-kt}$, where m_t is the percent AFDM remaining after time, t, m_0 is the initial percent

AFDM (from 0 d bags), and t is time in days or degree-days. These breakdown coefficients will be used to apply the index proposed by Gessner and Chauvet (2002; Table 2).

Table 2. Framework for assessing stream functional integrity from leaf litter breakdown (from Gessner and Chauvet 2002).

Method	Assessment parameter	Criterion	Score
Comparison with reference	Ratio of breakdown coefficients at impacted (k_i) and reference (k_r) site	k_i:k_r = 0.75–1.33	2
		k_i:k_r = 0.5–0.75 or 1.33–2.0	1
		k_i:k_r < 0.5 or >2.0	0
Absolute value	Breakdown coefficient at impacted site (k_i)	k_i = 0.01–0.03/d	2
		k_i = 0.005–0.01/d or 0.03–0.05/d	1
		k_i < 0.005/d or >0.05/d	0
Absolute value of ratio	Ratio of breakdown coefficients in coarse (k_c) and fine (k_f) mesh bags†	k_c:k_f = 1.2–1.5	2
		k_c:k_f = 1.5–2.0 or <1.2	1
		k_c:k_f > 2.0	0

† If sizable numbers of shredders are predicted to occur in the stream.

Benthic microbial biomass accrual rates. The periphyton assemblage is composed of both heterotrophs and autotrophs. Periphytic biomass, as distinct from exclusively algal biomass, may therefore be indexed by ash free dry mass (AFDM) of a sample. Consequently, benthic algal standing crop may be overestimated by AFDM determination. Determination of chl *a*, the sole photopigment common to all algal taxa, provides an alternative to AFDM when quantification of algal standing crop is of interest.

Accrual rates of periphyton on sterile substrate incorporate rates of colonization, growth, as well as loss rates due to scour, sloughing, and grazing. Gross primary production (GPP) is equivalent to the sum of community respiration (CR) and net primary production (NPP). Primary production is the rate at which C is fixed by autotrophs when corrected for loss due to respiration of these primary producers. Biomass accrual rates, measured as changes in chl *a* concentrations over time, should therefore be roughly equivalent to NPP in these streams when effects of scour, sloughing, and herbivory losses are controlled.

Artificial substrates will be used to measure periphyton biomass accrual (AFDM) rates and algal biomass (as chl *a*) accrual rates. Though artificial substrates do not accurately replicate natural conditions in streams, if standardized, reproducible results have been more readily obtained from artificial substrates than from natural substrates (Tuchman and Stevenson 1980). Relatively high levels of reproducibility facilitate comparison among streams differing in substrate composition. Ceramic tiles glued to blocks will be deployed in the upstream sub-reach of each site, arrays will include a total of 20 tiles per reach; ten tiles each for chl *a* and AFDM determination. Arrays will be retrieved following *in situ* incubation for approximately one month during September and October of 2010 and 2011.

In a review of periphyton field methods, Aloi (1990) noted that several incubation periods ranging from 1 d to several years have been used, though exposure periods of two weeks and one month are standard periods of exposure. Trophic status has been used to determine the appropriate incubation period, with more highly oligotrophic (i.e., nutrient-poor environments) environments requiring longer times for incubation (Aloi 1990). Further complicating the choice of incubation period are factors such as grazing, scour, and the purpose of the investigation. The longer the substrate is exposed to the environment, the more susceptible is the periphytic assemblage to grazing and scour; thus, biomass accrual rates as estimates of NPP may become less reliable over time. Because the purpose of this study is a comparative analysis of biomass accrual rates, rather than finer resolution taxonomic comparison, successional development stage (e.g., mature vs. developing) of the assemblage may not be a necessary criterion for selecting the appropriate exposure.   The dependent variable (biomass accrual rates) is of relatively coarse resolution, and given that mountain headwater streams are relatively nutrient poor compared to downstream reaches, an incubation period of one month will be used.

Although Kevern et al. (1966) suggest instantaneous growth rate collected at two- to four-day intervals in laboratory streams as a better estimator of primary productivity than mean growth rate, retrieval of periphytometers across such a large spatial extent is not practicable. The alternative of a shorter incubation period defeats the practical nature of this investigation—to evaluate functional parameters that integrate changes over a relatively large temporal scale.

Concentrations of chl *a* on substrata will be determined using either flourometry, spectrophotometry, or high-performance liquid chromatography (HPLC) according to standard methods (APHA 2005). Algal biomass accrual rates will be determined by dividing final chl *a* concentration by incubation time (two weeks to one month). Periphyton biomass accrual rates will be determined by the quotient of AFDM and incubation time. Additionally, the ratio of AFDM to chl *a* will be used to determine the autotrophic index (AI) for each site as a potential indicator of carbon cycling and relative ecological condition of each stream.

Data Analysis.

Given that the majority of restored/reconstructed streams encountered in the central Appalachian coalfields are typically $\leq$ 5-7 years old, multiple regression and multivariate techniques will be used to elucidate factors that may control C function of these streams. Though a more comprehensive age distribution is ideal, sufficient variation is expected to exist among streams to determine factors controlling functional variation among streams. Comparison of restored streams to relatively undisturbed reference conditions will facilitate the evaluation of C dynamics relative to a standard.

