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I am submitting herewith a dissertation written by Latha Malar Baskaran entitled "Effects of Switchgrass Related Land-Use Changes on Aquatic Macroinvertebrates." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Geography.

Liem Tran, Major Professor

We have read this dissertation and recommend its acceptance:

Virginia Dale, Carol Harden, Shih-Lung Shaw

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(Original signatures are on file with official student records.)

Effects of Switchgrass Related Land-Use Changes on Aquatic Macroinvertebrates

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Latha Malar Baskaran
May 2017

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DEDICATION

I dedicate this dissertation to my mother and mother-in-law, the strongest and most compassionate women in my life, whose endless support made this journey possible.

ACKNOWLEDGEMENTS

I have many people to thank in the path towards my Ph.D. First and foremost, I would like to offer my greatest gratitude to my advisor Dr. Liem Tran who guided and supported my work. His scientific and technical advice ensured my work was technically sound and helped me cross many roadblocks I faced in my research. In spite of my many delays and breaks, he always supported me when I came back to pick up the pieces and continue with my research. Without his support and understanding, I couldn't have made it to this stage.

I would like to sincerely thank Dr. Virginia Dale, my mentor, role model, committee member, and supervisor at work. She encouraged me to start my Ph.D., and she was always ready with support and advice when I needed it. I am grateful to have learnt most of my research and scientific approaches from her. I would like to thank my committee members Dr. Carol Harden and Dr. Shih-Lung Shaw for their comments and valuable advice that helped me focus my work early on. The lessons I learnt in their classes and seminars still resonate with me today,

My path to this Ph.D. was in conjunction with my work at the Oak Ridge National Laboratory (ORNL), and without the support of my work place and colleagues, this would have not been possible. I am grateful for the educational assistance program at ORNL which provided financial support for my course work. Further, the flexible work hours and ability to work part-time offered by ORNL, and supported by my supervisor, was critical for me to balance work, home and school. I would like to thank Dr. Yetta Jager, a mentor and close colleague, who was very supportive of my Ph.D. work and allowed me to shift project priorities when I had to focus on my dissertation, I would like to acknowledge my colleagues Esther Parish, Jasmine Kreig, Teresa Matthews, and John Smith, who through discussions, reviews and advice supported my work.

I could not have sanely made it this far without my friends, who in spite of my lack of phone calls, text messages, and missed social events, were always there for me. I would like to thank Udaya Kalluri, my dear friend who inspired, encouraged and supported me. Chats with her over walks, coffee, or lunch always gave me the boost of energy to move forward. I would like to thank my friends Priya and Giri, who are more like family, and have stood by me from the beginning of this journey and through various life events in between. I am grateful for my friends Geetha, Babu, Amudhan, Praveena, Aarthy, Pauline, Bhargavi, Manasi, Gayatri, Padma, Sriram, Bharat, Kartik and Raj who encouraged me through various ways.

My parents and family are a big part of who I am, and I would not be where I am without their love and support. My father, my hero, is my biggest role model and continues to inspire and make me proud today. Though I did not follow his path into physics, I like to believe that I might have inherited a small fraction of his scientific rigor, which helped me get this far in my research. My mother is my biggest supporter to this date. Her unconditional love has helped me sustain through every stage of my life. My sister Ezhil, my best friend, has listened to countless gripes and has always believed in me. My

brother Satish, though not near, has always been a supportive force while showing his concern for my wellbeing. I am very grateful for my mother-in-law and father-in-law, who treat me like a daughter and have been constant supporters of my Ph.D. I knew I could always rely on them for any help, and without their support, I could not have balanced family and work.

I am thankful every day for my greatest blessings, my boys Aadhi, Nilan and Niray, who came into my life at various points in this Ph.D. journey and have been my biggest strengths and joys. They constitute my happy place and give meaning and purpose to everything I do. Last, but certainly not least, my dissertation would not have come this far if not for my husband, Suresh. I am grateful for everything he is and for everything he has done for me. Suresh was truly the force behind my work from day 1. From sharing every chore and responsibility, to encouraging me to work more efficiently, his love and support helped me overcome several obstacles and made this long path to my Ph.D. possible.

ABSTRACT

This research examines if switchgrass-based land-management practices have the potential to influence aquatic macroinvertebrates through changes in stream flow and water quality. The number of taxa in Ephemeroptera, Plecoptera, and Trichoptera orders (EPT taxa richness/EPT-TR) is analyzed as an aquatic macroinvertebrate bioindicator in the context of regional environmental effects, and changes in stream flow and water quality. This dissertation is structured as three manuscripts that link together to address the overall research question.

The first manuscript focuses on identifying regional environmental variables that influence EPT-TR across ecoregions in Tennessee. The influences of temperature, precipitation, geology, soil, stream flow and velocity on EPT-TR differ among ecoregions and also set the context for local-scale factors.

The second manuscript uses multilevel regression models to evaluate the effects of stream flow and water quality on EPT-TR in the midst of regional environmental factors in Tennessee. Stream flow is found to be statistically significant in influencing EPT-TR across ecoregions, and total nitrogen, phosphorus and sediment are statistically significant within specific ecoregions. However, the magnitude of these effects is very small in the midst of the effects from regional factors. By testing the significance of EPT-TR in explaining water quality, EPT-TR is not found to be a strong indicator of water-quality changes in Tennessee under the conditions of this study.

The third manuscript uses the Soil and Water Assessment Tool (SWAT) to compare stream flow and water quality from a baseline scenario and switchgrass management scenario at the Nolichucky watershed in Tennessee. Stream flow increased and nitrogen and phosphorus concentrations decreased under the switchgrass scenario. Regression models relating EPT-TR and monthly stream flow and water quality from SWAT showed increase in EPT-TR in the switchgrass scenario, but these increases are within the margin of error of monthly estimates. The influence of switchgrass management on EPT-TR cannot be detected under current model assumptions.

Overall, results of the whole study show that EPT taxa are affected by factors that operate at different spatial and temporal scales, and impacts due to switchgrass-management related stream flow and water quality changes cannot be detected in the current spatial context.

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CHAPTER 1 – INTRODUCTION

The need to identify alternative sources of energy has been given increased attention in the past few decades. The Energy Independence and Security Act (EISA) of 2007 was enacted “to move the United States toward greater energy independence and security, to increase the production of clean renewable fuels…” (U. S. Department of Energy 2007). An assessment by the U. S. Department of Energy (2016) found that U.S. agriculture and forest resources have the capability to potentially produce at least one billion dry tons of biomass annually in a sustainable manner. Currently, grain based ethanol from corn is widely produced and is one of the major sources of biofuel in the United States. Though the environmental effects of intensive corn production for ethanol are well documented, grain-based biofuels remain a significant part of the bioenergy system due to their well-established infrastructure (Robertson et al. 2008). However, the Renewable Fuels Standard (RFS2), mandated by EISA, calls for increased use of cellulosic ethanol derived from energy crops. These energy crops include switchgrass, miscanthus, short rotation woody crops, agricultural residues, and forestry materials and residues. Among these energy crops, the cellulosic feedstock switchgrass has gained more attention due to its growth and yield properties that make it a better environmental choice rather than obtaining biofuels from crops such as corn. Switchgrass has been considered as a ”model” bioenergy crop owing to its wide range and adaptability (McLaughlin and Walsh 1998). Switchgrass is a perennial crop, and compared to corn, it has lower fertilizer requirements, improved soil conservation, improved energy gain and improved reductions in emissions of carbon dioxide (McLaughlin and Walsh 1998).

Based on various scenarios considered by the U. S. Billion Ton Report (U. S. Department of Energy 2016), by 2040 energy crops can contribute between 34% and 48% of the billion ton goal, and up 27 million acres of cropland and 37 million acres of pasture land could be managed for energy crops to meet the biofuel demand. The consequent land-use and land-cover change associated with biofuel expansion would have environmental effects that need to be given forethought and consideration (Ramney and Mann 1994; McLaughlin and Walsh 1998; Tolbert and Wright 1998; Borjesson 1999). Given that existing agricultural activities in the form of crop production are one of the top sources of impairment in assessed rivers and streams in the United States (U. S. EPA 2009), it is important to minimize negative consequences and to understand the environmental effects of bioenergy systems due to the large-scale management of land for bioenergy production.

To address environmental sustainability of the bioenergy system, Robertson et al. (2008) identified urgent research needs, which include a systems approach to assess the full impact of bioenergy systems, focus on ecosystem services, and understanding the implications of policy and management. Biodiversity is a vital component of these research efforts, and there is a growing need to better our understanding of bioenergy crop production and the effects it may have on biodiversity (Dale et al. 2010). Bioenergy systems can affect the habitats of species that rely on the land used to grow bioenergy crops and the streams that drain from these lands. Effects on habitat may stem from changes in land management for bioenergy crops. Such habitat alterations directly

affect biodiversity at different scales and are a function of the type of bioenergy crop being considered and how the crops are managed on the ground as well as prior management practices (Fletcher et al. 2011). For example, dependence on grain-based biofuels such as ethanol from corn can result in agricultural intensification and negatively impact stream habitat and biodiversity (Williams et al. 2009).

McBride et al. (2011) identified 19 indicators in six categories, including water quality and quantity, and biodiversity, for assessing environmental sustainability of bioenergy systems. ”Taxa of special concern” is one of the biodiversity indicators recommended by McBride et al. (2011). These taxa of special concern include “bioindicators”, which are used to monitor the condition of an environmental system (Hodkinson and Jackson 2005). Aquatic macroinvertebrates have been considered to be good bioindicators and are frequently used as environmental, ecological biodiversity indicators (Holt and Miller. 2011). Aquatic macroinvertebrates are sensitive to water quality changes and habitat changes and have recognized responses to such changes (e.g., Johnson et al. 1993; Kerans and Karr 1994). They are affected by human-induced alterations through changes in their food source, habitat structure and biotic interactions. Mechanisms that cause these changes to the stream ecosystems include sedimentation, nutrient enrichment, contaminant pollution, hydrologic alteration, riparian clearing and loss of large woody debris (Allan 2004). Currently, all 50 states of the United States use aquatic macroinvertebrates to assess the biological health of streams and rivers (Holt and Miller 2011). Bioindicators, such as aquatic macroinvertebrates, also have the ability to indicate indirect biotic effects of pollutants and nutrients in the stream without having to make chemical measurements in the stream. This property makes aquatic macroinvertebrates a potential indicator to assess both water quality/quantity and biodiversity in the categories identified by McBride et al. (2011) for assessing environmental sustainability of bioenergy systems.

This research evaluates the potential effects of switchgrass-related land management on an aquatic macroinvertebrate metric. This study also fills the gap in the current understanding of the effects of switchgrass related land-use changes on aquatic biodiversity by focusing on the number of taxa in Ephemeroptera, Plecopter and Trichoptera (EPT taxa richness) as the potential bioindicator. EPT richness is a standard community-level indicator since it includes insect orders that are very sensitive to environmental perturbations and can associate benthic assemblages to complex ecosystems and disturbance regimes (e.g., Feminella 1996; Maxted et al. 2000). Further, stream benthic macroinvertebrate metrics such as EPT taxa richness are useful indicators of the impacts of disturbances on catchment and stream conditions because they integrate many catchment-scale ecological processes (Maloney and Feminella 2006).

Research objective

The overall goal of this research is to understand if switchgrass-based land-management can affect aquatic macroinvertebrates through changes in stream flow and water quality. Towards this goal, the spatially variable regional environmental factors

that influence aquatic macroinvertebrates are identified. The potential of EPT taxa richness as a bioindicator to assess the effects of water quality changes in Tennessee is also evaluated. The key hypothesis is that aquatic macroinvertebrates are affected by factors and processes at different spatial scales and switchgrass related land-management influences some of those factors and hence can potentially affect EPT taxa richness. The specific objectives of this research are

1. Determine the key natural factors at different scales that affect EPT taxa richness across ecoregions in Tennessee
2. Evaluate the influence of water quality on EPT taxa richness in the midst of regional environmental variables (temperature, precipitation, soil, and slope) in Tennessee
3. Evaluate if switchgrass-based land-management influences EPT taxa richness within the Nolichucky watershed in Tennessee

Dissertation organization

This dissertation is organized into six chapters. The next chapter (chapter 2) provides background information on switchgrass, EPT taxa richness and the models used by this study. Chapter 2 addresses how switchgrass is managed and how land-management changes to switchgrass are expected to affect water quality and aquatic habitat in streams. Background information on factors affecting aquatic macroinvertebrates, and how EPT taxa richness as a bionindicator responds to changes in streams, is also presented in chapter 2. Since there are many models to address land-management changes and their impacts to the stream, chapter 2 also addresses the specific model chosen for this study.

