Quantifying macroinvertebrate structural and functional response to stream acidification and subsequent recovery in Shenandoah National Park

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Quantifying macroinvertebrate structural and functional response to stream acidification and subsequent recovery in Shenandoah National Park

Kelly Christine McIntyre

Abstract (Academic)

Acid rain alters freshwater pH and ion composition, preventing organisms from performing essential bodily functions causing mortality. Macroinvertebrate communities in acidified streams are characterized by species loss in response to physiological stress and altered food quality resulting from the degradation of microbial (e.g., fungi on leaves) communities. Although freshwater acidification in the U.S. is lessening following reduced industrial emissions, little is known about macroinvertebrate recovery. Often, biotic recovery is assessed by looking at changes to what taxa and how many individuals are present in the community (e.g., richness, density). While providing a metric for change, changes in "who" is there (i.e., richness) doesn't necessarily tell us changes in "what" they are doing (i.e., function). The relationship between diversity and function requires linking a "who" to their "what" with direct measurements or as indicated by their traits. Traits are attributes of an organism that aggregate biological, morphological, and behavioral information and may relate to their success in a particular environment. For example, taxa that cannot survive with stream drying (not desiccation resistant) may only be found in streams with permanent water. Trait-based taxonomic metrics could bridge "who" and "what" and expand the impact of stream recovery assessments. My objective was to assess trends over time in water chemistry and macroinvertebrate taxonomic and trait richness and density following reduced industrial emissions. To do so, I studied two long-term data sets from Shenandoah National Park to assess trends in water chemistry and macroinvertebrate taxa and trait composition over a 30-year period to identify taxa and traits that are sensitive to acidification. I also measured how much biomass macroinvertebrates produced in a year (i.e., secondary production) in two streams (1 acidified; 1 not acidified) to determine taxa and traits that are functionally sensitive to acidification. I used these

structural and functional measures of sensitivity to determine if changes in trait richness or density predict changes in the function of that trait (e.g., secondary production).

Changes over time show that streams have some recovery from acid rain with increasing stream pH and a greater number of taxa and traits present in the community. Changes in taxa were greater than changes in traits over time. While this result was expected as multiple taxa make up each trait category, it may also suggest minimal or delayed functional recovery over time. Still, macroinvertebrate secondary production indicated that function did differ with differences in acidification. Therefore, observed small changes in traits over time mirror prior studies that found other variables, such as competition for food or space, delay or inhibit macroinvertebrates from returning to the recovering streams. Additionally, there were similarities between traits changing over time and the secondary production of traits that differed between more and less acidified streams. Taxa characterized by long life spans and large body size (e.g., semivoltine, long adult life, slow seasonal development) appeared to be the most sensitive to changes in acidification. These findings suggest that some compositional attributes, like taxonomic or trait richness, may predict functional changes measured as secondary production while others, such as density, do not.

Quantifying macroinvertebrate structural and functional response to stream acidification and subsequent recovery in Shenandoah National Park

Kelly Christine McIntyre

Abstract (Public)

Acid rain causes the acidification of freshwater stream water observed as decreased stream pH and decreased ability of watershed soils to neutralize or “soak up” acidic inputs before they enter the stream. These changes in stream water chemistry inhibit the ability of aquatic animals to perform essential bodily functions resulting in mortality. The total number of aquatic insect taxa and total number of individuals in the community have been found to decrease in response to acidification. Although freshwater acidification is lessening following reduced industrial emissions, little is known about if and how aquatic insects can recover. Often, aquatic insect recovery is assessed through looking at changes to what taxa and how many individuals are present in the community. While providing a metric for change, changes in “who” is there (i.e., richness) doesn’t necessarily tell us changes in “what” they are doing (i.e., function). The relationship between diversity and function requires linking a “who” to their “what”. This is often done by assessing changes in the taxa present but also looking at how the composition of traits associated with those taxa also change. Traits or taxa-specific characteristics aggregate biological, morphological, and behavioral information and may relate to their ability to live in a given environment. For example, taxa that rely on filtering and consuming fine particles may only be found in faster flowing streams where they function to remove fine particulates. Trait-based assessments could link “who” and “what” to expand our understanding of how stream ecosystems change with a stressor. My objective was to assess recovery of streams in Shenandoah National Park following reduced industrial emissions. To do so, I studied two long-term (1987-2017) data sets from to assess trends in water chemistry and macroinvertebrate taxa and traits. This enabled me to determine “who” is changing in these streams over time. I also measured how much insect biomass s produced in a year (i.e., secondary production) in two

streams (1 more acidic; 1 less acidic) to determine what taxa and traits alter “what” (e.g., secondary production) they are doing.

I found that long-term trends indicate some recovery from acid rain with improving water quality and an increased number of insect taxa and traits present in the community over time. Changes in insect taxa were greater than changes in their traits suggesting that stream acidification did not greatly alter “what” the insects are doing greatly to begin with or that recovery may be delayed. In contrast, my secondary production study showed that “what” macroinvertebrates are doing does differ in more or less acidic streams. Therefore, our observed minimal changes in traits over time suggest that other variables, such as competition for food or space, delay or inhibit macroinvertebrates from returning to the recovering streams. Additionally, there were similarities between traits changing over time and traits that were different in the more or less acidic streams. Taxa characterized by long life spans and large body size (e.g., semivoltine, long adult life, slow seasonal development) appeared to be the most affected by differences or changes in stream water acidity. These findings suggest that changes in “who” can predict changes in “what” to some degree though static metrics of abundance do not always reflect the function of the taxa or community

Dedication

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Chapter 1: Introduction and literature review

1.1 Acid Deposition (General)

Atmospheric acidification of freshwater systems is a historical and ongoing facet of human alteration of the natural world. It is the transfer of strong acids and acid-forming substances from the atmosphere to the Earth's surface. Acid deposition comprises primarily sulfuric and nitric acids derived from sulfur dioxide (SO_2) and nitrogen oxides (NO_x) emitted during fossil fuel combustion and react with water and oxygen in the atmosphere to form sulfuric and nitric acids (Burns et al. 2008; Driscoll et al. 2001). These acids can then be transported by wind hundreds of miles before falling to the ground as acid rain. Typical rainwater has a pH around 5.7 while acid rain produces pH values between 3 and 5 (Likens, Bormann, and Johnson 1972). Acidification of precipitation has resulted in subsequent freshwater acidification. Background stream pH of 6.5-8 have been driven down around 4 in some instances (Herlihy et al. 1993; Hogsden and Harding 2011; Valett and Ely 2019). A drastic shift in abiotic conditions causes decreased biodiversity and ecosystem processing (e.g., decomposition) in stream ecosystems (Hildrew et al. 1984). Aquatic macroinvertebrates are central to the functional capacity of freshwater ecosystem, playing an integral role in food-web dynamics and energy flow (Wallace and Webster 1996). Acid-induced changes to macroinvertebrate community composition and function (e.g., secondary production) may result in diminished ecosystem function (e.g., carbon cycling) and the services upon which we rely.

Although atmospheric acid deposition has been regulated and its effects extensively studied in historically impacted areas, it is an emerging problem in developing areas with increasing industrial emissions. From 2005-2014, high SO_2-emitting regions included the United States, Europe, Asia, South Africa, Russia, Mexico, and the Middle East. During this timeframe anthropogenic emissions declined by about 80% in the United States and Europe, whereas emissions in India nearly doubled (Fioletov et al. 2016). Areas susceptible to the deleterious impacts of acidification are not limited to centers of industrialization. Regions downwind of emitters can also be threatened by acid deposition as prevailing winds the transport air pollution (Driscoll et al. 2001)(Figure 1). Particularly vulnerable landscapes are characterized by high precipitation, thin soils, and insoluble exposed bedrock, often associated with mountainous regions, which afford little buffering capacity for neutralization of acids (Kimmel 1985 (Ormerod and Durance 2009).

Historically, areas most impacted by acid deposition are located in the Northeastern United States and Europe due to the large number of cities and high concentrations of industry and power plants (Driscoll et al. 2001). Acid deposition and its effects on freshwater ecosystems has been a major scientific and public policy issue in the United States since the 1972 publication “Acid Rain” brought attention to large-scale and long-term changes in water quality and the resulting loss of biota (Likens et al. 1972). Public concern surrounding the issue resulted in the 1990 Clean Air Act Amendments, which reduced sulfur dioxide (SO_2) and nitrogen oxides (NO_x) emissions. For example, the United States Environmental Protection Agency (EPA) found a 91% and 61% reduction in average maximum SO_2 and $NO_{2,}$ respectively, across the United States from 1980-2018 (EPA 2018). Historically acidified regions that have experienced reduced emissions provide an opportunity to investigate if and how ecosystems change with the amelioration of acid-induced stress. Understanding how biota and their related ecosystem processes are altered by and potentially recover from acidification is important to inform how industrializing nations may choose to develop and how nations currently addressing acid deposition may maintain or alter regulations.

1.2 Acid Deposition (Biotic Alterations)

Atmospheric acid deposition has been shown to impair soil, forest, and freshwater ecosystems, all of which are interacting components of a watershed (Dangles and Guérold 2000; Junk, Bayley, and Sparks 1989; Vannote et al. 1980). Streams and their adjacent riparian zones are closely linked by hydrology and reciprocal flows of materials and organisms (Baxter, Fausch, and Carl Saunders 2005; Elwood and Mulholland 1989; Meegan et al. 1996) Headwater streams comprise 79% of river length and 70% of land drainage and are characterized by interaction with the riparian area (Colvin et al. 2019).

Riparian connection contributes large amounts of allochthonous detritus, which forms the base of the food web in forested headwater ecosystems (Dangles and Guérold 2000; Vannote et al. 1980). These inputs are processed by macroinvertebrates that play an integral role in food-web dynamics by recycling macronutrients (nitrogen, phosphorus, and organic carbon) and micronutrients (e.g., metals and metalloids) (Covich, Palmer, and Crowl 1999; Wallace and Webster 1996). Headwater connections between the terrestrial and aquatic environment render them particularly vulnerable to acidification because they are directly impacted by precipitation as well as indirectly due to cation depleted soil and riparian vegetation.

Acidification changes the chemical composition of soils by depleting base cations (ex. Ca^{2+} and Mg^{2+}) increasing aluminum mobility, and increasing concentrations sulfate and nitrogen (Driscoll et al. 2001; Rochelle and Church 1987). If the concentration of base cations is sufficiently diminished, H^+ neutralization will be incomplete resulting in acidified soil water (Cronan and Schofield 1990; Haines 1981; Kimmel et al. 1985). In the mid 1950's, Likens et al (1996) found acid rain enhanced the release of Ca^{2+} from forest soils in the northeastern United States. This release of the most weakly bound or absorbed forms of Ca^{2+} from the soil were followed by reductions of Ca^{2+} in stream water indicating declines in inputs of Ca^{2+} from the atmosphere and concentrations of soil Ca^{2+} (Likens, Driscoll, and Buso 1996). Acidic deposition in regions with base-poor soils has resulted in the acidification of soil waters, shallow groundwaters, streams, and lakes (Driscoll et al. 2001). Acidified aquatic systems are characterized by declines in pH and in base cations concentrations and increases in aluminum (Al^{3+}) concentrations (Ferreira and Guérold 2017).

The general responses of biotic community structure and activity to the abiotic changes (decreased pH and ANC; increased Al^{3+}) associated with stream acidification may reflect an 'acidification syndrome' (Ferreira and Guérold 2017). Biological symptoms of this syndrome include declines in biodiversity across taxonomic groups including microbial, algal, macroinvertebrate, and fish communities

(Baudoin et al. 2008; Ferreira and Guérold 2017; Ledger and Hildrew 2005; Murphy et al. 2014). In addition, ecosystem processes, such as rates of production and decomposition are generally reduced potentially having cascading effects for higher trophic levels, such as reduced resource availability for downstream and riparian communities (Hildrew et al. 1984; O. Dangles et al. 2004; K. Simon, Simon, and Benfield 2009; Jenkins, Woodward, and Hildrew 2013). Central to the functional capacity of freshwater ecosystems are macroinvertebrates that serve as food resources for higher trophic levels and facilitate nutrient cycling (Wallace and Webster 1996). Therefore, changes to macroinvertebrate communities, resultant from direct and indirect stressors associated with acidification, may result in changes to the functional capacity of stream ecosystems.

Microbial communities are an essential food source for macroinvertebrates (Wallace and Webster 1996). For example, some macroinvertebrates scrape epilithic algae off submerged surfaces in streams while others ingest hyphomycete fungi associated with detritus. (Ledger and Hildrew 2005). Fungal, bacterial, and algal assemblages differ between acidified and circumneutral streams because acidification disrupts microbial ion homeostasis, membrane transport, and metabolic processes (Clivot et al. 2013). Acidified microbial communities are characterized by decreased taxon richness and evenness (Baudoin et al. 2008; Ledger and Hildrew 2005; Müller 1980). Functional changes to microbial communities display a less distinct patterns with acidification. Declines in community respiration indicate overall microbial stress but biomass has been shown to increase, decrease, or show no response to acidification (Allard and Moreau 1985; Clivot et al. 2013; Mulholland et al. 1987; Simon et al. 2009; Valett and Ely 2019). For example, Ledger and Hildrew (2005) found cyanobacteria to be virtually absent from most acidic sites (pH 4.8 and 5.1) but dominated the biofilm assemblage even at only a slightly higher mean annual pH (5.2) potentially because the pH minimum of cyanobacteria is around 5.0. They also found increases in algal density and biomass with acidification, presumably from decreased macroinvertebrate herbivory (Ledger and Hildrew 2005).

Macroinvertebrates are indirectly impacted by acidification due to these observed changes in the quantity and quality of microbial resources. For example, Sjostrom et al (1990) found acid-sensitive mayfly (*Baetis rhodani*) survival decreased to the equivalent of starving individuals when fed biofilm from an acidic stream even when maintained in circumneutral water. Ledger and Hildrew (2001) found the growth of the acid-tolerant stonefly (*Numurella pictetii*) did not differ when fed biofilm from an acidic stream. Instead, they were required to increase their ingestion rate to compensate for the lower quality diet (Ledger and Hildrew 2001; Sjöström 1990).

Macroinvertebrates are also directly impacted by acidification because increases in H^+ and Al^{3+} ions and reductions in Ca^{2+} ions result in biochemical and physiological failures. In macroinvertebrates, gills or gill equivalents (e.g., chloride cells, anal papillae) are the primary organ for respiration, ion regulation, osmoregulation, acid-base balance, and excretion of nitrogenous waste (Havas and Rosseland 1995). Increased hydrogen concentrations cause Ca^{2+} to be lost from binding sites in the gill epithelium, diminishing the ability of the gill to control membrane permeability. Decreased control of permeability results in ion-regulatory disturbance and loss of ions (Na and Cl) as well as blood acidosis (Havas and Rosseland 1995; Morris, Taylor, and Brown 1989; Sharpe et al. 1983).

Physiological stress associated with acidification requires organisms to reallocate energy (Griffith, Perry, and Perry 1994). Griffith and Perry (1994) found production to biomass (P/B) ratios of acid-tolerant stoneflies, *Leuctridae* and *Nemouridae*, decreased with acidification while P/B ratios of acid-tolerant stonefly, *Capniidae*, increased, suggesting changes in energetic demands with acidification. Increased energetic demands associated with maintaining biogeochemical and physiological process in acidified environments can reduce taxon fitness and survival, potentially resulting in local extirpations.

Macroinvertebrate communities in acidified streams are characterized by a simplified community comprising taxa able to cope with acidic conditions (Allard and Moreau 1987). Taxa richness consistently declines with acidification (Bowman et al. 2006; Burton, Stanford, and Allan 1985; Dangles and Guérold

1999, 2000; Giberson and Mackay 1991; Hall et al. 1980, 1980; Hall and Ide 1987; Harriman and Morrison 1982; Hildrew et al. 1984; Kimmel et al. 1985, 1985; Kobuszewski and Perry 1994; Layer et al. 2011; Mulholland et al. 1992; Rosemond et al. 1992; Traister et al. 2013). For example, Rosemond et al (1992) observed taxa richness declined from sixty-nine taxa at the highest pH (6.8) site to thirty-three species at the lowest pH (4.5) site. Total abundance of macroinvertebrates may decrease or remain the same with acidification because tolerant taxa may experience reduced competition for resources or predation (Burton et al. 1985; Hall et al. 1980; Harriman and Morrison 1982; Kimmel et al. 1985; Mulholland et al. 1992; Müller 1980; Traister et al. 2013; Weatherley and Ormerod 1987; Winterbourn and Collier 1987; Zischke et al. 1983)**.**

Community composition of acidified streams is typically characterized by elimination of acid-sensitive groups, including *Ephemeroptera, Amphipoda, Orthocladinae*, and the grazer functional feeding guild (algal feeders)**.** Baker and Christensen (1991) found that a pH change from 6.13 to 5.13 completely eradicated the mayfly genus, *Hexagenia*, in an experimentally acidified lake. In addition, Simpson et al. 1985 found a 40–70% reduction in *Chironomidae*, the most ecologically diverse macroinvertebrate family, with stream acidification (pH 5.5–7.0). Macroinvertebrate communities tend to instead be dominated by Filipalpian stoneflies (i.e., *Leuctridae, Nemouridae, Capniidae, and Taeniopterygidae*), acid-tolerant *Chironomidae* (e.g. *Microtendipes, Chironomini and Tanypodinae*), and the shredder functional feeding guild (Allard and Moreau 1987; Baker and Christensen 1991; Baldigo et al. 2009; Bowman et al. 2006; Griffith et al. 1994; Groom and Hildrew 1989; Jenkins et al. 2013; Kobuszewski and Perry 1994; Lepori, Barbieri, and Ormerod 2003; Mulholland et al. 1992; Simpson, Bode, and Colquhoun 1985; Traister et al. 2013)**.** Mulholland (1992) found that Plecopteran density was more highly corelated with benthic organic matter stocks than pH or Al indicating a general tolerance to acidification.

Acid-tolerant taxa tend to be dietary generalists able to opportunistically switch among resources (Dangles 2002; Ledger and Hildrew 2001, 2005; Traister et al. 2013). Filipalpian stoneflies have been found to graze upon epilithic biofilm as well as consume detritus (Dangles 2002; Ledger and Hildrew 2000, 2005). This generalist behavior could potentially maintain herbivore-algal linkages lost with the extirpation of acid-sensitive grazers (Ledger and Hildrew 2005). On the other hand, loss of specialist species which drive discrete processes in communities could lead to functional impairment (Ledger and Hildrew 2005).

While it is well established that macroinvertebrate abundance and diversity respond to acidification, less is known about how macroinvertebrates communities functionally (e.g., secondary production) change with acidification. A limited number of studies have demonstrated a reduction in macroinvertebrate biomass and secondary production in acidic environments though tolerant taxa may experience relative increases in abundance, biomass, and production (France 1996; Griffith et al. 1994; Krueger and Waters 1983; Pretty, Giberson, and Dobson 2005; Stead, Schmid-Araya, and Hildrew 2005; Uutala 1981) (Table 1). For example, Griffith and Perry (1994) found that shredder (detrital consumers) secondary production was three times lower in an acidified stream than a circumneutral streams (pH 4.3 vs. 7.5). While overall production was lower, they found that production of an acid-tolerant stonefly (*Paracapnia angulata*) was three times higher in the acidic stream because of its higher abundance and biomass. Changes in macroinvertebrate biomass and production with regards to acidification appear to be related to the geochemistry of a stream's underlying bedrock. The carbonate and bicarbonate composition of the bedrock reflects the ability of the system to buffer acidic inputs, thus determining rate and extent of stream acidification observed with acid deposition.

1.3 Recovery

To date, emission reductions have translated into improving chemical conditions, or "recovery", of freshwater ecosystems from atmospheric acid deposition (Ormerod & Durance, 2009). Although accounts of chemical recovery have been widespread, biological recovery has been less observed and understood (Malcolm, Bacon, et al., 2014; Malcolm, Gibbins, et al., 2014). While the trajectory of recovery from acidification is not characterized by a simple reestablishment of acid-sensitive taxa, broader patterns of recovery have been observed in multiples systems (Hildrew, 2009; Layer et al., 2011; Monteith et al., 2005; Murphy et al., 2014; Ormerod & Durance, 2009). Studies typically report increased taxonomic richness and changes in relative abundances of taxa in response to recovering water quality. For example, Monteith et al., (2005) found that streams displaying recovery from 1988-2008 typically had an increase in macroinvertebrate abundances and richness of 1-3 taxa. Additionally, in a recovery assessment of historically acidified streams (1988-2008), Murphy et al., (2014) determined that the stream with the clearest indication of biological recovery had dramatic increases in macroinvertebrate abundance and richness as acid sensitive taxa (e.g., mayflies: *Baetis* and *Electrogena)* returned to the community and acid-tolerant taxa *(e.g.,* stoneflies*: Leuctra* and *Nemoura)* decreased in abundance. Ultimately, most studies do not find evidence of biotic recovery or that it lags behind chemical recovery. Potential explanations for slow recovery patterns include dispersal limitations, recurring acidic episodes, and indirect food web effects (Gray et al., 2016; M. Ledger & Hildrew, 2005; Monteith et al., 2005). While studies document some change in macroinvertebrate communities over time, the extent of, controls on, and trajectory of ecosystem recovery from acid deposition has yet to be fully examined.

1.4 Biodiversity-Ecosystem Function (BEF theory)

Human-driven elimination of taxa, such as that observed with soil and water acidification from atmospheric acid deposition, has motivated research to understand the role biodiversity plays in ecosystem function (Gagic et al. 2015; Gray et al. 2014; Lecerf and Richardson 2010). Greater biodiversity is often assumed to enhance ecosystem processes (e.g., production, decomposition) and is expected to enhance ecosystem stability and resilience to disturbance (McKie and Malmqvist 2009). While we understand that taxon diversity is significant to ecosystem function, we lack a complete understanding of the mechanistic relationship between biodiversity and macroinvertebrate secondary production (McKie and Malmqvist 2009; Statzner and Lévêque 2007).Although most research linking biodiversity and production has been conducted in plant communities, the mechanisms that drive this relationship, such as complementary resource use and facilitation, are expected to be the same for macroinvertebrate secondary production (Caldeira et al. 2001).

Taxonomically based biodiversity indicators are incomplete in that taxonomic identity does not provide insight into the roles of individuals in an ecosystem and neglect the reality that species loss is often non-random based upon those roles (Elmqvist et al. 2003; Fritschie and Olden 2018; Lecerf and Richardson 2010). Understanding the relationship between identity-based responses and ecosystem function requires linking taxon identity the role that they play in the environment (Craig et al. 2017; Nock, Vogt, and Beisner 2001). To understand the mechanistic underpinnings between organisms and their environments, ecologists explain and predict species distributions by linking species traits in a community to environmental drivers that influence taxa fitness (Poff et al. 2006, 2007; Southwood 1977; Townsend and Hildrew 1994). Variation in spatiotemporal conditions will influence the ecological trait composition of a community. Thus, habitats with similar environmental conditions should have taxa with similar trait attributes (Poff 1997).

1.5 Trait-based Ecology

A trait is a physiological, morphological, or ecological attribute of a taxa that describes their physical characteristics, ecological niche, or functional role within an ecosystem (Baird, Rubach, and Van den Brinkt 2008; McGill et al. 2006; Poff et al. 2006). Traits reflect adaptations to dominant environmental characteristics thus providing insight into the mechanisms structuring communities (McGill et al. 2006; Nock et al. 2001; Poff et al. 2006; Statzner et al. 1997; Usseglio-Polatera et al. 2000; Violle et al. 2007). For example, the trait type functional feeding group (FFG) designations are assigned to macroinvertebrates based on their morphology and behavior used to acquire food (Merritt, Cummins, and Berg 2008). The FFGs that often match the dominant food resources are: scrapers (algae), shredders (microbial conditioned riparian plant litter); filtering collectors (water column particulate organic material); gathering collectors (stream bottom particulate organic material); herbivore (live aquatic plants), and predators (live prey) (Cummins 2016).

Each taxon possesses a combination of traits, referred to as a functional trait niche (FTN). FTN's reflect and organism's relationship with the environment and other taxa that is constrained by evolutionary processes reflected in their phylogeny (Bonada et al. 2006; Poff et al. 2006) For example, the genus *Baetis sp.* has a FTN that includes bi/multivoltinism, fast seasonal development, unsynchronized emergence, short adult life spans, low female dispersal, low adult flight strength, no body armoring, streamlined body shape, swimming habit, collector-gatherer feeding group, and several other trait attributes (Poff et al. 2006). Within these FTN's, traits often occur in "syndromes" and respond to a stressor in concert (Poff et al. 2006, 2007). An instance of this is demonstrated in habitats with high temporal and spatial heterogeneity. Taxa in these locations are often characterized by fast development, small body size, and short lifespans, while more stable environments are characterized by long life spans, slow development, and large body size (Townsend and Hildrew 1994; Usseglio-Polatera et al. 2000).

A functional ecology approach thus focuses not only on interactions between taxa and their environments but their physical characteristics in order to better understand their influence on ecosystem processes and services (Nock et al. 2001; Poff et al. 2007). This approach offers a way to mechanistically link traits and associated functions present in a community to environmental conditions (Baird et al. 2008; Feio and Dolédec 2012; Poff et al. 2006). For example, Fritsche et al. (2018) investigated the use of body size of fish taxa to relate individuals to ecosystem function (per capita nutrient cycling). They found that trait-based models agreed with species-specific models about community structure variables influencing aggregate recycling rates and ratios; however, there was much variation for site-specific metrics between trait-based and species-specific models. These results highlight the potential utility of linking of traits to environmental conditions to enable a more predictive community ecology as well as a need for further research (Fritschie and Olden 2018; McGill et al. 2006; Nock et al. 2001; Poff et al. 2006).

Trait-based metrics are useful in bridging structural and functional characteristics by serving as a proxy for how ecosystem function is changing without directly measuring function. Traits have been shown to have greater explanatory power and better correlation to ecosystem function than taxonomically-based metrics (Craig et al. 2017; Fritschie and Olden 2018; Gagic et al. 2015; Lecerf and Richardson 2010; McGill et al. 2006). However, the ability of functional diversity measures to explain ecosystem function largely remains untested (Gagic et al. 2015; Lecerf and Richardson 2010).Measuring functional response will expand the capacity of trait-based metrics to link changes in structure and function. Improved linking structural and functional responses will be advantageous to developing the capacity for traits to be used to characterize and forecast ecosystem response to a stressor (Craig et al. 2017). For example, Gagic (2015) found that indices solely based on the numbers and abundances of species were consistently poor at predicting ecosystem functioning across eight field studies that included five animal groups (bees, carabid beetles, earthworms, soil nematodes and dung beetles) and seven ecosystem functions (pollination, biocontrol of crop pests, biocontrol of weeds, bioturbation, nutrient cycling, dung removal and seed burial). Instead, trait-based indices were better predictors of function, suggesting that functional capacity is underpinned by the combination of trait identities in the community (Gagic et al. 2015).

1.7 Study Overview and Objectives

The goal of this study is to understand how atmospheric acid deposition and subsequent water chemistry recovery (increases in pH and ANC through time) impacts macroinvertebrate composition and function. While abundance-based measures of macroinvertebrate communities provide some context for change, they do not support a direct assessment of functional changes that might occur. As a result, we may lack a comprehensive understanding of how acid deposition affects aquatic ecosystems.

This study explores the effects of stream acidification and subsequent recovery on macroinvertebrate taxa, trait composition, and function in streams. I worked in Shenandoah National Park, an area characterized by historically high levels of sulfate deposition and vulnerable geology that has resulted in stream acidification (Bulger et al. 1995). My research expanded previous long-term (1984-2009) analyses of water chemistry and macroinvertebrate communities within the park to include additional years of data. I broadened macroinvertebrate community analyses to include entire suites of macroinvertebrate traits to investigate how macroinvertebrates have changed over time and across geologic classes within the park. I identified acid-sensitive traits through changes in taxa richness or abundance over time to determine if there is an indication of biotic recovery in streams within the park. I then quantified secondary production in two streams within the park that have experienced differing magnitudes of acidification and recovery to determine how production within macroinvertebrate traits changes with varying levels of acidification. Traits identified as sensitive in long-term and secondary production analyses were compared to determine if abundance-based sensitivities align with sensitivities measured as production. This study is a first-step towards the goal of testing if observed changes in macroinvertebrate trait composition in the presence of a stressor can be used to predict changes in macroinvertebrate secondary production.

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1.9 Figures

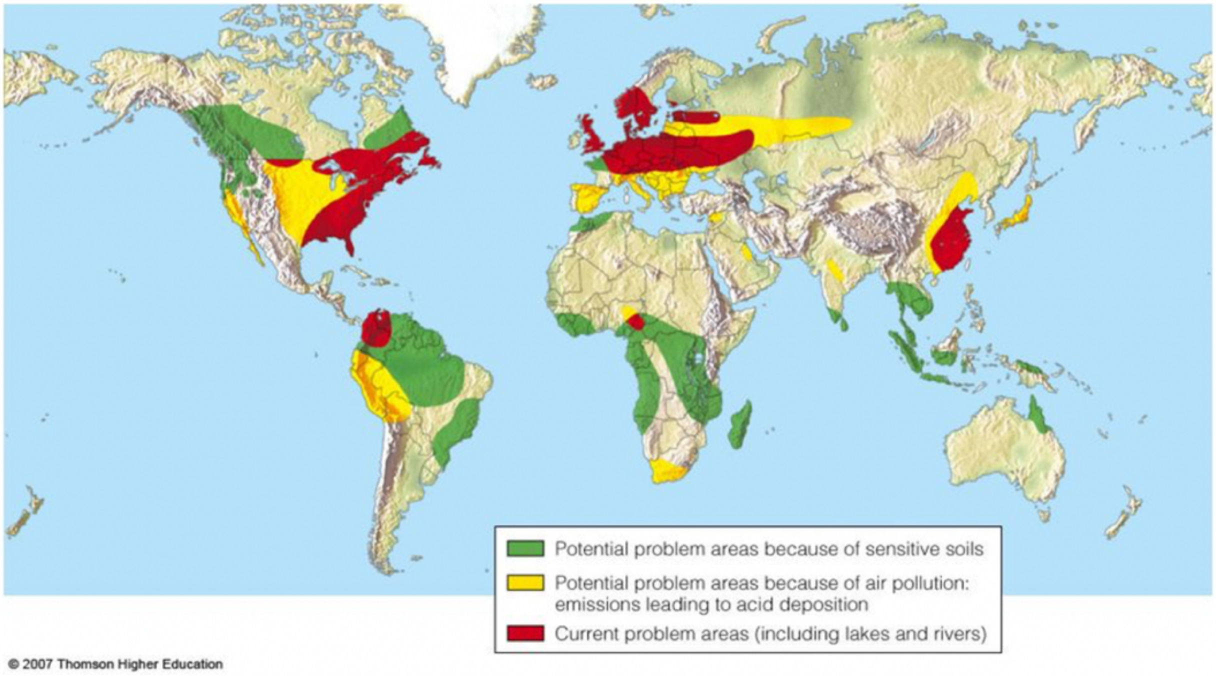

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Figure 1. Global map of areas impacted by acid deposition as well as areas that have the potential to become acidified due to increasing industrial emissions or vulnerable location or topography (Image from Thompson Education).

Chapter 2: Taxonomic and biologic trait differences among stream macroinvertebrates suggest modest biotic recovery from acidification over time (1987-2017) in Shenandoah National Park

Abstract:

Biodiversity loss under acidic conditions is among the most intensely researched global change effects. Yet, the process of biological recovery in neutralizing freshwaters remains to be understood. We analyzed two long-term data sets (1987-2017) from Shenandoah National Park (SNP) to determine trends in water chemistry and macroinvertebrate taxa and trait composition following reduced industrial emissions. We predicted that (1) stream sulfate decreased and pH and acid neutralizing capacity (ANC) increased in all streams over time due to reductions in sulfur and nitrogen oxide emissions. (2) All streams decreased in sulfate and increased in pH and ANC but with varying levels of increase depending on their underlying geology. (3) Total macroinvertebrate density, taxa and trait richness, diversity, and evenness increased in all streams over time with decreasing stream sulfate and increasing pH and ANC. (4) The rate of macroinvertebrate metric responses differed depending on the rate of change in pH, ANC, and sulfate concentration. (5) Large-bodied, semivoltine, long-lived, and high dispersing taxa increased in density and representation as pH increases. We found that changes in water chemistry suggested modest recovery over time with streams decreasing in sulfate and increasing in pH over time. ANC trends were insignificant and varied with geology, suggesting continued impacts of acidification. Changes in macroinvertebrate community metrics were suggestive of biotic recovery over time with increasing density and taxonomic and trait richness. Taxonomically-based metrics showed greater differentiation over time suggesting functional changes may be minimal or delayed. Taxa driving temporal changes were collector-gathering and scraping Ephemeroptera, net spinning and case-making Trichoptera, and predaceous Plecoptera. Traits that increased in richness and density over time were characterized by long-lived taxa with high dispersal ability. We conclude that streams in SNP have experienced modest recovery from acidification with biotic recovery primarily observed through taxonomic responses. Dampened response of traits over time suggest that acidification may not have significantly altered trait composition or trait-based recovery is more delayed than taxonomic recovery.

Key words: *Acid deposition, macroinvertebrate, recovery, trait, function*

2.1 Introduction

Freshwater acidification “syndrome” lowers pH and alkalinity and mobilizes metals that decrease microbial, algal, macroinvertebrate, and fish diversity followed by diminished ecosystem processes (e.g., production and decomposition, Ferreira & Guérold, 2017). Acid deposition in regions with base-poor soils has resulted in the acidification of freshwater ecosystems, driving stream pH below 4 in some instances and contributing to regional extirpation of freshwater species (Driscoll et al., 2001; Kahl et al., 1992). Headwater streams often experience relatively greater changes from acidification because of direct inputs of acidified precipitation and indirect effects from cation depleted soil and vegetation. Indirect impacts include loss of ability to neutralize acidic soil water, increased mobility of aluminum, and diminished quantity and quality of allochthonous inputs into streams from altered microbial activity (Driscoll et al., 2001).