With regard to the stream population chosen, we will assemble a set of continuous independent variables (Table 3), which may influence both C function and biotic structure. Because measures of richness, density, evenness, and function are continuous dependent variables, regression and multivariate techniques are well suited to this study. In addition, we will have categorical benthic macroinvertebrate taxonomic data and streams will be divided into a minimum of two *a priori* classes (e.g., UFR and MRR; Table 2). Correlations among continuous variables will be explored to determine relationships between explanatory (independent) and response (dependent) variables. In addition, the means of structural and functional variables will be compared among *a priori* classes using two-way analysis of variance (ANOVA; SAS v.9.1.3, SAS Institute, Cary, North Carolina). Using catchment as the first factor and *a priori* class as the second, results will be used to determine differences among classes of stream given that they occur in different catchments. Bonferroni correction (α/N) will be applied to determine significance for results of multiple pairwise comparisons.

Table 3. Continuous independent and dependent variables to be used in regression and multivariate analysis, as well as *a priori* stream and catchment categories.

Independent Variables (x)	Dependent Variables (y)
Reach Scale Continuous	**Benthic Macroinvertebrate Structure**
Total alkalinity (mg L^{-1} as $CaCO_3$)	Total density (#/g litter remaining)
TDS (mg L^{-1})	Total Taxon richness
TSS (mg L^{-1})	EPT taxon density (#/g litter remaining)
SRP (mg PO_4-P L^{-1})	EPT taxon richness
DIN (mg L^{-1})	Shredder density (#/g litter remaining)
DOC (mg L^{-1})	Shredder richness
Dissolved NH_4-N (mg L^{-1})	Diversity Index Scores
Dissolved NO_2+NO_3-N (mg L^{-1})	
Dissolved Mn (mg L^{-1})	**Riparian Inputs***
Dissolved Fe (mg L^{-1})	Annual litterfall input rate (g AFDM m^{-2} y^{-1})
Dissolved Ca^{2+} (mg L^{-1})	Annual litterblow input rate (g AFDM m-2 d-1)
Dissolved Mg^{2+} (mg L^{-1})	Relative contribution by species (g leaf species / g total)
SO_4^{2-} (mg L^{-1})	
Br^- (mg L^{-1})	**Leaf Litter Breakdown**
Cl^- (mg L^{-1})	$\|k\|$ (d^{-1} and degree d^{-1}) **
Specific Conductance (µS cm^{-1})	$k_i : k_r$
Dissolved Oxygen (mg L^{-1})	$k_c : k_f$ ***
Temperature (°C)	
pH	**Biomass Accrual**
Canopy cover	Periphytic biomass accrual rate (mg AFDM cm^{-2} d^{-1} and degree d^{-1})
Sinuosity (thalweg dist/channel dist)	Algal biomass accrual rate (mg chl *a* cm^{-2} d^{-1} and degree d^{-1})
Channel slope (%)	Autotrophic index (mg AFDM cm^{-2} / mg chl *a* cm^{-2})
D_{50} (mm)	
Catchment Scale Continuous	
Age since mined (yr)	
Age since restored (yr)	
Age since restored (yr)	
Mining extent (% catchment mined)	
Forest extent (% forested)	
Reach and Catchment Scale Categorical	
UFR	
URI	
MRR	
Restoration Type (e.g., NCD vs. Other)	
Mining type (e.g., VF vs. strip vs. deep)	

*Riparian inputs will also serve as potential explanatory variables with respect to leaf litter breakdown and assemblage structure

** Will be calculated for all sites, but will only applyGessner and Chauvet (2002) index to those which are not forested reference (UFR).

*** Only for standardized (*Q. alba*) leaf bags

Conclusions

Based on observations from scouting study sites, and communications with several contacts, it is clear that there are a wide range of methods to construct and restore streams for mitigation purposes. In this context, development of this study has provided a clear framework to achieve the objectives outlined. Moreover, this research will benefit the scientific community, regulatory agencies, monitoring authorities, and industry by: (1) increasing the body of knowledge associated with functional assessment of stream ecosystems following restoration practices in areas subjected to mining activities, (2) investigating methods that may be applied for accurate assessment of the functional status of these streams, (3) assessing effectiveness of stream restoration efforts and the relationships between structural and functional integrity within these stream ecosystems, and (4) attempting to guide future functional assessment through dissemination of results via presentations at professional meetings and publication in peer-reviewed journals, as well as cooperation with industry, regulatory agencies, and consulting firms.

Future Directions

The C functions measured in this study are only components of the entire ecosystem. Future study may involve augmenting or changing the list of ecosystem processes and/or structures measured to obtain a more holistic view of these streams. Furthermore, long-term study of these and other processes may be warranted to track ecosystem recovery over time and to gain insight to the factors affecting the development of these systems. This approach has potential for determination of restoration/reconstruction “success trajectories” based on structural and functional characteristics of the system.

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