Chapters 3, 4, and 5 present research intended for publication as three separate manuscripts submitted to scientific journals. Chapter 3 presents the manuscript titled “Analyzing aquatic macroinvertebrate taxa richness indices across ecoregions in Tennessee”. This chapter evaluates three different ecoregion classifications and identifies the ecoregion classification that best provides context for studying aquatic macroinvertebrates. In chapter 3, I addresses the first objective by evaluating regional variables and spatially variable stream-scale factors that are significant with respect to EPT taxa richness by ecoregion.

Chapter 4, “Potential of aquatic macroinvertebrates as water quality indicators: An evaluation across Tennessee, USA”, evaluates the potential of EPT taxa richness as an effective indicator of stream flow and water quality variables in affecting EPT taxa richness using data collected across Tennessee. The ecoregion classification identified in Chapter 3 is used to separate regional effects across ecoregions in a multilevel model framework. This chapter directly addresses objective 2 by evaluating the influence of water quality on EPT taxa richness in the midst of the effects from regional environmental variables.

Chapter 5, “Assessing effects of switchgrass-based land-management practices in aquatic macroinvertebrate taxa”, addresses the third objective by evaluating the potential impacts from land managed for switchgrass on EPT taxa richness through changes in stream flow and water quality within the Nolichucky watershed in the Tennessee River Basin. This chapter uses a modeling approach to generate stream flow and water quality variables that help quantify relationships between EPT taxa richness and stream flow/water quality, previously not captured by results in Chapter 4.

Chapter 6 provides conclusions to this study and summarizes the results from the three different manuscripts. The lessons learnt and future research questions are also addressed.

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CHAPTER 2 – BACKGROUND

Aquatic macroinvertebrates

Macroinvertebrates are animals without backbones such as insects, crustaceans, molluscs, arachnids and annelids that can be seen with naked eye and are found in streams, rivers, wetlands and lakes. This analysis focuses on immature insects that are found in freshwater habitats. They are an important source of food for fish and other higher order species. Macroinvertebrates also have an important influence on nutrient cycles, primary productivity, and translocation of material (Wallace and Webster 1996). Macroinvertebrates are considered to be a crucial intermediate link between primary producers and predators higher up in the trophic hierarchy. The majority of these insects spend most of their immature lives in the water, and the adults emerge from the aquatic environment to mate and disperse.

Factors affecting aquatic macroinvertebrates

When using invertebrate communities as bioindicators, it is important to understand that such assemblages are subject to natural variation in addition to the independent deterministic changes resulting from pollution or disturbance, particularly over the long term (Hodkinson and Jackson 2005). In general, macroinvertebrate habitat is influenced by several factors ranging from local-scale physical habitat characteristics to regional-scale characteristics (Table 1). At the local scale, reach level properties such as channel dimensions, substrate characteristics, woody debris, and hydraulic characteristics can affect macroinvertebrate habitat (Lenat and Crawford 1994; Richards et al. 1997). At the regional and watershed scales, geomorphology and climate characteristics are important predictors of macroinvertebrate community impairment (Kennan 1999; Goldstein et al. 2007).

Suspended and deposited sediments can affect benthic invertebrates by changing the suitability of substrates for taxa (Wood and Armitage 1997). One of the biggest impacts of sedimentation is associated with the fines eroded from agricultural land (Walling et al. 1990). Sandy substrates are poor habitats because the shifting nature of the bed provides unsuitable attachment and poor food conditions (Merritt and Cummins 1996). Further, silt-based substrates have low concentrations of oxygen and result in inefficient oxygen consumptions by some macroinvertebrate taxa. In general, taxa richness increases with increase in substrate size (Quinn and Hickey 1990). Courser substrates provide suitable habitat for invertebrates having high oxygen requirements, such as many taxa in the Ephemeroptera, Plecoptera and Trichoptera orders (Quinn and Hickey 1990). Addition of fine particulate material can increase turbidity, decrease light penetration, reduce primary productivity, and change the natural faunal assembly in the streams (Wood and Armitage 1997). Some types of anthropogenic disturbance to the land surface, such as agriculture and mining, increase the load of fine particles in streams and can affect aquatic macroinvertebrate habitat.

Increased input of nutrients can cause increased primary productivity and periphyton concentrations, which in turn can affect higher trophic levels that rely on them for habitat

Table 1: Landscape and stream features affecting macroinvertebrates, their scales of influence, and the factors by which they affect macroinvertebrate health.

Landscape/stream features	Scale(s) of influence	Related factors that affect macroinvertebrates	Example References
Geomorphology	Regional	Flow regime, substrate, ion concentration	Richards et al. 1996
Climate	Regional	Stream flow, temperature	Beche et al. 2006
Sediments and Substrate	Reach	Turbidity, scouring and abrasion, substrate composition, in-filling interstitial habitat, stream depth heterogeneity	Wood and Armitage 1997
Nutrients	Reach	Nutrient enrichment, dissolved oxygen, changes to assemblage composition	Wang et al. 2007
pH	Reach	pH sensitivity, toxicity	Simpson et al. 1985
Stream flow	Reach	Flow volume, channel dynamics, erosion, floods and low flows	Statzner et al. 1988

and food. Changes in the food web may cause changes in the ecosystem and further alter stream physical habitat and water chemistry, e.g., decreasing dissolved oxygen. In some Ephemeroptera and Plecoptera taxa, the rate of respiration (physiology) fails to compensate for falling oxygen levels within the water (Merritt and Cummins 1996). Wang et al. (2007) found that EPT taxa richness was strongly correlated with nutrient measures in Wisconsin streams. Overall, increased nutrient concentrations can cause macroinvertebrate community structure to shift from having sensitive species to more tolerant species. Nutrient enrichment can also alter other physical sources of ecological change that affect ecosystem properties such as resilience and resistance (Gafner and Robinson 2007). Lemly (1982) found that excess nitrates and phosphates, in association with sedimentation, resulted in growth of the filamentous bacterium *Sphaerotilus natans* on the body and respiratory surfaces of macroinvertebrates, as well as on the surface of stones in the stream. These bacteria resulted in a net-like formation due to the overlapping of filaments over the macroinvertebrates and greatly augmented the accumulation of sediments, with the eventual blanketing and smothering of the insect (Lemly 1982). Nutrient concentrations also vary by season. They are generally higher in summer than in autumn. Johnson et al. (1997) found that influence of land use on water chemistry was less in autumn compared to spring owing to low flow conditions and reduced fertilizer application and runoff in fall that diminished the connection between land use and stream chemistry.

Reductions of pH in stream water can result in low invertebrate diversity and density (Simpson et al. 1985; Rosemond et al. 1992). Low pH values (4 to 5.5) in the streams have been found to eliminate many Ephemeroptera taxa, while some other species decline gradually (Courtney and Clements 1998). Ernst et al. (2008) found that acidic headwaters resulted in fewer numbers of Ephemeroptera and Plecoptera taxa in the Neversink River of the Catskill Mountains in south-eastern New York. The toxic effects of acidic pH are associated with disruption of ion regulation, particularly NaC- and Cl−, increased membrane permeability, and respiratory stress (Courtney and Clements 1998). In the Obey River in Tennessee, acid mine drainage has been known to cause drastic reductions in benthic macroinvertebrate abundance and diversity because of decreased pH, increased concentrations of dissolved metals, and a high amount of metal precipitation (Nichols and Bulow 1973).

Accumulation of metals in aquatic organisms can be toxic. It is caused by the transport of dissolved metal species across external membranes, adsorption on body surfaces, and intake of particulate forms of metals (Hare 1992). For example, metal ions such as iron, can precipitate on gill surfaces under acidic conditions and affect respiration for some Ephemeroptera taxa (Gerhardt 1992). Studies have found that bioaccumulation of heavy metals such as mercury, lead, cadmium and aluminum is influenced by the water pH (Wren and Stephenson 1991). The pH of the water is strongly related to metal speciation because it affects the sorption process, remobilization, and solubility of metals (Gerhardt 1993). DeNicola and Stapleton (2002) found that acid mine drainage, in addition to affecting the water quality, also results in precipitation of metals in the substrate, which can affect macroinvertebrate habitat.

Flow is correlated with most important characteristics of stream systems (e.g., current, water depth, hydraulics, channel geomorphology, substratum stability) and is of critical importance to the range of potential microhabitats available to benthic macroinvertebrates (Statzner et al. 1988). Hydraulic stress associated with foraging, maintaining position, and organic matter retention in coarse substrata are some mechanisms through which the flow affects the spatial distribution of macroinvertebrates (Rempel et al. 2000). A reduction in flow velocity can lead to an increase in deposition of fines and decaying matter onto the riverbeds (Wood and Armitage 1997). This problem is prevalent during low flow conditions in summer, and it is acute in groundwater-fed streams (Wood and Armitage 1997). Flow changes can also affect taxa richness by causing changes in amount of nonpoint source runoff or the dilution of point source discharge (Lenat 1988).

Major floods can reduce the macroinvertebrate abundance and richness. Floods can wash away periphyton attached to the substrate material, and this can impact the periphyton-associated macroinvertebrates (Quinn and Hickey 1990). However, floods need to exceed a certain threshold relative to the median flow to have significant effects on invertebrate abundance and richness. Previous studies have found that this threshold ranges from 20X to 45X the median flow (Quinn and Hickey 1990). Geomorphic parameters, such as surface geology and topographic heterogeneity influence the flow regimes and physical habitat of streams and can hence affect the structure and composition of macroinvertebrate assemblages (Richards et al 1997). Seasonal and monthly changes in precipitation and solar radiation result in within-year changes in flow and temperature in aquatic systems (Beche et al. 2006). Similarly, year-to-year changes in precipitation produce variation in stream discharge and habitat quality and quantity (McElravy et al. 1989). These features influence the timing of emergence, reproduction, growth and development of macroinvertebrates, which in turn influences the seasonal recolonization of organisms after disturbances (Beche et al. 2006). Beche and Resh (2007) found that the stability and persistence of macroinvertebrate communities were correlated with climatic variation (precipitation and El Niño Southern Oscillation) and stream size. Seasonal habitat variability of macroinvertebrates was also found to be higher in intermittent streams than in perennial streams (Beche et al. 2006).

Processes such as land-use change can affect several of the stream and regional features, thereby affecting aquatic macroinvertebrates. Aquatic macroinvertebrate systems that vary in space and time need to be addressed at multiple scales by considering appropriate relationships between environmental effects and species traits at each scale (Poff 1997). Gergel et al. (2002) found that water quality parameters are sensitive to changes in the riparian zone, and a spatial view of a watershed can help in understanding where aquatic ecosystems are more vulnerable to land-use changes. Further, the spatial scale of landscape influence on aquatic ecosystems may be similar across systems or spatially variable (Gergel et al. 2002). Comprehending this spatial variation of vulnerability can help identify scales at which changes occur, such as those caused by climate (Poff et al. 2010) and also bioenergy based regional land-use changes.

Switchgrass related land-use changes and effects on the stream

Switchgrass is a perennial warm season grass with a range covering most of the eastern United States. It has been used as a forage crop for the past few decades and has also been investigated as a bioenergy crop (Parrish and Fike 1995). The extensive root system and dense canopy of switchgrass makes it suitable for erosion control, and hence switchgrass has been used in streamside buffers and vegetative strips for erosion control (Belden and Coats 2004; Blanco-Canqui et al. 2004). The perennial ground cover of switchgrass helps to dissipate the energy from direct impact of raindrops and reduce surface runoff and sediment transportation from the land (Nyakatawa et al. 2006). Surface runoff can remove large quantities of both dissolved and sediment-bound nutrients from the soil, which can affect water quality.

Land planted with switchgrass has reduced nutrient input into the streams and sediment loads (e.g., Lee et al. 2003). Switchgrass is also effective in its utilization of nitrogen, and the nitrogen removed in harvest is usually greater than the amount applied (Parrish and Fike 2005). Though switchgrass requires some nitrogen fertilizers for its growth, the requirements are less than those for corn, which requires almost twice as that required by switchgrass (McLaughlin and Walsh 1998). Miller et al. (2007) suggest that compared to corn, switchgrass-based ethanol offers climate change benefits as well as a low eutrophication impact due to high yields per acre and low nitrate emissions per mass of crops. With appropriate fertilizer management that accounts for the rate of N application, previous legume crops, and manure application, perennial cropping systems have the potential to reduce nitrate losses (Randall and Mulla 2001).