As predicted by the syndrome, macroinvertebrate communities in acidified streams are simplified, comprising taxa able to cope with acidic conditions (Baudoin et al., 2008; Ferreira & Guérold, 2017; M. Ledger & Hildrew, 2005; Murphy et al., 2014). Taxa richness consistently declines by as much as 30-70% with freshwater acidification (Feldman & Connor, 1992; Rosemond et al., 1992; Simpson et al., 1985). Community composition is typically characterized by elimination of acid-sensitive groups, including Ephemeroptera, Amphipoda, Orthocladinae (Chironomidae), and the grazer functional feeding group (Bowman et al., 2006; Feldman & Connor, 1992; Rosemond et al., 1992). Macroinvertebrate communities tend to instead be dominated by acid- tolerant Plecoptera, Chironomidae (non-Orthocladinae), and the shredder functional feeding group (Mulholland et al., 1992; Simpson et al., 1985). In acidified systems, plecopteran density has been shown to have greater positive correlation with organic matter availability than pH or Al, indicating acid-tolerance (Mulholland et al., 1992).

Increased energetic demands and diminished resource quality and quantity in acidified environments can reduce taxon fitness and survival, resulting in competitive release for acid-tolerant taxa or local extirpation for acid-sensitive taxa (Griffith et al., 1994; Groom & Hildrew, 1989). For example, some acid-tolerant stoneflies (Leuctridae and Nemouridae) increased production compared to biomass (P/B) suggesting an increase in growth while yet another (Capniidae) showed a decrease in P/B (Griffith et al., 1994). Clearly, acidification alters the bioenergetics of aquatic insects but the direction and reason for response is not always evident. Explanations for altered bioenergetics include direct effects like toxicity-induced suppression of feeding behavior and indirect effects like altered quantity and quality of microbial resources that operate to mediate insect responses. For example, Sjostrom et al (1990) found acid-sensitive mayfly (Baetidae: *Baetis rhodani*) survival decreased to the equivalent of starving individuals when fed biofilm from an acidic stream even when maintained in circumneutral water. While algae composition differed among neutral and acid streams, nymph mortality was related to a lack of grazing on any algae when reared in acidic water (pH 4.5; Sjöström, 1990). Alternatively, the acid tolerant stonefly (Nemouridae*: Numurella pictetii)* was able to compensate for lower quality food in acidified streams by increasing their ingestion rates (Ledger & Hildrew, 2000). Differences in the metabolic response of taxa to acidification suggest that tolerant taxa may possess traits that illicit the necessary compensatory response. For example, Griffith and Perry (1994) found that P/Bs of the most abundant shredders in acidified streams were greater than P/Bs of the most abundant shredders in circumneutral streams. Changes in P/B with acidification suggest traits such as multivoltinism, fast seasonal growth, and small body size may be favored in acidified streams.

Total abundance of macroinvertebrates may decrease or remain the same with acidification because tolerant taxa may experience reduced competition for resources or predation and compensate for losses of sensitive taxa (Traister et al., 2013). Acid-tolerant taxa tend to be dietary generalists able to opportunistically switch among resources (Dangles, 2002; M. E. Ledger & Hildrew, 2000). For example, acid-tolerant stoneflies can graze epilithic biofilm and eat detritus (Dangles, 2002; Ledger & Hildrew, 2000; Ledger & Hildrew, 2005). This generalist behavior helps to explain the maintenance of herbivore-algal linkages in acidified streams even with the extirpation of acid-sensitive grazers (Ledger & Hildrew, 2005). On the other hand, loss of specialist species that drive specific functions could lead to functional impairment (Ledger & Hildrew, 2005). For example, loss of the superfamily Ephemeroides (burrowing mayflies), which influence processes such as respiration and nutrient cycling, may alter the physio-chemical conditions of hyporheic substrates (Boeker et al., 2016).

To date, emission reductions have translated into improving chemical conditions, or “recovery”, of freshwater ecosystems from atmospheric acid deposition (Ormerod & Durance, 2009). Although accounts of chemical recovery have been widespread, biological recovery has been less observed and understood (Malcolm, Bacon, et al., 2014; Malcolm, Gibbins, et al., 2014). Potential explanations for slow recovery patterns include dispersal limitations, recurring acidic episodes, and indirect food web effects (Gray et al., 2016; M. Ledger & Hildrew, 2005; Monteith et al., 2005). While studies document some change in macroinvertebrate communities over time, the extent of, controls on, and trajectory of ecosystem recovery from acid deposition has yet to be fully examined.

Because aquatic invertebrates show a range of responses and compensatory mechanisms to changes in acidification, predicting functional shifts as an indication of recovery is challenging. Trait-based metrics can approximate ecosystem function without direct functional measures. For example, more individuals but fewer shredder taxa predict more interannual variation in leaf litter processing (Griffith et al., 1994). Additionally, in most disturbed ecosystems, taxa with small-bodied and fast-growing traits overtake the longer-lived food and habitat specialists (Bonada et al., 2006). Therefore, indicating a shift in emergence patterns and transfer of carbon within the stream and across the watershed. Because acidification reduces taxa but promotes greater density and biomass of some, like Plecoptera, we predict recovery will be indicated by relatively greater abundance of medium and large-bodied, univoltine and semivoltine, specialist feeders.

I aim to understand if and how macroinvertebrate communities have responded to fluctuating water chemistry that is related to effects from atmospheric acid deposition. We examined macroinvertebrate communities sampled in 15 streams from 1987-2017 in Shenandoah National Park to assess temporal trends in water chemistry and macroinvertebrate taxa and trait composition. Changes in macroinvertebrate richness and abundance were used to identify acid-sensitive taxa and traits which may be indicative of biotic recovery in streams within the park. We predicted that (1) stream sulfate decreased and pH and acid neutralizing capacity (ANC) increased in all streams over time due to reductions in sulfur and nitrogen oxide emissions. (2) All streams decreased in sulfate and increased in pH and ANC but with varying levels of increase depending on their underlying geology. (3) Total macroinvertebrate density, taxa and trait richness, and taxa and trait evenness increased in all streams over time with decreasing stream sulfate and increasing pH and ANC. (4) The rate of macroinvertebrate metric responses will differ depending on the rate of change in pH, ANC, and sulfate concentration. (5) Large-bodied, semivoltine, long-lived, and high dispersing taxa increased in density and representation as pH increases.

2.2 Materials and methods

2.2.1 Study area

Shenandoah National Park (SNP) is an approximately 800 km^2 strip of National Park Service land located in the Blue Ridge Mountains of western Virginia (National Park Service, 2017a). Climate is humid temperate with annual air temperatures ranging from -23°C to 37°C (mean 9°C -12 °C; National Park Service, 2017b). Mean annual rainfall ranges from 100 to 150cm with storms creating high runoff conditions occurring year-round (National Park Service, 2017b). Landcover is predominately deciduous and coniferous forest (95%) with elevations ranging from 170m in the northwest to 1230m in the central part of the park (National Park Service, 2017a). Three major geologies underlie the park, each differing in their ability to neutralize acidic inputs (Figure 1; Riscassi et al., 2019; Riscassi et al., 2020). Siliciclastic watersheds are in the most western regions of the park and are characterized by very low acid-neutralizing capacity (ANC) waters (-4.88-225.50 µeq/L). Granitic watersheds are in the eastern most areas of the park and have intermediate ANC values of the three geologic formations (Jastram et al., 2013) (29.36-489.54 µeq/L). Lastly, Basaltic watersheds are located in the central region of the park and are characterized by the highest ANC capacities (38.34-554.56 µeq/L; Supplemental 1-3).

2.2.2 Water quality

Water quality sampling has been conducted as part of the Shenandoah Watershed Study (SWAS) since 1979; however current analysis begins in 1987. Quarterly grab samples (January, April, July, October) were collected by program personnel and analyzed in the SWAS laboratory at the University of Virginia. Standard operating procedures for analyses are available through the National Park Service's NPSTORET database. Analysis results are compiled into the Mountain Stream Database by University of Virginia and are available for export from the National Water Quality Monitoring Council's Water Quality Portal for analyses. We selected 13 sites (3 Basaltic, 5 Granitic, 5 Siliciclastic) located within SNP with data from 1987-2017 for temporal analyses (Figure 1; Table 1).

Water quality parameters predicted to show biologically meaningful changes through time (pH, acid neutralizing capacity (µeq/L), and sulfate concentrations (mg/L) were analyzed for temporal and spatial trends. Water temperature (℃) was also assessed over time to determine changes in water quality in relation to climate change. Temporal trends in water quality parameters were evaluated using the Seasonal-Kendall Trend (S-KT), a nonparametric test of monotonic trends that accounts for seasonal variation using the kendallSeasonalTrendTest function in the EnvStats package in R. S-KT slope trends in water quality were compared across the 3 geologic classes to determines differences in the rate of change (i.e., recovery) over time. Slope comparisons were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test followed by a Tukey HSD or Wilcoxon Rank Sum post-hoc test if model results were less than $\alpha = 0.05$ for parametric and nonparametric data, respectively. Analyses were completed with the aov and TukeyHSD or kruskal.test and pairwise.wilcox.test functions in the stats package in R.

2.2.3 Macroinvertebrates

Macroinvertebrate sampling was conducted by National Park Service personnel as part of the Shenandoah Long-Term Ecological Monitoring (LTEM) Program. Annual spring (April- June) samples were collected annually since 1984, however current analysis begins in 1987. Three quantitative samples were taken in shallow, wadable riffles of a 100-meter reach of stream at each site using a Portable Invertebrate Box Sampler (PIBS) or surber in low flow, both outfitted with 363-µm mesh. Samples were stored in 70% ethanol (EtOH) and sent to a by a third-party contractor (Virginia Polytechnic Institute and State University or EcoAnalysts, Inc.) to be sorted from the debris, identified to genus or lowest practical taxonomic level, with the exception of Chironomidae (family level), and counted per taxonomic unit (Jastram et al., 2013; Wofford et al., 2018). Values from the three samples per site were averaged for analyses. We selected 13 sites (3 Basaltic, 6 Granitic, and 4 Siliciclastic) located within SNP with data from 1987-2017 for temporal and spatial analysis (Figure 1; Table 2; Supplemental 2.4).

Changes in macroinvertebrate taxa and trait composition were used to assess temporal and spatial trends. Twenty traits were assigned to each taxa from 59 potential trait states for trait-based analyses (e.g., the trait size at maturity contains three potential states: small, medium and large; Poff et al., 2006). Genera not included in this database were assigned traits associated with their next lowest taxonomic designation (i.e., a new genus not represented in database will be assigned its respective family level traits; Merritt et al., 2008). Temporal trends in macroinvertebrate metrics (richness, density, Shannon diversity and Pileou’s evenness) were assessed using a Mann-Kendall trend test using the kendallTrendTest function in the EnvStats package in R. Trend slopes from Mann-Kendall assessments were quantified and compared among the 3 geologic classes to determine differences in rates of change (i.e., recovery) over time. Slope comparisons across geologic classes were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test followed by a Tukey HSD or Wilcoxon Rank Sum post-hoc test if model results were less than $\alpha = 0.05$ for parametric and nonparametric data, respectively. Analyses were completed with the aov and TukeyHSD or kruskal.test and pairwise.wilcox.test functions in the stats package in R.

2.2.4 Multivariate analyses: water chemistry, environment, and macroinvertebrates

To determine the influence of landscape and water quality attributes on macroinvertebrate community composition, water chemistry and macroinvertebrate sites within 6 km of stream length of one another were paired for multivariate analyses. A total of 10 corresponding sites (1 Basaltic, 5 Granitic, 4 Siliciclastic; Figure 1; Supplemental 2.5) were matched from 10 macroinvertebrate and 7 water chemistry sites. The combined data set included annual macroinvertebrate, quarterly water chemistry (e.g., pH, ANC), and watershed characteristic data (e.g., elevation, aspect). Spatial and temporal trends were assessed through multivariate analyses that related response variables (e.g., richness) to a suite of predictor variables.

Density and richness-based non-metric multidimensional scaling analysis (NMDS) were used to analyze temporal and spatial patterns across macroinvertebrate communities using the metaMDS function in the vegan package in R. Richness and densities were log $(x + 1)$ transformed and Wisconsin double standardized prior to multivariate analyses so that differences in measurement scale would not skew results (McCune et al., 2002). An NMDS representation was considered acceptable when Stress did not exceed 0.2. The best 2 or 3-dimensional ordination solution was determined using a scree plot for each group by running 100 random-start configuration analyses with Bray-Curtis distance as the dissimilarity measure. A secondary matrix was fit to NMDS plots to identify environmental variables related to differences in macroinvertebrate taxa and trait composition using the envfit function in the ecodist package in R. Taxa correlation coefficients less than -0.6 or greater than 0.6 with a $p \leq 0.05$ were considered significantly correlated with NMDS axes.

Temporal tends were assessed across 5 -year blocks to determine changes in community composition overtime. Trends were also assessed for 3- and 10-year blocks and did not differ from 5-year trends. Differences among year groupings were determined using distance-based permutational multivariate analysis of variance (PERMANOVA) with the andonis2 function in vegan package. If composition differed among temporal designations an Indicator Species Analysis (ISA) was conducted to identify taxa and traits indicative of differences in water quality. ISA produced an indicator value between 0-100 that was the product of the relative abundance and relative frequency of taxa within a group. Indicator values attain a maximum value when all species occur in a single group of sites and all sites within the group (McCune and Grace 2002).

2.3 Results

2.3.1 Water chemistry: Decreased stream sulfate and increased pH indicate recovery from acidification while equivocal trends in ANC suggest modest recovery

As predicted with emission reductions following the Clean Air Act Amendments of 1990, water chemistry variables related to acidification changed over time suggesting a trajectory towards chemical recovery from acidification. While streams among the three geologic classes were characterized by differing degrees of acidification, temporal trends, when present, tended to be the same across streams in all geologic classes (Figures 2A-C; Table 3). Contrary to our predictions, stream sulfate had the same magnitude of change over time regardless of geologic class, decreasing 0.25 μeq L^{-1} $year^{-1}$ (0.35% $year^{-1}$) and yielding a change of about 15 mg L^{-1} (11%) from 1987-2017 (Supplemental 2.1, 2.2, 2.3). In addition, stream pH also had the same magnitude of change over time regardless of geologic class, increasing approximately 0.01 units $year^{-1}$ (0.12% $year^{-1}$) and yielding a change of about 0.24 units (3.5%) from 1987-2017 (Supplemental 2.2 and 2.6).

In contrast to consistent trends in stream sulfate and pH, temporal trends in stream ANC varied with geologic class (Figure 2C). ANC in Basaltic and Granitic streams increased by an average of 0.66 L^{-1} $year^{-1}$ (0.40% $year^{-1}$), yielding a change of about 20 µeq L^{-1} (12%) from 1987-2017 (Supplemental 2.2 and 2.6). Although average change did not differ between Basaltic and Granitic streams, Granitic streams were more variable in the directionality and magnitude. For example, all Basaltic streams had positive trends while Granitic streams had a mix of positive and negative trends in stream ANC through time (Figure 2F). In contrast, Siliciclastic streams had primarily negative trends with average ANC decreasing by 0.02 µeq L^{-1} $year^{-1}$ (-0.55% $year^{-1}$), yielding a change around 0.6 µeq L^{-1} (-16%) from 1987-2017 (Supplemental 2.2 and 2.6).

2.3.2 Water temperature: similar values across geologic classes with some indication of increase over time.

Stream water temperatures were similar among all geologic classes ranging from 0-28°C with an annual average of 11°C (Supplemental 2.7 -2.8). Temporal trends were present at only half of the sites sampled. Basaltic streams showed no significant change while Granitic and Siliciclastic streams had a mix of no change or increase through time (Supplemental 2.8-2.9). When present, temporal trends in water temperature showed an average increase of 0.05°C $year^{-1}$ (0.5% $year^{-1}$), yielding a change over time of 1.6 °C (15%) from 1987-2017 (Supplemental 2.8-2.9).

2.3.3. Macroinvertebrate: richness and density increased while diversity and evenness did not change over time with decreasing stream acidification

Temporal trends, when present, tended to be across all streams and geologic classes. For example, average macroinvertebrate density increased by an average of 11% or 17 individuals per sample across all streams, while average taxa richness increased by 54% or 15 unique taxa (Figure 3 A and C; Supplemental 11-13). Trait richness increased the least by an average of 3% or 2 trait groupings across all geologic classes from 1987-2017 (Figure 3B; Supplemental 11-13). In contrast, macroinvertebrate diversity (Shannon-Weiner) and evenness (Pileous) did not significantly change at most sites through time despite changes in richness and density (Figure 4; Supplemental 2.10-2.12). When significant trends were present, taxa diversity increased, trait diversity decreased, and taxa and trait evenness decreased over time (Figure 4; Supplemental 2.12).

Changes in macroinvertebrate identity and density across time was assessed across all sites and years as well as separately for each geologic class to deduce differences among general and geology-specific trends. Taxa density-based NMDS for each geologic class analyses explained a greater amount of variation (47-75%) among sampling events than analyses based on trait density (4-6%) or trait richness (3-4%; Figures 5 and 6; Supplemental 2.13-2.16). As expected, macroinvertebrate community composition varied across time in all analyses (all-stream and geology-specific; Table 5; Figures 5-6;). The most consistent and distinct differences observed in macroinvertebrate identity and density were among the earliest grouping of years (1987-1991) and all groupings of following years (1992-2017) (Table 5; Figures 5-6).

Most changes in macroinvertebrate identity and density across time were correlated with greater sulfate and potassium in earlier years and greater pH and ANC in later years in all analyses except in Granitic streams (Figures 5-6; Supplemental 2.17-2.20). Interestingly, temporal changes of trait density in Granitic were driven by increasing potassium, a variable often seen in association with increasing sulfate concentrations (Supplemental 2.19). Temporal changes in community composition included a shifting of points along the ordination axis mirroring these environmental gradients. Interestingly, temporal changes were also evident in differences in the sizes of area that encompassed each grouping of year where the area of points (polygons) associated with year groupings decreased in size over time suggesting homogenization of macroinvertebrate communities with decreasing acidification (Figures 5-6).

Across all analyses, later years were characterized by increased densities of collector-gathering and scraping Ephemeroptera, net spinning and case-making Trichoptera that are sensitive to changes in water chemistry, and predaceous Plecoptera (Figures 5A, 6A,6D, and 6G; Supplemental 2.13-2.16; 2.21-2.24). Trends in macroinvertebrate traits were less consistent across analyses. When all streams were assessed, traits indicative of temporal changes were increased richness of depositional and clinger taxa and increased density of taxa with slow seasonal growth, stream-exiting adults, well synchronized emergence, and heavy sclerotization (Figures 5 B-C,6B-C, 6E-F, and 6G-H; Supplemental 2.13-2.16; 2.21-2.24). When broken up by geologic class, temporal patterns in trait richness were consistent and characterized by increased clingers, collector-filterers, strong fliers, and long adult life across all geologic classes Figure 6B, 6E, and 6G; Supplemental 2.13-2.16; 2.21-2.24). Trends in trait density varied with geology, Basaltic and Siliciclastic streams increased in taxa with slow seasonal growth and aerial respiration while Granitic streams increased in taxa characterized by well synchronized emergence and clinging habit (Figure 6C, 6F, and 6H; Supplemental 2.13-2.16; 2.21-2.24)

2.4 Discussion

Our goal was to evaluate changes in macroinvertebrate community structure in headwater streams in response to changes in water chemistry from 1987-2017. Analysis of temporal trends in water suggested modest chemical recovery from acidification with sulfate decreasing 0.25 µeq L^{-1} $year^{-1}$ and pH increasing 0.01 units $year^{-1}$regardless of geologic class. Meanwhile, ANC trends were variable and often insignificant, increasing 0.66 µeq L^{-1} $year^{-1}$ in Basaltic and Granitic and decreasing 0.02 µeq L^{-1} $year^{-1}$ in Siliciclastic streams. Temporal trends in macroinvertebrate communities were similar to those indicative of biological recovery observed in other studies of acidified streams (Hildrew, 2009; Layer et al., 2011, 2011; Murphy et al., 2014; Ormerod & Durance, 2009). All streams increased in density by 11% (17 individuals), taxa richness by 54% (15 unique taxa), and increased in trait richness by 3% (2 trait categories) regardless of geologic class. Our findings are discussed for water chemistry and macroinvertebrate community composition (taxa and traits) as we attempt to build on the current body of knowledge regarding recovery of freshwater systems from human-induced acidification, specifically changes in macroinvertebrate traits that may be indicative functional changes in freshwater systems.

2.4.1 Temporal trends in water chemistry

Decreasing atmospheric sulfur and nitrogen oxide emissions following the implementation of the Clean Air Act Amendments have led to reduced levels of sulfur deposition and an expectation for recovery of acidified surface waters (Webb et al., 2004). As predicted, we observed declines in stream sulfate and increased stream pH over time in SNP. In contrast, we saw often insignificant and variable trends in stream ANC. These changes suggest only modest recovery when compared to chemical recovery from acidification in other regions (Kahl et al., 1992; Webb et al., 2004). For example, from Khal et al (2003) found that annual declines in sulfate were about 10 times greater (2.5 μeq L^{-1} $year^{-1}$) and increases in ANC were about 1.5 times (1 μeq L^{-1} $year^{-1}$) greater from 1990-2000 in the northeastern United States than we observed from 1987-2017 in SNP. Regional differences in chemical are determined by sulfate retention processes in watershed soils (Riscassi et al., 2019). In this study, unglaciated regions within SNP are thicker, more weathered, and consequently more efficient sulfate absorbers than glaciated soils in the northeast United States, thus slowing the rate of recovery (Riscassi et al., 2019). Still, small changes are likely biologically relevant. For example, because acidified watersheds in SNP have a pH as low as 4.93 and ANC as low as 4.88 μeq/L, small changes over time may eventually surpass toxic thresholds, such as a pH of 5.5, potentially resulting in microbial and macroinvertebrate reestablishment (Murphy et al., 2014; Sutcliffe & Hildrew, 1989).

2.4.2 Comparison of water chemistry trends by geology

Acid-base chemistry of stream water in SNP is closely tied to sulfate absorption potential of soil and underlying bedrock geology (Riscassi et al., 2019). Contrary to our prediction, trends in stream sulfate and pH were the same across geologic classes. Equivalent trends in sulfate and pH may be related to trends in ANC, which varied with geology as predicted. Observed subdued and variable patterns of ANC recovery, particularly in comparison with decreasing stream sulfate, is consistent with other assessments in SNP, North America, and the UK (Kahl et al., 1992; Riscassi et al., 2019; Webb et al., 2004). Possible explanations for the small magnitude and variability of ANC compared to sulfate and pH include decreasing trends in base cations, mobilization of dissolved organic carbon, local-scale processes that affect nitrogen (e.g., Gypsy Moth induced forest defoliation), and long-term climate patterns (McHale et al., 2017; Riscassi et al., 2019). Comparing ANC trends among streams with different ranges, particularly low ANC levels, may not inform biological recovery from acidification because of the non-linear relationship between pH and ANC where pH is more sensitive to changes in ANC as it approaches 0 (Riscassi et al., 2019). This relationship may also explain why streams in SNP have equivalent trends for stream pH but not ANC. For example, Riscassi at al. (2019) found that small ANC increases (0.19 µeq L^{-1} $year^{-1}$) in a low ANC stream in SNP (0–50 µeq L^{-1}) and an order of magnitude greater ANC increases (1.75 µeq L^{-1} $year^{-1}$) at a circumneutral stream in SNP (ANC range 300–400 µeq L^{-1}) resulted in similar rates of pH improvement (0.02 pH unit $year^{-1}$). Still, the observed absence of and negative trends in ANC suggest that acidification may still be impacting streams, thus inhibiting biological recovery.

2.4.3 Temporal trends in macroinvertebrate communities

While the trajectory of recovery from acidification is not characterized by a simple reestablishment of acid-sensitive taxa, broader patterns of recovery have been observed in multiple systems (Hildrew, 2009; Layer et al., 2011; Monteith et al., 2005; Murphy et al., 2014; Ormerod & Durance, 2009). As predicted, macroinvertebrate density, taxa richness, and trait richness increased over time, which are consistent with other studies documenting recovery. For example, Monteith et al., (2005) found that streams displaying recovery from 1988-2008 typically had an increase in macroinvertebrate abundances and richness of 1-3 taxa. Additionally, in a recovery assessment of historically acidified streams (1988-2008), Murphy et al., (2014) determined that the stream with the clearest indication of biological recovery had dramatic increases in macroinvertebrate abundance and richness as acid sensitive taxa (e.g., mayflies: *Baetis* and *Electrogena)* returned to the community and acid-tolerant taxa *(e.g.,* stoneflies*: Leuctra* and *Nemoura)* decreased in abundance. Another possible indication of macroinvertebrate recovery in these streams was the declining densities of *Leuctra*, an acid-tolerant stonefly that tends to dominate macroinvertebrate communities in acidified streams. Although these trends were often statistically insignificant and only present in more acidified streams, they could be an early indication of biological recovery (Siliciclastic and Granitic).

Temporal assessments of historically acidified streams are often more variable than space-for-time assessments because of variation in other environmental conditions like precipitation and temperature (Gray et al., 2016). Contrary to our predictions, trends in macroinvertebrate diversity and evenness were often insignificant and inconsistent in directionality. Regardless of statistical significance, Siliciclastic streams trended towards recovery whereas Granitic and Basaltic streams had often trended towards declining evenness and diversity. In comparison, Jastram et al., (2013) found that their assessments of diversity and evenness (Simpson diversity and percent dominant two taxa) showed significant park-wide declines, but only for the 10-year time period (1999-2009), whereas other time periods had no significant trend.

A pH of 5.5 is considered a significant biological threshold where alkalinity falls to zero and inorganic aluminum concentration rises to become toxic to many forms of life (Sutcliffe & Hildrew, 1989). For this reason, we speculate that patterns of diversity and evenness that differ with geologic class may be related to historic levels of acidification. For example, Bowman et al., (2006) found that streams with acidification to pH <5, but not pH 5.5-6.0 resulted in significant changes in stream communities. Within SNP, only Siliciclastic streams were acidified below the pH threshold of 5.0 whereas observed minimum values in Granitic and Basaltic streams did not go below 5.5. Surpassing significant biological thresholds for stream pH may result in a different trajectory of recovery from acidification then less acidified streams even if richness is increasing at a similar rate. Recovering streams often contain a core community of acid-tolerant taxa (e.g., Plecoptera, Chironomidae) that persist or increase in abundance as acidic conditions improve, hindering the recolonization capacity of acid-sensitive taxa (Davy-Bowker et al., 2005; Murphy et al., 2014). As a result, recovering communities are often characterized by stable food webs that may be fundamentally altered by acidification (Gray et al., 2016). So, while abiotic conditions may become favorable for more acid-sensitive taxa to recolonize the community, their return may be sporadic or characterized by low densities as their functional niches are occupied by more acid-sensitive generalists (Ledger & Hildrew, 2005). Such changes in community composition have the potential to decrease measures of diversity and evenness. Alternatively, if acidification was so persistently low that taxa richness and density decreased to a level where functional niches remained open, perhaps values below the biological threshold 5.5, it may be possible for acid-sensitive taxa to recolonize in greater densities because of reduced competition thus increasing diversity or evenness (Szkokan-Emilson et al., 2010).

2.4.4 Comparison of macroinvertebrate community trends by geology

Bedrock geology is a strong determinant of stream acidification and trajectory of recovery because of differences in their ionic strengths, buffering capacity against acidic inputs, and retention of sulfate (Jastram et al., 2013; Webb et al., 2004). Contrary to our prediction, the magnitude of change in total density and taxa and trait richness did not vary with geologic class. Meanwhile, as predicted, changes in macroinvertebrate diversity and evenness differed with geology. These findings are similar to an assessment of 24 sites within SNP by Jastram et al., (2013) that found park-wide trends for total richness did not differ among geologic classes even though other macroinvertebrate metrics were related to geology. Major differences in park-wide and geology-dependent trends in Jastram et al., (2013) appeared to be related to the generality or specificity of the metrics used to draw inferences. For example, park-wide trends were more general (e.g., increased total and EPT richness) or highly specific to acid-sensitive or acid-tolerant taxa (e.g., % Ephemeroptera and % *Leuctra*). Alternatively, metrics that differed by geology tended towards categories that contain taxa with varying acidification tolerances but do not encapsulate the entire community (e.g., percent EPT, percent Trichoptera, stream condition index value, and pollution tolerance value). These differences may suggest that on a broad scale recovery is occurring but the taxa or traits that constitute recovery in a given geologic class differ.

2.4.5 Taxa that change with stream recovery

Acidification inhibits macroinvertebrate ion exchange causing decreased individual fitness and survival (Havas & Rosseland, 1995; Morris et al., 1989; Sharpe et al., 1983). As predicted, sites becoming less acidified over time experienced changes in community composition with increases in richness or density of collector-gathering and scraping Ephemeroptera, net spinning and case-making Trichoptera, and predaceous Plecoptera. These taxonomic patterns are consistent with trends in other studies documenting biologic recovery from acidification (Hildrew, 2009; Monteith et al., 2005; Murphy et al., 2014). In particular, the recolonization of recovering streams by large-bodied predators, such as Plecoptera (Basasltic *Isoperla;* Granitic: *Hansoperla and Malirekus*, Siliciclastic: *Acroneuria),* is a common indicator of biotic recovery (Hildrew, 2009; Layer et al., 2011).

Biotic recovery is often observed to be inconsistent, patchy, or delayed despite widespread improving water quality trends(Murphy et al., 2014; Ormerod & Durance, 2009). Trajectories of biotic recovery are often stream-specific and influenced by abiotic factors such as periodic acidic episodes, underlying geology, and rising organic acids as well as biotic factors like acid-tolerant species that dominate niche space and top-down predation (Gray et al., 2016; Murphy et al., 2014). While taxa richness increased at the same rate across streams in SNP, the genera increasing within each order varied with geology. Differences among genera could be a result of different species pools (regional gamma diversity) or dispersal limitation. Additionally, the progression of recovery from acidification that differs among geologies may influence the genera present. For instance, Basaltic streams already have the taxa that Siliciclastic streams are gaining.

2.4.6 Traits that change with stream recovery

A trait is a physiological, morphological, or ecological attribute of a taxa that reflect adaptations to dominant environmental characteristics thus indicating the mechanisms structuring communities (McGill et al., 2006; Poff et al., 2006; Usseglio-Polatera et al., 2000). As predicted, trait composition changed over time with increasing trait richness (presence/absence of a trait category), increasing response diversity (# taxa within a trait category), and increasing density (# individuals within a trait category). Taxa that became more prevalent through time tended to be large in size, semivoltine, have nonseasonal development, long adult life, and well synchronized emergence. Correspondingly, they also tend to disperse far as strong flyers, drift readily, and resist drying. In addition, there was an increase in the breadth of ecological traits present as the community expanded beyond shredder-dominated plecopteran taxa to include burrowing, sprawling, clinging, collector-filtering, predator, and herbivore macroinvertebrates. We observed increases in phylogenetically labile traits such as high crawl speed, strong swimming ability, depositional, warm water dwelling, aerial respiration, and heavy sclerotization that are potentially more responsive to environmental change (Poff et al., 2006). Changes associated with more labile traits were represented by rare occurrences of low numbers of taxa. For example, within our 30-year dataset, aerial respiration was characterized by 1 taxon occurring in 1 stream on 2 instances (1994 and 2017). Yet, it parsed out as significant trait. We speculate that sensitivity of analyses to outliers may obscure actual temporal patterns related to acidification.

While both taxonomic and trait community composition differed over time, trait-based metrics had less explanatory power, highlighting that acidification can produce large changes in taxonomic but rather weak changes in trait composition (Bonada et al., 2007). For example, we found that taxa-based ordinations explained 65-70% of the variation in community composition where trait-based ordinations explained only 4-6%. Other studies have also found traits to be less variable than taxonomic position, because traits aggregate biological information among different taxa (Bonada et al., 2007; Charvet et al., 2000; Statzner et al., 2004). Alternatively, other studies have found pronounced differences in trait composition (Charvet et al., 2000; Chessman, 2015; DÍAZ et al., 2008; Gutiérrez-Cánovas et al., 2019). We speculate these differences are related to the gradient or stressor being assessed. For example, studies that found relatively stable distributions of traits tended to be across naturally occurring environmental gradients (e.g., geology; climate) where a continuum of conditions allow for replacement of trait groupings by different taxa along the gradient. Alternatively, significant differences were found in studies assessing anthropogenic alterations (e.g., sewage, salinization, drying) vs. reference conditions where binary conditions can select for discrete communities. Perhaps patterns in this study fall somewhere in the middle. While acidification is an anthropogenic stressor that has undoubtedly altered macroinvertebrate communities, all streams in this study have been acidified. Thus, we are not comparing streams to a reference condition. Additionally, recovery from acidification is not characterized by the replacement of acid-tolerant taxa by acid-sensitive taxa. Instead, the core community of acid-tolerant taxa persists as more acid-sensitive taxa slowly recolonize, increasing the breadth of traits present in the community.

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2.6 Tables and figures

Table 1. Thirteen sites from the Shenandoah Watershed Study (SWAS) monitoring stations used to assess spatial and temporal trends of water chemistry in SNP.

Geology	Stream	Site	Latitude	Longitude	Elevation (m)
Basaltic	Madison Run (upper)	MAD2	38.25151	-78.7471	1450
Basaltic	Jeremys Run	VT51	38.71577	-78.38157	950
Basaltic	Rose River	VT66	38.51547	-78.36677	1120
Granitic	North Fork of Dry Run	NFDR	38.63372	-78.35773	1600
Granitic	Staunton River	STAN	38.44461	-78.3708	1010
Granitic	Brokenback Run (upper)	VT58	38.5705	-78.3044	1080
Granitic	North Fork of Thornton River	VT61	38.69297	-78.27406	1150
Granitic	Hazel River	VT62	38.6162	-78.263855	1080
Siliciclastic	Deep Run	DR01	38.27964	-78.76391	1430
Siliciclastic	Paine Run	PAIN	38.1986	-78.7934	1390
Siliciclastic	Meadow Run	VT36	38.1587	-78.8059	1480
Siliciclastic	Twomile Run	VT53	38.33388	-78.67119	1220
Siliciclastic	White Oak Run (lower)	WOR1	38.25083	-78.74853	1480

Table 2. Thirteen sites from the Shenandoah Long-Term Ecological Monitoring (LTEM) Program used to assess spatial and temporal trends of macroinvertebrate communities in SNP.

Geology	Stream	Site	Latitude	Longitude	Area (ha)	Elevation (m)	Aspect (deg)	Slope (deg)
Basaltic	Jeremy's Run	1F118	38.7483578	-78.32445066	564	554	331	14
Basaltic	Piney River	1F308	38.7451702	-78.28169717	72	764	286	6
Basaltic	Hogcamp Branch	2F055	38.5227522	-78.42057407	193	800	67	5
Granitic	Land's Run	1F002	38.8280348	-78.18881118	171	517	259	10
Granitic	Whiteoak Canyon Run	2F009	38.5406878	-78.34966912	1,405	343	185	12
Granitic	Rose River	2F015	38.5154189	-78.3663898	2,368	341	100	2
Granitic	Hazel River	2F090	38.6305652	-78.29403047	275	667	350	3
Granitic	NF Dry Run	2F131	38.6329374	-78.35798738	220	493	331	18
Granitic	Staunton River	2F306	38.4671526	-78.41823183	128	900	77	7
Siliciclastic	Twomile Run	3F103	38.3342201	-78.67156204	561	370	253	3
Siliciclastic	Paine Run	3F123	38.1987237	-78.79337203	1,270	425	302	2
Siliciclastic	Paine Run	3F300	38.2091423	-78.75341012	177	557	144	14
Siliciclastic	Twomile Run	3F302	38.3111852	-78.64962791	143	506	55	10

Table 3. Statistical results comparing water chemistry trend mean (ANOVA) or median (Kruskal-Wallis) values among geologic classes from 1987-2017. Shapiro results at α ≤ 0.05 were considered different. Comparisons were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α = 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. All comparisons had 2 degrees of freedom. Bold p-values were significant at α ≤ 0.05.

		ANOVA/ Kruskal-Wallis		Tukey HSD/ Mann-Whitney Wilcoxon		
Parameter	**Shapiro (p)**	**F / chi-sq**	**p-value**	**B vs. G (p)**	**B vs. S (p)**	**G vs. S (p)**
SO_4^{2-} tau	0.881	1.11	0.366	NA	NA	NA
SO_4^{2-} slope	**0.099**	1.00	0.404	NA	NA	NA
pH tau	0.514	1.33	0.307	NA	NA	NA
pH slope	**<0.001**	0.65	0.721	NA	NA	NA
ANC tau	0.499	8.21	**0.008**	0.334	**0.008**	**0.049**
ANC slope	**0.020**	6.75	**0.034**	0.250	0.110	0.190

Table 4. Statistical results comparing macroinvertebrate trend mean (ANOVA) or median (Kruskal-Wallis) values among geologic classes from 1987-2017. A Shapiro test was used to assess the normality of data. Comparisons were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α = 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. All comparisons had 2 degrees of freedom. Bold p-values were significant at α ≤ 0.05.

		ANOVA/ Kruskal-Wallis		Tukey HSD/ Mann-Whitney Wilcoxon		
Parameter	**Shapiro (p)**	**F / chi-sq**	**p-value**	**B vs. G (p)**	**B vs. S (p)**	**G vs. S (p)**
Density tau	0.436	3.920	**0.055**	NA	NA	NA
Density slope	0.614	0.399	0.681	NA	NA	NA
Taxa richness tau	0.251	1.086	0.374	NA	NA	NA
Taxa richness slope	0.251	0.691	0.523	NA	NA	NA
Taxa Shannon tau	**0.029**	0.860	0.651	NA	NA	NA
Taxa Shannon slope	0.187	2.404	0.140	NA	NA	NA
Taxa evenness tau	0.714	2.943	0.099	NA	NA	NA
Taxa evenness slope	0.756	1.83	0.210	NA	NA	NA
Trait richness tau	0.223	1.255	0.326	NA	NA	NA
Trait richness slope	0.617	3.415	0.074	NA	NA	NA
Trait Shannon tau	0.224	4.045	**0.052**	0.636	0.314	**0.042**
Trait Shannon slope	0.199	4.932	**0.032**	0.979	0.095	**0.033**
Trait evenness tau	0.714	3.329	0.078	NA	NA	NA
Trait evenness slope	0.756	1.857	0.206	NA	NA	NA

Table 5. Permutational multivariate analysis of variance (PERMANOVA) results testing for differences among the centroids of year groupings (e.g., 1-6) for all streams and streams by geologic classes. If PERMANOVA p-values were α ≤ 0.05, pairwise comparisons were run to determine differences among year groupings. **Year groupings:** 1 = 1987-1991; 2 = 1992-1996; 3 = 1997-2001; 4 = 2002-2008; 5 = 2009-2013; 6 = 2014-2017. Bold p-values were significant at α ≤ 0.05.

	PERMANOVA		Pairwise (Adj. p-values)														
Parameter	F	p-value	1 vs 2	1 vs 3	1 vs 4	1 vs 5	1 vs 6	2 vs 3	2 vs 4	2 vs 5	2 vs 6	3 vs 4	3 vs 5	3 vs 6	4 vs 5	4 vs 6	5 vs 6
All taxa density	18.32	**0.001**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	0.495	**0.015**	0.06	**0.015**	0.285	**0.015**
All trait density	38.79	**0.001**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	0.33	**0.015**	0.33	**0.015**	0.465	**0.015**	1	**0.015**	1	**0.015**
All trait richness	66.66	0.001	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	1	**0.015**	1	**0.015**	0.375	0.51	**0.03**	**0.015**	1	**0.015**
Basaltic taxa density	4.93	0.002	1	1	0.3	0.42	0.255	1	1	1	0.42	1	1	0.915	1	1	0.105
Basaltic trait density	8.65	0.004	1	1	0.27	0.885	0.39	1	1	1	0.975	1	1	0.975	1	1	0.57
Basaltic trait richness	14.36	0.002	**1**	1	0.09	0.195	0.09	1	1	1	0.54	1	1	1	1	1	0.48
Granitic taxa density	14.59	**0.001**	**0.045**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.03**	**0.015**	**0.015**	0.48	**0.03**	0.09	**0.015**	1	**0.015**
Granitic trait density	34.40	**0.001**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.765**	**0.045**	**0.465**	**0.03**	1	**0.045**	0.915	**0.015**	1	**0.015**
Granitic trait richness	37.54	**0.001**	0.135	**0.015**	**0.015**	**0.015**	**0.015**	1	0.12	1	**0.015**	1	1	**0.015**	**0.015**	1	**0.015**
Siliciclastic taxa density	7.44	**0.001**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	**0.135**	**0.015**	**0.015**	**0.015**	1	**0.015**	0.39	**0.015**	0.135	**0.015**
Siliciclastic trait density	7.10	**0.002**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	1	**0.045**	0.6	1	1	**0.03**	1	**0.015**	1	0.06
Siliciclastic trait richness	28.57	**0.001**	**0.015**	**0.015**	**0.015**	**0.015**	**0.015**	1	0.18	1	0.405	1	0.57	1	**0.015**	1	**0.09**

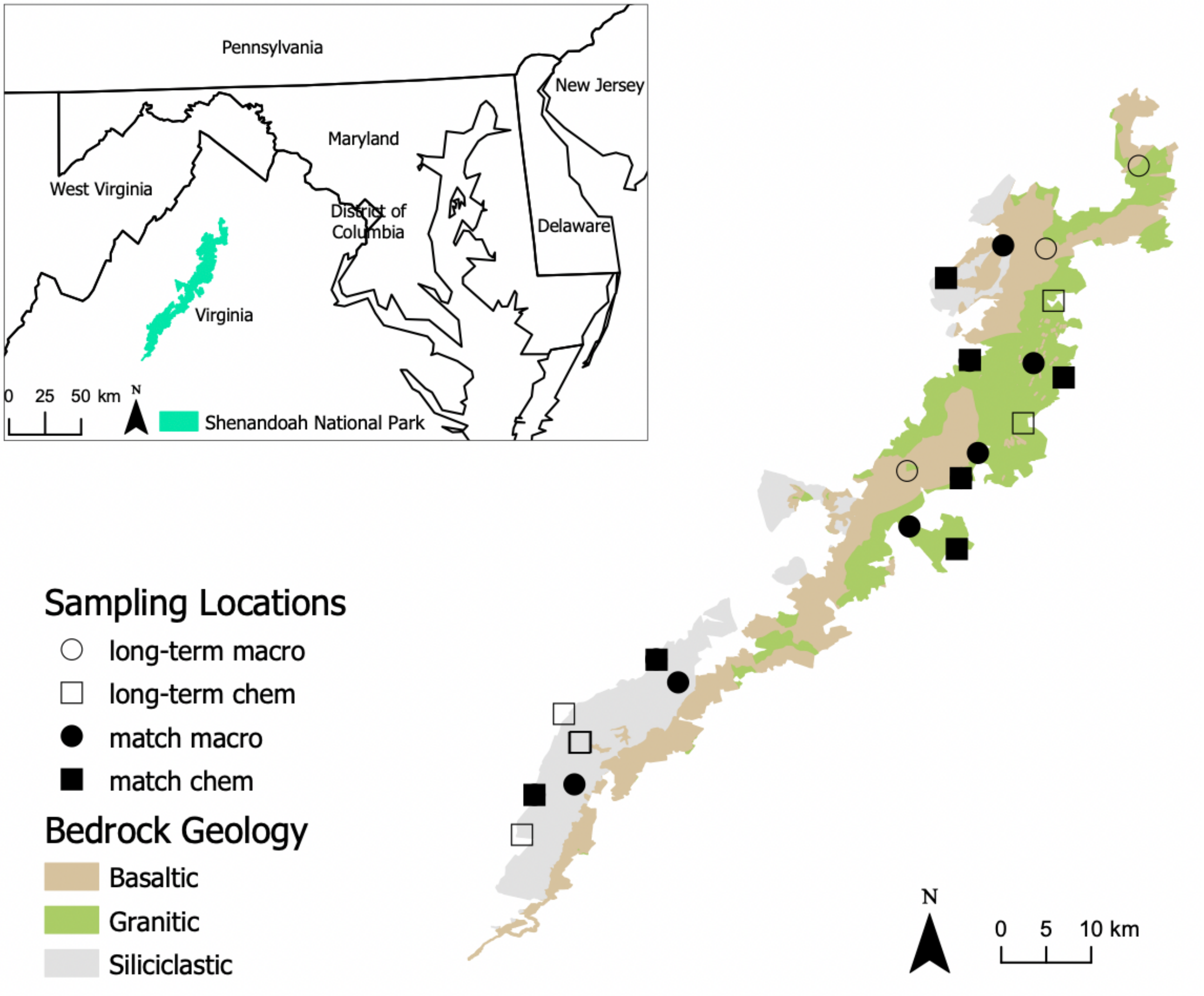


Figure 1. Water chemistry and macroinvertebrate sampling locations in Shenandoah National Park. Colors represent the three geologic classes within the park characterized by their differing ability to neutralize acidic inputs. Sites represented by filled shapes indicate water chemistry and macroinvertebrate sites used only for trend analyses. Sites represented by open shapes indicate water chemistry and macroinvertebrate paired with one another for multivariate analyses. A total of 13 water chemistry (3 Basaltic, 5 Granitic, 5 Siliciclastic) and 13 macroinvertebrate (3 Basaltic, 6 Granitic, and 4 Siliciclastic) sites were used for spatial and temporal analyses. Seven water chemistry and 10 macroinvertebrate sites were paired for a total of 10 sites (1 Basaltic, 5 Granitic, 4 Siliciclastic) used for multivariate analyses.

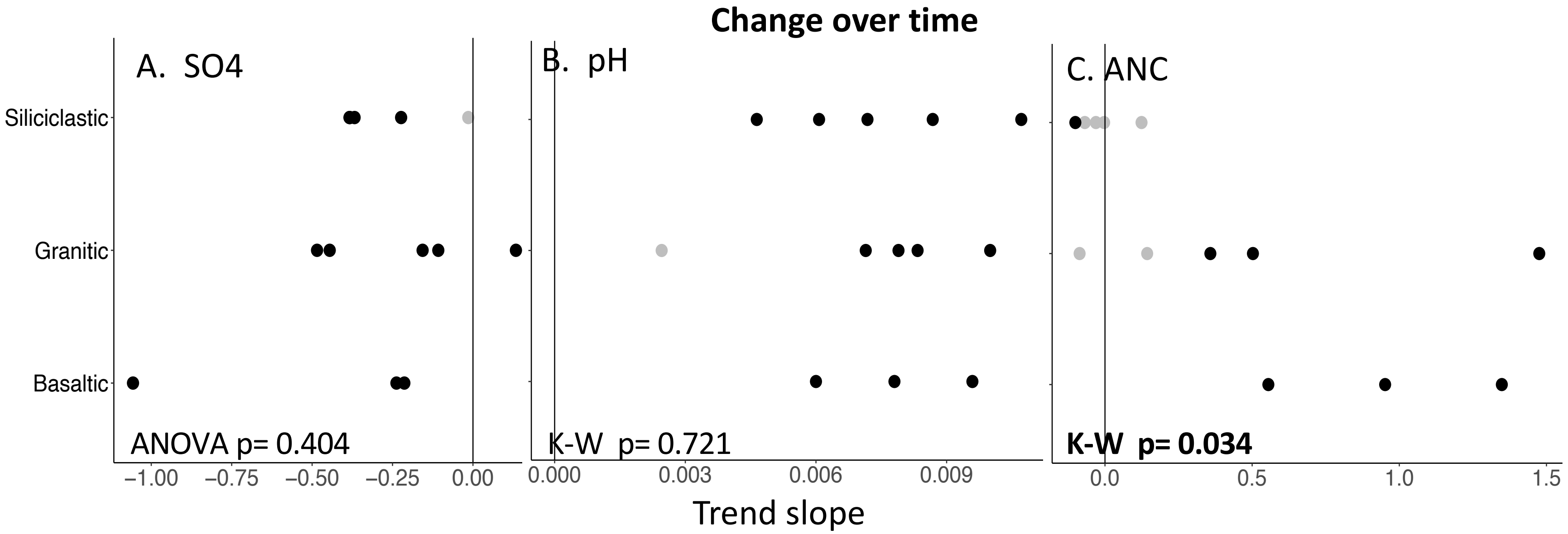


Figure 2. Water chemistry (sulfate, pH, and acid neutralizing capacity) temporal trends (1987-2017) across all sites by geologic class. Temporal trends were assessed with a Seasonal-Kendall Trend Test and Sen slope estimates. Sen slope values are displayed in strip charts and colored by Tau significance. Sites with slope p ≤ 0.05 are designated with by black points while sites with p > 0.05 are designated by gray points. A positive slope corresponds with increasing values over time and a negative slope corresponds to decreasing values over time. Comparisons of absolute values and slope trends (recovery) among geologic classes were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α ≤ 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. Differences in absolute or trend values among geologic classes are denoted by differing letters.

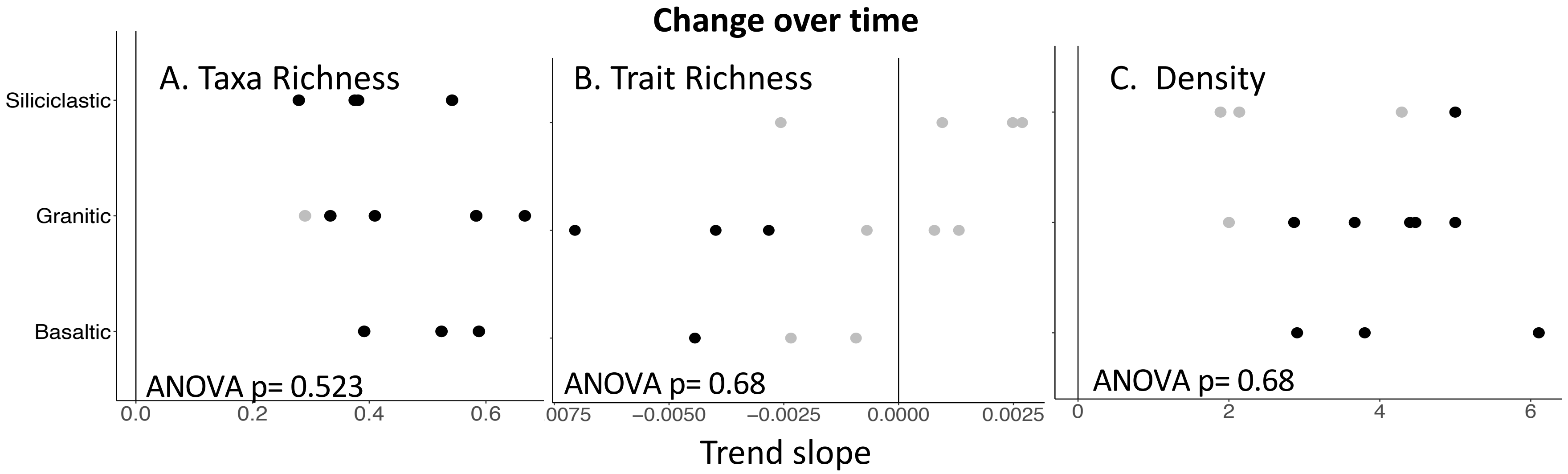


Figure 3. Macroinvertebrate community metric (taxa richness, trait richness, and density) temporal trends (1987-2017) by geologic class. Trends were assessed with a Mann-Kendall Trend Test and Sen slope estimates. Sen slope values are displayed in strip charts and colored by Tau significance. Sites with Tau ≤ 0.05 are designated with by black points while sites with Tau > 0.05 are designated by gray points. A positive slope corresponds with increasing values over time and a negative slope corresponds to decreasing values over time. Comparisons of absolute values and slope trends (recovery) among geologic classes were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α ≤ 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. Differences in absolute or trend values among geologic classes are denoted by differing letters.

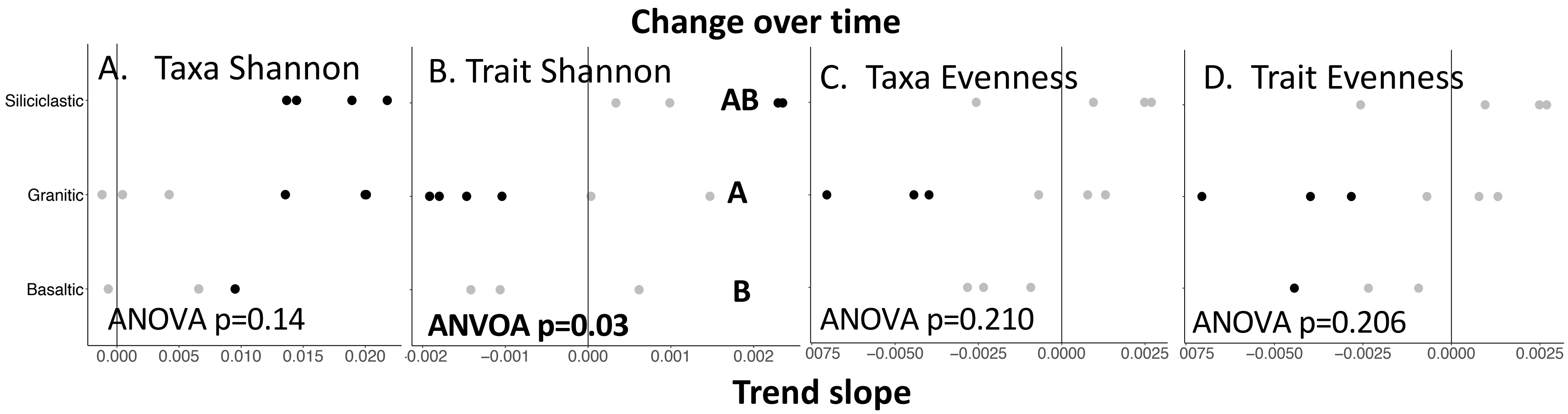


Figure 4. Macroinvertebrate community diversity metric (taxa Shannon, trait Shannon, taxa evenness, trait evenness) temporal trends (1987-2017) by geologic class. Temporal trends were assessed with a Mann-Kendall Trend Test and Sen slope estimates. Sen slope values are displayed in strip charts and colored by Tau significance. Sites with Tau ≤ 0.05 are designated with by black points while sites with Tau > 0.05 are designated by gray points. A positive slope corresponds with increasing values over time and a negative slope corresponds to decreasing values over time. Comparisons of absolute values and slope trends (recovery) among geologic classes were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α ≤ 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. Differences in absolute or trend values among geologic classes are denoted by differing letters.

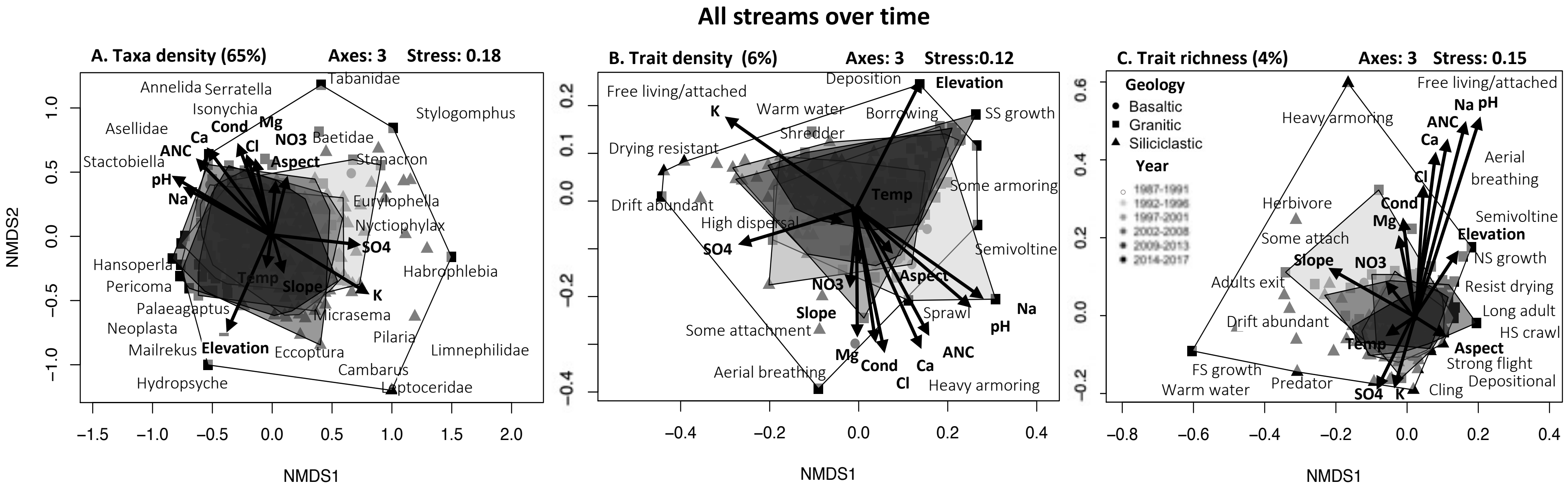
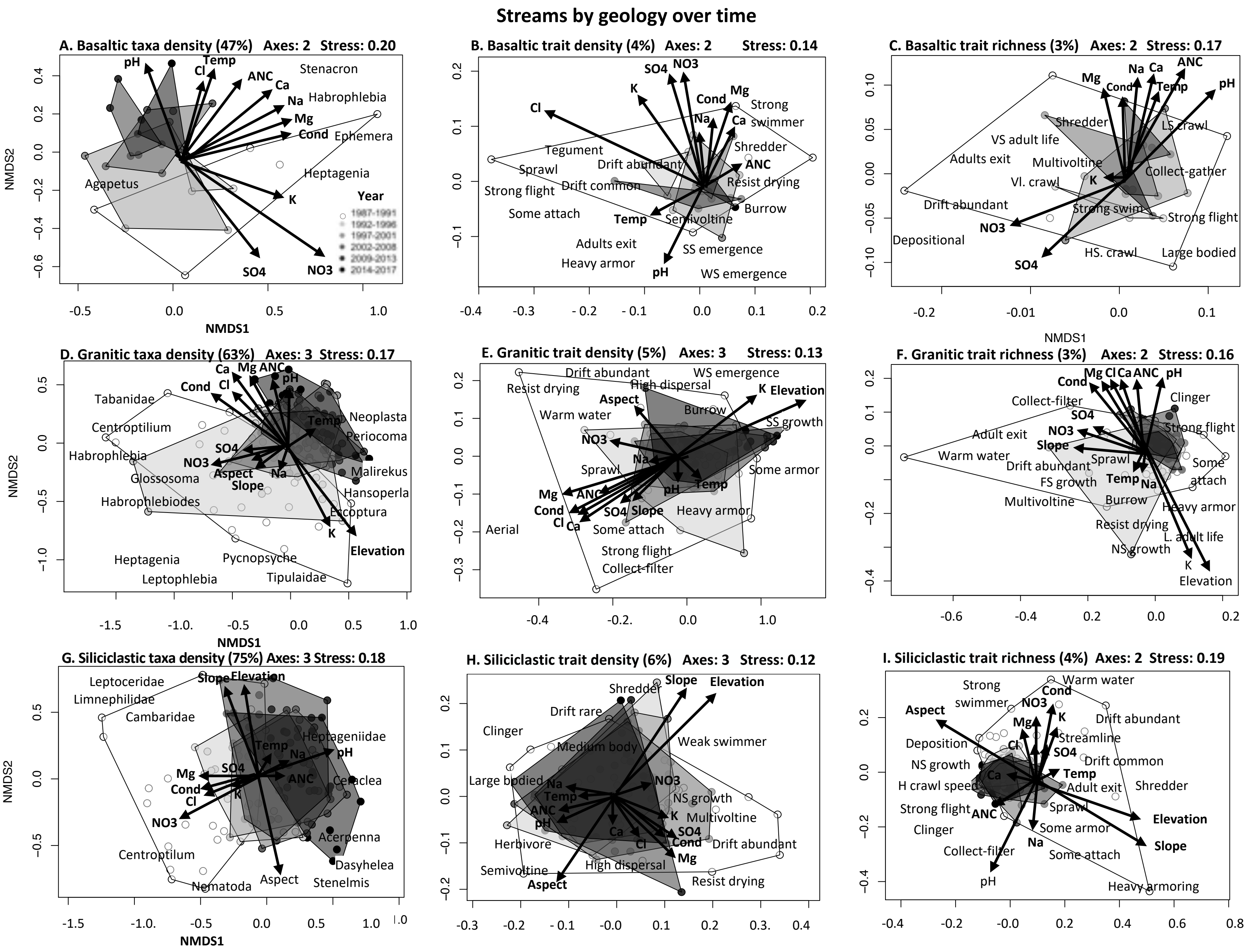


Figure 5. Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate **(A.)** taxonomic density, **(B.)** trait density, and **(C.)** trait richness for all streams, all years. Symbols differentiate sites by geologic class (Basaltic = circle; Granitic = square; Siliciclastic = triangle). Colors of points and hulls around groups of points differentiate groupings of years. Darkening of color corresponds to temporal progression. Arrows correspond to correlations of secondary matrix of environmental variables. Insect names listed are the 10% most positively and 10% most negatively correlated taxa or traits with each NMDS axis. Total variation explained by all axes of NMDS analyses displayed in parentheses next to figure title.



Streams by geology over time

A. Basaltic taxa density (47%) Axes: 2 Stress: 0.20

B. Basaltic trait density (4%) Axes: 2 Stress: 0.14

C. Basaltic trait richness (3%) Axes: 2 Stress: 0.17

D. Granitic taxa density (63%) Axes: 3 Stress: 0.17

E. Granitic trait density (5%) Axes: 3 Stress: 0.13

F. Granitic trait richness (3%) Axes: 2 Stress: 0.16

G. Siliciclastic taxa density (75%) Axes: 3 Stress: 0.18

H. Siliciclastic trait density (6%) Axes: 3 Stress: 0.12

I. Siliciclastic trait richness (4%) Axes: 2 Stress: 0.19

Figure 6. Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate taxonomic density, trait density, and trait richness for streams in Basaltic **(A-C.)**, Granitic **(D-F.)**, and Siliciclastic **(G-I.)** geology over time. Symbols differentiate sites by geologic class (Basaltic = circle; Granitic = square; Siliciclastic = triangle). Colors of points and hulls around groups of points differentiate groupings of years. Darkening of color corresponds to temporal progression. Arrows correspond to correlations of secondary matrix of environmental variables. Insect names listed are the 10% most positively and 10% most negatively correlated taxa or traits with each NMDS axis. Total variation explained by all axes of NMDS analyses displayed in parentheses next to figure title.

Chapter 3: Stream acidification reduces macroinvertebrate functional diversity and secondary production in Shenandoah National Park

Abstract

Human-driven elimination of taxa has motivated research to understand the role biodiversity plays in ecosystem function. In aquatic systems, biomonitoring has emerged as a frequent and effective means of detecting anthropogenic stressors through observed changes in macroinvertebrate taxonomic and trait structure. However, these structural analyses are rarely paired with corresponding assessments of ecological functions. As a result, we lack a mechanistic link between macroinvertebrate community structure and functions. We estimated macroinvertebrate secondary production in two streams (1 acidified; 1 circumneutral) in Shenandoah National Park. Secondary production was calculated for all taxa and categorized by functional groups to determine traits sensitive to acidification and compared with traits identified as acid-sensitive through changes in community structure. We predicted that (1) macroinvertebrate density and taxa richness would be greater in the circumneutral stream because of lower osmoregulatory stress. (2a) Trait richness would not differ between streams because they aggregate biological information among different taxa. Alternatively, (2b) functional diversity (# taxa within a trait), trait density, and trait biomass will generally be greater in the circumneutral stream in correspondence with increased taxonomic richness. We also predicted functional differences between streams represented by (3) greater macroinvertebrate production in circumneutral streams because of reduced osmoregulatory stress that allows more taxa to persist and allocate energy towards growth. Differences among the dominant traits in each stream will be functionally evident as (4) relative contribution of each trait state (e.g., multivoltine, univoltine) to total production within a trait category (e.g., voltinism) will differ between streams due to higher proportions of acid-tolerant traits in the acidified stream. Additionally, (5) production to biomass ratios (P/B) will be greater in low pH streams because the community is dominated by small-bodied, fast developing, multivoltine taxa. Lastly, we predicted that (6) the circumneutral stream will show greater functional redundancy than the acidified stream with a greater number of taxa significantly contributing to production within a trait state. We found that density did not differ between the two streams but taxa richness, trait richness and functional diversity were greater the circumneutral stream. Additionally, density and biomass within each trait were often greater in the circumneutral stream. Secondary production was also greater in the circumneutral stream. Traits characteristic of the circumneutral stream were semivoltine, slow seasonal development, long adult life span, large body size, and gill respiration. Traits characteristic of the acidified stream were univoltine, fast seasonal development, short adult life span, medium body size, and tegument respiration. Average P/B ratios did not differ between streams though trait analysis suggested differences in dominant development and body size traits. Lastly, greater functional diversity did not always correspond to greater functional redundancy. We concluded that a more acidified stream in SNP has different macroinvertebrate trait composition as well as ecosystem function (secondary production). Additionally, this study indicates that richness-function relationships may not align with patterns of density and evenness of macroinvertebrate communities. Therefore, changes in macroinvertebrate community structure do not necessarily correspond with changes in macroinvertebrate function.

Keywords: macroinvertebrate, acid deposition, secondary production, biomass, traits, function

3.1 Introduction

Biodiversity is the quantification of variation of life forms in a given ecosystem, accounting not only the number of taxa present but also relative abundance among species (Hillebrand et al., 2008; Lecerf & Richardson, 2010). Human-driven elimination of taxa has motivated research to understand the role biodiversity plays in ecosystem function (Gagic et al., 2015; Gray et al., 2014; Lecerf & Richardson, 2010). Greater biodiversity can enhance ecosystem processes (e.g., production, decomposition) and ecosystem stability and resilience to disturbance but not always (McKie & Malmqvist, 2009). This relationship between biodiversity and functioning (BEF) is of particular concern for ecosystems either supporting high levels of biodiversity or vulnerable to species loss. Freshwater systems host tremendous biodiversity, containing almost 9.5% of Earth's animal species while comprising only 2.3% of the Earth's surface (Reid et al., 2019). These systems are also disproportionately and directly threatened by anthropogenic activity (Outhwaite et al., 2020; Reid et al., 2019). As a result, freshwater systems have experienced a greater degree of species loss population declines than marine and terrestrial systems (Reid et al., 2019; Strayer & Dudgeon, 2010).

As human-induced stress to aquatic systems increases, biomonitoring has emerged as a frequent and effective means of assessing resultant changes in water quality (Bonada et al., 2007; Huryn et al., 2002; Wallace et al., 1996) The primary task in biomonitoring is to look for "indicators" whose presence, abundance, and/or behavior reflects the impact of an anthropogenic stressor (Bonada et al., 2007). Most methods use changes in community structure of aquatic invertebrates to assess water quality because of their ubiquitous occurrence, high species richness, sedentary nature, well described taxonomy, and affordability (Bonada et al., 2007; Wallace et al., 1996). While assessing changes community structure has proven successful in determining changes in water quality, less is known on how these changes relate to ecosystem function (Huryn et al., 2002).

Trait-based metrics are useful in bridging structural and functional characteristics by serving as a proxy for how ecosystem function is changing without directly measuring function. In fact, functional feeding groups are a common trait used in bioassessment metrics. Traits have been shown to have greater explanatory power and better correlation to ecosystem function than taxonomically-based metrics because of shared environmental selective pressures (Craig et al., 2017; Fritschie & Olden, 2018; Gagic et al., 2015; Lecerf & Richardson, 2010; McGill et al., 2006). However, these structural analyses are rarely paired with corresponding assessments of ecological functions (but see Huryn et al., 2002; Wallace et al., 1996). As a result, correlations between macroinvertebrate community structure and functions are inferred rather than demonstrated (Huryn et al., 2002). If trait diversity measures are going to be used to assess ecosystem function their predictive ability needs to be tested as the two may not necessarily be linked due to ecological redundancy among species (Gagic et al., 2015; Lecerf & Richardson, 2010). Measuring functional response will expand the capacity of trait-based metrics to link changes in macroinvertebrate structure and function. Structure-function relationships will support the use of traits as indicators of functional responses to stressor (Craig et al., 2017).