Compared to annual agricultural crops such as corn, switchgrass has been found to have reduced sediments and erosion to streams (e.g., Wu and Liu 2012; U.S. Department of Energy 2017). Brown et al. (2000) evaluated the feasibility of a large-scale conversion of land to grow switchgrass in the place of corn, sorghum, soybeans or winter wheat in the Missouri–Iowa–Nebraska–Kansas region. Using the Erosion Productivity Impact Calculator (EPIC) model, they found that under the baseline scenario, the surface runoff and erosion from switchgrass was lower than that under the traditional crops. Similarly, in a modified climate scenario, erosion under switchgrass cultivation decreased, and erosion tended to increase under corn. Switchgrass has increased growth and is able to reduce the erosion risk by providing continuous cover throughout the year (Brown et al. 2000). Brown et al. (2000) also concluded that a large-scale conversion of corn to switchgrass can affect water resources by the decreases in surface runoff. The higher levels of runoff and water export from conventional crops by themselves are not critical issues with respect to water quality; however, when the water also carries agricultural pollutants, it poses a significant risk to the streams and estuaries downstream (Schilling et al. 2008). The lower rates of sediments entering streams draining switchgrass fields, compared to rates from row crops, indicate the potential to improve channel characteristics and reduce suspended sediments in streams to recover or protect macroinvertebrate habitat.

Models – Why SWAT?

To understand the potential effects of bioenergy-based land-use changes, the hydrologic foundation for the study region needs to be established using a hydrologic model that can capture the bioenergy/land-use/water relationships in the region and relate them to mechanisms influencing macroinvertebrates. Changes to sediment and nutrients in the stream are some of the important factors caused by bioenergy-based land-use changes that can directly impact macroinvertebrates. These changes are also driven by change in stream flow, which was identified as a factor contributing to change in macroinvertebrate structure of Tennessee (Arnwine et al. 2011). Switchgrass management operations that are relevant to water quality and need to be represented in a model include perennial crop management, modified fertilizer routine, and a modified harvest schedule.

Several hydrologic models that can simulate land-use changes have been developed and implemented for small to large-scale applications (Breuer et al. 2009). These models range from complex distributed models such as DHSVM (e.g., Storck et al. 1998) and MIKE-SHE (e.g., Al-Khudhairy et al. 1999) to lumped models such as IHACRES (e.g., Croke and Jakeman 2004). Hybrid mechanistic/empirical, basin scale models such as the SPARROW (SPAtially Referenced Regression On Watershed attributes) model use spatially distributed water-quality measurements and watershed characteristics to estimate N and P delivery to streams (Smith et al. 1997). In between distributed and lumped hydrologic models are semi-distributed models that are spatially explicit to the scale of sub-watersheds and model hydrologic response units as their basic unit of analysis. The Soil and Water Assessment Tool (SWAT) is an example of such a model.

SWAT is a physically based, semi-distributed model operating on a daily time scale that has been used extensively around the world for different applications ranging from Total Maximum Daily Loadanalysis at the local scale to macro-scale analysis of the entire US (Gassman et al. 2007). SWAT was developed as an integration of several models – EPIC, CREAMS, GLEAMS and a weather generator model (Krysanova and Arnold 2008). SWAT models overland processes and channel processes by a mass water balance equation within a watershed (Neitsch et al. 2005). The crop-growth component of SWAT, which helps model various crop properties and their effects on the hydrological cycle, is part of the overland component of the hydrologic cycle and was originally derived from the EPIC model. The crop growth component of SWAT simulates all crops with a single crop-growth model with the use of unique parameter values for each crop (Neitsch et al. 2005). The crop-growth model is also used to assess removal of water and nutrients from the root zone, transpiration, and biomass production (Neitsch et al. 2005). Switchgrass growth has been parameterized in SWAT and has been applied in several studies (e.g., Baskaran et al. 2010).

Models that can support assessment of detailed management and within-region water quality can help study water quality impacts in large regional studies. An evaluation of models to support efforts to study nutrient transport to the Gulf of Mexico contributing to

hypoxia found that SWAT, with its ability to target watersheds, placement of conservation practices and timing of nutrient flows, was one of the useful models (Dale et al. 2010). Given the detailed crop module in SWAT, the overland and in-stream nutrient and sediment transportation mechanisms modeled by SWAT, and the model's ability to identify temporal changes in stream flow, SWAT was selected as the appropriate model for this study.

Modeling framework

The wide adaptation, tolerance to drought, reduced nutrient and sediment runoff, and good yield properties of switchgrass have also made it a favorable bioenergy crop. Based on the land management of switchgrass and the various pathways by which it affects streams, the potential of switchgrass to affect aquatic macroinvertebrates is depicted in Figure 1. The influence of scale-based factors affecting aquatic macroinvertebrates is also depicted as boxes around the modeling framework. Land-management changes associated with switchgrass affect land processes, which, in turn affect stream processes and potentially cause instream stress. The spatial scales operating in this framework are reach-scale macroinvertebrate responses nested within watershed-scale land-management processes, which are constrained within a regional framework identified by environmental processes (Figure 1). Accounting for such nesting of scales is critical to address the objectives of this research.

The established use of EPT richness as an integrative water quality and aquatic habitat indicator, and the ability of SWAT to model interactions among switchgrass production and conditions affecting benthic macroinvertebrates provide the means to predict the effects of switchgrass production on aquatic habitats. This research is focused in Tennessee, where there is potential for switchgrass production (Baskaran et al. 2010). Further, the diverse geomorphological features in Tennessee provide a background to study the spatial distribution of macroinvertebrates as a function of different environmental conditions.

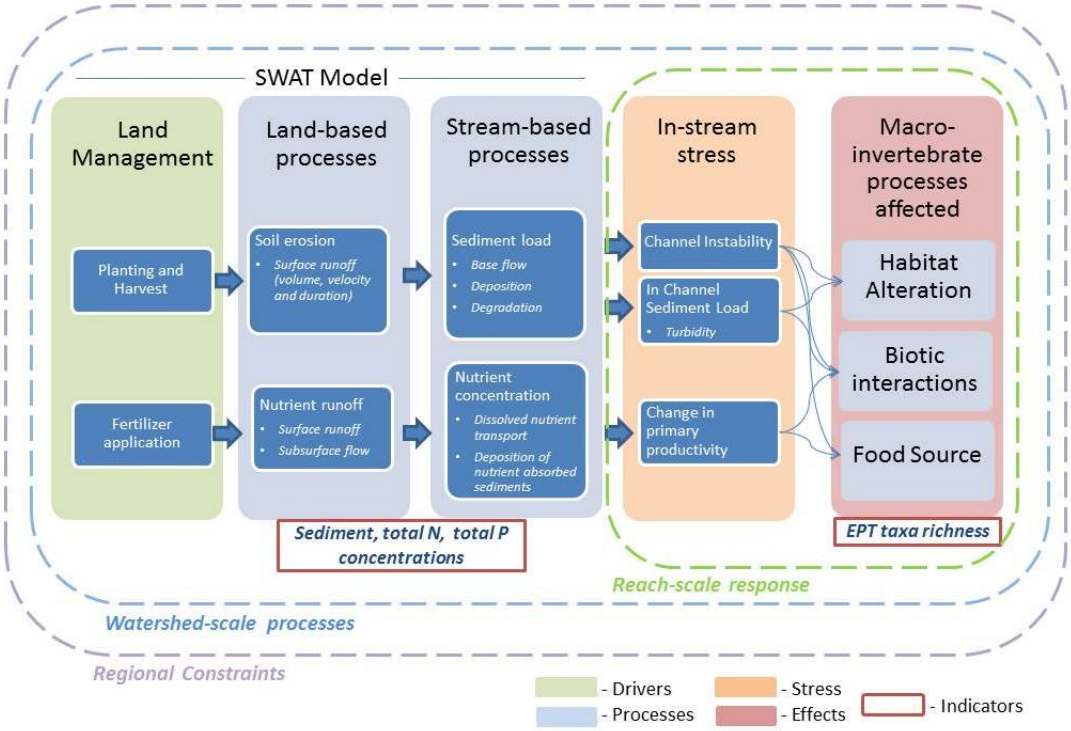

Figure 1: Linkages between switchgrass-based land management and sources of stress to macroinvertebrates (roughly adapted from Nietch et al. 2005).

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CHAPTER 3 – ANALYZING AQUATIC MACROINVERTEBRATE TAXA RICHNESS INDICES ACROSS ECOREGIONS IN TENNESSEE

A version of this chapter was submitted to the American Midland Naturalist by Latha M. Baskaran, Virginia H. Dale and Liem Tran:

Latha M. Baskaran, Virginia H. Dale and Liem Tran. “Analyzing Aquatic Macroinvertebrate Taxa Richness Indices across Ecoregions in Tennessee” *American Midland Naturalist*.

This article was prepared and written primarily by Latha Baskaran. Liem Tran and Virginia Dale advised on the work described here. They also reviewed early revisions of this manuscript.

Abstract

Aquatic macroinvertebrates are important components of stream ecosystems and are intimately linked to stream quality and land use and management in the regions that drain the streams. Understanding the factors that affect aquatic macroinvertebrate community structure can help develop predictive tools to assess landscape-level changes. The aquatic macroinvertebrate community structure is spatially variable and constrained by a hierarchy of factors ranging from regional climate, geology and soils, to local stream and substrate characteristics, so it is critical to establish a baseline against which changes are compared. This study analyzes the regional variation of EPT taxa richness (aquatic macroinvertebrate in the Ephemeroptera, Plecoptera and Trichoptera orders) across the state of Tennessee under the Omernik, Bailey, and Freshwater ecoregion classification schemes. Of the three schemes considered, silhouette analysis found Omernik’s ecoregion classification scheme to best characterize EPT taxa richness in Tennessee. The influence of regional factors, such as climate and soils, on EPT taxa richness varied by location and by Omernik’s ecoregion class. EPT taxa richness was sensitive to bedrock and soil variables but not to climate variables in Tennessee’s Interior Plateau. EPT taxa in the Ridge and Valley ecoregion in the eastern part of the state were correlated to temperature and soil variables. However, the ecoregion classification alone could not completely characterize EPT taxa richness. Other finer-scale factors such as stream discharge and velocity were also found to be significant influences on the macroinvertebrate community. Ecoregions provide the context for studying aquatic macroinvertebrates, but this study found inclusion of spatially variable, finer-scale factors to be necessary for an effective understanding of the macroinvertebrate community response to changes in land use and management.

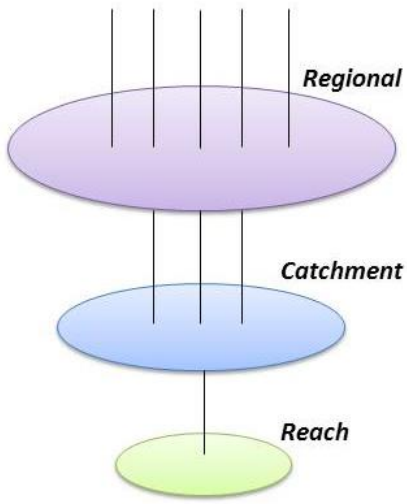
Introduction

Changes on the land affect many facets of the aquatic environment including habitat for aquatic species. Aquatic macroinvertebrates are often used as indicators of stream condition because they are abundant and, short lived, and can be sensitive to ecosystem disturbances and water quality changes (Rosenberg and Resh 1993; Sharma and Rawat 2009). For this reason different metrics to describe aquatic macroinvertebrate community structure (abundance, taxa richness, structural, functional, and feeding assemblages) can be useful because they are easy to measure

or calculate and are applicable to various systems (Carlisle and Clements 1999; Roy et al. 2003). Among these metrics, EPT taxa richness (the number of taxa in Ephemeroptera , Plecoptera and Trichoptera orders) has become a standard indicator, for it includes insects that are very sensitive to environmental perturbations and can associate benthic assemblages with complex ecosystems and disturbance regimes (e.g., Feminella 1996; Maxted et al. 2000). For example, excessive sedimentation has been shown to cause a decrease in EPT taxa abundance and richness due to alteration of the habitat of these species (Richards et al. 1996; Yuan and Norton 2003). Nutrient enrichment has also been found to decrease EPT taxa richness (Lenat 1984).