A drastic shift in abiotic conditions results in a change in the biotic composition of stream ecosystems (Hildrew et al., 1984). Macroinvertebrate communities are directly impacted by acidification because increases in H^+ and Al^{3+} ions and reductions in Ca^{2+} ions result in biochemical and physiological failures (Havas & Rosseland, 1995). Additionally, macroinvertebrate communities can be indirectly impacted by acidification due to changes in the quantity and quality of microbial resources that mediate leaf litter quality (Baudoin et al., 2008; Clivot et al., 2013; Ledger & Hildrew, 2005). As a result, macroinvertebrate communities in acidified streams are characterized by a simplified community comprising taxa able to cope with acidic conditions (Allard & Moreau, 1987). Taxa richness consistently declines while abundances may or may not decrease with increasing acidification (Dangles & Guérold, 1999; Elwood & Mulholland, 1989; Mulholland et al., 1992).

While it is well established that macroinvertebrate diversity declines with freshwater acidification, less is known about how stream functions change (e.g., macroinvertebrate secondary production). A limited number of studies have demonstrated a reduction in macroinvertebrate biomass and secondary production in acidic environments though tolerant taxa may experience relative increases in abundance, biomass, and production (France, 1996; Griffith et al., 1994; Krueger & Waters, 1983; Pretty et al., 2005; Stead et al., 2005; Uutala, 1981) (Table 1). For example, Griffith and Perry (1994) found that shredder (detrital consumers) secondary production was three times lower in an acidified stream than a circumneutral streams (pH 4.3 vs. 7.5). While overall production was lower, they found that production of an acid-tolerant stonefly (*Paracapnia angulata*) was three times greater in the acidic stream because of its greater abundance and biomass. Disparate effects of acidification on secondary production among taxa suggest a more complex relationship between biodiversity and ecosystem function that has yet to be understood.

The goal of this study is to understand how atmospheric acid deposition impacts macroinvertebrate community composition and function. While abundance-based measures of macroinvertebrate communities provide some context for change, they do not support a direct assessment of functional changes. As a result, we may lack a comprehensive understanding of how acid deposition affects aquatic ecosystems. First, we measured secondary production for all macroinvertebrate taxa. Then, secondary production for functional groups were summed across taxa to identify traits sensitive to acidification. We then examined the relationship between community structure (e.g, trait richness) and secondary production. To do this, we compared traits identified as acid-sensitive through changes in community structure with traits deemed sensitive through secondary production. We predicted that (1) macroinvertebrate density and taxa richness would be greater in the circumneutral stream because of reduced osmoregulatory stress (Havas & Rosseland, 1995). (2a) Trait richness (presence or absence of each of the 59 trait states) would not differ between streams because trait categories are represented by multiple taxa and thus vary less than taxonomic composition (Bonada et al., 2007). Alternatively, (2b) functional diversity (# taxa within a trait state), trait density (# of individuals within a trait state), and trait biomass (biomass within a trait state) will typically be greater in the circumneutral stream in correspondence with increased taxonomic richness. We also predicted functional differences between streams represented by (3) greater macroinvertebrate production in circumneutral streams because of reduced osmoregulatory stress that allows more taxa to persist and allocate energy towards growth (Griffith et al., 1994). Differences among the dominant traits in each stream will be functionally evident as (4) relative contribution of each trait state (e.g., multivoltine, univoltine) to total production within a trait category (e.g., voltinism) will differ between streams due to higher proportions of acid-tolerant traits in the acidified stream (see Table 2; (Dangles & Guérold, 1999; Elmqvist et al., 2003; Jastram et al., 2013). Additionally, (5) production to biomass ratios (P/B) will be greater in low pH streams because the community is dominated by small-bodied, fast developing, multivoltine taxa (Kolss et al., 2009; Lepori et al., 2003). Lastly, we predicted that (6) the circumneutral stream will show greater functional redundancy than the acidified stream with a greater number of taxa significantly contributing to production within a trait state (Huryn et al., 2002).

3.2 Materials and methods

3.2.1 Study area

Shenandoah National Park (SNP) is an approximately 800 km^2 strip of National Park Service land located in the Blue Ridge Mountains of western Virginia (National Park Service, 2017a). Climate is humid temperate with annual air temperatures ranging from -23°C to 37°C (mean 9°C -12 °C; (National Park Service, 2017b). Mean annual rainfall ranges from 100 to 150cm with storms creating high runoff conditions occurring year-round (National Park Service, 2017b). Landcover is predominately deciduous and coniferous forest (95%) with elevations ranging from 170m in the northwest to 1230m in the central part of the park (National Park Service, 2017a). Three major geologies underlie the park, each differing in their ability to neutralize acidic inputs (Figure 1; Riscassi et al., 2019; Riscassi et al., 2020). Siliciclastic watersheds are in the most western regions of the park and are characterized by very low acid-neutralizing capacity (ANC) waters, Granitic watersheds are in the eastern most areas of the park and have intermediate ANC values, and Basaltic watersheds are located in the central region of the park and are characterized by the highest ANC capacities. For this study, we measured secondary production in one Siliciclastic and one Basaltic stream (Figure 1). Watershed area, nutrients, elevation, substrate, riparian cover, and aspect were as similar as possible between sites to reduce other factors known to influence macroinvertebrate community structure (Table 3-4).

3.2.2 Habitat variables

Water temperature (℃) was collected on an hourly basis using a HOBO® pendant logger. Each logger was sheltered in an airtight container constructed out of Polyvinyl chloride (PVC) and a clear solvent cement. The logger was installed at the onset of sampling in July 2019 by hammering rebar into the streambed and attaching the logger to rebar with zip ties. Data was offloaded from the logger using a reader on an approximately monthly basis. HOBO® data were exported from the reader into HOBOware and saved as a.csv file. Times were adjusted as necessary to account for HOBO® readings consistently at standard time. Missing values (e.g., logger lost and had to be replaced) were estimated by adding or subtracting the annual average temperature difference between two streams and adding it the respective corresponding stream. Temperature data was used for calculations of production using the instantaneous growth rate method.

3.2.3 Macroinvertebrates

Quantitative benthic macroinvertebrate samples were collected approximately monthly from July 2019 – June 2020. Five quantitative samples were taken in shallow, wadable riffle habitat of a 100-meter reach of stream at each site using a 20.3-centimeter diameter Hess sampler outfitted with a 250-µm net driven approximately 10cm into the streambed as possible. Samples were stored in 95% ethanol (EtOH) and brought back to the lab for processing. In the laboratory, samples were decanted from EtOH and sieved at the 1000-micrometer and 250-micrometer level. All items in the 1000-micrometer sieve were hand sorted and 250-micrometer level sample were sorted under a dissecting microscope (~ 15x magnification). Macroinvertebrates were identified to genus or lowest practical taxonomic level with the exception of Chironomidae, which were identified as either Tanypododinae or non-Tanypodinae Merritt et al., 2008). Twenty traits were assigned to each taxa from 59 potential trait states for trait-based analyses (e.g., the trait size at maturity contains three potential states: small, medium and large; Poff et al., 2006). Genera not included in this database were assigned traits associated with their next lowest taxonomic designation (i.e., a new genus not represented in database was assigned its respective family level traits (Merritt et al., 2008).

Secondary production was computed for taxa representing 90% of the total biomass using the size-frequency method with a correction for cohort production interval (CPI) calculated from size-frequency histogram (Benke et al., 1984; Benke & Huryn, 2017). Production for rare taxa was calculated from published production to biomass (P/B) ratios or estimated from size-frequency data from this study (P/B; Krueger & Waters, 1983). Chironomidae production was quantified using temperature and size-specific growth rates (Walther et al., 2006).

3.2.4 Data Analysis

Organic material and macroinvertebrate richness, density, and biomass:

We evaluated mean annual differences in macroinvertebrate richness, density, and biomass between streams using Welch's t-tests (stats package in R). Data were log transformed (ln [1+x]), if necessary, to meet assumptions of normality. If data did not meet assumptions post transformation, a Kruskal-Wallis test was used to determine differences. All data analysis was visualized using the ggplot2 package in R.

Secondary Production:

Secondary production was calculated for each taxa for one year to ensure that at least one generation could be followed. Because secondary production integrates growth over a single generation and most taxa are univoltine, only a single production estimate for each taxa can be generated per sampling location. Therefore, comparisons of production between Meadow and Piney were made primarily with visual comparisons and relied on biomass data to inform the potential variance that may lie within production estimates. Data were visually represented using the ggplot2 package in R.

3.3 Results

3.3.1 Macroinvertebrate density, richness, and biomass

Contrary to our first prediction, macroinvertebrate density differed between streams (Table 5 and 7; Figure 2A). Piney had a mean density of 327 individuals per sample while Meadow had an average of 264. As predicted with differences in water quality, macroinvertebrate taxa richness was greater in the circumneutral stream. Piney had an average of 56 taxa while Meadow had 41 (Table 5 and 7; Figure 2B). Trait-based measures of richness also differed between streams. Contrary to prediction 2a, trait richness was greater in the circumneutral stream (Table 5 and 7; Figure 2C). Piney had an average of 56 traits present while Meadow had 55. Neither stream had the trait categories of free-ranging and sessile attachment and the skate habit. Additionally, plastron respiration had limited representation in both streams.

Increased functional diversity (# of taxa within a trait state), trait density, and trait biomass in the circumneutral stream supported our second prediction (2b). Functional diversity was greater in the circumneutral stream for 50 of 57 trait states present in both streams (Table 8; Figures 5 and 6; Supplemental 3.2-3.3). The remaining 7 traits (exit ability present, desiccation resistance, plastron respiration, sprawlers, clingers, swimmers, and shredders) were the same between streams and were characterized by low richness (1-9 taxa) in relation to other trait categories. Density within trait categories was greater at Piney for about half (26 of 57) of the traits present (Table 9; Figures 5 and 6; Supplemental 3.2-3.3). Additionally, density within trait categories was greater at Meadow for 5 of the traits present. The remaining 26 traits did not differ between streams. Also as predicted, biomass was 3 times greater in the circumneutral compared to the acidified stream (Table 7 and 10; Figure 3A). Piney had and average biomass of 6292 mg AFDM m^{-2} while Meadow had an annual average of 1849 mg AFDM m^{-2}. Biomass within trait categories was also greater in Piney than meadow for 47 of 57 trait states present in both streams (Table 10; Figures 5 and 6; Supplemental 3.2-3.3). Biomass of the remaining 10 traits (multivoltine, fast seasonal development, exit ability present, desiccation resistant, weak swimming ability, strong swimming ability, poor armoring, plastron respiration, sprawlers, and swimmers) did not differ between streams.

3.3.2 Macroinvertebrate secondary production

Total annual macroinvertebrate secondary production was greater in the circumneutral stream supporting our third prediction (Figure 3B). Piney had a total production of 112,242 mg AFDM m^{-2} $year^{-1}$ and Meadow had a total annual production of 32, 947 mg AFDM m^{-2} $year^{-1}$. The percentage of secondary production contributed by each trait (e.g., multivoltine, semivoltine, univoltine) to each trait category (e.g., voltinism) differed between streams for several trait categories with our fourth prediction (Figure 4; Supplemental 3.1). Trait categories that differed in their relative contributions of each trait state to production were voltinism, development, adult life span, body size, respiration, desiccation resistance, armoring, and functional feeding group. Trait categories with a priori predictions that did not differ in relative contributions of trait states were drift, emergence, female dispersal, and flight strength.

In alignment with our fourth prediction, traits that had greater relative contributions to total trait category production in Piney, suggesting sensitivity to acidification, were semivoltine, slow seasonal development, long adult life span, large body size, and gill respiration (Figure 4; Supplemental 3.1). Traits that had greater relative contributions to total trait category production in Meadow, suggesting tolerance to acidification, were univoltine, fast seasonal development, short adult life span, medium body size, and tegument respiration (Figure 4; Supplemental 3.1). In contrast to our fourth prediction, not desiccation resistant, good armored, and shredder taxa were also dominant at Piney while desiccation resistant, no armored, and collector-filterer taxa were dominant at Meadow. Interestingly, in contrast with our fifth prediction and what would be assumed from differences in dominant traits between streams (e.g., development, body size), P/B ratios did not differ between streams (Table 7; Figure 3).

Contrary to our sixth prediction, the relationship between functional diversity, functional redundancy, and function (e.g., secondary production) remains unclear. Patterns were particularly difficult to elucidate because there were almost no trait categories where Meadow had greater functional diversity, density, biomass, or production. Trait states that were dominant in Piney tended to have greater functional diversity, density, and biomass than Meadow (Figures 5 and 6; Supplemental 3.2-3.3). Alternatively, traits dominant in Meadow tended to have lower functional diversity but displayed variable differences in density (Figures 5; Supplemental 3.2). Often, there were dominant taxa that significantly contributed to production within a trait. Thus, there did not appear to be an observable pattern between a trait dominating the relative contribution within a trait category and a more even distribution of production among taxa within a trait (i.e., increased functional redundancy). For example, traits that were dominant at Piney displayed functional redundancy for semivoltine, slow seasonal development, gill respiration and non-desiccation resistant taxa (Figures 6; Supplemental 3.3). Alternatively, production was dominated by 1 or 2 taxa for long-living, large-bodied, and well-armored taxa. This distinction was less prevalent for traits dominant at Meadow (Figures 5; Supplemental 3.2).

3.4 Discussion

3.4.1 Macroinvertebrate structural metrics

Similarities in density of macroinvertebrates between Piney and Meadow was in opposition to our first hypothesis. While we anticipated greater densities of macroinvertebrates at Piney, equivocal responses of macroinvertebrate density have been observed in other studies (Feldman & Connor, 1992; Mulholland et al., 1992; Rosemond et al., 1992). For example, Mulholland et al (1992) found total macroinvertebrate density to be weakly or not correlated with stream pH across acidified streams in the eastern United States (e.g., Adirondack and Blue Ridge mountains). Instead, they found a strong positive relationship between pH and the density of acid-sensitive taxa like Ephemeroptera. In SNP, Feldman and Connor (1992) found reduced overall abundance at pH 5.7 vs 7.1. Declines were most significant for 18 of 47 taxa (30%) in the study while another 10 of 47 (20%) were completely absent from the acidic streams but did not exhibit statistically significant lower abundances. This pattern holds true for many studies as macroinvertebrates may decrease or remain the same with acidification because tolerant taxa may experience reduced competition for resources or predation and compensate for losses of sensitive taxa (Benke et al., 1984; Benke & Huryn, 2017). For example, Jastram et al., (2013) found acidified streams in SNP to be dominated by the acid-tolerant stonefly, *Leuctra* sp., while it was present in much lower densities in circumneutral streams

Greater taxonomic richness at Piney confirmed our second hypothesis. These findings are in agreement with extensive research demonstrating decline in taxa richness by as much as 30-70% with freshwater acidification (Feldman & Connor, 1992; Rosemond et al., 1992; Simpson et al., 1985). These differences may be explained by differences in watershed geology. The bedrock control on stream acid neutralizing capacity and subsequent acidification has been long recognized in SNP (Jastram et al., 2013). Therefore, lower taxonomic richness in Meadow may be related to a mix of direct (e.g., osmoregulatory stress) and indirect (e.g., altered resource quality and quantity) effects of acidification. Additionally, regardless of acidification, Meadow is expected to have lower production because of lower baseline watershed alkalinity which has been shown to influence macroinvertebrate secondary production (Kreuger and Waters 1983). While Meadow did have lower species richness than Piney, differences in taxonomic richness were not limited to a simple elimination of taxa from Meadow. Instead, Meadow had species that Piney did not and vice versa. Most taxa unique to Meadow were acid-tolerant Ephemeroptera (*Ephemera*), Plecoptera (*Allocapnia*), and Trichoptera (*Cyrnellus* and *Nyctiophylax*) (Davy-Bowker et al., 2005; Kobuszewski & Perry, 1994; Lonergan & Rasmussen, 1996). Interestingly, acid-sensitive Amphipods were also present at Meadow. Taxa unique to Piney were often moderately acid-sensitive Ephemeroptera (*Heptagenia, Drunella, Leucrocuta,* and *Cinygmula*), Plecoptera (*Isogenoides, Pteronarcys,* and *Taeniopteryx*), Coleoptera (*Dubiraphia* and *Promoresia*), and Diptera (*Limonia,* and *Tipula*; (Kowalik & Ormerod, 2006; Simpson et al., 1985)Kowalik et al 2007, Smit et al 1990, Pimpson et al 1985). Acid-tolerant Ephemeroptera (*Habrophlebiodes, Paraleptophlebia*, and *Centroptilium*) as well as taxa not typically associated with changes in stream pH like Diptera (*Dixa,* and *Blephariccera*) and Trichoptera (*Goera, Ochrotrichia, Pycnopsyche*, and Glossosomatidae) were also distinctive of Piney (Allard & Moreau, 1987; Kowalik & Ormerod, 2006). Differences in taxonomic diversity between streams may expand beyond acid tolerance to include differences in regional gamma diversity from large-scale geologic events (e.g., glaciations) or covariates such as slope, elevation, meteorology, temperature, hydrology, inorganic detritus, and organic detritus (Bonada et al., 2007; Feldman & Connor, 1992).

Traits tend to be more stable than taxonomic position because they aggregate biological information among different taxa (Bonada et al., 2007; Charvet et al., 2000; Statzner et al., 2004). In contrast to our second prediction, trait richness did differ between the circumneutral and acidified stream suggesting that functional change does occur in response to acidification in SNP. Studies that have found stable distributions of traits tend to be across a natural gradients (e.g., geographic regions; Bonada et al., 2007; Charvet et al., 2000). In these systems, local taxonomic richness tended to be similar, tough specific taxa may differ, suggesting baseline assembly or regulation of traits by environmental characteristics (Bonada et al., 2007). This shift in community composition without loss of richness allows for the maintenance of taxa within a trait along the gradient. Alternatively, studies that found differences in trait assemblages were associated with anthropogenic or naturally occurring stressors (e.g., drought, salinization; Charvet et al., 2000; Chessman, 2015; Gutiérrez-Cánovas et al., 2019). In these systems taxonomic richness differs between a reference and altered stream. Charvet et al. (2000) captured both of these relationships. First, they observed that taxonomic composition exhibited a gradient from lowlands to uplands and from calcareous to granitic geology while traits remained stable. They also compared trait composition between a reference and wastewater effluent impacted stream where they found greater abundances of taxa that were multivoltine, tegument respiring, and preferred stagnant or slow flowing water. While both streams in our study have been impacted by acid deposition, Meadow has lower pH and ANC values (Table 4). This suggests thresholds exist where magnitude of stressor experienced becomes great enough to alter the trait composition and potentially functional capacity of a stream. Perhaps the biological threshold of pH 5.5, where alkalinity falls to zero and inorganic aluminum concentration rises to become toxic to many forms of life (Sutcliffe & Hildrew, 1989).

3.4.2 Macroinvertebrate functional metrics

Macroinvertebrate secondary production has been shown to correlate with geologic attributes (Krueger & Waters, 1983). Krueger and Waters (1983) found secondary production to be greater in more alkaline streams. As predicted, we found greater secondary production in our circumneutral stream which is characterized by greater pH and acid neutralizing capacity (unfiltered alkalinity). Annual secondary production was estimated to be 32.9 g AFDM m^{-2} $year^{-1}$ in our acidified stream and 112.2 g AFDM m^{-2} $year^{-1}$ in the circumneutral stream. These estimates are greater than other studies in eastern deciduous forests of the south east United States which yielded values between 1.8 and 13.3 g AFDM m^{-2} $year^{-1}$ (GRUBAUGH et al., 1997; Huryn & Wallace, 1987; Smock et al., 1985; Wohl et al., 1995). Differences may be a result of different sampling techniques or variables such as geology, temperature, stream order, slope, elevation, organic matter standing stock, etc. (Grubaugh et al., 1997; Krueger & Waters, 1983). For example, most studies listed above used habitat-weighted sampling techniques (e.g., cobble, depositional, bedrock) whereas all samples in this study were taken in riffle areas with cobble. Grubaugh et al (1997) demonstrated production estimates vary with habitat type in the southern Appalachian Mountains. Cobble habitat yielded the greatest estimates ranging 3-364 g AFDM m^{-2} $year^{-1}$, whereas depositional and bedrock habitus yielded lesser estimates ranging 1-7 g AFDM m^{-2} $year^{-1}$ and 10-168 g AFDM m^{-2} $year^{-1}$, respectively. Interestingly, our shredder production estimates were similar to estimates from four streams with varying degrees of acidification (pH 4.3-7.5) in West Virginia (Griffith et al., 1994). Production estimates for shredders in Griffith and Perry (1994) ranged from 1.19 -3.372 g AFDM m^{-2} $year^{-1}$ with production decreasing with increasing acidification. Estimates of shredder production were 5.2 g AFDM m^{-2} $year^{-1}$ at out acidified stream and 30.5 g AFDM m^{-2} $year^{-1}$ at our circumneutral stream. While 30.5 g AFDM m^{-2} $year^{-1}$ is much greater than 1.19 -3.372 g AFDM m^{-2} $year^{-1}$, 24.9 g AFDM m^{-2} $year^{-1}$ of production at Piney as contributed by the giant stonefly *Pteronarcys sp,* which was not present in Griffith and Perry (1994).

Abiotic and anthropogenic filters are strong determinant of biotic composition in freshwater ecosystems (Heino, 2008; Townsend & Hildrew, 1994). In SNP, we observed decreased taxonomic and trait richness with stream acidification. While differences in the relative contribution of traits to production suggest tolerance or sensitivity to acidification, there were also traits that did not differ between streams. Trait categories with a priori predictions that did not differ in their relative contribution to production related to insect dispersal (drift, emergence, female dispersal, and flight strength). While dispersal limitations have been thought to delay biotic recovery in historically acidified streams, mixed results have been found (Bitušík et al., 2017; Masters et al., 2007) Masters et al (2006) found evidence of acid-sensitive adult taxa at geographically isolated acidified streams suggesting that other factors like mating and oviposition behavior may be affecting colonization.

Traits displaying acid-sensitivity were semivoltine, slow seasonal development, long adult life span, large body size, gill respiration, as well as the shredder and herbivore functional feeding groups. Traits more prevalent in the acidified stream were univoltine, fast seasonal development, short adult life span, small body size, tegument respiration and the as well as the collector-filter and predator functional feeding groups. Most of the differing trait categories are correlated trait suites often constrained by phylogeny (Poff et al., 2006). For example, most large-bodied organisms tend to have slower development rates, live longer, and reproduce less (Poff et al., 2006; Woodward et al., 2005). Differences in the prevalence of life history strategies between streams was anticipated as disturbance tends to have disproportionate negative effects on larger species (Woodward et al., 2005). Additionally, studies have found that another ionoregulatory disturbance, salinization, selects for multivoltine life cycles and aerial respiration (Gutiérrez-Cánovas et al., 2019). Increased incidence of gilled and herbivore taxa in the circumneutral stream was anticipated because of their association with the acid-sensitive order Ephemeroptera. Piney had an additional six Ephemeropteran genera not found at Meadow (*Centroptilum, Drunella, Habrophlebiodes, Heptagenia, Leucrocuta, and Paraleptophlebia*). Greater dominance of predator production at the acidified stream was expected as predators tend to have greater richness and relative abundance in acidified streams (Dangles & Guérold, 1999; Alan G Hildrew, 2009). We did not anticipate shredder production to be dominant in the circumneutral stream as acidified streams tend to be dominated by shredder stoneflies (Davy-Bowker et al., 2005; Murphy et al., 2014). Differences in relative shredder production was related to production of the genus *Pteronarcys.* This large-bodied stonefly was absent from Meadow yet amassed 24.9 of the 112.2 g AFDM m^{-2} year^{-1} of production at Piney, contributing not only the majority of shredder production but a significant amount of total production.

Differences in P/B ratios between stream can reflect changes in species composition or environmental condition. Shieh (2003) found changes in community composition to dominance by smaller and faster growing taxa in urbanized and agricultural streams resulted in a greater community P/B ratio than in reference streams. Griffith and Perry (1994) found changes in P/B rations of individual taxa in acidified streams. Acid-tolerant stoneflies (Leuctridae and Nemouridae) increased production compared to biomass (P/B) while another (Capniidae) showed a decrease in P/B suggesting alterations in resource allocation with acidification. Contrary to our fifth prediction, we did not find a difference in community P/B ratio between streams. This result was surprising considering differences in life history strategies of taxa between streams. With further consideration, both streams were comprised primarily of univoltine taxa of medium body size. Additionally, published literature values were used to calculate production for many taxa. Using the shortcut P/B method does not allow differences in the bioenergetics of taxa between streams in the same way as the size-frequency method. Significant differences were undetected for size-frequency calculations as well though Piney appears to have increased P/B ratios though not statistically significant. Differences in P/B ratios of taxa calculated via size-frequency method may be difficult to deduce because more taxa had P/B estimate using this method in Piney (Meadow: 17 taxa; Piney: 26 taxa). It may be useful to conduct this analysis on only taxa that overlap between the two streams to infer further information about changing bioenergetics.

Greater functional diversity should result in a greater diversity of community responses and resilience to a stressor (Elmqvist et al., 2003). Contrary to our sixth prediction, increased functional diversity was not always associated with increased functional redundancy within a trait. We found that while more dominantly contributing traits to secondary production in Piney had greater functional diversity, density, and biomass, they often lacked an even distribution of production among taxa. Alternatively, while traits more dominant in Meadow had lower functional diversity than Piney, they often had greater functional redundancy. So, while Piney had greater functional diversity and production than

Meadow, large differences in production were sometimes attributed to individual taxa (e.g., *Pteronarcys)* making function within this trait vulnerable to an alternative stressor such as increasing temperature. This concept was also supported in a metanalysis of biodiversity-ecosystem function research in streams by Lecerf et al., (2010). They found that richness-function relationships are altered by a multitude of factors, such as evenness, non-taxonomic diversity (genetic/phenotypic diversity), species extinction order, environmental context, and study design.

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3.6 Tables and figures

Table 1. Summary of studies investigation macroinvertebrate function in relation to stream acidification (changes in pH and Acid Neutralizing Capacity). Under response, BM = biomass and P= production. RD is the response direction of measured variables with increasing acidification (↓ decreases as pH decreases and streams become more acidified, ↑ increases as pH decreases and streams become more acidified).

Location	# Sites	Type	Taxa or trait	Predictor	Predictor range	Response	Response range	RD	Reference
Ontario, CA	10	lake	taxa - Hyalella	Alkalinity	0.2 to 58.1 mg liter^{-1}	BM	0.4 -1.4 (g m^{-2})	↓	France et al. 1996
Ontario, CA	10	lake	taxa - Hyalella	Alkalinity	0.2 to 58.1 mg liter^{-1}	P	1.2-3.6 (g m^{-2} year $^{-1}$)	↓	France et al. 1996
West Virginia, USA	4	stream	shredders	Alkalinity; pH	0-40.8 mg liter^{-1}; 4.3-7.5	P	1.19-3.77 (g m^{-2} year $^{-1}$)	↓	Griffith and Perry 1994
West Virginia, USA	3	stream	shredders on leafpacks	Alkalinity; pH	0-33.5 mg liter^{-1} ; 4.2-7.6	BM	~0-60 (g m^{-2})	↓	Meegan et al. 1996
Minnesota, USA	3	stream	benthos by FFG	Alkalinity; pH	34-245 mg liter^{-1} ;6.8-8.3	P	Herbivore: 27 - 119.6 (g m^{-2} year $^{-1}$) Predator: 5.-12.8 (g m^{-2} year $^{-1}$)	↓	Krueger and Waters 1983
Ashdown Forest, UK	1	stream	benthos + meiofauna	pH	Min ave: 4.0; Max ave: 7.0	p	Macros: 4.46 (g m^{-2} year^{-1}) Meiofauna: 0.76 (g m^{-2} year^{-1})	-	Stead et al. 2005
Derbyshire Peak, UK	1	stream	trait- shredders	pH	Min ave: 3.5; Max ave: 5.0	P	1997: 1.67 (g m^{-2} year $^{-1}$) 1998: 1.99 (g m^{-2} year $^{-1}$)	-	Pretty et al. 2005
South Westland, NZ	4	stream	taxa - mayflies	pH	4.8-7.5	BM	0.020-0.376 (g m^{-2})	↓	Collier and Winterbourn 1990
South Westland, NZ	4	stream	taxa - mayflies	pH	4.8-7.5	P	2.49-10.35 (g m^{-2} year $^{-1}$)	↓	Collier and Winterbourn 1990
West Virginia, USA	4	stream	*Rhyacophila, Ameletus,* and *Isonychia*	pH	4.5-6.7	P	Rhyacophila: 15.8-49.6 (mg m^{-2} y^{-1}) ↑ Ameletus: 7.4-208.3 (mg m^{-2} y^{-1}) ↑ Isonychia: 0 -116.6 (mg m^{-2} y^{-1}) ↓	↓ or ↑	Kobuszewski and Perry 1994

Table 2. A priori hypotheses and rationale of expected differences in traits between acidified and circumneutral streams. This list includes 12 of 20 trait categories (e.g., voltinism) and 28 of 59 trait states (e.g., multivoltine, semivoltine, univoltine) for which we provided hypotheses with rationales.

Trait group	Trait category	Acid tolerant trait state	Acid-sensitive trait state	Rationale
Life history	Voltinism	Multivoltine	Univoltine	Taxa with shorter life spans and an increased number of cohorts are more likely to develop adaptations to chronic stressors like acidification (Kloss et al 2019).
Life history	Development	Fast-seasonal	Slow/non-seasonal	Taxa with fast development have a lower risk of exposure to high-stress events, such as episodic acidification, that result in increased osmoregulatory stress (Kowalik et al 2006)
Life history	Emergence	Poorly Synchronized	Well Synchronized	Poorly synchronized emergence will have all individuals at their most vulnerable life stages at different times rending them less vulnerable to extirpation in the event of a temporary stressor (Bell 1970).
Life history	Adult life span	Very short (<1 week)	Long (>1 month)	Insects with a shorter adult life span have less chances to disperse to different streams with more favorable conditions (Poff et al 2006).
Life history	Desiccation resistance	Absent	Present	Non-resistant organisms have a higher likelihood of inhabiting the hyporheic zone where conditions may be less acidic (Allard and Moreau 1987).
Mobility	Female dispersal	Low (<1km)	High (>1km)	Low dispersing taxa are more constrained in their ability to move more suitable habitat within the river network (Sarremejane et al 2017).
Mobility	Flight strength	Weak	Strong	Weak flight strength is associated with a diminished dispersal ability to move to different streams with more favorable conditions (Brown et al 2010).
Mobility	Drift	Common/abundant	Rare	Greater drift occurrence corresponds to an increased ability to actively or passively move in search of microrefugia from stressful conditions (Kohler and McPeek; Naman et al 2015).
Morphology	Armoring	Poor (heavily sclerotized)	None (soft-bodied)	Sclerotization reduces potential surface area used for respiration and ion regulation (Hall et al. 1987).
Morphology	Respiration	Aerial	Gills/Tegument	Taxa with aerial respiration do not use vulnerable body surface for respiration (Hall et al. 1987).

Morphology	Body size	Small	Medium/large bodied	Small bodied organisms take less time to develop under chronically stressful conditions and have a higher likelihood of not being present for an episodic acidification spike (Lepori 2003).
Ecology	Functional feeding group (FFG)	Shredder	Scraper	Filipalpian stoneflies (shredders) already demonstrated to dominate acidified streams (Dangles et al 2002). Additionally, algal richness is diminished in acidified streams resulting in diminished resource quality and quantity for scrapers (Sutcliffe and Hildrew, 1989)

Table 3. Site attributes (general). Meadow is the acidified stream and Piney is the circumneutral stream.

Site	Geology	Latitude	Longitude	Area (ha)	Elevation	Aspect	Aspect (deg)	Slope (deg)
Meadow	Siliciclastic	38.1587	-78.8059	657	511	westerly	247	11
Piney	Basaltic	38.7452	-78.2817	72	764	easterly	286	6

Table 4. Mean site attribute (habitat) values for both sites. All data were collected between July 2019 – June 2020 unless otherwise noted. Discharge data provided by USGS. Water chemistry data from University of Virginia. CBOM = coarse benthic organic matter, AFDM = ash-free dry mass, FBOM = fine benthic organic matter, Chl *a* = chlorophyll *a*. Meadow is the acidified stream and Piney is the circumneutral stream.

Variable	Meadow	Piney
Discharge (L/s)	NA	NA
Bankfull width (m)	NA	NA
ANC (µeq/L)	4	261.9
pH	5.5	7.1
specific conductivity (µS/cm)	15.6	37.8
SO4 (µeq/L)	75.5	50.1
CBOM (g AFDM/ m2)	NA	NA
FBOM (g AFDM/ m2)	NA	NA
Chl a (gravel) (weighted for % cover gravel in the stream)	NA	NA
Chl a (rock) (weighted for % cover gravel in the stream)	NA	NA
Daily temp (℃)	11.38	11.42
Summer temp (℃)	17.31	17.79
fall temp (℃)	12.79	13.24
winter temp (℃)	6.11	5.1
spring temp (℃)	10.69	10.46

Table 5. Summary statistics of macroinvertebrate density and taxa and trait richness. cov= coefficient of variation. Meadow is the acidified stream and Piney is the circumneutral stream.

	Density				Taxa richness				Trait richness			
Site	**range**	**median**	**mean**	**cov**	**range**	**median**	**mean**	**cov**	**range**	**median**	**mean**	**cov**
Meadow	175-450	263.5	284	0.30	32-47	40	40.5	0.12	54-57	55	55.1	0.02
Piney	235-821	326.7	378	0.44	45-65	57	55.5	0.10	55-57	56	55.9	0.01

Table 6. Summary statistics of macroinvertebrate biomass and production. cov= coefficient of variation.

Meadow is the acidified stream and Piney is the circumneutral stream.