In addition to the independent deterministic changes in macroinvertebrate assemblages that can result from pollution or disturbance, macroinvertebrates are subject to natural variation, particularly over the long term (Hodkinson and Jackson 2005). In general, macroinvertebrate habitat is influenced by several factors, ranging from local-scale physical habitat characteristics to regional-scale land-use characteristics (Poff 1997). At the local scale, reach-level properties such as channel dimensions, substrate characteristics, presence of woody debris, water quality, water chemistry, and hydraulic characteristics can affect macroinvertebrate habitat (Lenat and Crawford 1994; Richards et al. 1997). At the regional and watershed scales, geomorphology and land-use characteristics have been shown to be important predictors of the macroinvertebrate community (Kennan 1999; Goldstein et al. 2007). For example, geology influences the physical and chemical attributes of aquatic systems, thus making stream habitat erosional or depositional, which, in turn, constrains the type of macroinvertebrate species that can occur based on their habit-trait group – climbers and burrowers in depositional habitat versus clingers in erosional habitat (Neff and Jackson 2011).

Local richness and species turnover of benthic macroinvertebrates are controlled by both regional and local factors that influence how the assemblages respond to anthropogenic stressors (Maloney et al. 2011). Poff's hierarchical-filtering model is based on the assumption that an organism must pass through a series of biotic and abiotic filters before becoming part of a local community (Poff 1997). Habitat filters may operate hierarchically so that processes at different scales constrain species traits from the regional scale to the microhabitat scale (Figure 2) (Poff 1997). These factors can influence macroinvertebrate habitat by affecting physiological constraints (e.g., oxygen acquisition), trophic considerations (e.g., food acquisition), physical habitat constraints (e.g., substrate), and biotic interactions (e.g., predation) (Merritt and Cummins 1996). In addition to hierarchical effects, the environmental factors that have the most influence on macroinvertebrates vary regionally and influence the response of aquatic systems to other changes (Wang et al. 2003; Johnson and Host 2010). Stewart et al. (2001) found variation in how macroinvertebrate and fish species respond to watershed, riparian, and reach characteristics. The distribution of aquatic biota, scale dependencies in environmental features, size of the study region, background disturbance conditions, and landscape heterogeneity influence the relative contribution of local, reach, and watershed variables (Johnson and Host 2010). Hence the relative importance of each variable with respect to the habitat of macroinvertebrates typically varies by region.



Landscape/ stream features	Related factors that affect macroinvertebrates
Geology	Flow regime, substrate, ion concentration
Climate	Stream flow, temperature
Land Cover	Land disturbance, increased impervious surface area, altered hydrology, transport of non-point source pollutants (e.g. sediment, nutrients) to streams, input of organic matter in the stream
Stream Size	Channel characteristics, food resources
Sediments and Substrate	Turbidity, scouring and abrasion, substrate composition, in-filling interstitial habitat, stream depth heterogeneity
Stream flow	Flow volume, channel dynamics, erosion, floods and low flows

Figure 2: Hierarchical relationships between select landscape and stream features and habitat “filters” that affect macroinvertebrates (adapted from Poff 1997). The colors from the diagram on the left correspond to various scales and the colors in the table.

Further, the spatial scale of influences on aquatic ecosystems may be similar across riverine systems or may be spatially variable (Gergel et al. 2002). Analyzing this spatial variation across environmental gradients can help identify places and scales at which large environmental changes, such as those caused by climate (Poff et al. 2010), and also large-scale resource management changes are most prominent.

Ecoregions can be a useful basis for monitoring and management of streams. The underlying premise of this approach is that ecoregion classifications provide a first-level filter in assessing influence on the habitats of aquatic macroinvertebrates and, in turn, can help improve understanding about how the spatial variation in aquatic macroinvertebrates respond to other changes. Ecoregions are based on patterns of a combination of causal and integrative factors including land use, resource management, land-surface form, potential natural vegetation, and soils (Omernik 1987). Ecoregions reflect the distribution of species and communities across a region and have been used to study biodiversity and aid in environmental conservation (Olson et al. 2001). Aquatic ecosystems are also influenced by ecoregion classifications since the characteristics of a stream depend on the characteristics of the land it drains (Larsen et al. 1986). Feminella (2000) found ecoregions were useful to classify benthic invertebrates and their habitats within catchments in the southeastern U.S. Ecoregions, therefore, can be very useful in management of large watersheds by identifying areas within a watershed with similar aquatic ecosystems (Rohm et al. 1987, Bailey 2004). Characterizing species by ecoregion limits the variability of natural regional-scale forces, which helps improve understanding about how differences between regions influence the effects of stressors on biota (Hawkins et al. 2000). For example, knowing if a stream is in a catchment with highly erodible soils or one with low erosion potential provides insight regarding vulnerability of aquatic biota to otherwise similar disturbance (Hawkins et al. 2000).

Different ecoregion classifications have been developed based on the management need and resource under consideration (McMahon et al. 2001). Of these classifications, the most frequently used for resource analysis and environmental conservation are Omernik's Ecoregions of the United States (Omernik 1987), Bailey's Ecoregions of the World (Bailey 1983), and Freshwater Ecoregions of the World (Abell et al. 2008). Omernik's ecoregion classification, adopted by U. S. Environmental Protection Agency, incorporates the spatial correlation of both physical and biological factors and is based on the premise that relatively homogenous areas can be identified by simultaneously analyzing causal and integrative factors including land-surface form, soils, land use, and potential natural vegetation (Omernik 1987; Omernik and Griffith 2014). Omernik's ecoregion classification scheme is based on vegetation, physiography, land use and soils, and has four hierarchical classification levels. Omernik's classification was found to be useful in classifying aquatic species in streams in Oregon (Van Sickle and Hughes 2000). Bailey's ecoregion classification, primarily focused on terrestrial systems, has been adopted by the U.S. Forest Service and The Nature Conservancy (Olson et al. 2001). Climate plays a key role in Bailey's ecoregion differentiation. For broad-scale subdivision of the continent, the large ecological climate zones based on temperature and precipitation are used in identifying categories called Divisions (Bailey 1983).

Beyond climate, vegetation and other natural land cover, which usually coincide with major relief units, are used to identify more detailed ecoregion classifications called Provinces. Provinces are further divided into Sections, based on the terrain defined by elevation and soils, in Bailey's scheme (Bailey 1983). Benthic invertebrate and fish assemblages in Missouri were found to be coincident with Bailey's Sections and Omernik's ecoregions (Rabeni and Doisy 2000). The Freshwater Ecoregions of the World (here called "Freshwater"), which depict a global biogeographic regionalization of Earth's freshwater system, are based on the distributions and compositions of freshwater fish species and incorporate major ecological and evolutionary patterns (Abell et al. 2008). Freshwater ecoregions are based on large regional watersheds around major rivers, and have been used for global and regional conservation efforts related to fish species (Abell et al. 2008).

The objectives of the present study were: (1) to evaluate which ecoregional classification scheme (Omernik, Bailey, or Freshwater) provides a better framework for understanding the spatial distribution of aquatic macroinvertebrates in the EPT taxa in the state of Tennessee, and (2) to analyze how EPT taxa respond to variations in regional environmental and stream-based factors. To address the objective of this study, the distribution of statewide benthic macroinvertebrate data was analyzed with the three ecoregion classifications, and the differences in EPT taxa richness across ecoregions and the regional factors that could cause such differences were evaluated. The ecoregion classification schemes were used to partition naturally occurring variation in the aquatic biota and in the effects of regional-scale stressors effects on aquatic macroinvertebrates. Evaluating these classification schemes and understanding the ecoregion-scale factors affecting EPT taxa richness can help identify the baseline and context under which several other factors, such as those caused by land-use change, can affect EPT taxa.

Study area and methods

Study area

This study focuses on the diversity and distribution of aquatic macroinvertebrates in Tennessee, which has great variation in topography, climate, and geography, and hence has one of the highest freshwater species diversities of any inland state in the United States (Stein 2002). Several distinct landforms exist in Tennessee – from the Appalachian Mountains in the east to the Mississippi Plains in the west (Figure 3). The unique geomorphological and geological structures of the area provide different stream characteristics and substrates that are expected to affect macroinvertebrate distribution. Since one of the goals of this research is to understand the spatial distribution of macroinvertebrates as a function of different environmental variables, the diverse geomorphological structure of Tennessee offers an ideal case study.

The Blue Ridge Mountains (part of the Appalachian range) of Tennessee, extending to parts of the Great Smoky Mountains, are characterized by forested slopes; high gradient, cool, clear streams with bedrock and boulder substrates; and rugged terrain on primarily metamorphic bedrock (Griffith 2010). West of the Blue Ridge Mountains is

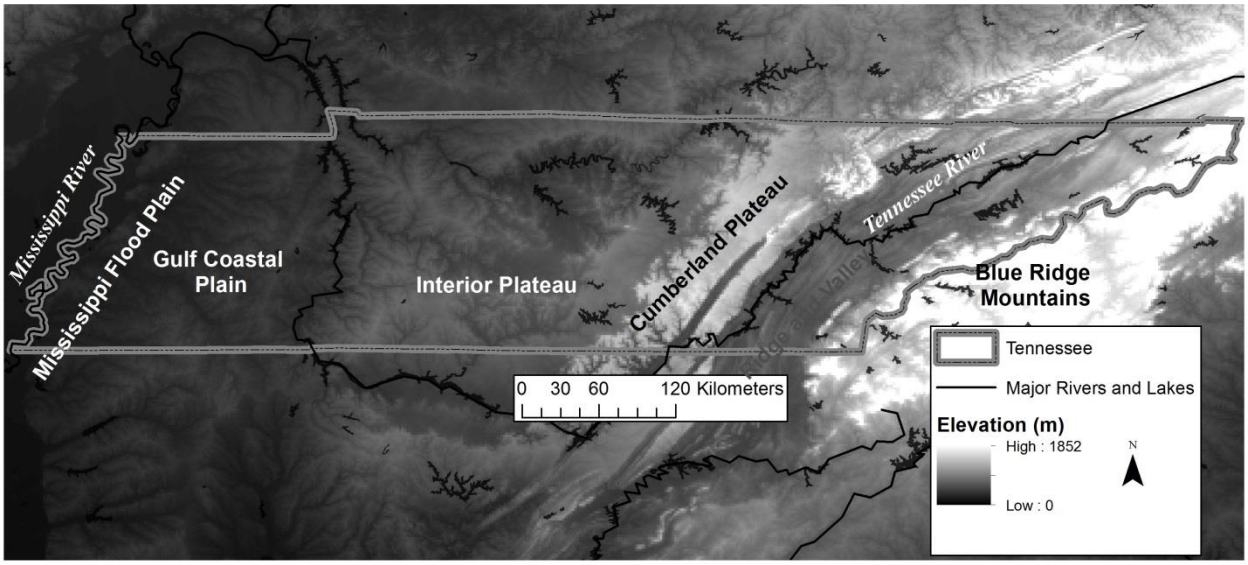


Figure 3: Physical map of Tennessee illustrating elevation gradient, major landforms and rivers.

the Ridge and Valley Province, which is characterized by parallel ridges and valleys representing a variety of widths, heights, and geologic materials, including limestone, dolomite, shale, siltstone, sandstone, chert, mudstone, and marble. Small streams drain the ridge slopes and join at right angles with larger lower-gradient stream courses that meander along the parallel valley floors (Griffith 2010). The Southwestern Appalachian Mountains are low mountains to the west of the Ridge and Valley ecoregion that have a moderate to high density of small perennial streams, mostly on moderate to high gradients. The Interior Plateau is a diverse ecoregion with Mississippian to Ordovician-age limestone, chert, sandstone, siltstone, and shale underlying the landforms of open hills, irregular plains, and tablelands. This region has perennial to intermittent streams on low to moderate gradients. Close to the western end of the state, the Gulf Coastal Plain has Cretaceous or Tertiary-age sands, silts, and clays that contrast geologically with the older limestone, chert, and shale found in the Interior Plateau. Streams in this area have relatively low gradients with sandy substrates (Griffith 2010). The Mississippi Flood Plains at the far west end of the state are flat broad floodplains with river terraces and levees. The soils there are generally fine textured and poorly drained.