Site	Biomass range	Biomass median	Biomass mean	Biomass cv	Production total
Meadow	792-3232	1716	1849	0.418415	32947
Piney	1907-15915	5013	6292	0.682692	112242

Table 7. Statistical results comparing macroinvertebrate metric mean (Welch's t-test) or median (Kruskal-Wallis) values between streams. Test values: tt= t-test; tt (log) = t-test on log transformed (ln [1+x]) data, kw = Kruskal-Wallis for data that were still not normally distributed after transformation. Bold p-values were significant at α ≤ 0.05.

		t.test/ Kruskal-Wallis			mean	
Parameter	**test**	**t / chi-sq**	**df**	**p-value**	**Meadow**	**Piney**
Density (n)	tt	-1.7794	22	0.089	274	327
Taxa richness (n)	tt	-7.0356	1	**<0.001**	41	56
Trait richness (n)	kw	5.3332	0.02092	**0.021**	55	56
Biomass (mg AFDM m-2)	tt (log)	4.9318	22	**<0.001**	1850	6292
P/B (all)	kw	0.34414	1	0.5574	4.5	4.7
P/B (sf)	tt	-1.1531	41	0.255	2.5	3.3

Table 8. Statistical results comparing monthly mean functional diversity (# of taxa within each trait state) mean (Welch's t-test) or median (Kruskal-Wallis) values between streams. Test values: tt= t-test; tt (log) = t-test on log transformed (ln [1+x]) data, kw = Kruskal-Wallis for data that were still not normally distributed after transformation. Bold p-values were significant at α ≤ 0.05.

		t.test/ Kruskal-Wallis			mean	
Parameter (richness)	**test**	**t / chi-sq**	**df**	**p-value**	**Meadow**	**Piney**
semivoltine	kw	14.08	1	**<0.001**	6	10
univoltine	tt	-5.95	21	**<0.001**	27	35
multivoltine	tt	-3.62	21	**0.002**	6	8
fast seasonal development	tt	-3.50	22	**0.002**	14	18
slow seasonal development	tt	-5.63	19	**<0.001**	22	32
nonseasonal development	kw	6.18	1	**0.013**	2	3
poorly synchronized emergence	tt	-7.49	21	**<0.001**	12	19
well synchronized emergence	tt	-3.85	17	**0.001**	26	34
very short adult life	tt	-5.52	22	**<0.001**	16	23
short adult life	tt	-4.36	22	**<0.001**	20	25
long adult life	kw	11.95	1	**<0.001**	3	5
exit ability absent	tt	-7.81	21	**<0.001**	34	49
exit ability present	tt (log)	0.71	22	0.485	4	4
not desiccation resistant	tt	-7.68	21	**<0.001**	31	44
desiccation resistant	tt	-2.01	21	0.058	7	9
low dispersal	tt	-7.48	20	**<0.001**	29	43
high dispersal	kw	4.30	1	**0.038**	9	10
weak flight	tt	-6.65	20	**<0.001**	32	44
strong flight	kw	13.54	1	**<0.001**	6	9
drift rare	tt	-4.51	21	**<0.001**	24	31
drift common	tt	-7.43	21	**<0.001**	11	18
drift abundant	kw	6.16	1	**0.013**	3	4
very low speed crawl	tt	-3.60	21	**0.002**	11	14
low speed crawl	tt	-6.16	21	**<0.001**	22	30
high speed crawl	tt	-5.10	18	**<0.001**	6	9
no swim	tt	-6.26	22	**<0.001**	18	27
weak swim	tt	-4.02	20	**<0.001**	17	22
strong swim	tt	-3.82	20	**0.001**	3	4

no attach	tt	-7.99	22	**<0.001**	28	40
some attach	tt	3.03	22	**0.001**	10	13
no armor	tt	-3.77	22	**0.001**	27	34
poor armor	kw	11.50	1	**<0.001**	9	13
good armor	tt	-6.60	18	**<0.001**	2	6
streamlined	kw	17.49	1	**<0.001**	10	17
not streamlined	tt	-4.09	21	**<0.001**	29	36
tegument	tt	-2.11	20	**0.048**	17	20
gills	kw	17.37	1	**<0.001**	21	33
plastron	kw	0.00	1	1	0	0
small-bodied	tt	-5.07	22	**<0.001**	14	19
medium-bodied	tt	-5.47	19	**<0.001**	20	29
large-bodied	tt	-3.33	20	**0.003**	4	5
depositional	kw	4.72	1	**0.030**	1	2
depositional/erosional	tt	-5.94	22	**<0.001**	23	33
erosional	tt	-4.63	21	**<0.001**	14	19
cool	tt	-4.71	18	**<0.001**	12	16
cool/warm	tt	-5.97	22	**<0.001**	25	35
warm	kw	15.44	1	**<0.001**	1	2
burrow	tt	-6.00	22	**<0.001**	8	12
climb	tt	-5.26	18	**<0.001**	25	34
sprawl	tt	-1.41	20	0.175	3	3
cling	kw	1.09	1	0.297	1	2
swim	kw	0.16	1	0.691	1	1
collect-gather	tt	-7.82	21	**<0.001**	10	15
collect-filter	tt	-2.38	21	**0.027**	6	8
herbivore	kw	17.35	1	**<0.001**	5	11
predator	tt	-3.17	19	**0.005**	14	16
shredder	tt	-0.70	18	0.493	5	5

Table 9. Statistical results comparing monthly mean density within each trait state mean (Welch's t-test) or median (Kruskal-Wallis) values between streams. Test values: tt= t-test; tt (log) = t-test on log transformed (ln [1+x]) data, kw = Kruskal-Wallis for data that were still not normally distributed after transformation**.** Bold p-values were significant at α ≤ 0.05.

		t.test/ Kruskal-Wallis			mean	
Parameter (richness)	test	t / chi-sq	df	p-value	Meadow	Piney
semivoltine	tt (log)	-5.11	22	**<0.001**	9	36
univoltine	tt	-0.86	21	0.401	142	159
multivoltine	tt (log)	-1.08	22	0.293	135	173
fast seasonal development	tt (log)	-0.58	22	0.567	190	221
slow seasonal development	tt (log)	-7.16	22	**<0.001**	40	124
nonseasonal development	tt	5.81	17	**<0.001**	55	23
poorly synchronized emergence	tt	-1.28	20	0.214	72	85
well synchronized emergence	tt (log)	-1.38	22	0.182	214	283
very short adult life	tt (log)	-1.29	22	0.211	185	256
short adult life	tt	-4.0623	21	**<0.001**	46	87
long adult life	tt	5.34	18	**<0.001**	55	25
exit ability absent	tt (log)	-2.90	22	**0.008**	150	222
exit ability present	tt (log)	-0.07	22	0.948	135	146
not desiccation resistant	tt (log)	-2.81	22	**0.010**	212	335
desiccation resistant	tt (log)	4.97	22	**<0.001**	73	33
low dispersal	tt (log)	-1.81	22	0.084	141	186
high dispersal	tt (log)	-1.25	22	0.224	145	182
weak flight	tt (log)	-0.83	22	0.418	271	332
strong flight	tt (log)	-5.17	22	**<0.001**	15	46
drift rare	tt	-2.91	18	**<0.001**	68	113
drift common	tt (log)	-1.46	22	0.159	83	110
drift abundant	tt (log)	-0.01	22	0.988	135	145
very low speed crawl	tt (log)	-0.77	22	0.449	144	175
low speed crawl	tt (log)	-2.00	22	0.058	123	163
high speed crawl	tt	-2.09	21	**0.049**	19	31
no swim	tt (log)	-0.38	22	0.708	209	229

weak swim	tt (log)	-3.5212	22	**0.002**	68	131
strong swim	kw	0.85	1	0.356	9	8
no attach	tt (log)	-0.86	22	0.402	261	313
some attach	tt (log)	-3.65	22	**0.001**	24	55
no armor	tt (log)	-1.78	22	0.089	215	289
poor armor	tt	0.74	21	0.470	69	63
good armor	kw	15.89	1	**<0.001**	2	16
streamlined	tt (log)	-6.69	22	**<0.001**	23	102
not streamlined	tt (log)	-6.69	22	**<0.001**	262	266
tegument	tt	4.08	20	**<0.001**	113	71
gills	tt (log)	-3.02	22	**0.006**	173	297
plastron	kw	0.00	1	1.000	1	1
small-bodied	tt (log)	0.40	22	0.691	224	223
medium-bodied	tt (log)	-4.46	22	**0.002**	57	122
large-bodied	tt (log)	-6.82	22	**<0.001**	5	24
depositional	kw	0.38	1	0.539	1	1
depositional/erosional	tt (log)	-0.50	22	0.623	255	291
erosional	tt	-5.40	16	**<0.001**	30	76
cool	tt (log)	-1.46	22	0.159	167	226
cool/warm	tt (log)	22.00	22	0.698	114	122
warm	kw	14.54	1	**<0.001**	3	20
burrow	tt	1.12	20	0.276	69	60
climb	tt (log)	-1.95	22	0.063	206	291
sprawl	tt (log)	-0.98	22	0.339	8	11
cling	tt	-5.25	16	**<0.001**	1	4
swim	kw	0.81	1	0.367	1	2
collect-gather	tt	-0.82	17	0.424	198	223
collect-filter	tt	-2.49	16	**0.024**	15	32
herbivore	tt	-4.10	23	**0.001**	9	46
predator	tt (log)	-3.13	22	**0.005**	44	67
shredder	tt	3.61	16	**0.002**	30	10

Table 10. Statistical results comparing monthly mean biomass within each trait state mean (t-test) or median (Kruskal-Wallis) values between streams. Test values: tt= t-test; tt (log) = t-test on log transformed (ln [1+x]) data, kw = Kruskal-Wallis for data that were still not normally distributed after transformation. Bold p-values were significant at α ≤ 0.05.

		t.test/ Kruskal-Wallis			mean	
Parameter (richness)	test	t / chi-sq	df	p-value	Meadow	Piney
semivoltine	tt (log)	-11.54	20	**0.003**	156	636
univoltine	tt (log)	-7.53	20	**<0.001**	1380	5140
multivoltine	tt (log)	-7.28	21	0.239	307	483
fast seasonal development	tt (log)	-6.83	22	0.116	654	1061
slow seasonal development	tt (log)	-6.12	20	**<0.001**	980	5065
nonseasonal development	tt	3.15	22	**0.005**	209	132
poorly synchronized emergence	tt (log)	-6.06	22	**<0.001**	550	1499
well synchronized emergence	tt (log)	-5.21	19	**<0.001**	1293	4760
very short adult life	tt (log)	-5.21	21	**0.001**	433	1574
short adult life	tt (log)	-5.20	22	**0.007**	1127	2215
long adult life	kw	8.00	1	**0.005**	283	2470
exit ability absent	tt (log)	-5.09	19	**<0.001**	1620	6048
exit ability present	tt (log)	-4.95	21	0.835	222	210
not desiccation resistant	tt (log)	-4.93	21	**<0.001**	1476	5480
desiccation resistant	tt (log)	-4.89	19	0.105	367	779
low dispersal	tt (log)	-4.72	21	**0.002**	1275	3179
high dispersal	tt (log)	-4.69	20	**0.001**	567	3080
weak flight	tt (log)	-4.52	21	**0.003**	1392	3266
strong flight	tt (log)	-4.40	22	**<0.001**	451	2993
drift rare	tt (log)	-4.34	21	**0.002**	1038	2463
drift common	tt (log)	-4.22	20	**<0.001**	714	3617
drift abundant	tt (log)	-4.17	16	**0.036**	90	179
very low speed crawl	tt (log)	-4.13	22	**<0.001**	191	969
low speed crawl	tt (log)	-4.10	22	**0.013**	1113	2276
high speed crawl	tt (log)	-4.01	18	**0.002**	539	3015
no swim	tt (log)	-3.93	18	**<0.001**	671	4210
weak swim	tt (log)	-3.78	21	0.070	1058	1931

strong swim	kw	0.75	1	0.387	114	117
no attach	tt (log)	-3.64	19	**<0.001**	1293	5099
some attach	tt (log)	-3.59	22	**0.003**	550	1160
no armor	tt (log)	-3.51	22	**0.008**	1104	2376
poor armor	tt (log)	-3.36	21	0.183	727	1038
good armor	kw	17.28	1	**<0.001**	13	2845
streamlined	tt (log)	-3.34	19	**0.001**	473	1752
not streamlined	tt (log)	-3.33	18	**<0.001**	1370	4506
tegument	tt (log)	-3.25	22	0.252	932	759
gills	tt (log)	-3.13	22	**<0.001**	911	5500
plastron	kw	0.00	1	0.952	0	0
small-bodied	tt (log)	-3.01	22	**0.049**	474	817
medium-bodied	tt (log)	-2.91	22	**0.004**	1095	2685
large-bodied	tt (log)	-2.79	21	**<0.001**	274	2756
depositional	kw	5.90	1	**0.015**	2	8
depositional/erosional	tt (log)	-2.66	19	**<0.001**	1081	4836
erosional	tt (log)	-2.29	22	**0.005**	759	1415
cool	tt (log)	-2.18	19	**<0.001**	647	3382
cool/warm	tt (log)	-2.09	22	**0.003**	1184	2503
warm	kw	16.81	1	**<0.001**	12	374
burrow	kw	12.00	1	**<0.001**	525	2941
climb	tt (log)	-1.63	20	**0.011**	1219	2824
sprawl	tt (log)	-1.38	20	0.270	24	65
cling	kw	14.09	1	**<0.001**	67	420
swim	kw	0.34	1	0.562	8	8
collect-gather	tt (log)	-1.13	20	**0.001**	366	1001
collect-filter	tt (log)	-0.51	17	**0.016**	263	445
herbivore	tt (log)	-0.21	18	**<0.001**	248	1079
predator	tt (log)	0.54	22	**0.041**	705	1222
shredder	tt (log)	1.18	16	**0.002**	264	2539

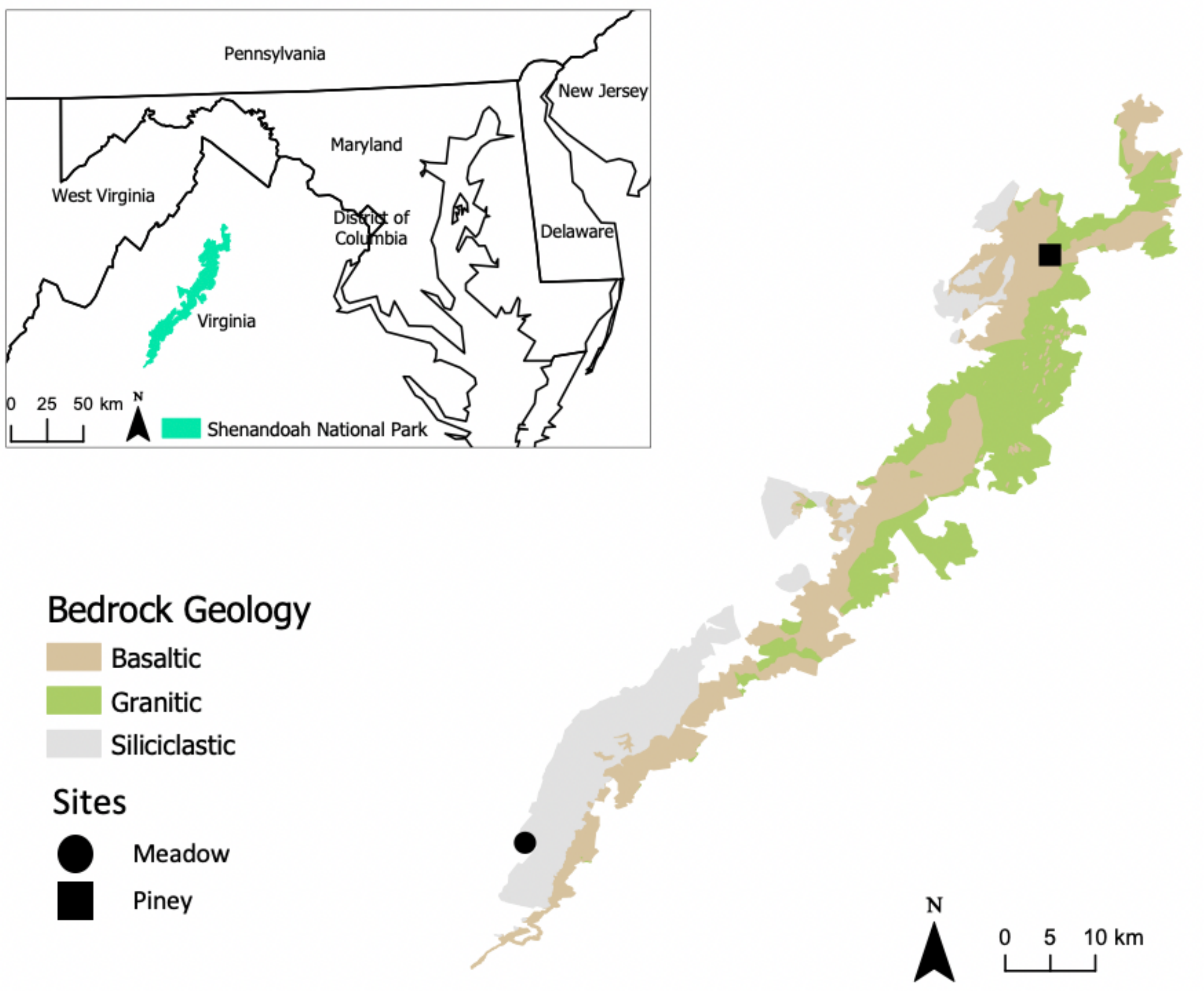


Figure 1. Sampling locations within Shenandoah National Park. Colors represent the three geologic classes within the park characterized by their differing ability to neutralize acidic inputs. Meadow is the acidified stream and Piney is the circumneutral stream.

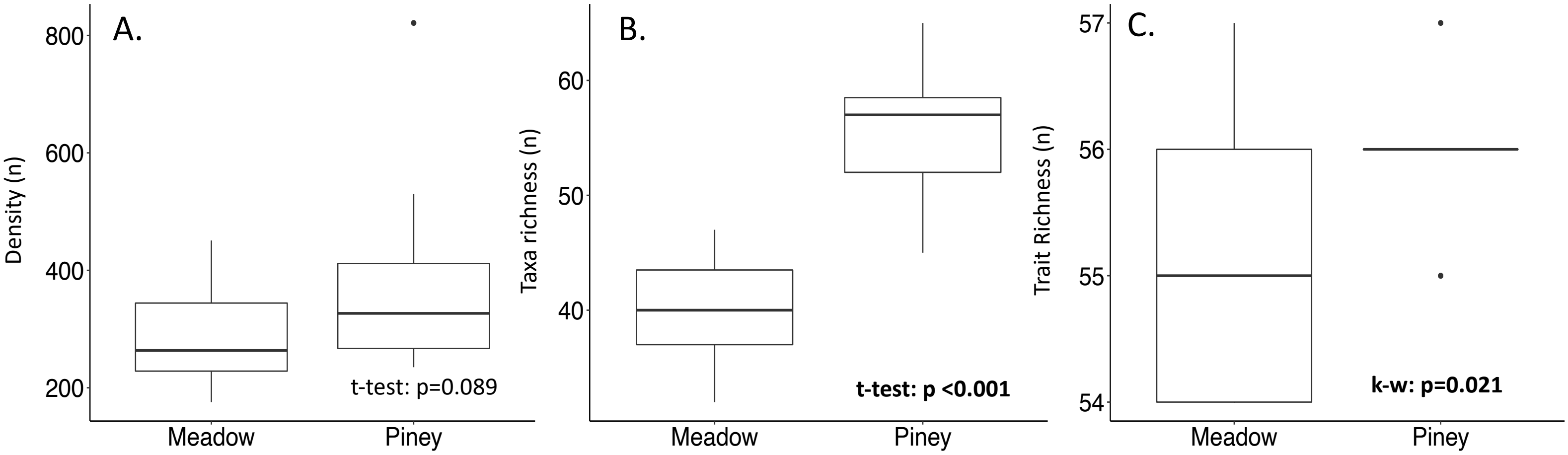


Figure 2. Differences in macroinvertebrate structural metrics averaged across sample months: A. total density, B. taxa richness, and C. trait richness between streams. Comparisons of values between sites were completed with a Welch’s t-test or a Kruskal-Wallis test for parametric and nonparametric data, respectively. Meadow is the acidified stream and Piney is the circumneutral stream.

Figure 3.

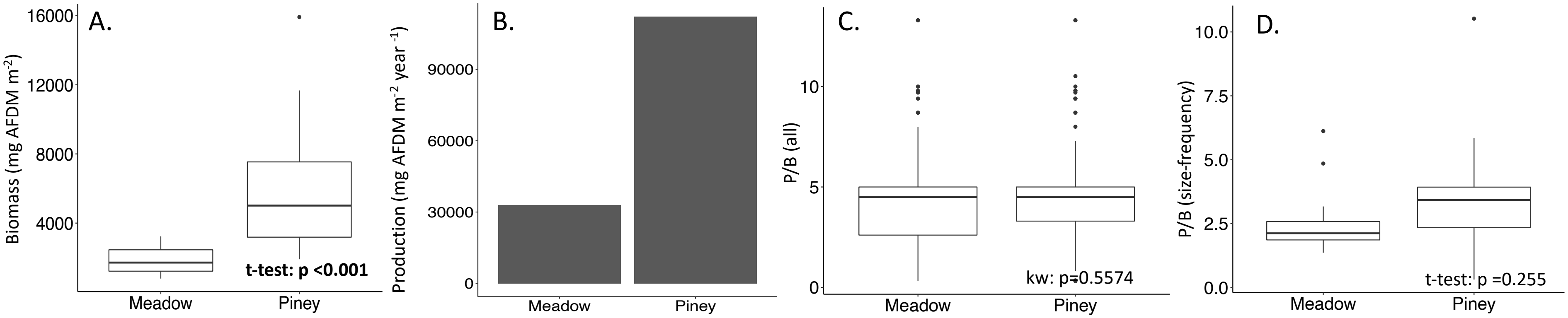


Figure 3. Differences in macroinvertebrate functional metrics: A. total biomass averaged across sample months, B. total secondary production, C. P/B averaged across all taxa, D. P/B averaged from all taxa estimated using the size-frequency histograms between streams. Comparisons of values between sites were completed with a Welch's t-test or a Kruskal-Wallis test for parametric and nonparametric data, respectively. Meadow is the acidified stream and Piney is the circumneutral stream.

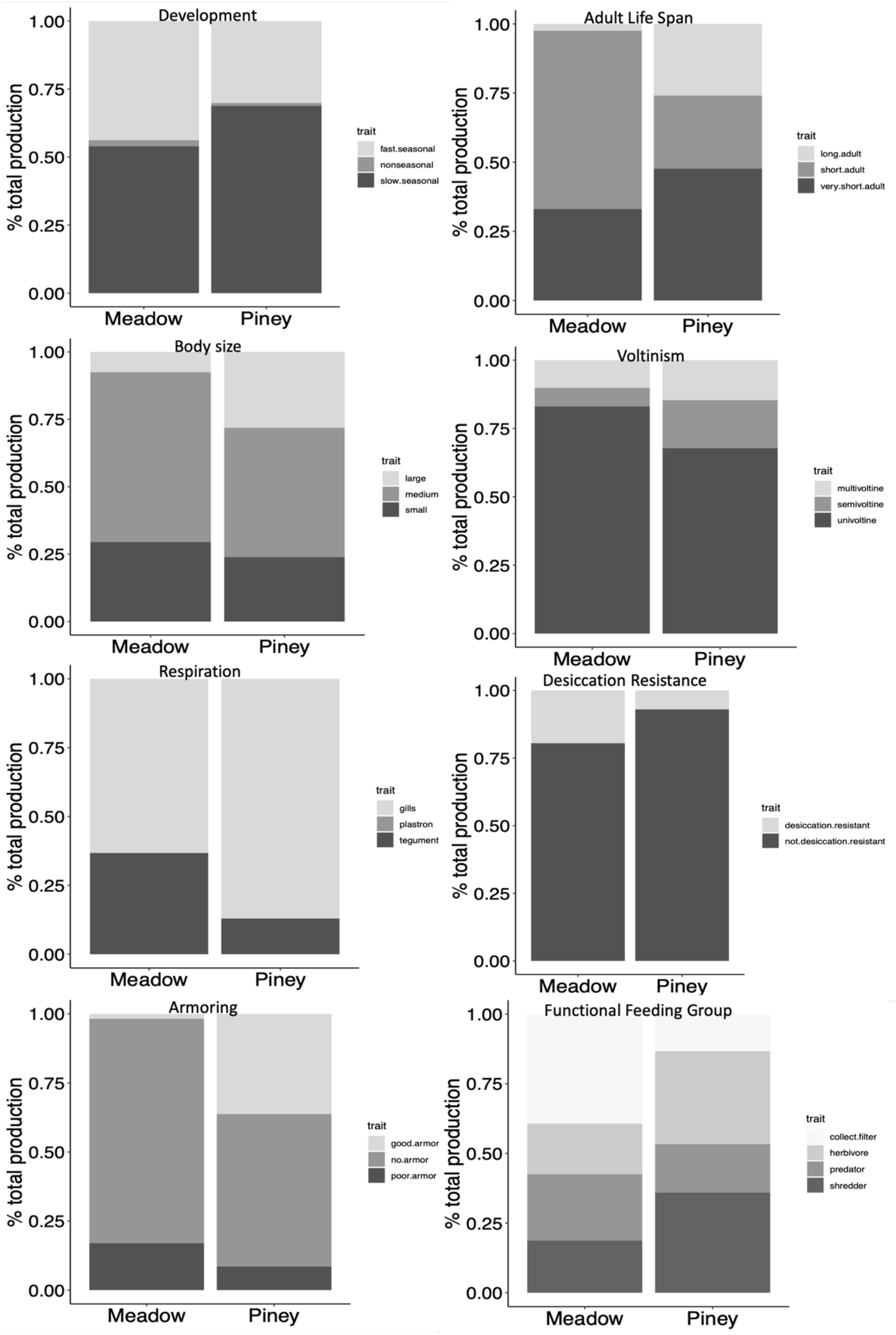


Figure 4. Percentage of secondary production contributed by each trait state for selected trait categories that differed between streams. Meadow is the acidified stream and Piney is the circumneutral stream.

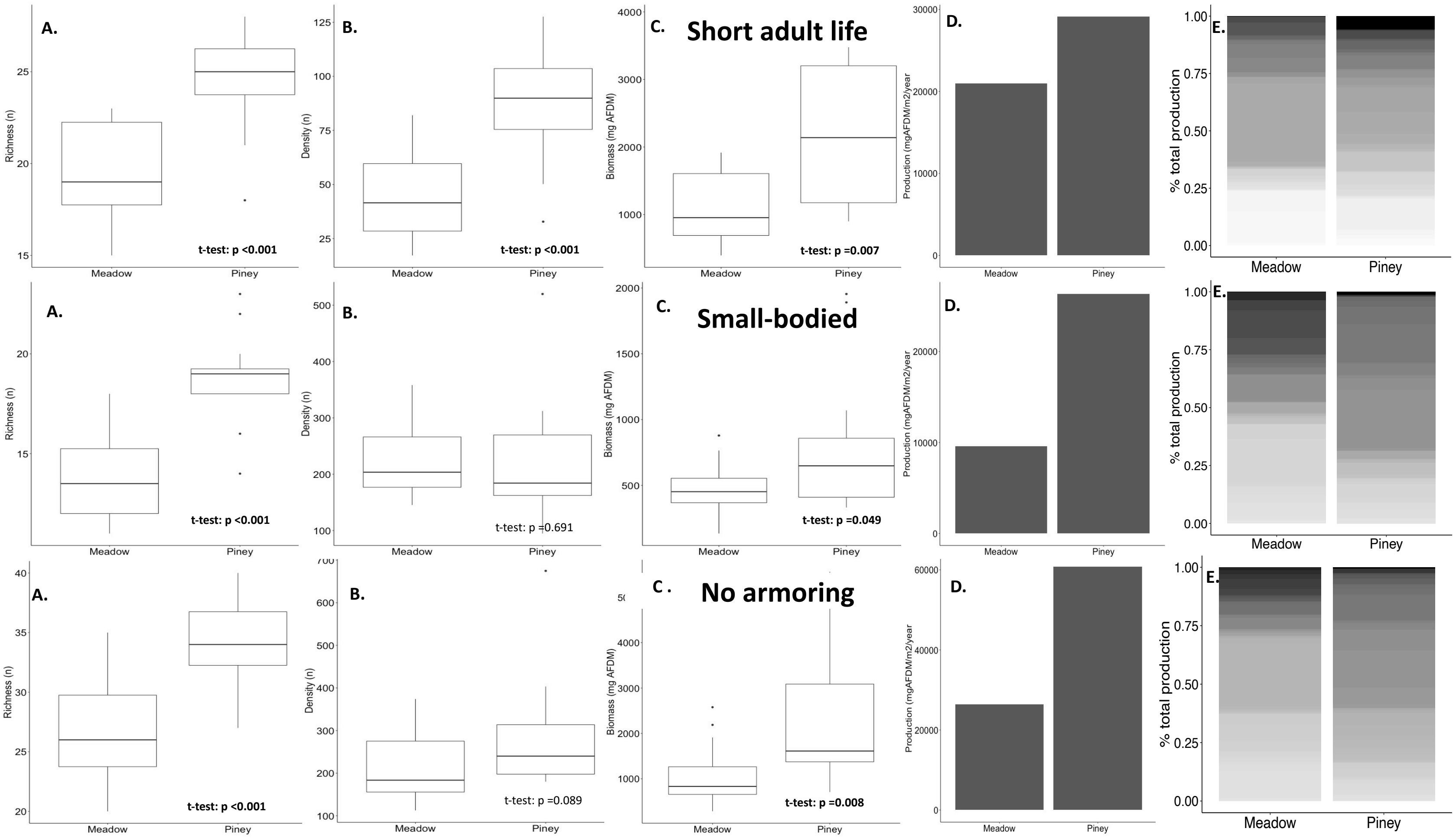


Figure 5. Differences in structural metrics averaged across sample months for traits dominant in the **acidified** stream (Meadow). Each row represents a trait. Each column represents comparisons of **A.** functional diversity, **B.** density, **C.** biomass, **D.** secondary production, **E.** relative contribution of each taxa to secondary production (functional redundancy). Taxa are represented by a unique color in bar graphs (**E.**). Meadow is the acidified stream and Piney is the circumneutral stream.

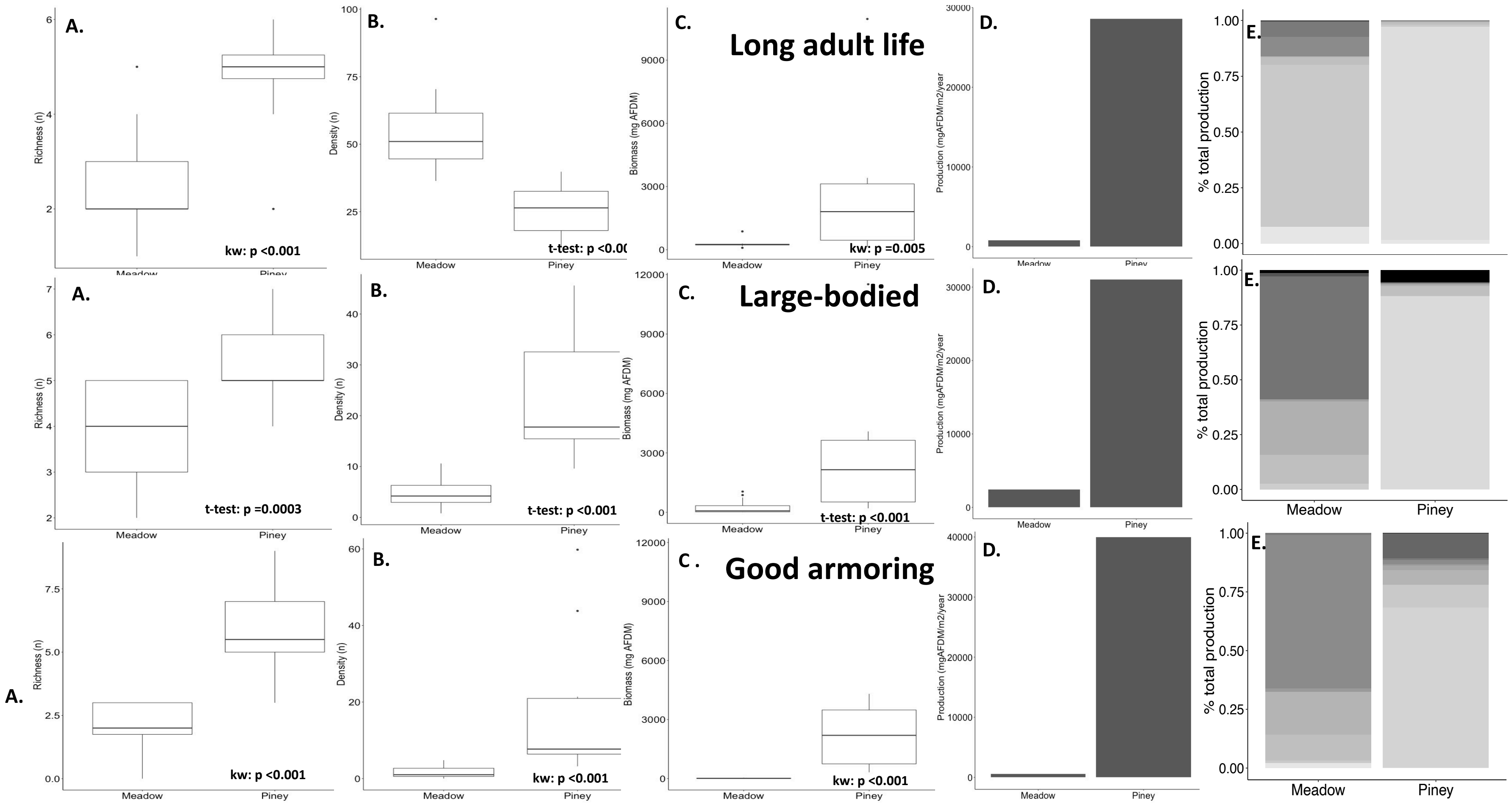


Figure 6. Differences in structural metrics averaged across sample months for traits dominant in the **circumneutral** stream (Piney). Each row represents a trait. Each column represents comparisons of **A.** functional diversity, **B.** density, **C.** biomass, **D.** secondary production, **E.** relative contribution of each taxa to secondary production (functional redundancy). Taxa are represented by a unique color in bar graphs (**E.**). Meadow is the acidified stream and Piney is the circumneutral stream.