Methods: Data and analysis

As a first step towards the research goal, the EPT taxa richness values across streams in Tennessee were categorized by different ecoregion classification schemes. We used Omernik's level III classification, Bailey's sections, and Freshwater ecoregion classes as the classification schemes with which to evaluate EPT taxa richness. Omernik's level III classification was selected for this study since the number of Omernik's classes at the level III hierarchical level (8 classes in Tennessee) was comparable to the number of classes in Bailey's sections (9 classes in Tennessee). Omernik's and Bailey's classifications have similar classes that capture the east-west geomorphological gradient in Tennessee (Figure 4). Bailey's classification includes additional classes that separate north-south regions such as the Northern Ridge and Valley section and Central Ridge and Valley Section. The Freshwater ecoregion classification follows hydrologic regions that drain major rivers (Lower Mississippi, Tennessee and Cumberland rivers). The distribution of EPT taxa richness across different ecoregions in each classification was analyzed to evaluate whether EPT taxa richness values were similar across the study region or different based on ecoregion.

EPT taxa richness measures were obtained from data collected by the Tennessee Department of Environmental Conservation (TDEC). TDEC surveys and monitors hundreds of streams in Tennessee to conduct benthic macroinvertebrate assessments using standard operating protocols (TDEC 2008). Based on semi-quantitative single habitat surveys (SQSH) in riffles or banks, taxa are identified to the genus level within a 200-organism subsample, and indices such as total taxa, EPT taxa richness, percentage of organisms in EPT order, and intolerant taxa are calculated (TDEC 2011). The information from TDEC used in this study included data from the Wadeable Streams Assessment (WSA) (Arnwine et al. 2011); reference stream assessment; and biological, chemical, bacteriological, and physical data collected as part of the following programs: routine watershed monitoring, 303(d) monitoring, antidegradation monitoring, and permit compliance/complaint investigation (Denton et al. 2010). TDEC conducted

probabilistic monitorings of about 90 wadeable streams in 2007 and 2010 as part of a national monitoring effort (Arnwine et al. 2011). TDEC's wadeable stream assessment is based on one site visit per station and includes information on macroinvertebrates, habitat, nutrients, and metals. The reference stream assessment by TDEC evaluates habitat at reference sites using standardized numeric assessment approaches. These reference sites are the least-impacted, yet representative, streams in each of the Omernik's ecoregions within the state. There are 162 reference stream sites in Tennessee, and these sites are monitored on a 5-y rotation period. As part of TDEC's routine watershed monitoring program, 55 hydrologic watershed boundaries were identified within Tennessee, and each of these watersheds was classified into one of five groups that were monitored on 5-y cycles scheduled in different years. Macroinvertebrate sampling and habitat assessments were conducted by TDEC at these sites. We assembled 741 data samples between the years 2007 and 2010 from TDEC. Ninety-eight of these samples were from reference streams and the rest of the samples were from "non-reference" stream sites. The EPT taxa richness values were between 0 and 25 and not normally distributed. Hence we used the Kruskal-Wallis test, a nonparametric statistical procedure, to evaluate if EPT taxa richness was different by ecoregion as defined by each of the three ecoregion classification schemes.

The distribution of EPT taxa richness values were analyzed based on Bailey's, Omernik's, and the freshwater ecoregion classification schemes (Figure 4). To select the classification that performed the best with respect to explaining EPT taxa richness patterns in Tennessee, the regional variables that influence macroinvertebrate habitat (climate, geology, soils) were analyzed as covariates along with EPT taxa richness. Based on a review of studies evaluating factors affecting macroinvertebrate habitat at various scales (Figure 2), climate, geomorphology, stream flow, and stream order were identified as potential important factors influencing macroinvertebrates at the regional scale (e.g., Lenat and Crawford 1994; Poff 1997). We obtained data for each of these factors at every macroinvertebrate stream sampling site. Long-term average precipitation and temperature (over the period 1971-2000) were obtained from the NHDplus dataset for each stream segment that contained the macroinvertebrate sampling site (McKay et al. 2012). Average annual precipitation values, corresponding to the year of macroinvertebrate data collection, were obtained from PRISM climate data at the macroinvertebrate sampling site (PRISM climate group 2013) (Table 2).

Soil variables, geology, and slope were used to describe geomorphology. Soil, silt, and clay texture compositions were derived from the Soil Survey Geographic (SSURGO) database (Soil Survey Staff 2013). Based on a weighted average of the soil texture fractions by component in ArcGIS® (Environmental Systems Research Institute 2012), we mapped the soil texture values to SSURGO map units for Tennessee. Soil texture percentages for the SSURGO map units corresponding to the macroinvertebrate stream sampling site were extracted for each macroinvertebrate sample. Predominant lithology categories were obtained from the geologic maps of Tennessee and used to identify primary and secondary rock types at each stream sampling site (USGS 2005). Stream-based variables such as stream order, stream velocity, and stream flow were obtained from NHDplus for each stream segment that contained the macroinvertebrate sampling

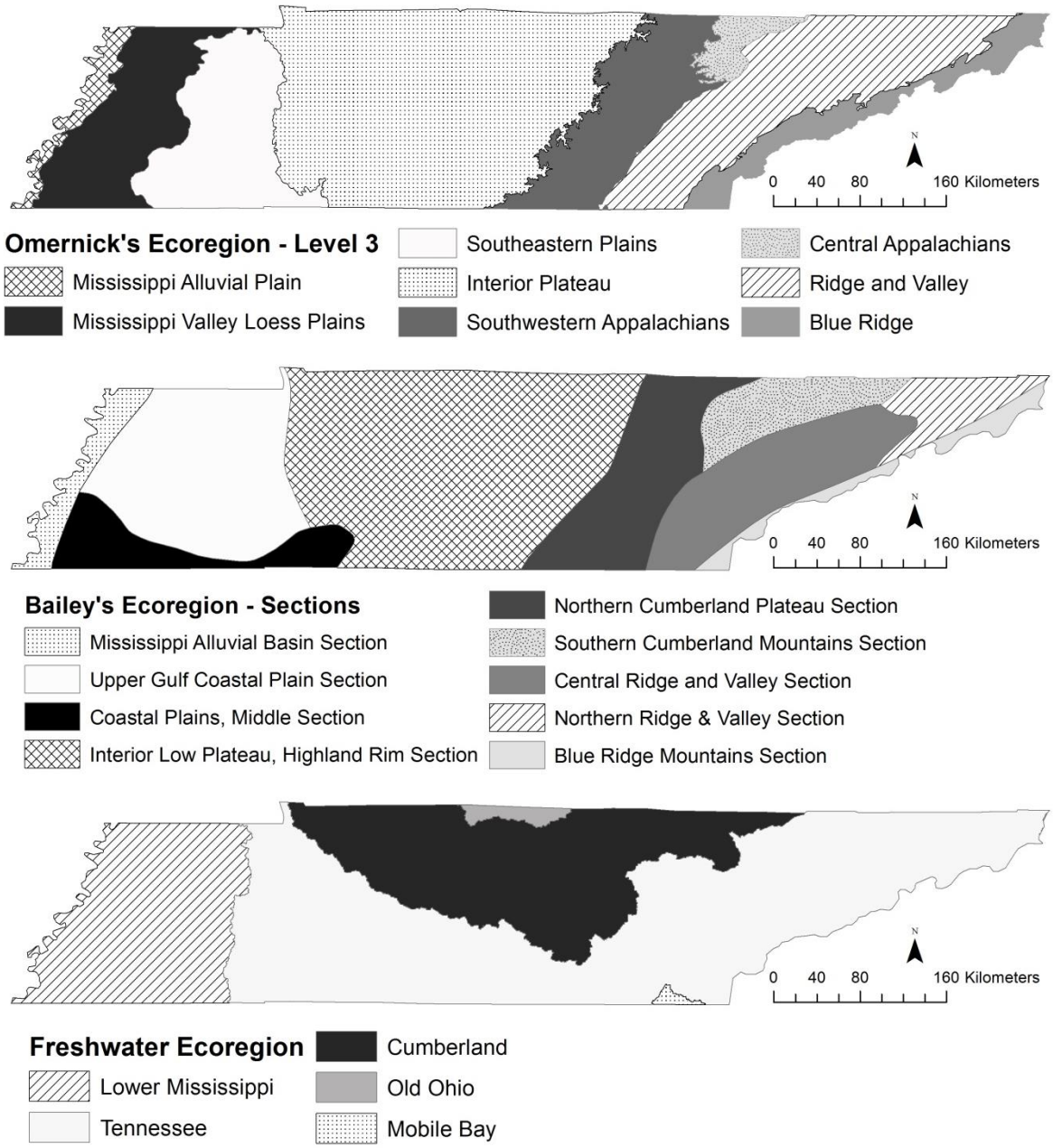


Figure 4: Ecoregion classification schemes for Tennessee.

Table 2: List of regional variables considered to be potentially important in affecting macroinvertebrate taxa richness, with sources of data and statistical tests. NHDplus refers to the hydrologic framework dataset developed by the US Environmental Protection Agency and the US Geological Survey (McKay et al. 2012). The PRISM Climate Group develops spatial climate datasets to reveal short- and long-term climate patterns (PRISM climate group 2013). The SSURGO database contains information about soil collected by the National Cooperative Soil Survey (Soil Survey Staff 2013).

Category	**Variable**	**Unit**	**Source**	**Statistical test used to test significance**
Climate	Average temperature at the stream sampling site	C	NHDplus	Correlation
	Average precipitation over several years at the stream sampling site	mm	NHDplus	Correlation
	Mean annual precipitation during the year of data collection	mm	PRISM Climate group	Correlation
Geomorphology	Rock Type	Categorical	US Geological Survey Geology data	Kruskal-Wallis/ one-way ANOVA
	Depth to bedrock	m	State Soil Geographic (STATSGO) dataset	Correlation
	Soil (*e.g.* % clay)	%	SSURGO	Correlation
	Slope	Degrees	NHDplus	Correlation
Stream flow	Stream Flow	Cubic meters per second	NHDplus	Correlation
	Velocity	Meters per second	NHDplus	Correlation
Stream Order	Stream order	Categorical (nominal)	NHDplus	Kruskal-Wallis/ one-way ANOVA

site (McKay et al. 2012). Stream velocity and stream flow from NHDplus represent long-term velocity and flow estimates based on data from 1971 to 2000. Slope of the stream segment, which represents the change in elevation by the length of the stream segment, was also obtained from the NHDPlus dataset. NHDplus uses 10 m resolution National Elevation Dataset (NED) to calculate elevation and slope of each stream segment.

In order to focus on the regional environmental influences on EPT taxa richness and to prevent masking of such influences by other anthropogenic factors, data from the reference stream sites were used to determine which ecoregion classification would be more appropriate for the purpose of this study (e.g., to differentiate the most EPT taxa richness from one ecoregion to others). Silhouette analysis, commonly used to evaluate clusters, was applied to data from the reference stream sites. Silhouette analysis computes the within-cluster compactness and between-cluster dissimilarity to obtain a silhouette value between -1 and 1. The silhouette value for the ith point, Si, is defined as

$$Si = \frac{bi - ai}{\max(ai, bi)}$$

where ai is the average distance from the ith point to the other points in the same cluster as i, and bi is the minimum average distance from the ith point to points in a different cluster, minimized over clusters (Rouseeuw 1987). High silhouette values above 0 indicate a tight cluster that is well matched to its own cluster and well differentiated from its neighboring clusters. Low silhouette values can be due to too many or too few clusters (Kaufman and Rouseeuw 2009). A negative silhouette value indicates that an object may have been placed in a cluster that is heterogeneous, since the average within-cluster distance is large and/or the distance to the next closest cluster is small. As the silhouette value gets closer to -1, it is clearer that the object has been misclassified and its placement will be more appropriate in a different cluster. However values closer to zero indicate that the within cluster distance and the distance to the next closest cluster is about the same and the object can belong to both clusters (Rousseeuw 1987). The ecoregion classification that showed the highest silhouette value assessed using EPT taxa richness and regional variables was selected as the one suitable for further analysis.