Chapter 4: Final conclusions and future research

Our spatial and temporal assessment of historically acidified streams of SNP suggested modest recovery from atmospheric acid deposition from 1987-2017. Changes in water chemistry suggested recovery over time with streams decreasing in sulfate and increasing in pH. ANC trends were insignificant and varied with geology, suggesting continued impacts of acidification. Changes in macroinvertebrate community metrics were suggestive of biotic recovery with increasing density and taxonomic and trait richness. Changes in taxa were greater than changes in traits over time. While this result was expected as multiple taxa make up each trait category though it may also suggest minimal or delayed functional recovery over time. Taxa and their associated traits driving temporal changes were collector-gathering and scraping Ephemeroptera, net spinning and case-making Trichoptera, and predaceous Plecoptera that also tended to belong-lived taxa with high dispersal ability. We concluded that streams in SNP have experienced modest recovery from acidification with biotic recovery primarily observed through taxonomic responses. Dampened response of traits over time suggest that acidification may not have significantly altered trait composition or trait-based recovery is more delayed than taxonomic recovery.

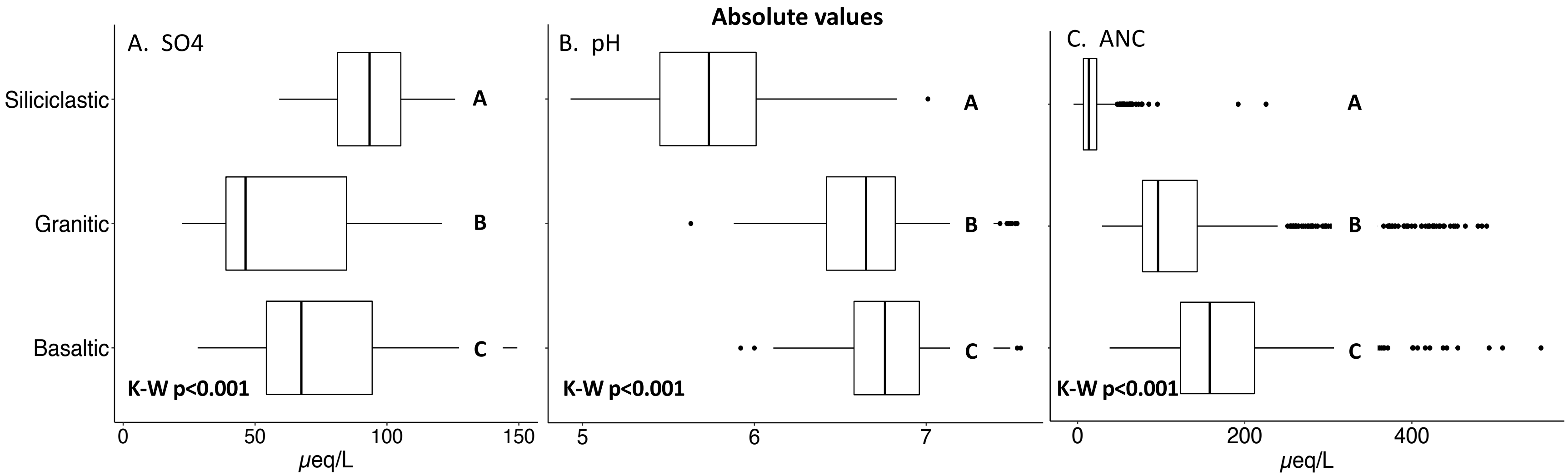
Our secondary production study showed differences in macroinvertebrate community structure and function between a more and less acidified stream. Density did not differ between the two streams but taxa richness, trait richness, and functional diversity were greater in the circumneutral stream. Additionally, density and biomass within each trait were often greater in the circumneutral stream. Traits more prevalent in the circumneutral stream were semivoltine, slow seasonal development, long adult life span, large body size, and gill respiration. Traits more dominant in the acidified stream were univoltine, fast seasonal development, short adult life span, medium body size, and tegument respiration. Average P/B ratios did not differ between streams even though trait analysis suggested differences in dominant development and body size traits. Lastly, dominant traits in each stream did not always display greater functional redundancy than the other stream. In fact, greater relative production of a trait was sometimes the result of one dominant taxa in either stream. We concluded that stream acidification in

SNP has altered macroinvertebrate trait composition as well as ecosystem function (secondary production). This study indicates that richness-function relationships are altered by variables such as the densities and evenness of macroinvertebrate communities.

The findings of our production study demonstrate that acidification may alter trait composition and functional capacity (secondary production of streams) of streams in SNP. Additionally, while almost all trait categories had greater functional diversity and secondary production in the circumneutral stream, there were similarities between traits changing over time with stream recovery and traits that displayed particular sensitivity to acidification in our production study. Taxa characterized by long-lived life history strategies (e.g., semivoltine, long adult life, slow seasonal development) appeared to be the most responsive to changes in acidification in both studies. These findings suggest that changes in trait composition (e.g., functional diversity) do predict changes in function to some degree but the relationship is complicated by community structure attributes such as density and evenness. Additionally, increased functional diversity does not always correspond to increased functional redundancy or resilience within a trait.

Future directions for this study include tracking changes in macroinvertebrate production over time with recovery from acidification. Samples from 1987-2017 have been preserved and could conceivably be processed to estimate biomass. Additionally, the applicability of BEF theory need to be tested further. Results of this study suggest that increased functional diversity leads to increased secondary production while density had a less clear relationship. Parsing out factors that alter richness-function relationships in a variety of settings (e.g., acidification, salinization, urbanization) will help to improve our ability to predict changes in ecosystem function through changes in community structure.

Appendix



Supplemental 2.1: Water chemistry parameter (sulfate, pH, and acid neutralizing capacity) absolute values (across all sites and years by geologic class. Comparisons among geologic classes were completed with a Kruskal-Wallis Test. If model results were significant at α ≤ 0.05, post-hoc analyses were completed using a Wilcoxon Rank Sum test. Differences in absolute or trend values among geologic classes are denoted by differing letters.

Supplemental 2.2. Summary statistics of water chemistry parameters related to acidification in SNP from 1987-2017 by site and geologic class. Summaries for geologic classes are either entire ranges or average values for all sites within a geologic class. Values: SO_4^{2-} = sulfate concentration; ANC = acid neutralizing capacity; raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values.

Geology	Site	ANC range	ANC median	ANC mean	ANC cov	ANC % Δ	pH range	pH median	pH mean	pH cov	pH % Δ	SO_4^{2-} range	SO_4^{2-} median	SO_4^{2-} mean	SO_4^{2-} cov	SO_4^{2-} % Δ
Basaltic	MAD2	38.34-238.39	118.07	127.75	0.35	14.10	5.92-6.90	6.60	6.56	0.03	4.36	50.49-98.64	67.45	68.48	0.13	-10.58
Basaltic	VT51	101.17-554.56	202.72	236.35	0.42	19.95	6.41-7.55	6.96	6.96	0.04	2.59	63.04-155.57	112.69	110.10	0.19	-28.13
Basaltic	VT66	93.44-269.26	156.16	165.92	0.25	18.29	6.36-7.24	6.80	6.79	0.03	3.44	28.22-72.36	49.83	49.77	0.18	-12.84
Basaltic		**38.34-554.56**	**158.13**	**177.24**	**0.46**	**18.06**	**5.92-7.55**	**6.67**	**6.78**	**0.04**	**3.51**	**28.22-155.57**	**67.65**	**76.34**	**0.39**	**-22.29**
Granitic	NFDR	29.36-196.31	63.81	71.00	0.39	6.74	5.93-6.93	6.43	6.42	0.03	3.89	68.99-117.45	95.64	95.41	0.10	-15.20
Granitic	STAN	53.19-197.92	90.01	93.14	0.21	-2.87	5.99-7.00	6.69	6.60	0.04	3.54	32.98-56.06	42.38	43.04	0.10	9.45
Granitic	VT58	41.58-166.65	91.17	98.31	0.29	11.78	5.63-7.00	6.66	6.59	0.04	4.50	23.77-69.36	40.09	40.37	0.19	-8.06
Granitic	VT61	162.98-489.54	290.14	308.15	0.28	15.26	6.39-7.53	6.98	6.97	0.04	3.07	40.34-120.77	77.71	77.36	0.23	-17.19
Granitic	VT62	50.15-183.32	102.38	106.87	0.26	14.73	5.89-7.06	6.71	6.64	0.04	1.10	22.25-62.95	35.77	36.70	0.23	-13.13
Granitic		**29.36-489.54**	**96.22**	**135.56**	**0.72**	**14.92**	**5.63-7.53**	**6.65**	**6.54**	**0.05**	**3.24**	**22.25-120.77**	**46.38**	**58.61**	**0.44**	**-13.72**
Siliciclastic	DR01	-0.02 - 31.27	7.84	8.98	0.56	-11.83	5.10-5.79	5.43	5.43	0.02	3.35	76.75-124.84	100.07	97.86	0.11	-11.51
Siliciclastic	PAIN	3.59-26.33	12.94	13.10	0.41	-15.89	5.13-6.27	5.78	5.80	0.04	2.41	89.26-125.74	110.48	109.77	0.06	-10.40
Siliciclastic	VT36	-4.88-12.89	4.51	4.60	0.82	-66.99	4.93-5.74	5.41	5.40	0.03	4.81	61.45-111.15	85.87	84.63	0.13	-7.80
Siliciclastic	VT53	3.26-45.62	19.66	22.16	0.40	-0.43	5.32-6.30	5.99	5.94	0.03	3.60	59.14-125.83	96.20	93.84	0.14	-11.48
Siliciclastic	WOR1	10.27-225.50	29.26	40.39	0.72	12.73	5.23-7.01	6.09	6.09	0.04	5.28	59.31-93.54	79.62	78.81	0.07	-0.55
Siliciclastic		**-4.88-225.50**	**13.37**	**17.83**	**1.05**	**-3.54**	**4.93-7.01**	**5.74**	**5.73**	**0.06**	**3.90**	**59.14-125.83**	**93.40**	**93.02**	**0.16**	**-8.82**

Supplemental 2.3: Supplemental 3. Statistical results comparing water chemistry parameter mean (ANOVA) or median (Kruskal-Wallis) values among geologic classes from 1987-2017. A Shapiro test was used to assess the normality of data. Shapiro results at $\alpha \leq 0.05$ were considered non-parametric. Comparisons were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at $\alpha = 0.05$, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. All comparisons had 2 degrees of freedom. Values: SO_4^{2-} = sulfate concentration; ANC = acid neutralizing capacity; raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values. Bold p-values were significant at $\alpha \leq 0.05$.

		ANOVA/ Kruskal-Wallis		Tukey HSD/ Mann-Whitney Wilcoxon		
Parameter	**Shapiro (p)**	**F / chi-sq**	**p-value**	**B vs. G (p)**	**B vs. S (p)**	**G vs. S (p)**
SO_4^{2-} raw	**< 0.001**	460.57	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
SO_4^{2-} cov	0.448	2.80	0.108	NA	NA	NA
SO_4^{2-} Δ	0.178	1.17	0.349	NA	NA	NA
pH raw	**< 0.001**	1058.30	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
pH cov	**0.002**	2.20	0.333	NA	NA	NA
pH Δ	0.911	0.42	0.669	NA	NA	NA
ANC raw	**< 0.001**	1127.00	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
ANC cov	**0.043**	7.44	**0.024**	0.571	0.286	**0.024**
ANC Δ	**0.001**	7.21	**0.027**	0.190	0.110	0.190

Supplemental 2.4. Years in which samples were collected for 13 macroinvertebrate sites used in temporal analyses. An 'X' corresponds to a sample being collected in a year while a blank corresponds to a year without a sampling event.

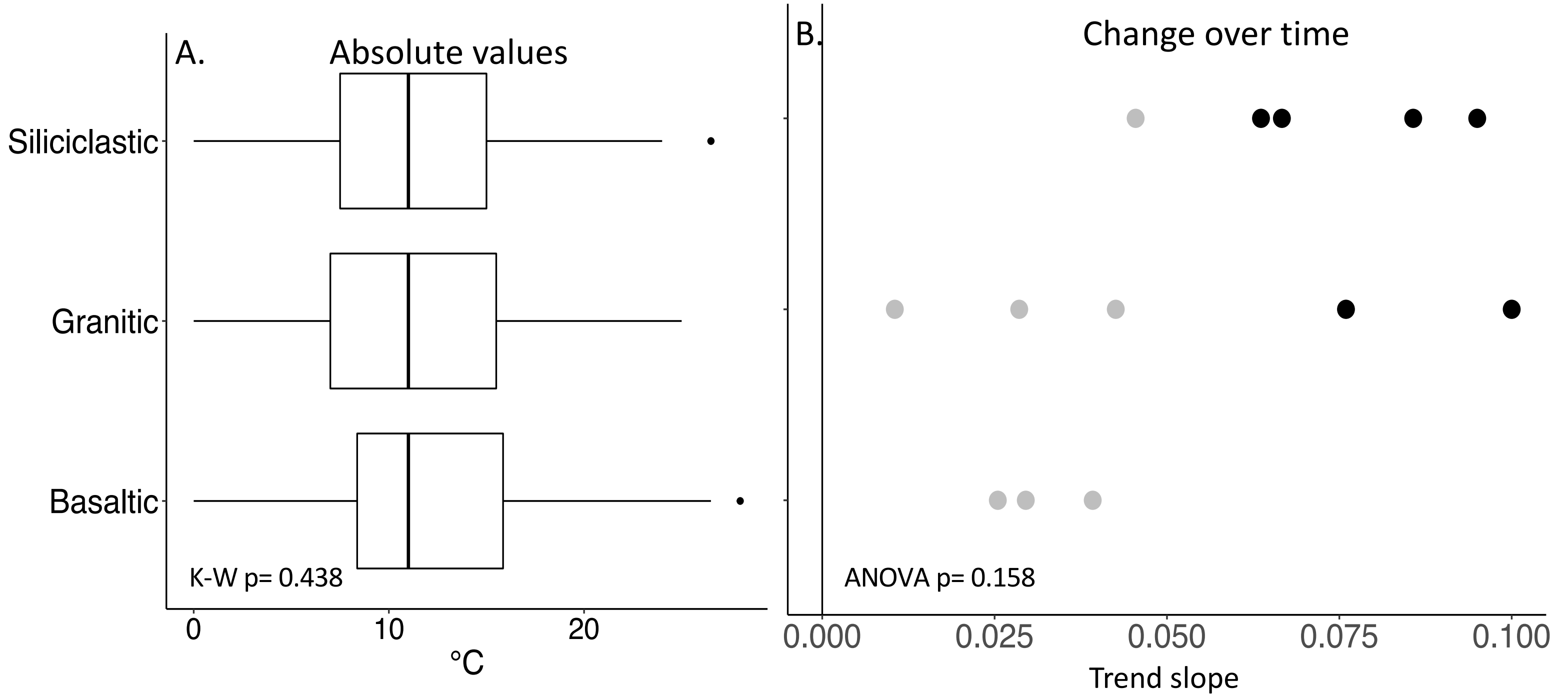
Site	1987	1988	1989	1990	1991	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
1F002	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
1F118	x	x	x	x	x	x			x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
1F308	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F009	x	x	x	x	x	x	x		x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F015	x	x	x	x	x	x	x		x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F055	x	x	x	x	x	x			x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F090	x	x	x	x	x	x			x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F131	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
2F306	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
3F103	x	x	x	x	x	x			x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
3F123	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x		x	x	x	x	x	x	x
3F300	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x
3F302	x	x	x	x	x	x			x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x

Supplemental 2.5. 10 macroinvertebrate and 7 water chemistry sites used for multivariate analyses. Not all macroinvertebrate sampling locations had an associated or nearby water chemistry monitoring location and were subsequently dropped from this data set. Some water chemistry sites were used for more than one macroinvertebrate sampling location if they were on the same stream reach. Distance (m) refers to the stream length between the macroinvertebrate and water chemistry sampling locations. Tributaries refer to any tributaries that enter the stream reach in between the macroinvertebrate and water chemistry sampling locations.

Geology	Macroinvertebrate site	Water chemistry site	Distance (m)	Tributary (Y/N)	# of tributaries
Basaltic	1F118	VT51	8031	N	0
Granitic	2F009	VT66	518	N	0
Granitic	2F015	VT66	5359	Y	2
Granitic	2F090	VT62	4522	Y	1
Granitic	2F131	NFDR	182	N	0
Granitic	2F306	STAN	6759	Y	1
Siliciclastic	3F103	VT53	366	N	0
Siliciclastic	3F123	PAIN	0	0	0
Siliciclastic	3F300	PAIN	3685	Y	1
Siliciclastic	3F302	VT53	4023	N	0

Supplemental 2.6. Statistics from trend analysis of water chemistry variables related to acidification in SNP from 1987-2017 using a Seasonal-Kendall Test (S-KT) for monotonic trends by site and geologic class. Summaries for geologic classes are the average values for all sites within a geologic class. Tau estimates for the S-KT range form -1-1 depending on the strength and direction of the trend; -1 indicating a negative trend and 1 indicating a positive trend. Sen slope estimates correspond to the concentration change per year. Chi p corresponds to the seasonal equality of tau estimates. Z p corresponds to the significance of the monotonic trend. Bold p values were significant at $\alpha \le 0.05$. Values: SO_4^{2-} = sulfate concentration; ANC = acid neutralizing capacity. Bold p-values were significant at $\alpha \le 0.05$.

Geology	Site	ANC tau	ANC slope	ANC chi p	ANC z p	pH tau	pH slope	pH chi p	pH z p	SO_4^{2-} tau	SO_4^{2-} slope	SO_4^{2-} chi p	SO_4^{2-} z p
Basaltic	MAD2	0.20	0.56	**0.028**	**0.003**	0.42	0.010	0.772	**< 0.001**	-0.22	-0.24	**0.046**	**0.001**
Basaltic	VT51	0.24	1.35	0.466	**< 0.001**	0.31	0.006	0.704	**< 0.001**	-0.57	-1.06	0.971	**< 0.001**
Basaltic	VT66	0.31	0.95	0.202	**< 0.001**	0.28	0.008	0.712	**< 0.001**	-0.18	-0.21	**0.077**	**0.005**
Basaltic		**0.25**	**0.95**			**0.34**	**0.008**			**-0.32**	**-0.50**		
Granitic	NFDR	0.09	0.14	0.386	0.183	0.37	0.008	0.255	**< 0.001**	-0.35	-0.48	0.738	**< 0.001**
Granitic	STAN	-0.06	-0.09	0.145	0.356	0.26	0.008	0.189	**< 0.001**	0.21	0.13	**0.015**	**0.001**
Granitic	VT58	0.18	0.36	0.559	**0.005**	0.26	0.010	0.765	**< 0.001**	-0.11	-0.11	**0.002**	0.092
Granitic	VT61	0.26	1.48	0.742	**< 0.001**	0.26	0.007	0.433	**< 0.001**	-0.24	-0.45	0.637	**0.000**
Granitic	VT62	0.19	0.50	0.547	**0.003**	0.09	0.002	0.743	0.168	-0.15	-0.16	**0.038**	**0.021**
Granitic		**0.13**	**0.48**			**0.25**	**0.007**			**-0.13**	**-0.21**		
Siliciclastic	DR01	-0.06	-0.03	**0.066**	0.268	0.27	0.006	0.215	**< 0.001**	-0.37	-0.38	0.604	**< 0.001**
Siliciclastic	PAIN	-0.11	-0.07	**0.029**	**0.071**	0.20	0.005	0.175	**0.001**	-0.40	-0.38	0.750	**< 0.001**
Siliciclastic	VT36	-0.20	-0.10	0.843	**0.001**	0.31	0.009	0.352	**< 0.001**	-0.19	-0.22	0.664	**0.003**
Siliciclastic	VT53	-0.01	0.00	0.355	0.937	0.22	0.007	0.982	**0.001**	-0.31	-0.37	0.434	**< 0.001**
Siliciclastic	WOR1	0.10	0.12	0.218	0.139	0.30	0.011	0.754	**< 0.001**	-0.02	-0.01	0.521	0.827
Siliciclastic		**-0.06**	**-0.02**			**0.26**	**0.007**			**-0.26**	**-0.27**		



Supplemental 2.7. Water temperature absolute values **(A.)** and temporal trends (1987-2017) **(B.)** across all sites and years by geologic class. Temporal trends were assessed with a Seasonal-Kendall Trend Test and Sen slope estimates. Sen slope values are displayed in strip charts and colored by Tau significance. Sites with Tau ≤ 0.05 are designated with by black points while sites with Tau > 0.05 are designated by gray points. A positive slope corresponds with increasing values over time and a negative slope corresponds to decreasing values over time. Comparisons of absolute values and slope trends (recovery) among geologic classes were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at $\alpha \leq 0.05$, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively.

Supplemental 2.8. Statistical results comparing water temperature absolute value and trend mean (ANOVA) or median (Kruskal-Wallis) values among geologic classes from 1987-2017**.** Shapiro results at α ≤ 0.05 were considered non-parametric. Comparisons were completed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis Test for parametric and nonparametric data, respectively. If model results were significant at α = 0.05, post-hoc analyses were completed using a Tukey HSD or Wilcoxon Rank Sum test for parametric and nonparametric data, respectively. All comparisons had 2 degrees of freedom. Values: raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values. Bold p-values were significant at α ≤ 0.05.

		ANOVA/ Kruskal-Wallis		Tukey HSD/ Mann-Whitney Wilcoxon		
Parameter	Shapiro (p)	F / chi-sq	p-value	B vs. G (p)	B vs. S (p)	G vs. S (p)
Temp raw	**<0.001**	1.65	0.438	NA	NA	NA
Temp tau	0.145	1.79	0.216	NA	NA	NA
Temp slope	0.573	2.23	0.158	NA	NA	NA
Temp cov	**<0.001**	1.6	0.449	NA	NA	NA
Temp Δ	0.523	4.94	0.523	NA	NA	NA

Supplemental 2.9. Summary and trend analysis statistics of water temperature in SNP from 1987-2017 by site and geologic class. Summaries for geologic classes are either entire ranges or average values for all sites within a geologic class. Tau estimates for the S-KT range form -1-1 depending on the strength and direction of the trend; -1 indicating a negative trend and 1 indicating a positive trend. Sen slope estimates correspond to the concentration change per year. Chi p corresponds to the seasonal equality of tau estimates. Z p corresponds to the significance of the monotonic trend. Bold p values were significant at α ≤ 0.05. Values: raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values. Bold p-values significant at α ≤ 0.05.

Geology	Site	Temp range	Temp median	Temp mean	Temp cov	Temp% Δ	Temp tau	Temp slope	Temp chi p	Temp z p
Basaltic	MAD2	0-23	11.8	11.7	0.5	7.5	0.09	0.03	0.834	0.174
Basaltic	VT51	0-28	11.0	11.7	0.6	10.7	0.11	0.04	0.794	0.099
Basaltic	VT66	0-23	11.0	11.2	0.5	6.9	0.11	0.03	0.924	0.082
Basaltic		**0-28**	**11.3**	**11.6**	**0.5**	**8.4**	**0.11**	**0.03**		
Granitic	NFDR	0-23	10.1	10.7	0.5	8.5	0.09	0.03	0.491	0.154
Granitic	STAN	0-24	11.0	11.6	0.5	2.9	0.07	0.01	0.692	0.329
Granitic	VT58	0-24.5	11.0	11.2	0.6	11.6	0.11	0.04	0.479	0.107
Granitic	VT61	0-25	11.5	11.4	0.6	26.1	0.25	0.10	0.416	**<0.001**
Granitic	VT62	0-23.5	10.0	10.8	0.6	22.8	0.18	0.08	0.845	**0.010**
Granitic		**0-25**	**10.7**	**11.1**	**0.5**	**14.4**	**0.14**	**0.05**		
Siliciclastic	DR01	0-24	11.5	11.4	0.5	16.6	0.16	0.06	0.262	**0.016**
Siliciclastic	PAIN	0-26.5	11.0	11.8	0.5	23.5	0.22	0.09	0.170	**0.001**
Siliciclastic	VT36	0-23	10.4	10.7	0.5	27.4	0.24	0.10	0.261	**<0.001**
Siliciclastic	VT53	0-23	10.0	10.9	0.6	13.6	0.11	0.05	0.926	0.122
Siliciclastic	WOR1	2-24	11.7	11.8	0.5	17.2	0.19	0.07	0.565	**0.005**
Siliciclastic		**0-28**	**10.9**	**11.3**	**0.5**	**19.7**	**0.18**	**0.07**		

Supplemental 2.10. Summary statistics of macroinvertebrate density and taxa-based metrics from 1987-2017 by site and geologic class. Summaries for geologic classes are either entire ranges or average values for all sites within a geologic class. raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values

							Taxa														
Geology	**Site**	**Density range**	**Density median**	**Density mean**	**Density cov**	**Density Δ**	**Richness range**	**Richness median**	**Richness mean**	**Richness cov**	**Richness Δ**	**Shannon range**	**Shannon median**	**Shannon mean**	**Shannon cov**	**Shannon Δ**	**Evenness range**	**Evenness median**	**Evenness mean**	**Evenness cov**	**Evenness Δ**
Basaltic	1F118	42-437	196	193±117	0.61	8	16-53	28	30±9	0.31	57	1.3-2.9	2.6	2.5±0.3	0.13	8	0.44-0.88	0.75	0.75±0.09	0.12	-9
Basaltic	1F308	28-362	124	144±86	0.60	14	11-47	28	30±10	0.32	63	2-3	2.6	2.6±0.2	0.08	11	0.67-0.86	0.78	0.78±0.06	0.07	-4
Basaltic	2F055	22-295	134	132±72	0.54	9	12-41	29	28±8	0.28	40	2.1-2.9	2.5	2.5±0.2	0.08	-1	0.63-0.89	0.74	0.75±0.07	0.10	-18
Basaltic		**22-437**	**151**	**156**	**0.58**	**10**	**11-53**	**28**	**29**	**0.30**	**54**	**1.3-2.9**	**2.6**	**2.5**	**0.10**	**6**	**.44-.89**	**0.76**	**0.76**	**0.10**	**-10**
Granitic	1F002	27-254	115	111±57	0.52	8	13-42	25	27±8	0.29	35	2.1-3.1	2.6	2.6±0.2	0.08	5	0.71-0.94	0.81	0.81±0.06	0.08	-10
Granitic	2F009	14-379	112	144±98	0.68	16	5-43	27	26±10	0.40	65	1.2-2.8	2.4	2.3±0.4	0.17	25	0.62-0.98	0.71	0.74±0.09	0.12	3
Granitic	2F015	44-323	148	158±90	0.57	7	18-43	29	30±8	0.25	34	2-3	2.6	2.6±0.2	0.09	16	0.57-0.92	0.79	0.78±0.08	0.10	5
Granitic	2F090	23-470	106	155±114	0.73	17	18-48	30	32±9	0.29	59	1.9-3	2.7	2.7±0.2	0.08	0	0.5-0.98	0.79	0.78±0.1	0.12	-15
Granitic	2F131	11-255	96	112±68	0.60	21	9-53	25	26±10	0.38	80	1.8-3	2.6	2.5±0.3	0.12	23	0.72-0.95	0.79	0.8±0.05	0.07	-3
Granitic	2F306	10-325	109	119±78	0.66	11	9-39	26	27±7	0.28	47	2.2-3.1	2.6	2.6±0.2	0.08	-1	0.68-0.99	0.80	0.82±0.09	0.11	-26
Granitic		**10-470**	**114**	**133**	**0.63**	**13**	**5-48**	**27**	**28**	**0.31**	**53**	**1.2-3.1**	**2.6**	**2.6**	**0.10**	**11**	**.50-.99**	**0.78**	**0.79**	**0.10**	**-8**
Siliciclastic	3F103	29-368	144	164±98	0.60	8	10-42	25	27±9	0.34	45	1.3-2.9	2.3	2.3±0.4	0.16	28	0.48-0.91	0.73	0.72±0.09	0.13	10
Siliciclastic	3F123	41-515	128	197±142	0.72	13	10-45	22	25±9	0.37	74	1.1-2.7	2.0	2±0.4	0.20	28	0.37-0.81	0.63	0.64±0.11	0.18	5
Siliciclastic	3F300	5-771	108	153±150	0.98	8	4-43	17	20±8	0.42	49	1-2.6	2.1	2±0.4	0.19	20	0.46-0.96	0.69	0.69±0.12	0.17	12
Siliciclastic	3F302	13-735	130	180±162	0.90	9	7-52	21	23±10	0.42	54	1.5-2.5	2.0	2±0.3	0.13	22	0.48-0.94	0.66	0.67±0.11	0.17	-12
Siliciclastic		**5-771**	**128**	**174**	**0.80**	**9**	**4-52**	**21**	**24**	**0.38**	**56**	**1.1-2.9**	**2.1**	**2.1**	**0.17**	**24**	**.37-.96**	**0.68**	**0.68**	**0.16**	**4**

Supplemental 2.11. Summary statistics of macroinvertebrate trait-based metrics from 1987-2017 by site and geologic class. Summaries for geologic classes are either entire ranges or average values for all sites within a geologic class. Values: raw = absolute values; cov = coefficient of variation; Δ = percent change from 1987-2017; calculated by multiplying slope values by 30 (1987-2017) and dividing by median values.

Trait																
Geology	Site	Richness range	Richness median	Richness mean	Richness cov	Richness Δ	Shannon range	Shannon median	Shannon mean	Shannon cov	Shannon Δ	Evenness range	Evenness median	Evenness mean	Evenness cov	Evenness Δ
Basaltic	1F118	48-55	53	53±2	0.029	3	3.4-3.7	3.6	3.6±0.1	0.02	-1	0.86-0.94	0.92	0.91±0.02	0.02	-8
Basaltic	1F308	51-56	54	54±1	0.022	4	3.6-3.8	3.7	3.7±0	0.01	1	0.9-0.93	0.92	0.92±0.01	0.01	-3
Basaltic	2F055	45-56	53	52±3	0.050	5	3.5-3.7	3.6	3.6±0.1	0.02	-1	0.87-0.94	0.91	0.91±0.02	0.02	-15
Basaltic		**45-56**	**53**	**53**	**0.03**	**4**	**3.4-3.8**	**3.6**	**3.6**	**0.01**	**-1**	**.86-.94**	**0.91**	**0.91**	**0.01**	**-8**
Granitic	1F002	49-56	53	53±2	0.029	0	3.6-3.7	3.7	3.7±0	0.01	-1	0.9-0.94	0.92	0.92±0.01	0.01	-9
Granitic	2F009	39-56	54	53±4	0.068	5	3.4-3.7	3.6	3.6±0.1	0.02	1	0.89-0.95	0.91	0.91±0.01	0.01	3
Granitic	2F015	52-55	54	53±1	0.019	2	3.6-3.7	3.6	3.6±0	0.01	-1	0.9-0.94	0.92	0.92±0.01	0.01	4
Granitic	2F090	51-56	53	54±1	0.025	0	3.5-3.8	3.7	3.7±0	0.01	-2	0.88-0.95	0.93	0.92±0.01	0.01	-13
Granitic	2F131	43-55	54	53±3	0.051	4	3.4-3.7	3.7	3.7±0.1	0.02	0	0.90-0.94	0.92	0.92±0.01	0.01	-2
Granitic	2F306	42-55	53	53±3	0.049	4	3.6-3.8	3.7	3.7±0	0.01	-1	0.90-0.95	0.92	0.92±0.01	0.01	-23
Granitic		**42-56**	**54**	**53**	**0.04**	**2**	**3.4-3.8**	**3.7**	**3.7**	**0.01**	**-1**	**.90-.95**	**0.92**	**0.92**	**0.01**	**-7**
Siliciclastic	3F103	45-55	53	53±3	0.048	4	3.5-3.7	3.6	3.6±0.1	0.02	2	0.87-0.93	0.91	0.91±0.01	0.02	8
Siliciclastic	3F123	46-55	53	53±2	0.035	2	3.4-3.7	3.6	3.6±0.1	0.02	2	0.86-0.93	0.90	0.9±0.02	0.02	3
Siliciclastic	3F300	40-55	51	50±4	0.071	3	3.3-3.7	3.5	3.5±0.1	0.02	0	0.87-0.97	0.90	0.9±0.02	0.02	9
Siliciclastic	3F302	41-55	51	50±3	0.066	6	3.4-3.7	3.5	3.5±0	0.01	1	0.87-0.95	0.90	0.9±0.02	0.02	-9
Siliciclastic		**40-55**	**52**	**52**	**0.05**	**4**	**3.4-3.7**	**3.6**	**3.6**	**0.02**	**1**	**.86-.97**	**0.90**	**0.90**	**0.02**	**3**

Supplemental 2.12. Statistics from trend analysis of taxa and trait-based macroinvertebrate community metrics from 1987-2017 using a Mann-Kendall Test (M-KT) for monotonic trends by site and geologic class. Summaries for geologic classes are the average values for all sites within a geologic class. Tau estimates for the M-KT range form -1-1 depending on the strength and direction of the trend; -1 indicating a negative trend and 1 indicating a positive trend. Sen slope estimates correspond to the concentration change per year. Chi p corresponds to the seasonal equality of tau estimates. Z p corresponds to the significance of the monotonic trend. Bold p-values were significant at α ≤ 0.05.

					Taxa									Trait									
Geology	Site	Density tau	Density slope	Density p	Richness tau	Richness slope	Richness p	Shannon tau	Shannon slope	Shannon p	Evenness tau	Evenness slope	Evenness p	Richness tau	Richness slope	Richness p	Shannon tau	Shannon slope	Shannon p	Evenness tau	Evenness slope	Evenness p
Basaltic	1F118	0.45	0.52	**0.001**	0.45	0.52	**0.001**	0.22	0.01	0.113	-0.26	-0.003	0.055	0.37	0.06	**0.006**	-0.16	-0.001	0.252	-0.19	-0.002	0.172
Basaltic	1F308	0.41	0.59	**0.002**	0.41	0.59	**0.002**	0.41	0.01	**0.002**	-0.19	-0.002	0.172	0.44	0.08	**0.001**	0.13	0.001	0.320	-0.14	-0.001	0.302
Basaltic	2F055	0.33	0.39	**0.019**	0.33	0.39	**0.019**	-0.03	0.00	0.860	-0.14	-0.001	0.302	0.31	0.09	**0.023**	-0.15	-0.001	0.290	-0.43	-0.004	**0.002**
Basaltic		**0.40**	**0.50**		**0.40**	**0.50**		**0.20**	**0.01**		**-0.20**	**-0.002**		**0.37**	**0.08**		**-0.06**	**-0.001**		**-0.26**	**-0.003**	
Granitic	1F002	0.21	0.29	0.127	0.21	0.29	0.127	0.15	0.00	0.277	0.04	0.001	0.802	0.07	0.00	0.596	-0.38	-0.002	**0.005**	-0.26	-0.003	**0.055**
Granitic	2F009	0.34	0.58	**0.013**	0.34	0.58	**0.013**	0.40	0.02	**0.004**	0.10	0.001	0.478	0.31	0.08	**0.019**	0.18	0.001	0.196	0.04	0.001	0.802
Granitic	2F015	0.31	0.33	**0.023**	0.31	0.33	**0.023**	0.40	0.01	**0.004**	-0.43	-0.004	**0.002**	0.26	0.04	**0.047**	-0.26	-0.001	**0.055**	0.10	0.001	0.478
Granitic	2F090	0.42	0.58	**0.003**	0.42	0.58	**0.003**	0.02	0.00	0.930	-0.27	-0.004	**0.052**	0.15	0.00	0.282	-0.33	-0.002	**0.019**	-0.27	-0.004	**0.052**
Granitic	2F131	0.42	0.67	**0.002**	0.42	0.67	**0.002**	0.40	0.02	**0.003**	-0.09	-0.001	0.511	0.28	0.07	**0.029**	0.00	0.000	0.985	-0.09	-0.001	0.511
Granitic	2F306	0.33	0.41	**0.011**	0.33	0.41	**0.011**	-0.03	0.00	0.807	-0.50	-0.007	**<0.001**	0.32	0.08	**0.013**	-0.29	-0.001	**0.031**	-0.50	-0.007	**<0.001**
Granitic		**0.34**	**0.48**		**0.34**	**0.48**		**0.22**	**0.01**		**-0.19**	**-0.002**		**0.23**	**0.04**		**-0.18**	**-0.001**		**-0.16**	**-0.002**	
Siliciclastic	3F103	0.30	0.38	**0.027**	0.30	0.38	**0.027**	0.42	0.02	**0.002**	0.19	0.002	0.182	0.27	0.07	**0.044**	0.24	0.002	**0.080**	0.19	0.002	0.182
Siliciclastic	3F123	0.41	0.54	**0.002**	0.41	0.54	**0.002**	0.32	0.02	**0.016**	0.04	0.001	0.750	0.25	0.04	**0.046**	0.23	0.002	**0.088**	0.04	0.001	0.750
Siliciclastic	3F300	0.22	0.28	**0.090**	0.22	0.28	0.090	0.29	0.01	**0.028**	0.15	0.003	0.268	0.14	0.06	**0.297**	0.04	0.000	0.778	0.15	0.003	0.268
Siliciclastic	3F302	0.31	0.38	**0.025**	0.31	0.38	**0.025**	0.28	0.01	**0.045**	-0.06	-0.003	0.677	0.25	0.11	0.066	0.11	0.001	0.453	-0.06	-0.003	0.677
Siliciclastic		**0.31**	**0.39**		**0.31**	**0.39**		**0.33**	**0.02**		**0.08**	**0.00**		**0.23**	**0.07**		**0.15**	**0.001**		**0.08**	**0.001**	

Supplemental 2.13. Ten most positive and negative correlation coefficients for each density or richness weighted macroinvertebrate taxa or trait-based NMDS ordination for all streams across all years.

Geology	Taxa/trait	Richness/density	NMDS 1 "species"	NMDS 1 score	NMDS 2 "species"	NMDS 2 score	NMDS 3 "species"	NMDS 3 score
all	**taxa**	**density**	**27%**		**20%**		**18%**	
			Hansonoperla	-1.60	*Parapsyche*	-2.27	Limnephilidae	-2.77
			Malirekus	-1.23	Leptoceridae	-1.95	*Stilobezzia*	-1.90
			Pericoma	-1.13	Limnephilidae	-1.42	*Ceraclea*	-1.73
			Parapsyche	-1.03	*Cambarus*	-0.92	*Baetidae*	-1.69
			Annelida	-1.00	*Pilaria*	-0.86	Taeniopterygidae	-1.57
			Neoplasta	-0.99	*Eccoptura*	-0.86	*Caecidotea*	-1.55
			Stactobiella	-0.86	*Micrasema*	-0.81	*Dasyhelea*	-1.46
			Palaeagapetus	-0.86	Cambaridae	-0.81	*Fattigia*	-1.43
			Bivalvia	-0.86	*Pseudolimnophila*	-0.77	Ostracoda	-1.42
			Paragnetina	-0.84	*Malirekus*	-0.74	*Cryptolabis*	-1.30
			Cambaridae	0.72	*Isopoda*	0.57	*Hansonoperla*	0.53
			Dixa	0.72	*Psilotreta*	0.58	*Cambarus*	0.70
			Nyctiophylax	0.75	Asellidae	0.58	*Heptagenia*	0.73
			Centroptilum	0.86	Baetidae	0.66	Elmidae	0.74
			Eurylophella	0.90	*Isonychia*	0.69	*Limnophila*	0.86
			Stenacron	0.99	*Serratella*	0.74	*Cyrnellus*	1.04
			Habrophlebia	1.22	*Stylogomphus*	0.84	*Pycnopsyche*	1.08
			Leptoceridae	1.24	Annelida	0.91	*Parapsyche*	1.36
			Stylogomphus	1.82	*Centroptilum*	1.35	*Peltoperla*	1.59
			Limnephilidae	2.29	Tabanidae	1.76	*Leptophlebia*	1.95
all	**trait**	**density**	**3%**		**2%**		**1%**	
			resist.dry	-0.23	aerial	-0.43	free.and.attach	-0.61
			warm	-0.21	heavy.armor	-0.21	tegument	-0.13

			shredder	-0.20	collect-filter	-0.16	ws.emergence	-0.13
			high.dispersal	-0.19	some.attach	-0.16	shredder	-0.13
			abundant.drift	-0.19	sprawl	-0.13	common.drft	-0.10
			free.and.attach	-0.19	stong.flight	-0.12	l.crawl	-0.09
			vl.crawl	-0.15	herbivore	-0.10	adults.exit	-0.09
			multivoltine	-0.13	ps.emergence	-0.05	ss.growth	-0.09
			ns.growth	-0.12	rare.drift	-0.05	univoltine	-0.08
			small	-0.12	streamline	-0.04	weak.swim	-0.08
			h.crawl	0.13	predator	0.05	vl.crawl	0.09
			vs.adult.life	0.16	vs.adult.life	0.10	strong.swim	0.09
			herbivore	0.17	some.armor	0.10	abundant.drift	0.10
			strong.swim	0.18	warm	0.11	large	0.10
			some.armor	0.19	burrow	0.12	high.dispersal	0.10
			semivoltine	0.22	ss.growth	0.13	resist.dry	0.10
			ss.growth	0.23	free.and.attach	0.15	herbivore	0.10
			heavy.armor	0.33	adults.exit	0.26	heavy.armor	0.14
			adults.exit	0.39	ws.emergence	0.32	deposition	0.17
			ws.emergence	0.44	deposition	0.40	aerial	0.45
all	**trait**	**richness**	**2%**		**1%**		**1%**	
			warm	-0.36	warm	-0.33	aerial	-1.09
			abundant.drift	-0.15	deposition	-0.26	ns.growth	-0.29
			fs.growth	-0.14	cling	-0.22	l.adult.life	-0.22
			shredder	-0.12	predator	-0.10	strong.swim	-0.16
			adults.exit	-0.11	stong.flight	-0.08	deposition	-0.15
			vl.crawl	-0.11	h.crawl	-0.06	resist.dry	-0.10
			cold	-0.10	ps.emergence	-0.05	ps.emergence	-0.10
			vs.adult.life	-0.09	tegument	-0.05	abundant.drift	-0.08
			small	-0.09	fs.growth	-0.04	herbivore	-0.06
			multivoltine	-0.08	univoltine	-0.04	burrow	-0.06
			large	0.14	no.swim	0.04	cold	0.04

some.armor	0.15	adults.exit	0.04	rare.drift	0.04
h.crawl	0.16	abundant.drift	0.04	sprawl	0.07
heavy.armor	0.18	ns.growth	0.05	shredder	0.08
resist.dry	0.18	some.attach	0.07	stong.flight	0.08
aerial	0.18	semivoltine	0.14	erosion	0.09
free.and.attach	0.25	herbivore	0.16	some.attach	0.10
l.adult.life	0.26	aerial	0.27	cf	0.16
ns.growth	0.26	heavy.armor	0.36	cling	0.17
deposition	0.29	free.and.attach	0.44	free.and.attach	1.25

Supplemental 2.14. Ten most positive and negative correlation coefficients for each density or richness weighted macroinvertebrate taxa or trait-based NMDS ordination for all Basaltic streams across all years.

Geology	Taxa/trait	Richness/density	NMDS 1 "species"	NMDS 1 score	NMDS 2 "species"	NMDS 2 score
Basaltic	**taxa**	**density**	**25%**		**22%**	
			Agapetus	-0.71	*Eurylophella*	-1.22
			Capniidae	-0.58	*Wormaldia*	-0.93
			Optioservus	-0.58	Annelida	-0.86
			Simuliidae	-0.58	*Suwallia*	-0.86
			Remenus	-0.55	Tipulidae	-0.86
			Annelida	-0.50	Tubificidae	-0.86
			Suwallia	-0.50	*Glossosoma*	-0.48
			Tipulidae	-0.50	*Stenonema*	-0.45
			Tubificidae	-0.50	*Prosimulium*	-0.32
			Prosimulium	-0.50	Perlidae	-0.31
			Chloroperlidae	0.37	Acari	0.54
			Cambaridae	0.44	Turbellaria	0.69
			Hydropsychidae	0.46	*Dicranota*	0.70
			Habrophlebiodes	0.47	Capniidae	0.83
			Glossosoma	0.52	*Optioservus*	0.83
			Stenacron	0.55	Simuliidae	0.83
			Habrophlebia	0.78	*Fattigia*	1.00
			Heptagenia	0.79	Glossosomatidae	1.00
			Ephemera	1.38	Nematoda	1.00
			Ectopria	1.60	Ostracoda	1.00
Basaltic	**trait**	**density**	**3%**		**1%**	
			stong.flight	-0.35	aerial	-0.39
			cf	-0.34	ws.emergence	-0.11
			sprawl	-0.28	heavy.armor	-0.11

	some.attach	-0.17	ss.growth	-0.09
	tegument	-0.16	adults.exit	-0.08
	common.drft	-0.10	semivoltine	-0.06
	cool	-0.08	rare.drift	-0.05
	no.swim	-0.07	herbivore	-0.05
	heavy.armor	-0.07	ps.emergence	-0.04
	univoltine	-0.07	medium	-0.04
	strong.swim	0.08	common.drft	0.02
	some.armor	0.08	cool	0.03
	high.dispersal	0.08	abundant.drift	0.04
	multivoltine	0.09	vs.adult.life	0.05
	burrow	0.09	sprawl	0.05
	abundant.drift	0.10	tegument	0.06
	resist.dry	0.10	shredder	0.07
	warm	0.18	strong.swim	0.13
	aerial	0.38	warm	0.21
	deposition	0.69	deposition	0.36
Basaltic trait richness	**1%**		**2%**	
	deposition	-0.24	aerial	-0.35
	abundant.drift	-0.12	cling	-0.11
	strong.swim	-0.10	large	-0.08
	vl.crawl	-0.09	deposition	-0.07
	adults.exit	-0.08	h.crawl	-0.05
	multivoltine	-0.07	stong.flight	-0.03
	vs.adult.life	-0.07	l.adult.life	-0.03
	semivoltine	-0.06	strong.swim	-0.02
	fs.growth	-0.05	s.adult.life	-0.02
	herbivore	-0.05	heavy.armor	-0.02
	some.armor	0.06	climb	0.01
	cf	0.07	resist.dry	0.01

large	0.07	fs.growth	0.01
resist.dry	0.11	common.drft	0.02
stong.flight	0.12	ns.growth	0.02
warm	0.16	shredder	0.02
cling	0.16	cg	0.02
l.adult.life	0.18	vs.adult.life	0.02
aerial	0.32	l.crawl	0.03
ns.growth	0.32	warm	0.35

Supplemental 2.15. Ten most positive and negative correlation coefficients for each density or richness weighted macroinvertebrate taxa or trait-based NMDS ordination for all Granitic streams across all years.

Geology	Taxa/trait	Richness/density	NMDS 1 "species"	NMDS 1 score	NMDS 2 "species"	NMDS 2 score	NMDS3 "species"	NMDS 3 score
Granitic	taxa	density	23%		21%		19%	
			Habrophlebia	-1.89	*Parapsyche*	-2.61	*Leptophlebia*	-1.70
			Centroptilum	-1.60	*Cyrnellus*	-1.33	Tipulidae	-1.37
			Tabanidae	-1.57	*Leptophlebia*	-1.20	*Centroptilum*	-1.13
			Heptagenia	-0.96	Tipulidae	-1.18	*Nyctiophylax*	-1.06
			Habrophlebiodes	-0.88	*Pycnopsyche*	-1.15	*Atherix*	-1.02
			Leptophlebia	-0.87	*Heptagenia*	-1.01	Nematoda	-0.95
			Glossosoma	-0.54	*Eccoptura*	-0.68	*Fattigia*	-0.94
			Dolophilodes	-0.49	*Perlesta*	-0.64	*Plauditus*	-0.94
			Psephenus	-0.45	*Nyctiophylax*	-0.63	Taeniopterygidae	-0.94
			Leptophlebiidae	-0.43	*Hansonoperla*	-0.53	Ostracoda	-0.91
			Bivalvia	0.71	*Acentrella*	0.88	*Stenonema*	0.56
			Yugus	0.74	Asellidae	0.89	*Prosimulium*	0.57
			Peltoperlidae	0.76	Philopotamidae	0.98	*Stenelmis*	0.60
			Tubificidae	0.81	Ephemerellidae	1.01	*Hansonoperla*	0.67
			Neoplasta	0.86	Gammaridae	1.05	Planariidae	0.70
			Pericoma	0.92	Leptoceridae	1.11	*Chimarra*	0.80
			Eccoptura	0.99	*Taeniopteryx*	1.12	*Isonychia*	0.83
			Parapsyche	1.01	Ostracoda	1.16	*Tipula*	1.13
			Malirekus	1.19	*Fattigia*	1.19	*Pycnopsyche*	1.25
			Hansonoperla	1.39	*Caecidotea*	1.37	*Peltoperla*	3.01
Granitic	trait	density	3%		1%		1%	
			aerial	-0.47	aerial	-0.23	sprawl	-0.27
			resist.dry	-0.17	cf	-0.21	strong.swim	-0.21
			warm	-0.17	stong.flight	-0.17	stong.flight	-0.14

			abundant.drift	-0.14	some.attach	-0.16	cf	-0.10
			high.dispersal	-0.14	heavy.armor	-0.11	aerial	-0.10
			vl.crawl	-0.12	sprawl	-0.11	vs.adult.life	-0.07
			cf	-0.09	herbivore	-0.09	tegument	-0.07
			multivoltine	-0.09	medium	-0.08	shredder	-0.06
			ns.growth	-0.07	rare.drift	-0.06	ws.emergence	-0.05
			l.adult.life	-0.06	ps.emergence	-0.06	adults.exit	-0.04
			h.crawl	0.08	some.armor	0.08	medium	0.04
			semivoltine	0.10	ss.growth	0.08	rare.drift	0.05
			vs.adult.life	0.13	burrow	0.09	ps.emergence	0.06
			free.and.attach	0.18	high.dispersal	0.11	semivoltine	0.09
			burrow	0.19	warm	0.12	deposition	0.09
			some.armor	0.19	abundant.drift	0.13	cling	0.11
			ss.growth	0.26	resist.dry	0.13	herbivore	0.14
			adults.exit	0.36	adults.exit	0.14	free.and.attach	0.15
			deposition	0.39	ws.emergence	0.17	warm	0.16
			ws.emergence	0.42	deposition	0.36	heavy.armor	0.18
Granitic	**trait**	**richness**	**2%**		**1%**			
			warm	-0.46	aerial	-0.73	-	-
			abundant.drift	-0.14	ns.growth	-0.21	-	-
			adults.exit	-0.14	l.adult.life	-0.14	-	-
			fs.growth	-0.13	deposition	-0.10	-	-
			multivoltine	-0.12	resist.dry	-0.10	-	-
			small	-0.10	burrow	-0.07	-	-
			vs.adult.life	-0.09	ps.emergence	-0.06	-	-
			cold	-0.07	cg	-0.05	-	-
			vl.crawl	-0.07	deposition.and.erosion	-0.04	-	-
			streamline	-0.07	semivoltine	-0.04	-	-
			burrow	0.12	warm	0.06	-	-
			h.crawl	0.13	adults.exit	0.06	-	-

ns.growth	0.13	high.dispersal	0.06	-	-
large	0.13	erosion	0.07	-	-
resist.dry	0.14	some.attach	0.08	-	-
l.adult.life	0.14	sprawl	0.08	-	-
aerial	0.16	stong.flight	0.12	-	-
heavy.armor	0.16	cf	0.15	-	-
free.and.attach	0.21	cling	0.16	-	-
deposition	0.33	free.and.attach	0.46	-	-

Supplemental 2.16. Ten most positive and negative correlation coefficients for each density or richness weighted macroinvertebrate taxa or trait-based NMDS ordination for all Siliciclastic streams across all years.

Geology	Taxa/trait	Richness/density	NMDS 1 "species"	NMDS 1 score	NMDS 2 "species"	NMDS 2 score	NMDS3 "species"	NMDS 3 score
Siliciclastic	**taxa**	**density**	**28%**		**26%**		**21%**	
			Pycnopsyche	-2.34	*Stylogomphus*	-1.35	Limnephilidae	-1.97
			Limnephilidae	-2.32	*Stilobezzia*	-1.20	*Agnetina*	-1.69
			Leptoceridae	-1.82	Baetidae	-1.03	*Chimarra*	-1.13
			Stylogomphus	-0.90	Nematoda	-0.92	Simuliidae	-0.94
			Centroptilum	-0.76	*Dasyhelea*	-0.92	*Optioservus*	-0.77
			Cambaridae	-0.69	*Stenelmis*	-0.88	*Hydropsyche*	-0.73
			Peltoperla	-0.64	Aeshnidae	-0.85	*Isonychia*	-0.71
			Heptagenia	-0.54	Empididae	-0.85	*Isoperla*	-0.71
			Habrophlebia	-0.53	Hydropsychidae	-0.81	*Pycnopsyche*	-0.64
			Eurylophella	-0.42	*Cryptolabis*	-0.74	*Pteronarcys*	-0.59
			Maccaffertium	0.91	*Wormaldia*	0.67	*Stilobezzia*	0.73
			Leuctridae	0.93	Polycentropodidae	0.69	*Ectopria*	0.75
			Cryptolabis	0.93	*Alloperla*	0.70	*Pseudolimnophila*	0.83
			Stilobezzia	0.94	*Peltoperla*	0.77	Polycentropodidae	0.87
			Heptageniidae	0.95	Leptoceridae	0.80	*Bezzia*	0.97
			Turbellaria	0.98	*Chimarra*	0.89	Taeniopterygidae	1.05
			Acerpenna	0.98	*Pycnopsyche*	0.90	*Remenus*	1.06
			Baetidae	1.00	*Yugus*	0.94	Glossosomatidae	1.19
			Dasyhelea	1.11	*Micrasema*	1.00	*Alloperla*	1.22
			Ceraclea	1.33	*Isonychia*	1.15	*Capniidae*	1.28
Siliciclastic	**trait**	**density**	**2%**		**2%**		**2%**	
			heavy.armor	-0.39	deposition	-0.41	free.and.attach	-0.35
			aerial	-0.26	adults.exit	-0.27	deposition	-0.31
			herbivore	-0.25	ws.emergence	-0.26	ws.emergence	-0.27

			cling	-0.25	semivoltine	-0.14	adults.exit	-0.26
			deposition	-0.24	heavy.armor	-0.13	stong.flight	-0.12
			large	-0.23	resist.dry	-0.12	warm	-0.10
			some.armor	-0.20	abundant.drift	-0.11	h.crawl	-0.10
			adults.exit	-0.17	high.dispersal	-0.11	cling	-0.08
			strong.swim	-0.16	herbivore	-0.11	vs.adult.life	-0.08
			ws.emergence	-0.15	no.swim	-0.09	cf	-0.07
			cg	0.09	cling	0.08	large	0.03
			l.adult.life	0.10	univoltine	0.08	rare.drift	0.03
			small	0.10	tegument	0.09	vl.crawl	0.03
			cold	0.10	l.crawl	0.09	ps.emergence	0.04
			ns.growth	0.10	medium	0.09	some.attach	0.04
			multivoltine	0.10	rare.drift	0.10	adults.no.exit	0.05
			high.dispersal	0.11	weak.swim	0.11	semivoltine	0.08
			abundant.drift	0.12	shredder	0.12	herbivore	0.09
			shredder	0.13	free.and.attach	0.41	aerial	0.18
			resist.dry	0.13	aerial	0.61	heavy.armor	0.56
Siliciclastic	**trait**	**richness**	**2%**		**2%**			
			deposition	-0.36	free.and.attach	-1.17	-	-
			l.adult.life	-0.34	heavy.armor	-0.48	-	-
			ns.growth	-0.33	some.attach	-0.13	-	-
			stong.flight	-0.20	cf	-0.11	-	-
			h.crawl	-0.18	cling	-0.10	-	-
			some.armor	-0.15	sprawl	-0.09	-	-
			predator	-0.14	erosion	-0.09	-	-
			resist.dry	-0.14	semivoltine	-0.09	-	-
			cling	-0.14	large	-0.09	-	-
			ps.emergence	-0.13	rare.drift	-0.07	-	-
			cg	0.07	herbivore	0.05	-	-
			fs.growth	0.08	small	0.06	-	-

vl.crawl	0.12	fs.growth	0.06	-	-
cold	0.13	ps.emergence	0.07	-	-
adults.exit	0.13	common.drft	0.07	-	-
warm	0.15	streamline	0.09	-	-
abundant.drift	0.16	deposition	0.10	-	-
free.and.attach	0.16	abundant.drift	0.10	-	-
shredder	0.22	strong.swim	0.17	-	-
heavy.armor	0.41	warm	0.33	-	-

Supplemental 2.17. Correlation coefficients between 14 categorized environmental variables and the scores of axes I and axes II of density or richness weighted macroinvertebrate taxa or traits NMDS for all streams across all years. Bold p-values were significant at α ≤ 0.05.

	Taxa density				**Trait density**				**Trait richness**			
Parameter	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**
elevation	-0.50	-0.87	0.40	**0.001**	0.54	0.84	0.40	**0.001**	0.62	0.78	0.05	**0.003**
aspect	0.11	0.99	0.08	**0.001**	0.51	-0.86	0.08	**0.001**	0.90	-0.43	0.01	0.282
slope	0.21	-0.98	0.03	**0.031**	-0.09	-1.00	0.03	**0.031**	-0.91	0.41	0.10	**0.001**
ANC	-0.79	0.62	0.50	**0.001**	0.57	-0.82	0.50	**0.001**	0.22	0.97	0.24	**0.001**
pH	-0.91	0.42	0.64	**0.001**	0.81	-0.58	0.64	**0.001**	0.38	0.92	0.35	**0.001**
Cond	-0.38	0.93	0.24	**0.001**	0.18	-0.98	0.24	**0.001**	-0.15	0.99	0.08	**0.001**
Temp	-0.37	-0.93	0.01	0.288	0.01	1.00	0.01	0.288	-0.73	-0.68	0.01	0.33
SO4	1.00	-0.09	0.43	**0.001**	-0.97	-0.26	0.43	**0.001**	-0.50	-0.86	0.29	**0.001**
NO3	0.35	0.94	0.11	**0.001**	-0.02	-1.00	0.11	**0.001**	-0.69	0.73	0.02	0.139
Cl	-0.43	0.90	0.35	**0.001**	0.24	-0.97	0.35	**0.001**	0.09	1.00	0.13	**0.001**
Ca	-0.69	0.73	0.47	**0.001**	0.49	-0.87	0.47	**0.001**	0.15	0.99	0.22	**0.001**
Mg	-0.25	0.97	0.20	**0.001**	0.02	-1.00	0.20	**0.001**	-0.26	0.97	0.05	**0.006**
K	0.90	-0.43	0.62	**0.001**	-0.87	0.49	0.62	**0.001**	-0.34	-0.94	0.39	**0.001**
Na	-0.92	0.38	0.49	**0.001**	0.86	-0.51	0.49	**0.001**	0.31	0.95	0.31	**0.001**

Supplemental 2.18. Correlation coefficients between 14 categorized environmental variables and the scores of axes I and axes II of density or richness weighted macroinvertebrate taxa or traits NMDS for Basaltic streams across all years. Bold p-values were significant at α ≤ 0.05.

	Basaltic taxa density				**Basaltic trait density**				**Basaltic trait richness**			
Parameter	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**
elevation	0	0	0	1	0	0	0	1	0	0	0	1
aspect	0	0	0	1	0	0	0	1	0	0	0	1
slope	0	0	0	1	0	0	0	1	0	0	0	1
ANC	0.61	0.79	0.21	0.141	0.85	0.53	0.02	0.862	0.37	0.93	0.29	0.062
pH	-0.34	0.94	0.40	**0.01**	-0.46	-0.89	0.10	0.423	0.70	0.72	0.28	0.076
Cond	0.96	0.29	0.23	0.123	0.02	1.00	0.07	0.529	-0.02	1.00	0.13	0.299
Temp	0.29	0.96	0.19	0.177	-0.87	-0.49	0.04	0.686	0.36	0.93	0.21	0.142
SO4	0.54	-0.84	0.39	**0.017**	-0.22	0.97	0.25	0.085	-0.73	-0.68	0.21	0.119
NO3	0.83	-0.56	0.65	**0.001**	-0.17	0.99	0.28	0.059	-0.93	-0.36	0.25	0.071
Cl	0.28	0.96	0.14	0.293	-0.91	0.42	0.39	**0.018**	0.24	0.97	0.05	0.623
Ca	0.78	0.62	0.26	0.083	0.36	0.93	0.07	0.53	0.18	0.98	0.22	0.134
Mg	0.94	0.35	0.26	0.097	0.30	0.95	0.10	0.424	-0.21	0.98	0.16	0.219
K	0.94	-0.33	0.24	0.09	-0.59	0.81	0.16	0.228	-0.97	-0.24	0.01	0.934
Na	0.88	0.47	0.25	0.089	-0.10	1.00	0.04	0.69	0.15	0.99	0.16	0.244

Supplemental 2.19. Correlation coefficients of 14 categorized environmental variables and the scores of axes I and axes II of density or richness weighted macroinvertebrate taxa or traits NMDS for Granitic streams across all years. Bold p-values were significant at α ≤ 0.05.

	Granitic taxa density				**Granitic trait density**				**Granitic trait richness**			
Parameter	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**	**NMDS1**	**NMDS2**	**R2**	**p**
elevation	0.62	-0.79	0.64	**0.001**	0.97	0.26	0.67	**0.001**	0.43	-0.90	0.46	**0.001**
aspect	-0.89	-0.46	0.03	0.171	-0.89	0.45	0.11	**0.003**	-0.35	0.94	0.01	0.607
slope	-0.91	-0.41	0.05	0.058	-0.90	-0.44	0.15	**0.001**	-1.00	0.09	0.10	**0.003**
ANC	-0.33	0.94	0.31	**0.001**	-0.96	-0.29	0.13	**0.002**	-0.29	0.96	0.17	**0.001**
pH	0.02	1.00	0.16	**0.002**	-0.78	-0.63	0.00	0.813	0.25	0.97	0.11	**0.002**
Cond	-0.73	0.68	0.54	**0.001**	-0.96	-0.27	0.57	**0.001**	-0.57	0.82	0.37	**0.001**
Temp	0.87	0.49	0.06	**0.025**	0.79	-0.62	0.03	0.224	-0.13	-0.99	0.00	0.796
SO4	-1.00	-0.05	0.09	**0.004**	-0.90	-0.43	0.15	**0.001**	-0.90	0.43	0.06	**0.029**
NO3	-0.97	-0.24	0.27	**0.001**	-0.99	0.11	0.18	**0.001**	-0.95	0.32	0.10	**0.01**
Cl	-0.70	0.72	0.40	**0.001**	-0.96	-0.29	0.53	**0.001**	-0.46	0.89	0.28	**0.001**
Ca	-0.64	0.77	0.50	**0.001**	-0.94	-0.34	0.44	**0.001**	-0.53	0.85	0.34	**0.001**
Mg	-0.59	0.81	0.55	**0.001**	-0.97	-0.23	0.47	**0.001**	-0.42	0.91	0.37	**0.001**
K	0.49	-0.87	0.41	**0.001**	0.90	0.45	0.29	**0.001**	0.40	-0.92	0.28	**0.001**
Na	-0.33	-0.94	0.03	0.19	-1.00	0.00	0.01	0.487	-0.42	-0.91	0.01	0.588

Supplemental 2.20. Correlation coefficients between 14 categorized environmental variables and the scores of axes I and axes II of density or richness weighted macroinvertebrate taxa or traits NMDS for streams across all years. Bold p-values were significant at α ≤ 0.05.

	Siliciclastic taxa density				Siliciclastic trait density				Siliciclastic trait richness			
Parameter	NMDS1	NMDS2	R2	p	NMDS1	NMDS2	R2	p	NMDS1	NMDS2	R2	p
elevation	-0.35	0.94	0.43	**0.001**	0.71	0.71	0.44	**0.001**	0.92	-0.39	0.27	**0.001**
aspect	0.24	-0.97	0.43	**0.001**	-0.56	-0.83	0.32	**0.001**	-0.81	0.58	0.28	**0.001**
slope	-0.32	0.95	0.48	**0.001**	0.54	0.84	0.44	**0.001**	0.90	-0.44	0.31	**0.001**
ANC	1.00	-0.02	0.01	0.523	-0.97	-0.26	0.03	0.295	-0.69	-0.73	0.01	0.607
pH	0.97	0.25	0.18	**0.001**	-0.93	-0.37	0.08	**0.039**	-0.43	-0.90	0.11	**0.006**
Cond	-0.96	-0.29	0.05	0.077	0.78	-0.63	0.08	**0.04**	0.08	1.00	0.08	**0.022**
Temp	0.73	0.68	0.01	0.694	-0.98	-0.22	0.00	0.843	0.96	0.28	0.00	0.908
SO4	-0.98	0.22	0.00	0.916	0.82	-0.58	0.08	**0.036**	0.03	1.00	0.02	0.494
NO3	-0.92	-0.38	0.18	**0.001**	0.97	0.24	0.02	0.454	-0.07	1.00	0.06	0.08
Cl	-0.93	-0.36	0.06	0.071	0.62	-0.79	0.02	0.374	-0.23	0.97	0.02	0.396
Ca	0.62	0.78	0.01	0.813	-0.02	-1.00	0.01	0.55	-0.96	0.29	0.01	0.685
Mg	-0.95	-0.30	0.06	0.057	0.65	-0.76	0.07	**0.033**	-0.14	0.99	0.05	0.133
K	-0.94	-0.34	0.02	0.416	0.89	-0.46	0.06	0.073	0.10	0.99	0.05	0.136
Na	0.91	0.42	0.03	0.217	-1.00	-0.03	0.07	**0.033**	-0.06	-1.00	0.04	0.219

Supplemental 2.21. Indicator species analysis of all sites and years values for taxa and traits indicative of year groupings or specific combinations of year groupings. Year groupings: 1 = 1987-1991; 2 = 1992-1996; 3 = 1997-2001; 4 = 2002-2008; 5 = 2009-2013; 6 = 2014-2017. Bold p-values were significant at α ≤ 0.05.