The second part of this research focused on the regional factors that influence aquatic macroinvertebrates and how their influence varied across ecoregions associated with the ecoregion classification scheme selected earlier. First we used the Kruskal-Wallis test on each regional variable (α =0.05) to examine which factors varied across different ecoregions. Next we evaluated the association between regional factors and EPT taxa richness (Table 2). If a regional factor was a continuous variable, we utilized correlation analyses between EPT taxa richness and the regional factor for each ecoregion. Then we compared the strength of those associations to see if they varied by ecoregions. For a regional factor as a categorical variable, such as rock type and stream order, we used

the Analysis Of Variance (ANOVA) test to determine if there were significant differences in EPT taxa richness across groups of each variable.

Results

EPT taxa richness across ecoregions

Kruskal-Wallis tests showed that under all three ecoregion classification schemes considered in this study, the EPT taxa richness values were statistically different across ecoregions at a 0.001 level ($\chi 2$ (Freshwater ecoregion) = 75.638, p = 0.00; $\chi 2$ (Omernik's ecoregion) = 169.268, p = 0.00; $\chi 2$ (Bailey's ecoregion) = 122.359, p = 0.00). A graphical representation of the EPT taxa distribution for Omernik's and Bailey's ecoregions illustrates that higher EPT taxa richness was found in the eastern regions – Blue Ridge, Central and Southwestern Appalachians, and the Ridge and Valley ecoregions (Figure 5). Using the Freshwater ecoregion classification showed low EPT taxa richness in the Mississippi ecoregion and higher EPT taxa richness for reference streams in the Tennessee ecoregion. In all ecoregion classifications, the EPT taxa richness values of reference streams were higher than those of other stream sites.

Statistical analysis of the regional variables using Kruskal-Wallis tests indicated that temperature, rock depth, percentage of sand, silt and clay, slope, stream flow, stream velocity, and stream order were significantly different across all three ecoregion classifications (Table 3). Precipitation was significantly different across the Omernik's and Bailey's ecoregion classes, but not significantly different across Freshwater ecoregion classes.

Silhouette analysis of the EPT taxa richness and regional variables for the three different ecoregion classifications resulted in silhouette values of -0.0812 for Omernik's classification, -0.1672 for Bailey's classification, and -0.2953 for the freshwater ecoregion classification. Silhouette plots of the classification schemes (Figure 6) identified individual classes that had high negative or positive values. In general, values close to 0 indicate samples are close to boundaries between clusters, and negative values indicate heterogeneous classes that can be improved with further clustering. Overall, all classification schemes provided negative values, indicating that the classification could be improved with better clusters. However since the goal of this analysis was to compare the relative performance of three pre-existing classification schemes, the best among the three, Omernik's classification, was chosen for further analysis.

Regional factors among ecoregions

The results from the analyses testing the significance of relationship between the regional variables and EPT taxa richness across the Omernik's ecoregion indicated differences by ecoregion class (Table 4). For example, Pearson's correlation values between temperature and EPT taxa richness were found to be significant only in the Central Appalachians (-0.27, p=0.048), the Ridge and Valley (-0.301, p=0.0), and Blue Ridge (-0.419, p=0,002) ecoregions. However, the correlations between stream velocity

Figure 5: Box plots of EPT taxa richness (using data collected by TDEC between 2007 and 2010) grouped by Omernik (a), Bailey (b) and Freshwater(c) ecoregion classes of Tennessee (ordered from west to east) and separated by reference stream category (reference streams are light grey boxes and non-reference streams are dark grey boxes). The number of stream samples in each ecoregion class is provided in parenthesis. The boxes represent the interquartile (IQ) range, and the line across the boxes indicates the median value. The whiskers from the upper and lower edge of the box indicate the highest and lowest values within 1.5 times the IQ range. Outliers between 1.5 and 3 times the IQ range are indicated by the closed circles, and outliers with values more than 3 times the IQ range are indicated by the asterisk. Data from the Mississippi Alluvial Plain have been excluded due to insufficient data.

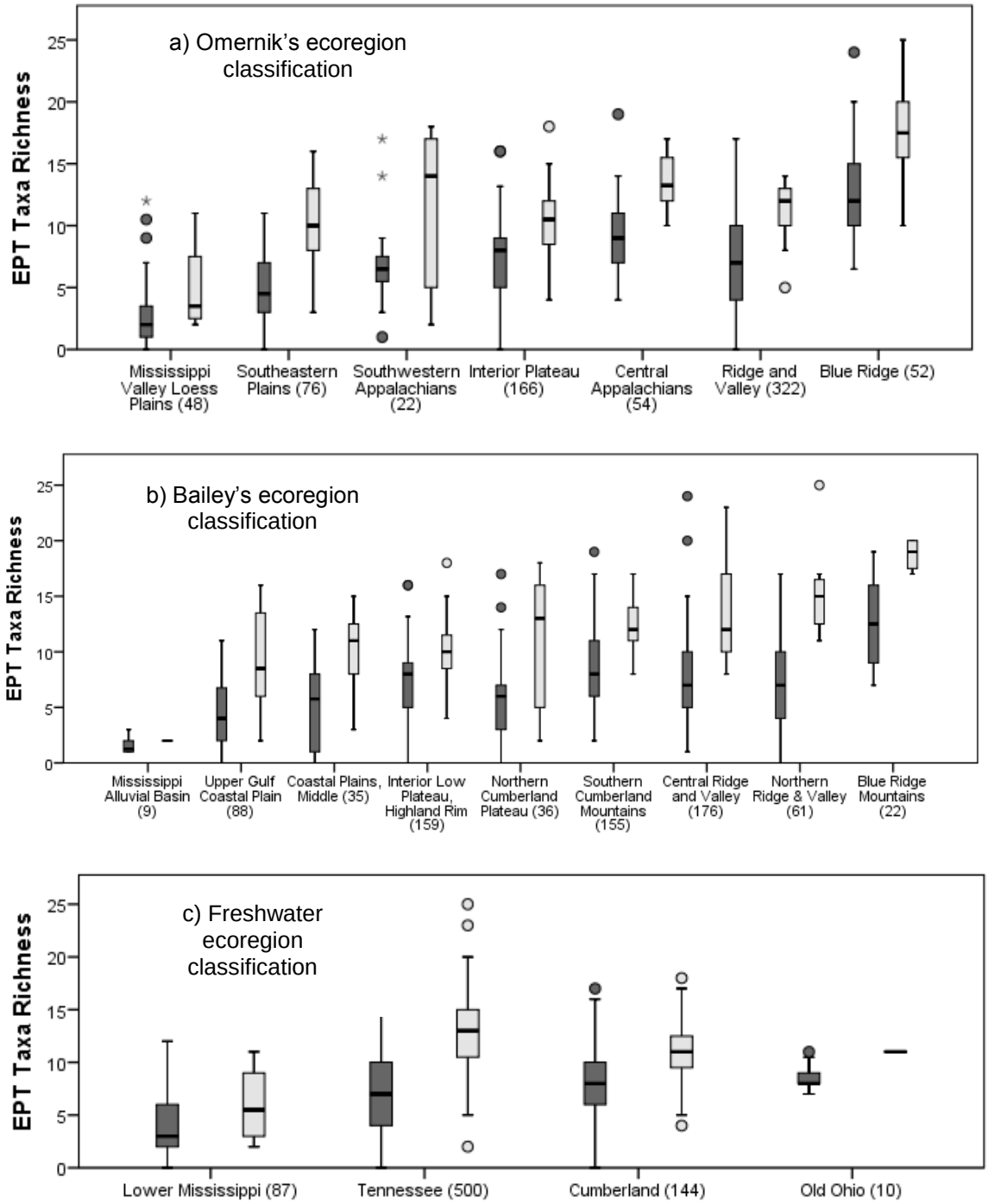


Figure 5: Continued

Table 3: Chi square values and p-values (in parentheses) of the Kruskal Wallis test to test significance of differences in regional variables across three ecoregion classifications.

Ecoregion Classification	Temperature	Precipitation	Rock Depth	Rock Type	% Sand	% Silt	% Clay	Slope	Stream Flow	Velocity	Stream order
Omernik's	461.04 (0.00)	177.88 (0.00)	292.03 (0.00)	47.49 (0.00)	99.4 (0.00)	84.24, (0.00)	167.5 (0.00)	84.47 (0.00)	30.433 (0.00)	131.23 (0.00)	24.47 (0.00)
Baileys	491.53 (0.00)	261.79 (0.00)	288.59 (0.00)	76.55 (0.00)	25.86, (0.00)	62.3 (0.00)	115.16 (0.00)	85.77 (0.00)	53.52 (0.00)	98.09 (0.00)	23.5 (0.00)
Freshwater	192.36 (0.00)	0.125 (0.98)	142.33 (0.00)	21.9 (0.00)	15.49 (0.00)	54.59 (0.00)	75.84 (0.00)	30.0 (0.00)	41.25 (0.00)	63.12 (0.00)	8.04 (0.05)

Figure 6: Silhouette plots for classification of EPT taxa richness using Omenik's (A), Bailey's (B), and Freshwater (C) ecoregion classification schemes. Classes on Omernik's plot (A) are: 1- Blue Ridge, 2-Central Appalachian 3-Interior Plateau, 4-Mississippi Valley Loess Plains, 5-Ridge and Valley, 6-Southeastern Plains, and 7-Southwestern Appalachians. Classes on Bailey's plot (B) are 1-Blue Ridge Mountains, 2-Central Ridge and Valley, 3-Coastal Plains middle section, 4-Interior Low Plateau, Highland Rim Section, 5-Mississippi Alluvial Basin Section, 6-Northern Cumberland Plateau Section, 7-Northern Ridge and Valley Section, 8-Southern Cumberland Mountains Section, and 9-Upper Gulf Coastal Plain Section. Classes on Freshwater ecoregion plot (C) are: 1-Lower Mississippi, 2-Old Ohio, 3-Cumberland, and 4-Tennessee.

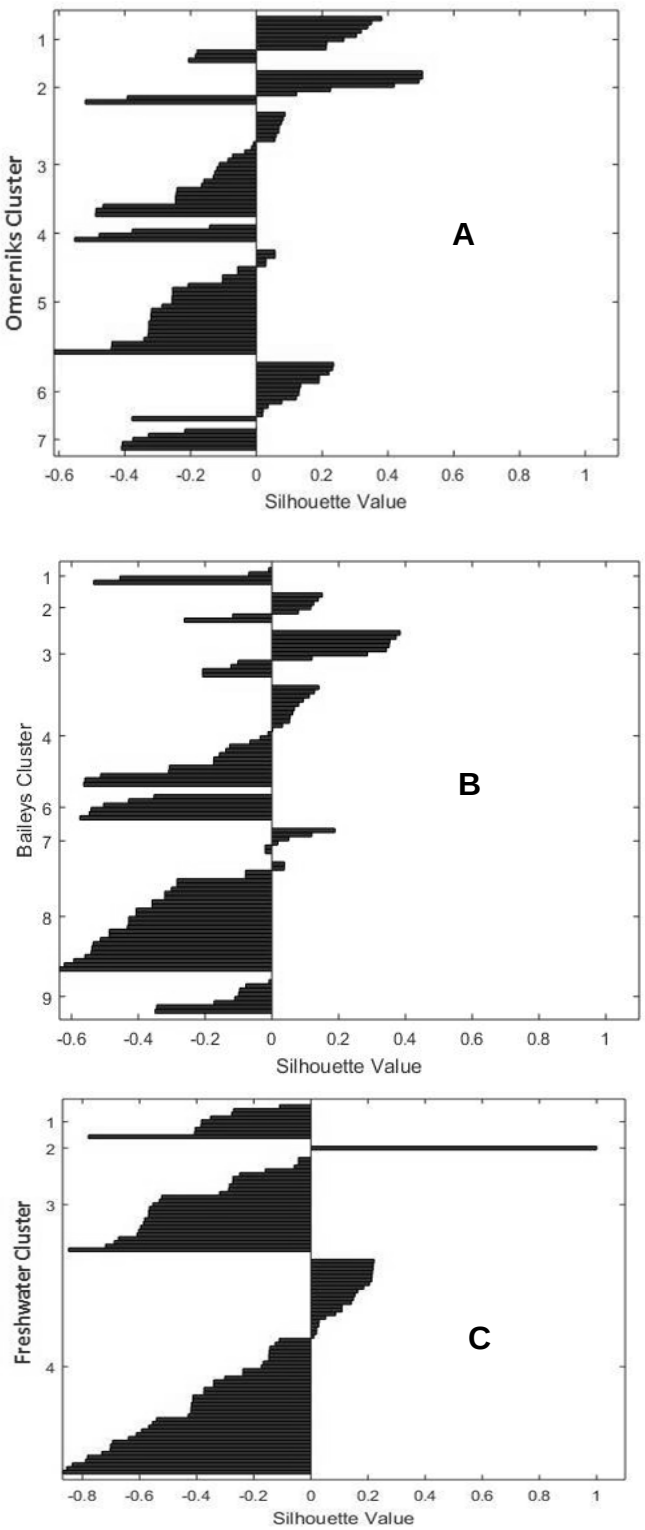


Figure 6: Continued

and EPT taxa richness were significant in most ecoregions, including the Mississippi Valley Loess Plain (0.539, p=0.0), Southeastern Plains (0.423, p=0.0), Interior Plateau (-0,213, p=0.006), Southwestern Appalachians (0.52, p=0.013), and Ridge and Valley (0.217, p=0.0). Two precipitation variables were considered for the analysis: mean annual precipitation for the year of macroinvertebrate data collection and overall average precipitation over the macroinvertebrate stream site. The mean annual precipitation, which was included to identify the potential influence of annual variations in precipitation on aquatic macroinvertebrates, was not significantly different among any of the ecoregion classes and hence has not been included in the results. The average precipitation over the stream, which helps differentiate the regional precipitation trends, was found to be significant in the western ecoregions of Mississippi Valley Loess Plains (0.336, p=0.02) and Southeastern Plains (0.308, p=0.007), and in the Central Appalachian (0.423, p=0.001) ecoregions.