All streams											
Taxa density				Trait density				Trait richness			
years	taxa	stat	p	years	trait	stat	p	years	trait	stat	p
	Heptagenia	0.557	**0.001**	2+3+4+5+6	slow seasonal growth	0.975	**0.001**	2+3+4+5+6	clingers	0.869	**0.001**
	Pycnopsyche	0.283	**0.009**		adults exit the stream	0.916	**0.001**		depositional	0.509	**0.029**
1	*Centroptilum*	0.245	**0.025**		clingers	0.875	**0.001**				
	Peltoperla	0.245	**0.03**		well synchronized emergence	0.868	**0.001**				
2	Perlodidae	0.475	**0.001**		heavily sclerotized	0.858	**0.001**				
	Suwallia	0.532	**0.001**		depositional	0.51	**0.042**				
3	Tubificidae	0.39	**0.001**								
	Heptageniidae	0.302	**0.012**								
	Ephemerellidae	0.234	**0.029**								
4	Pleuroceridae	0.374	**0.001**								
5	*Chimarra*	0.277	**0.005**								
6	*Maccaffertium*	0.791	**0.001**								
	Turbellaria	0.671	**0.001**								
	Ceratopogoninae	0.592	**0.001**								
	Bezzia	0.548	**0.001**								
	Acari	0.539	**0.001**								
	Leuctridae	0.5	**0.001**								
	Diphetor	0.474	**0.001**								
	Cryptolabis	0.418	**0.001**								
	Eurylophella	0.418	**0.001**								
	Gomphidae	0.416	**0.001**								

	Eccoptura	0.415	**0.001**
	Nematoda	0.415	**0.001**
	Acentrella	0.387	**0.001**
	Acerpenna	0.354	**0.001**
	Ostracoda	0.354	**0.001**
	Cambarus	0.316	**0.001**
	Dasyhelea	0.316	**0.002**
	Fattigia	0.316	**0.002**
	Malirekus	0.316	**0.002**
	Taeniopterygidae	0.316	**0.001**
	Glossosomatidae	0.313	**0.002**
	Psychomyia	0.299	**0.011**
	Pseudolimnophila	0.274	**0.013**
	Paragnetina	0.274	**0.007**
	Polycentropodidae	0.237	**0.025**
	Neoplasta	0.224	**0.034**
	Roederiodes	0.224	**0.036**
2+6	Gastropoda	0.316	**0.004**
3+4	*Lanthus*	0.443	**0.002**
	Gammaridae	0.384	**0.001**
3+6	Peltoperlidae	0.379	**0.004**
4+5	*Yugus*	0.299	**0.012**
4+6	*Hydroptila*	0.367	**0.002**
	Trichoptera	0.298	**0.005**
5+6	Capniidae	0.236	**0.045**
1+2+3	*Glossosoma*	0.502	**0.001**
1+2+6	*Perlesta*	0.447	**0.001**
1+4+6	*Stenacron*	0.387	**0.014**
2+3+4	*Chelifera*	0.324	**0.004**
3+4+5	Planariidae	0.545	**0.001**

	Oligochaeta	0.717	**0.001**
3+4+6	*Clinocera*	0.377	**0.001**
	Psilotreta	0.316	**0.02**
3+5+6	*Ameletus*	0.501	**0.002**
4+5+6	*Isonychia*	0.373	**0.004**
1+2+4+6	*Habrophlebia*	0.556	**0.027**
2+3+4+5	Ceratopogonidae	0.656	**0.001**
2+3+4+6	*Perlidae*	0.706	**0.001**
	Hemerodromia	0.455	**0.001**
3+4+5+6	*Amphinemura*	0.798	**0.001**
	Sweltsa	0.732	**0.001**
	Cinygmula	0.714	**0.001**
	Lepidostoma	0.611	**0.005**
1+2+3+4+5	*Stenonema*	0.704	**0.001**
1+2+3+4+6	*Polycentropus*	0.764	**0.001**
2+3+4+5+6	*Ephemerella*	0.97	**0.001**
	Epeorus	0.963	**0.001**
	Diplectrona	0.902	**0.001**
	Oulimnius	0.832	**0.001**
	Simulium	0.828	**0.001**
	Acroneuria	0.823	**0.001**
	Rhyacophila	0.821	**0.001**
	Hexatoma	0.806	**0.001**
	Chloroperlidae	0.801	**0.001**
	Paraleptophlebia	0.787	**0.001**
	Tallaperla	0.758	**0.001**
	Antocha	0.755	**0.001**
	Dolophilodes	0.729	**0.001**
	Isoperla	0.718	**0.001**
	Neophylax	0.703	**0.001**

Pteronarcys	0.664	**0.001**
Drunella	0.659	**0.011**
Hydropsyche	0.636	**0.004**
Blephariceral	0.558	**0.036**
Leucrocuta	0.555	**0.001**

Supplemental 2.22. Indicator species analysis of all Basaltic streams across all years to determine taxa and traits indicative of year groupings or specific combinations of year groupings. Year groupings: 1 = 1987-1991; 2 = 1992-1996; 3 = 1997-2001; 4 = 2002-2008; 5 = 2009-2013; 6 = 2014-2017. Bold p-values were significant at α ≤ 0.05.

Basaltic streams											
Taxa density				Trait density				Trait richness			
years	taxa	stat	p	years	trait	stat	p	years	trait	stat	p
1	*Heptagenia*	1	**0.001**	2+3+4+5+6	slow seasonal growth	0.985	**0.011**	-	-	-	-
2	*Tallaperla*	0.89	**0.012**		adults exit the stream	0.961	**0.034**				
4	*Isonychia*	0.775	**0.013**								
	Pleuroceridae	0.775	**0.017**								
6	*Ceratopogoninae*	0.866	**0.003**								
	Diphetor	0.866	**0.002**								
	Maccaffertium	0.866	**0.007**								
2+6	*Simulium*	0.913	**0.017**								
	Dolophilodes	0.896	**0.002**								
4+5	Planariidae	0.832	**0.003**								
5+6	*Neophylax*	0.823	**0.014**								
2+4+6	*Leucrocuta*	0.847	**0.024**								
2+3+4+6	*Pteronarcys*	0.922	**0.009**								
3+4+5+6	*Sweltsa*	0.872	**0.037**								
	Hexatoma	0.829	**0.027**								
2+3+4+5+6	*Epeorus*	0.989	**0.01**								
	Ephemerella	0.986	**0.003**								
	Blephariceraa	0.976	**0.012**								
	Isoperla	0.952	**0.007**								

Supplemental 2.23. Indicator species analysis of all Granitic streams across all years to determine taxa and traits indicative of year groupings or specific combinations of year groupings. Year groupings: 1 = 1987-1991; 2 = 1992-1996; 3 = 1997-2001; 4 = 2002-2008; 5 = 2009-2013; 6 = 2014-2017. Bold p-values were significant at α ≤ 0.05.

Granitic streams											
Taxa density				Trait density				Trait richness			
years	taxa	stat	p	years	trait	stat	p	years	trait	stat	p
1	*Heptagenia*	0.566	**0.001**	2+3+4+6	warm water	0.699	**0.002**	2+3+4+5+6	clingers	0.868	**0.001**
	Pycnopsyche	0.346	**0.025**	2+3+4+5+6	clingers	0.867	**0.001**		warm water	0.692	**0.005**
	Tipulidae	0.346	**0.024**								
2	Perlodidae	0.465	**0.02**								
3	*Suwallia*	0.577	**0.001**								
4	Planariidae	0.493	**0.001**								
	Pleuroceridae	0.4	**0.006**								
5	*Chimarra*	0.341	**0.026**								
6	*Maccaffertium*	0.742	**0.001**								
	Turbellaria	0.707	**0.001**								
	Bezzia	0.632	**0.001**								
	Acentrella	0.548	**0.001**								
	Diphetor	0.548	**0.001**								
	Acari	0.51	**0.002**								
	Ceratopogoninae	0.5	**0.001**								
	Leuctridae	0.5	**0.001**								
	Habrophlebia	0.493	**0.002**								
	Peltoperlidae	0.473	**0.002**								
	Malirekus	0.447	**0.001**								
	Ostracoda	0.447	**0.002**								
	Eccoptura	0.39	**0.008**								

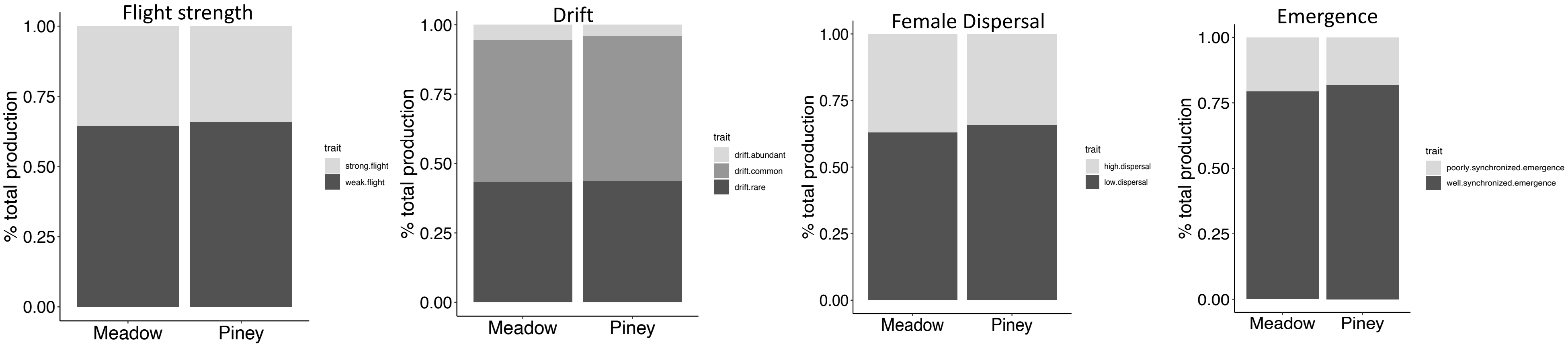
	Fattigia	0.387	**0.006**
	Paragnetina	0.386	**0.005**
	Psychomyia	0.368	**0.014**
	Alloperla	0.316	**0.013**
	Neoplasta	0.316	**0.024**
	Roederiodes	0.316	**0.021**
1+3	*Glossosoma*	0.506	**0.013**
1+6	*Perlesta*	0.483	**0.009**
2+6	Gastropoda	0.349	**0.018**
	Philopotamidae	0.271	**0.05**
3+4	*Hydroptila*	0.357	**0.018**
4+5	*Yugus*	0.402	**0.016**
4+6	Oligochaeta	0.674	**0.001**
	Promoresia	0.417	**0.049**
	Trichoptera	0.333	**0.028**
5+6	*Isonychia*	0.489	**0.008**
2+3+4	*Chelifera*	0.408	**0.01**
2+4+6	*Tallaperla*	0.734	**0.006**

Supplemental 2.24. Indicator species analysis of all Siliciclastic streams across all years to determine taxa and traits indicative of year groupings or specific combinations of year groupings. Year groupings: 1 = 1987-1991; 2 = 1992-1996; 3 = 1997-2001; 4 = 2002-2008; 5 = 2009-2013; 6 = 2014-2017. Bold p-values were significant at α ≤ 0.05.

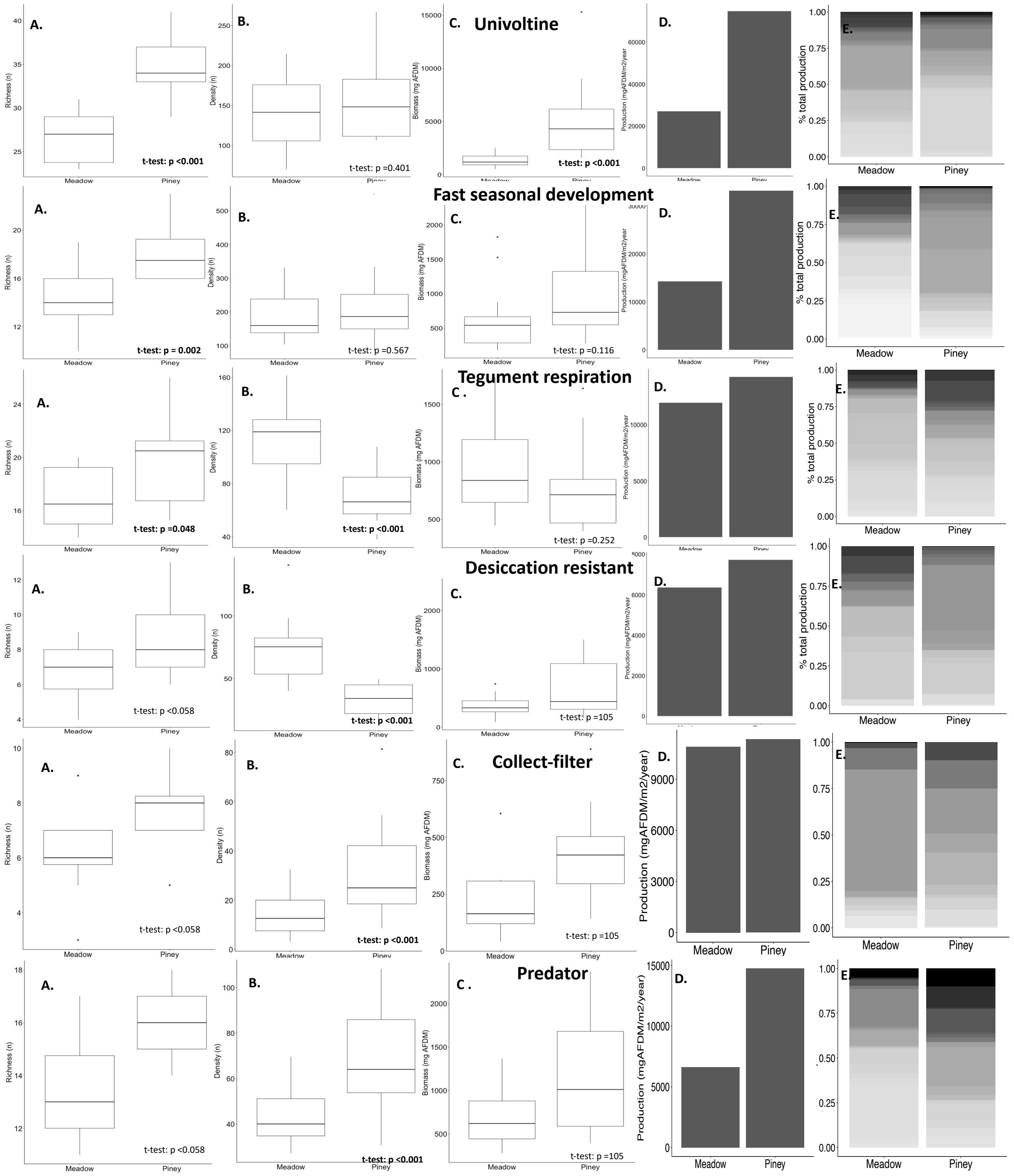
Siliciclastic streams											
Taxa density				Trait density				Trait richness			
years	taxa	stat	p	years	trait	stat	p	years	trait	stat	p
1	*Heptagenia*	0.365	**0.04**	4+6	depositional	0.619	**0.007**	4+5+6	depositional	0.603	**0.012**
2	Perlodidae	0.481	**0.009**	2+3+4+6	adults exit the stream	0.855	**0.001**	2+3+4+6	nonseasonal growth	0.797	**0.004**
	Hydropsychidae	0.354	**0.044**		well synchronized emergence	0.83	**0.001**	1+2+3+4+5	warm water	0.761	**0.019**
	Suwallia	0.481	**0.002**	1+2+3+4+5	warm water	0.777	**0.008**		collector-filter	0.976	**0.001**
3	Tubificidae	0.447	**0.004**		collector-filter	0.992	**0.001**	2+3+4+5+6	resist drying	0.975	**0.001**
	Oreogeton	0.387	**0.025**		strong adult flight	0.99	**0.001**		strong adult flight	0.968	**0.001**
4	*Lanthus*	0.546	**0.004**		slow seasonal growth	0.984	**0.001**		long adult life	0.835	**0.003**
6	*Maccaffertium*	0.829	**0.001**	2+3+4+5+6	sprawlers	0.979	**0.001**				
	Cryptolabis	0.661	**0.001**		high crawl speed	0.976	**0.001**				
	Eurylophella	0.613	**0.001**		large bodied	0.955	**0.001**				
	Ceratopogoninae	0.612	**0.001**		clingers	0.88	**0.004**				
	Turbellaria	0.612	**0.001**								
	Gomphidae	0.561	**0.001**								
	Acerpenna	0.559	**0.001**								
	Acari	0.543	**0.005**								
	Nematoda	0.519	**0.001**								
	Bezzia	0.5	**0.001**								
	Leuctridae	0.5	**0.002**								
	Eccoptura	0.49	**0.013**								
	Dasyhelea	0.433	**0.005**								
	Taeniopterygidae	0.433	**0.004**								

	Glossosomatidae	0.354	**0.03**
	Pseudolimnophila	0.354	**0.039**
	Remenus	0.354	**0.043**
2+3	*Glossosoma*	0.605	**0.001**
2+6	*Perlesta*	0.524	**0.002**
	Planariidae	0.566	**0.001**
3+4	Gammaridae	0.552	**0.001**
	Cinygmula	0.387	**0.029**
4+6	*Hydroptila*	0.508	**0.001**
2+4+6	*Perlidae*	0.73	**0.001**
	Leucrocuta	0.488	**0.012**
3+4+6	Oligochaeta	0.789	**0.001**
	Paraleptophlebia	0.623	**0.009**
	Clinocera	0.485	**0.009**
1+2+4+5	Cambaridae	0.554	**0.016**
2+3+4+5	Ceratopogonidae	0.675	**0.001**
2+3+4+6	Chloroperlidae	0.866	**0.001**
	Oulimnius	0.795	**0.001**
	Dolophilodes	0.756	**0.002**
	Antocha	0.614	**0.003**
	Hemerodromia	0.581	**0.007**
3+4+5+6	*Epeorus*	0.935	**0.001**
	Amphinemura	0.927	**0.001**
	Hexatoma	0.815	**0.001**
	Sweltsa	0.729	**0.001**
	Prosimulium	0.593	**0.004**
1+2+3+4+5	Leptophlebiidae	0.777	**0.017**
	Stenonema	0.771	**0.001**
1+2+3+4+6	*Polycentropus*	0.811	**0.007**
2+3+4+5+6	*Ephemerella*	0.959	**0.001**

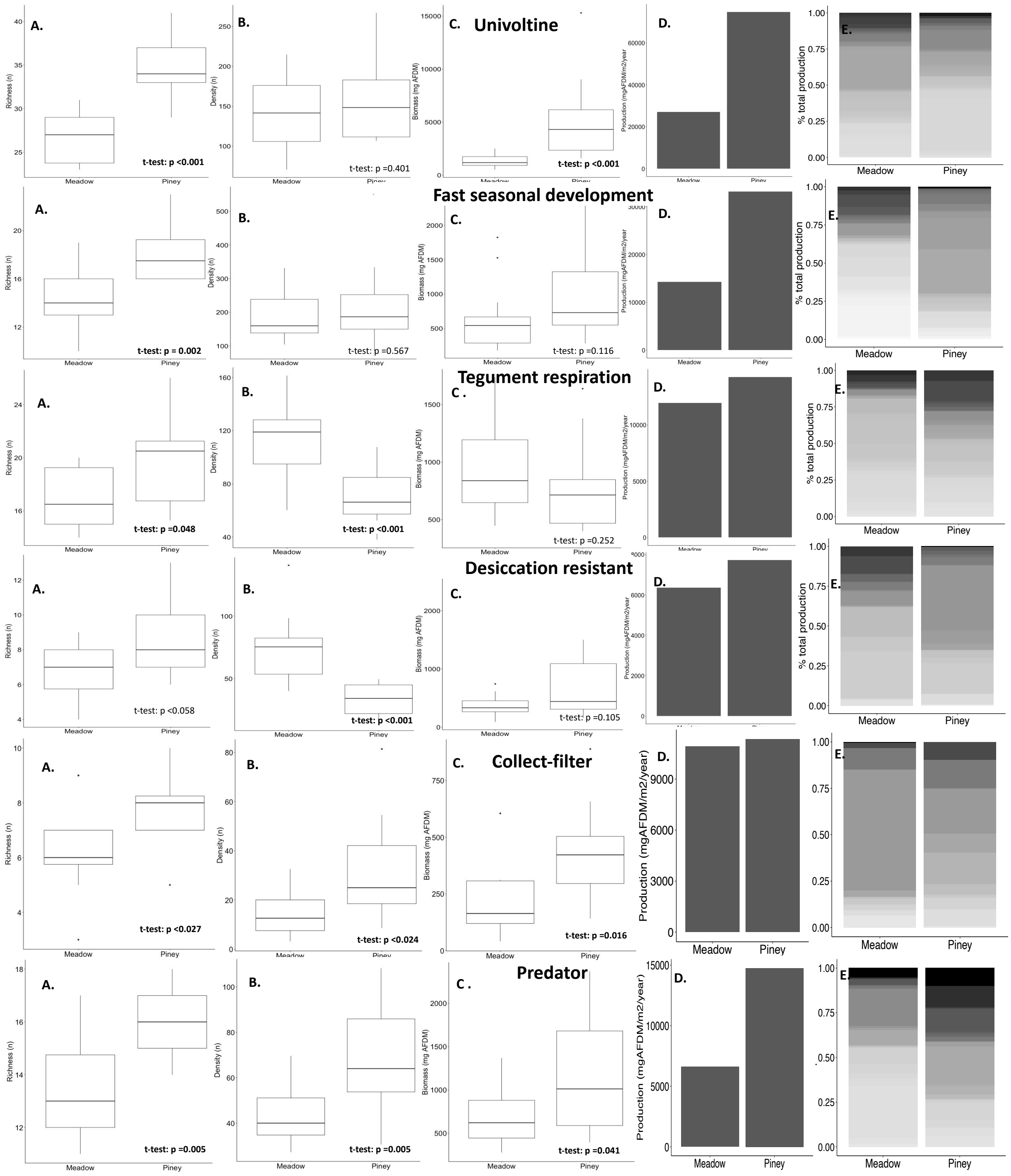
Simulium	0.935	**0.001**
Tallaperla	0.816	**0.002**
Rhyacophila	0.789	**0.004**



Supplemental 3.1: Percentage of secondary production contributed by each trait state for selected trait categories that did not differ between streams**.** Meadow is the acidified stream and Piney and the circumneutral stream.



Supplemental 3.2. Differences in structural metrics averaged across sample months for remaining traits dominant in the **acidified** stream. Each row represents a trait. Each column represents comparisons of **A.** functional diversity, **B.** density, **C.** biomass, **D.** secondary production, **E.** relative contribution of each taxa to secondary production (functional redundancy). Taxa are represented by a unique color in bar graphs (**E.**). Meadow is the acidified stream and Piney is the circumneutral stream.



Supplemental 3.3. Differences in structural metrics averaged across sample months for remaining traits dominant in the **circumneutral** stream. Each row represents a trait. Each column represents comparisons of **A.** functional diversity, **B.** density, **C.** biomass, **D.** secondary production, **E.** relative contribution of each taxa to secondary production (functional redundancy). Taxa are represented by a unique color in bar graphs (**E.**). Meadow is the acidified stream and Piney is the circumneutral stream.

Supplemental 3.4. Macroinvertebrate biomass (B; mg ash-free dry mass [AFDM]/m^{2}), secondary production (P; mg AFDM m-^{2} y^{-1}), and P/B. IGR = instantaneous growth rate method, SF = size-frequency method, P/B our data = secondary production calculated using P/B data from our study, P/B SM = secondary production calculated using P/B from Smock et al., (1984); P/B BH = secondary production calculated using P/B from Benke and Huryn (2017); P/B WH = secondary production calculated using P/B from Woodcock and Huryn (2007); P/B KW = secondary production calculated using P/B from Kreuger and Waters (1983); P/B LW = secondary production calculated using P/B from Lugthart and Wallace (1992) ; P/B KP = secondary production calculated using P/B from Kirk and Perry (1994);); P/B HW = secondary production calculated using P/B from Huryn and Wallace (1987);); P/B BW = secondary production calculated using P/B from Benke et al., (2001);); P/B SS = secondary production calculated using P/B from Smock and Smith (1992).

Meadow

Taxa	B	P	P/B	Method
Acari	34.94	349.30	10	P/B SM
Acentrella	1.22	6.10	5	P/B BH
Acerpenna	94.94	357.96	3.77	P/B WH
Acroneuria	536.24	31.21	1.96	SF
Agnetina	32.13	38.52	1.2	P/B KW
Allocapnia	99.70	398.80	4	P/B KW
Alloperla	107.66	538.25	5	P/B BH
Ameletus	348.21	1156.40	3.17	SF
Amphinemura	136.58	682.90	5	P/B BH

Piney

Taxa	B	P	P/B	Method
Acari	151.01	1510.10	10	P/B SM
Acentrella	62.15	310.75	5	P/B BH
Acerpenna	36.40	137.19	3.77	P/B WH
Acroneuria	4497.39	1717.91	2.02	SF
Agapetus	8.12	26.80	3.3	P/B KW
Agnetina	86.47	103.80	1.2	P/B KW
Alloperla	205.10	1025.55	5	P/B BH
Ameletus	29.59	93.83	3.17	P/B our data
Amphinemura	2.92	14.60	5	P/B BH

Amphipoda	7.50	37.50	5	P/B BH
Anthopotamus	10.91	62.19	5.7	P/B SM
Antocha	12.18	48.72	4	P/B KW
Atrichopogon	0.99	4.95	5	P/B SM
Baetidae	16.11	140.07	8.7	P/B KW
Baetis	22.69	197.40	8.7	P/B KW
Bezzia	35.90	190.16	5.3	P/B KW
Capniidae	68.62	274.48	4	P/B KW
Ceratopogon	5.76	30.53	5.3	P/B KW
Ceratopogonidae	1.13	5.94	5.3	P/B KW
Chelifera	3.06	7.65	2.5	P/B KW
Cheumatopsyche	78.71	298.31	3.7	P/B our data
Chironomidae	924.21	1084.24	NA	IGM
Chloroperlidae	422.49	101.14	2.1	SF
Clinocera	2.42	6.05	2.5	P/B KW
Cordulegastridae	0.88	4.75	5.4	P/B LW
Cyrnellus	2.58	15.93	6.2	P/B KP
Dicranota	93.92	309.94	3.3	P/B KW
Diphetor	8.38	41.90	5	P/B BH
Diplectrona	644.63	1243.33	2.58	SF
Eccoptura	1683.21	1377.71	1.2	P/B KW
Ectopria	0.87	4.35	5	P/B BH
Elmidae	11.03	55.20	5	P/B KW
Empididae	0.44	1.08	2.5	P/B KW
Epeorus	105.17	190.08	1.46	SF
Ephemera	0.51	2.91	5.7	P/B SM
Ephemerella	1.12	3.92	3.5	P/B our data
Ephemerellidae	38.17	190.90	5	P/B KW

Anchytarsus	0.20	1.00	5	P/B BH
Antocha	43.84	175.36	4	P/B KW
Baetidae	50.01	435.00	8.7	P/B KW
Baetis	81.79	711.49	8.7	P/B KW
Bezzia	367.69	1948.55	5.3	P/B KW
Bleph1aricera	1607.08	4309.53	5.19	SF
Capniidae	44.11	176.36	4	P/B KW
Centroptilum	252.76	1263.80	5	P/B BH
Ceratopogon	27.04	143.26	5.3	P/B KW
Ceratopogonidae	49.33	261.45	5.3	P/B KW
Chelifera	2.20	5.48	2.5	P/B KW
Cheumatopsyche	774.49	1106.35	3.7	SF
Chironomidae	1110.99	1356.39	NA	IGM
Chloroperlidae	846.19	369.11	3.89	AF
Cinygmula	1962.84	6602.49	3.75	SF
Clinocera	1.77	4.43	2.5	P/B KW
Dicranota	106.77	352.37	3.3	P/B KW
Diphetor	52.38	261.85	5	P/B BH
Diplectrona	988.57	1744.03	5.62	SF
Dixa	0.27	1.35	5	P/B BH
Dixidae	0.27	1.35	5	P/B BH
Drunella	857.69	5352.81	4.08	SF
Dubiraphia	6.28	31.40	5	P/B BH
Ectopria	45.43	227.20	5	P/B BH
Elmidae	5.29	26.45	5	P/B KW
Empididae	2.03	5.08	2.5	P/B KW
Epeorus	3574.94	9513.04	5.84	SF
Ephemera	51.53	293.66	5.7	P/B SM

Ephemeridae	0.21	1.20	5.7	P/B SM
Ephemeroptera	0.60	3.00	5	P/B BH
Eurylophella	38.17	278.50	7.3	P/B SM
Glossosoma	114.67	378.35	3.3	P/B KW
Glossosomatidae	2.76	9.08	3.3	P/B KW
Gomphidae	12.96	70.09	5.4	P/B LW
Habrophlebia	16.52	64.43	3.9	P/B HW
Haploperla	15.34	76.75	5	P/B BH
Hemerodromia	1.39	3.45	2.5	P/B KW
Heptageniidae	58.18	232.68	4	P/B KW
Heterocloeon	1.22	6.10	5	P/B BH
Hexatoma	527.45	563.15	1.42	SF
Hydropsyche	1711.16	7132.00	6.12	SF
Hydropsychidae	106.03	360.54	3.4	P/B KW
Isonychia	0.17	0.45	2.68	P/B our data
Isoperla	2.46	2.02	0.82	P/B our data
Lepidostoma	23.08	103.86	4.5	P/B KW
Leptophlebiidae	144.87	101.33	2.88	SF
Leuctra	40.01	199.95	5	P/B KW
Limnophila	5.56	54.53	9.79	P/B WH
Lype	2.45	12.94	5.28	P/B HW
Microvelia	0.57	2.80	5	P/B BH
Nemouridae	0.58	2.90	5	P/B KW
Neophylax	20.97	104.80	5	P/B KW
Nigronia	8.71	21.78	2.5	P/B SS
Nyctiophylax	2.58	9.51	3.7	P/B SM
Oemopteryx	319.58	1166.46	2.35	SF
Optioservus	5.63	28.15	5	P/B KW
Oulimnius	2468.71	585.99	4.85	SF

Ephemerella	998.71	4259.96	3.5	SF
Ephemerellidae	186.29	931.45	5	P/B KW
Ephemeroptera	1.88	9.40	5	P/B BH
Eurylophella	0.31	2.26	7.3	P/B SM
Gastropoda	165.51	529.66	3.2	P/B KW
Glossosoma	236.02	778.73	3.3	P/B KW
Glossosomatidae	44.66	147.38	3.3	P/B KW
Goera	169.24	846.20	5	P/B KW
Gomphidae	9.93	53.78	5.4	P/B LW
Gomphus	0.24	1.30	5.4	P/B LW
Habrophlebia	1.02	3.98	3.9	P/B HW
Habrophlebiodes	2.68	10.45	3.9	P/B HW
Haploperla	72.93	364.70	5	P/B BH
Hemerodromia	1.70	4.25	2.5	P/B KW
Heptagenia	107.53	430.12	4	P/B KW
Heptageniidae	114.51	458.04	4	P/B KW
Heterocloeon	2.69	13.45	5	P/B BH
Hexatoma	3896.53	3138.99	2.48	SF
Hydropsyche	1393.68	2762.36	3.39	SF
Hydropsychidae	341.27	1160.35	3.4	P/B KW
Hydroptilidae	1.17	11.00	9.4	P/B BW
Isogenoides	32.48	139.66	4.3	P/B KW
Isonychia	836.56	1936.79	2.68	SF
Isoperla	1677.57	627.14	0.82	SF
Lanthus	33.49	90.40	2.76	P/B HW
Lepidostoma	126.16	567.81	4.5	P/B KW
Leptophlebiidae	976.36	1252.85	10.52	SF
Leucrocuta	249.62	1273.11	5.1	P/B HW
Leuctra	85.13	425.65	5	P/B KW

Paracapnia	583.74	1880.13	2.12	SF
Peltoperlidae	2134.30	1154.34	1.96	SF
Perlesta	61.74	598.78	9.7	P/B BW
Perlidae	84.52	321.25	3.8	P/B SM
Perlodidae	52.91	201.06	3.8	P/B SM
Philopotamidae	10.64	99.92	9.4	P/B SM
Planariidae	0.82	4.10	5	P/B BH
Plecoptera	23.68	118.35	5	P/B BH
Polycentropodidae	90.70	353.69	3.9	P/B KW
Polycentropus	590.90	297.65	1.71	SF
Prosimulium	149.30	329.52	2.17	SF
Psephenus	8.94	2.77	0.31	P/B our data
Pseudolimnophila	6.53	63.93	9.79	P/B WH
Psilotreta	2.11	6.30	3	P/B our data
Rhyacophila	2815.80	1929.75	2.01	SF
Serratella	2.49	19.92	6	P/B HW
Simuliidae	23.78	316.27	13.3	P/B KW
Simulium	52.55	699.05	13.3	P/B KW
Stenelmis	18.15	59.86	3.3	P/B SM
Stenonema	2399.71	2894.29	2.2	SF
Sweltsa	616.21	197.94	1.86	SF
Taeniopterygidae	41.50	207.50	5	P/B KW
Tanypodinae	24.76	111.02	4.5	P/B KW
Trichoptera	2.41	12.00	5	P/B BH

Limnophila	32.43	317.39	9.79	P/B WH
Limoniidae	0.39	3.82	9.79	P/B WH
Lype	17.97	94.88	5.28	P/B HW
Neophylax	480.92	2404.60	5	P/B KW
Nigronia	29.77	74.48	2.5	P/B SS
Ochrotrichia	3.79	35.63	9.4	P/B BW
Oemopteryx	26.46	30.32	2.35	P/B our data
Optioservus	66.55	332.80	5	P/B KW
Oulimnius	1342.16	265.82	3.94	SF
Paracapnia	158.85	1475.84	3.45	SF
Paragnetina	15.36	18.43	1.2	P/B KW
Paraleptophlebia	2451.16	2020.74	4.49	SF
Peltoperlidae	254.81	95.92	0.96	SF
Perlesta	21.25	206.13	3.8	P/B SM
Perlidae	373.08	1417.89	3.8	P/B SM
Perlodidae	164.88	626.66	3.8	P/B SM
Philopotamidae	66.53	625.29	9.4	P/B SM
Planariidae	1.73	8.65	5	P/B BH
Plecoptera	2.63	13.20	5	P/B BH
Polycentropodidae	48.57	189.31	3.9	P/B KW
Polycentropus	217.15	48.46	0.35	SF
Promoresia	8.48	27.98	3.3	P/B SM
Prosimulium	257.28	701.84	3.49	SF
Psephenidae	0.05	0.25	5	P/B BH
Psephenus	3016.75	310.96	0.31	SF
Pseudolimnophila	6.20	60.80	9.79	P/B WH
Psilotreta	3508.03	3852.46	3	SF
Pteronarcys	#######	#######	1.67	SF

Pycnopsyche	4.99	24.95	5	P/B KW
Rhyacophila	506.03	664.06	2.65	SF
Serratella	265.99	2128.08	8	P/B HW
Simuliidae	77.17	1026.49	6	P/B HW
Simulium	6.73	89.51	13.3	P/B KW
Stenelmis	142.23	469.36	13.3	P/B KW
Stenonema	316.93	493.07	2.42	SF
Sweltsa	1530.97	832.64	2.32	SF
Taeniopterygidae	2.44	12.20	5	P/B KW
Taeniopteryx	11.42	57.10	5	P/B KW
Tanypodinae	12.27	54.81	5	P/B KW
Trichoptera	1.14	5.60	5	P/B BH