Discussion

Complex biological systems, such as aquatic ecosystems, which vary in space, need to be studied at multiple scales by considering appropriate relationships between environmental effects and species traits at each scale (Poff 1997). Ecoregions are intended to help partition such effects and capture the environmental framework within which other factors may influence aquatic systems. In this study, the variation of EPT taxa richness across ecoregions reflects the regional variation of macroinvertebrate community distribution as a response to different ecological, geological, and climatic conditions. All three ecoregion classifications showed significant differences in EPT taxa richness among ecoregions (Figure 5). However, there were differences in the significance of regional variables across the different classifications. The Freshwater classification had the lowest silhouette scores of the three classification schemes. This result could be because this scheme does not take into consideration climate or soil (Figures 4 and 7), which have been shown to be important indicators of macroinvertebrate community structure (Diaz et al. 2008). Bailey's ecoregion classification, being based on climate, captures the precipitation gradient but does not align with the east-west soil gradient seen in Tennessee. Omernik's ecoregions align with the soil gradient and follow the general east-west gradient of precipitation. However, the silhouette plots of the Omernik's ecoregion classification indicated that many regions had predominantly negative silhouette values. This observation could be attributed to the presence of other factors that influence the EPT taxa richness that are not captured by the ecoregion classification schemes. For example, silhouette values of the Ridge and Valley ecoregion were largely negative. The Ridge and Valley ecoregion is composed of roughly parallel ridges and valleys in a variety of widths, heights, and geologic materials. The diversity within the landscape is not captured by the ecoregion classification and requires other sub-regional variables such as stream bank characteristics, substrate, and stream bed information for a more effective classification. Such a classification is beyond the scope of the current study, which is to assess whether existing data could be used to differentiate macroinvertebrates by ecoregions. Considering Omernik's level IV classification, which has finer classes based on geomorphological features, could have provided different results and more detailed

Table 4: Significance of relationship between EPT taxa richness and regional variables by Omernik's ecoregion classification. Significance of the relationship between EPT taxa richness and rock type and relationship between EPT taxa richness and stream order are based on a one-way ANOVA tested at the 0.05 level (indicated with an asterisk). The Pearson's R correlation coefficient significant at the 0.05 level is presented for the rest of the variables.

Ecoregion	Temp-erature	Precip-itation	Rock depth	Rock type	% Sand	% Silt	% Clay	Slope	Stream Flow	Velocity	Stream order	Map
Mississippi Valley Loess Plains		.336			.301				.491	.539	*	
Southeastern Plains		.308		*			.355			.423	*	
Interior Plateau			.195	*	.187			.253	-.356	-.213	*	
Southwestern Appalachians										.52		
Central Appalachians	-.27	.423					.269					
Ridge and Valley	-.301				.193	-.213		.147		.217	*	
Blue Ridge	-.419				.394			.344	0.328		*	

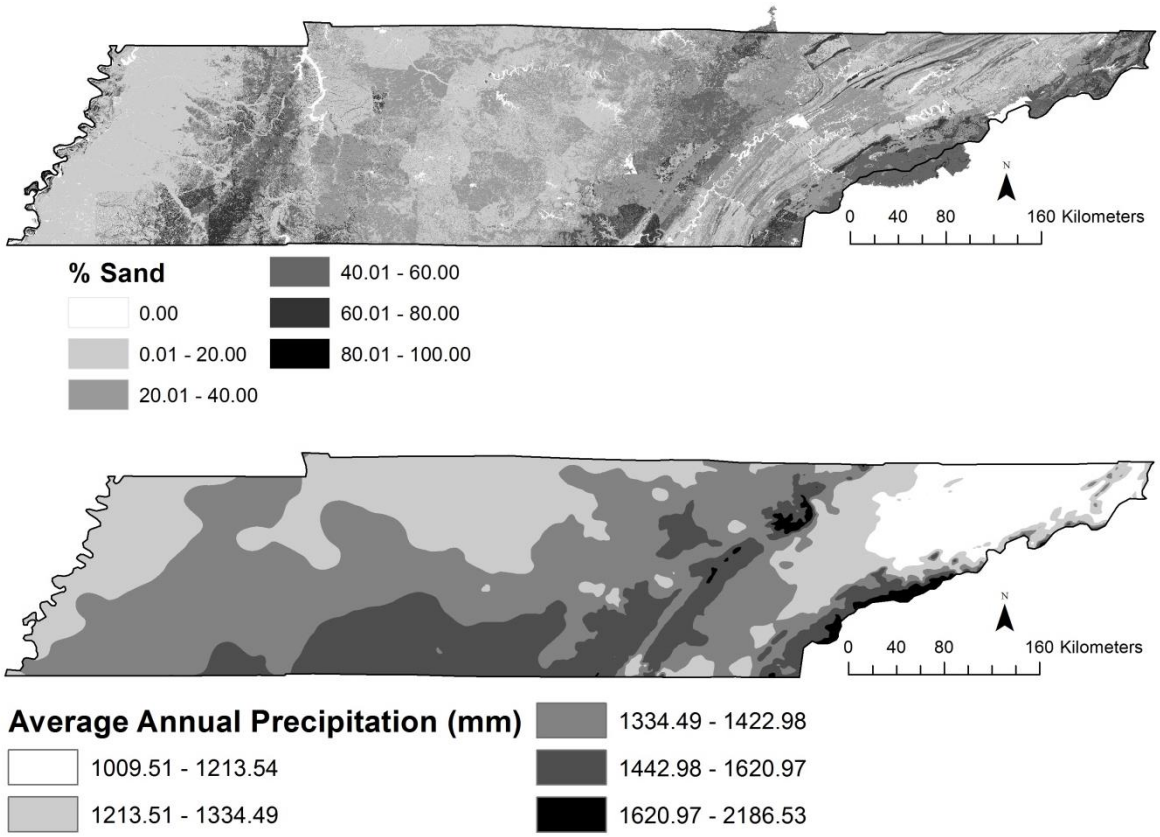


Figure 7: Soil (upper) and climate (lower) gradients across Tennessee based on soil composition and average annual precipitation (Source: SSURGO and PRISM data).

differentiation of the spatial distribution of EPT taxa richness. However, Omernik's level IV classification for Tennessee has 25 classes (Griffith et al. 2012), which is much higher, and hence not comparable, to the number of classes in Bailey's and Freshwater classifications. Since Omernik's level III classification was the better classification scheme among the three ecoregion classifications based on the silhouette analysis, it was chosen as the spatial framework for classifying aquatic macroinvertebrates and their naturally occurring regional variation in Tennessee. This result aligns with existing water quality management since EPA's ecoregions are based on Omernik's classification scheme, and are also used by TDEC to manage stream sampling protocols.

In addition to setting the regional constraints, analyzing the spatial distribution of EPT taxa in relation to environmental gradients such as temperature, precipitation, and geomorphology, and stream–based factors is critical for predicting how species and communities are affected by other external changes. Based on the regional factors correlated with EPT taxa richness across Omernik's ecoregions (**Error! Reference source not found.**), stream velocity was found to be a significant factor across all but Central Appalachian and Blue Ridge ecoregions. EPT taxa richness was positively correlated with stream velocity in the Mississippi Valley Loess Plains, Southeastern Plains, Southwestern Appalachians and Ridge and Valley ecoregions.

Aquatic macroinvertebrates need well-aerated streams, and a reduction in flow velocity can lead to an increase in deposition of fine sediments and decaying matter onto the riverbeds (Wood and Armitage 1997). This increase is prevalent during low flow conditions in summer, and it is acute in groundwater-fed streams (Wood and Armitage 1997). Flow changes can also affect taxa richness by causing changes in amount of nonpoint source runoff or the dilution of point source discharge (Lenat 1988). Further, a number of studies have found significant correlations between flow-related variables and macroinvertebrate habitat (e.g., Poff and Ward 1989). In the Interior Plateau ecoregion, stream flow and stream velocity were negatively correlated with EPT taxa richness. The Interior Plateau ecoregion, composed of hills and irregular plains, is relatively larger than the other ecoregions and has intermittent streams with low to moderate gradients. Macroinvertebrates in intermittent streams are influenced by zero flow conditions and seasonality of flow, which are of critical for persistence and stability of species (Poff and Ward 1989; Beche et al. 2006). In such intermittent streams, the stream flow components are not of primary importance with respect to species richness. The negative correlation of EPT taxa richness with stream flow and velocity in the Interior Plateau ecoregion could be a function of other confounding factors, not captured by the data within the single ecoregion class.

Differences in EPT taxa richness were significant across stream orders in the Mississippi Valley Loess Plains, Southeastern Plains, Interior Plateau, Ridge and Valley and Blue Ridge ecoregions. In all cases, EPT taxa richness was highest in the first order headwater streams, and then decreased in the second order streams (Figure 8). Headwater systems are critical for biodiversity and support higher macroinvertebrate diversity (Gomi et al. 2002). Previous studies have found taxa richness tends to

increase with stream order till the 5th order streams (Lenat 1988). A similar trend was observed for streams in the Mississippi Valley Loess Plains, Southeastern Plains and Ridge and Valley ecoregions (Figure 8). The Interior Plateau streams also show a gradual increase from 2nd order streams and then a drop in taxa richness beyond 5th order streams. This downstream shift in species richness conforms to the river continuum concept (Vannote et al. 1980), according to which the macroinvertebrate community structure changes along a continuum, from stream headwaters to mouths due to changes in stream flow, channel morphology, and temperature (Minshall et al. 1985). With an increase in stream order, there is a decrease in the physio-chemical fluctuations, mean annual turbidity, and water temperature, while mean annual flow, alkalinity, and conductivity increase (Harrel and Dorris 1968). These factors affect macroinvertebrate habitat and food resources. However the influence of stream order was different for Blue Ridge streams, which showed a decrease in taxa richness with increasing stream order. In the Blue Ridge, the highest EPT taxa richness values were observed in the first-order streams, which are pristine mountainous headwater streams.

The percentage of sand was positively correlated with EPT taxa richness in the Mississippi Valley Loess Plains, Interior Plateau, Ridge and Valley and Blue Ridge ecoregions. The type of soil influences the substrate type in streams and hence affects macroinvertebrate habitat. For example, clay soils are less permeable to water than sandy soils. In areas with sandy soils, larger proportions of sand are found in the stream reaches, and streams are generally shallow (Richards et al. 1996). Dissolved ion concentration, which is also an important determinant of stream macroinvertebrate composition, can be attributed to geology and soil conditions (Hynes 1975). Correlation of mean annual temperature with EPT taxa richness was statistically significant in the eastern ecoregions of Tennessee. This significance could be a function of the rugged terrain in these regions, which influences temperature variations and also precipitation changes. The EPT taxa richness in the Ridge and Valley and Blue Ridge streams was also positively correlated with slope, which can also be attributed to the mountainous terrain with varying elevation.

Conclusion

The local macroinvertebrate community structure in Tennessee is a result of continuous sorting processes through various environmental filters, ranging from regional processes (geology, climate) to local processes (substratum porosity, channel velocity) (Figure 2). Our analysis across ecoregions Tennessee indicates that EPT taxa richness varies by ecoregion, irrespective of the classification type used (Figure 5). However from a conservation or stream management perspective, Omernik's ecoregion classification was found to be the better classification for addressing local effects on aquatic macroinvertebrates in view of regional environmental influences. Differences in the regional factors that influence EPT taxa richness across ecoregions have implications for how the macroinvertebrate community responds to other changes, such as land-use changes. The spatial scales with which land cover and macroinvertebrate variables are associated vary depending on the regional and local conditions and mechanisms that link land cover and ecological conditions (Allan et al. 1997; Strayer et

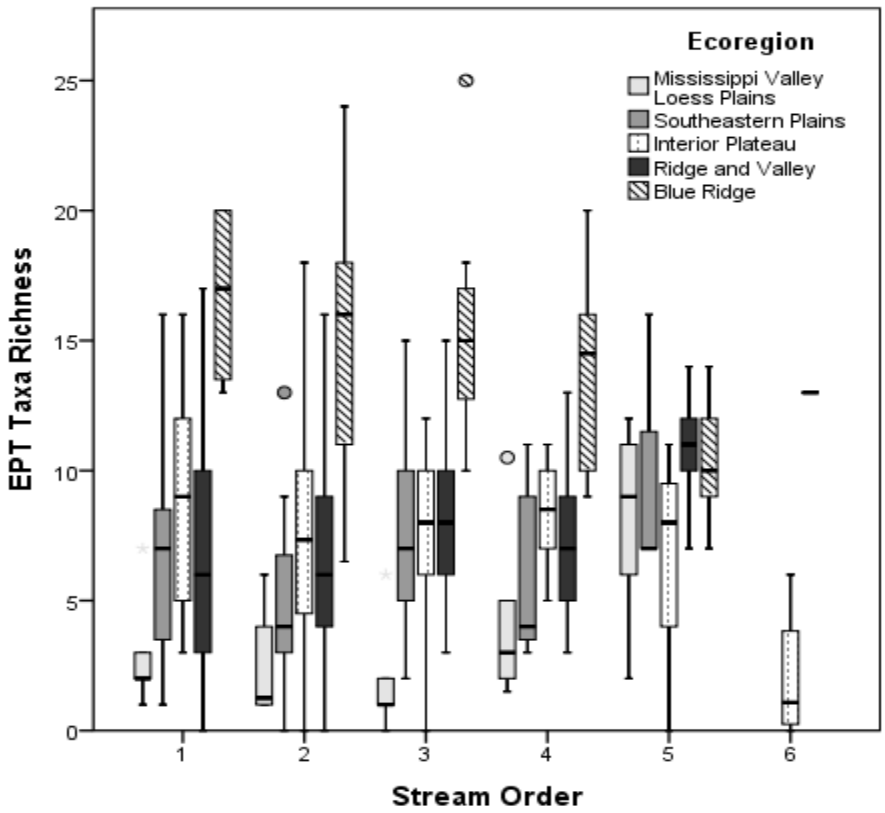


Figure 8: Distribution of EPT taxa richness by stream order for ecoregions found to have significant correlation between stream order and EPT taxa richness. The boxes represent the interquartile (IQ) range, and the line across the boxes indicates the median values. The whiskers from the upper and lower edge of the box indicate the highest and lowest values within 1.5 times the IQ range. Outliers between 1.5 and 3 times the IQ range are indicated by the closed circles.

al. 2003).

The processes that influence the structure and dynamics of communities occur on multiple spatial scales; thus, for many communities, focusing on processes at a single spatial scale does not provide understanding of the factors that shape communities (Swan and Brown 2011). Understanding the relative influence of catchment, reach, and riparian-scale factors in structuring aquatic biota can help prioritize the scale at which to rehabilitate, manage and derive policies for stream ecosystem integrity (Weigel et al. 2003). Although the correlative approach used in this study cannot be used to directly infer regional effects on macroinvertebrates, it helps identify factors that may indicate potential pathways by which changing landscapes can affect macroinvertebrate assemblages.

The results of this study are a function of the spatial and temporal scale of the data, and the effects inferred can vary with different spatial and temporal scales. For example, although short-term flow variations and hydrologic changes can affect aquatic macroinvertebrates, studies have shown that long-term flow records are useful to evaluate spatial patterns of stream systems (Poff and Ward 1989). Further, aquatic macroinvertebrates are resilient and can recover within weeks after flood or drought events (Angradi 1997; Fritz and Dodds 2004). Hence variables that describe long-term precipitation and stream characteristics, such as those used in this study, can be useful to understand interactions over larger temporal and spatial scales.

The regional variables used in this study are a function of the spatial scales at which they were collected. For example, soil textures were assembled from SSURGO data, which were collected at regional scales between 1:12,000 and 1:63,360. These data sources do not capture localized variations in soil texture that can influence runoff and input into streams. However, since one of the objectives of this study was to analyze how EPT taxa respond to variations in regional environmental and stream-based factors across Tennessee, the data collected at the regional spatial scale can address this objective at the spatial scale considered.

In this study we found that various regional controls constrain EPT taxa richness and hence can influence the susceptibility of EPT taxa to further abiotic changes. Understanding the regional controls is important since the usefulness of macroinvertebrate assemblages for monitoring water quality and biological integrity depends on the ability to distinguish human impacts from natural variability (Waite et al. 2000). We also found that though the regional variables that define ecoregions are important, they are not sufficient to characterize the EPT taxa richness across ecoregions. For example, based on the silhouette analysis, the regional variables are not sufficient covariates to characterize the EPT taxa richness in the Ridge and Valley ecoregion (Figure 6). EPT taxa richness in the Ridge and Valley streams was significantly correlated to soil, terrain, and stream velocity, and in these streams the effect of land-use change also depends on the location of the stream and its flow characteristics (also a function of the terrain).

One of the important conclusions of this study is identifying the need to address the regional environmental context while studying aquatic macroinvertebrates. Identifying the regional factors and accounting for them can help characterize land-use and land-management change effects on aquatic macroinvertebrates. Since aquatic macroinvertebrate indices are used as biological indicators, understanding the regional environmental controls and being able to separate them from other potential influences is critical to interpreting aquatic macroinvertebrate indicators. This can help land managers to select appropriate indicators based on the regional context and the potential local effects. Though other studies have considered regional and local factors together in their analyses, such methods can cause the effect of a variable to overshadow the effect of other significant, but smaller variables. A multi-scale analysis, which takes into consideration regional effects across ecoregions before considering other local–scale variables, such as stream water quality and sediment, can help assess the multiple processes operating on aquatic macroinvertebrates and also set the stage for analyzing anthropogenic influences in the context of defined regional/local variable influences.

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CHAPTER 4 – Potential of aquatic macroinvertebrates as water quality indicators: an evaluation across Tennessee, USA

A version of this chapter will be submitted to the journal Freshwater Science by Latha M. Baskaran, Virginia H. Dale and Liem Tran:

Latha M. Baskaran, Virginia H. Dale, and Liem Tran. “Potential of aquatic macroinvertebrates as water quality indicators: an evaluation across Tennessee, USA” *Freshwater Science*.

This article was prepared and written primarily by Latha Baskaran. Liem Tran and Virginia Dale advised on the work described here. They also reviewed early revisions of this manuscript.

Abstract

Water-quality indicators for stream nutrient and sediment changes provide critical information on the condition of stream and are important to assess environmental sustainability of a system. We evaluated whether the number of taxa in Ephemeroptera, Plecoptera and Trichoptera orders (EPT taxa richness) can be used as an indicator to assess stream condition with respect to changes in water quality in Tennessee, USA. Using multilevel regression models we accounted for regional environmental effects on EPT taxa richness as fixed effects and assessed the local influence of stream flow, total nitrogen, total phosphorus and sediment variables on EPT taxa richness as random effects within ecoregions. Owing to the absence of direct sediment measurements, we used a quantitative estimate of sediment on the stream bed from stream habitat surveys. We found that the influence of stream flow on EPT taxa richness was statistically significant across all ecoregions. The influence of nitrogen (N) and phosphorus (P) concentrations and sediment in the stream bed was statistically significant within several ecoregions, after accounting for a suite of regional effects. However, the magnitudes of these effects were small with respect to the variance in EPT taxa richness explained by regional effects. We also evaluated the potential of EPT taxa richness as an independent variable in explaining variations in water quality using a second set of multilevel models. Our results indicated that the relationship between EPT taxa richness and the water quality variables was not statistically significant across the entire study region. However, by accounting for the within-ecoregion variance, EPT taxa richness was found to be statistically significant within some ecoregions. The magnitudes of these relationships were very small in the context of other regional effects and water quality thresholds for aquatic macroinvertebrates. Overall, our analysis from both sets of multi-level models (EPT taxa richness as dependent variable and EPT taxa richness as independent variable) found that the relationships between N and P with EPT-taxa richness are statistically significant within certain ecoregions. Relationships between sediment in the stream bed and EPT taxa richness could not be conclusively derived, highlighting the need for a different indicator to quantify sediment. Our results suggest that, at the spatial scale considered, EPT taxa richness is not a good indicator of water quality changes, specifically nutrient changes, in Tennessee.

Introduction

Monitoring stream health on a regular basis is important for understanding stream conditions and assessing any potential changes that may affect stream water quality and aquatic biodiversity. With many changes occurring on the land, including urbanization, land-management shifts, and agriculture and livestock changes (Wear et al. 2013), it is critical that the streams draining these lands be monitored to assess stream degradation and loss of habitat. For example, agricultural lands are one of the biggest sources of degradation in streams in the United States (US) (US EPA 2006). Sediments and nutrients drained from agricultural lands can affect water quality and stream condition, which, in turn, cause flow alteration, contamination, habitat loss and biodiversity changes in the stream (e.g., Lenat and Crawford 1994; Zimmerman et al. 2003). One of the key components to monitoring such changes due to land management is the selection of appropriate indicators with which to assess stream condition and potential improvements (Meals et al. 2010).

Physical and chemical water quality measurements are useful, but are costly and it is not always possible to get reliable measurements at the desired frequency and resolution (Chapman 1996). The equipment, expertise and cost associated with measurement are functions of the component being assessed (Cuffney et al. 2014). Furthermore, in flowing waters, where changes in hydrology are rapid and difficult to estimate, physical and chemical indicators cannot reflect the integration of numerous environment factors and long-term sustainability of river ecosystems (Li et al. 2010). Biological monitoring or biomonitoring using living organisms has been a useful tool to assess water quality conditions. Monitoring of biological communities can be relatively inexpensive, compared to the cost of directly measuring pollutants in the environment, either chemically or with toxicity tests (Ohio EPA 1987, Yoder and Rankin 1995). State water resource agencies in the US, for example in California and Florida, and agencies in other countries, such as Australia, have relied on rapid bioassessment protocols, which include macroinvertebrate sampling as an inexpensive screening tool to determine if a stream is impaired and needs restoration (Lenat and Barbour 1994; McCarron and Frydenborg 1997; Barbour et al. 1999; Davies 2000). Rapid bioassessments are cost effective, scientifically valid, and can provide quick turn-around of results (Barbour et al. 1999). However, it is not clear if such bioindicators can be effective in the context of detecting water quality changes such as changes in nitrogen and phosphorus concentrations in the water, which can be of concern in streams draining agricultural lands.

Macroinvertebrate richness metrics are straightforward to calculate and useful in studying the macroinvertebrate response to environmental changes (Roy et al. 2003). Some of the widely used metrics include taxa richness, diversity metrics, and metrics based on functional feeding groups (Rosenberg and Resh 1993). Kerans and Karr (1994) found that community structure indicators such as total taxa richness and Ephemeroptera and Trichoptera richness measures are useful for identifying streams with poor water quality in the Tennessee Valley. Such macroinvertebrate taxa richness indicators are highly sensitive to ecosystem disturbances and also have relatively low

temporal variation (Carlisle and Clements 1999). Among the richness indicators, EPT richness (the number of taxa in Ephemeroptera, Plecoptera, and Trichoptera orders) has become a standard indicator, for it includes insect orders that are very sensitive to environmental perturbations and can associate benthic assemblages with complex ecosystems and disturbance regimes (e.g., Feminella 1996; Maxted et al. 2000). Organisms in these orders are at the base of the food web and are highly sensitive to stream nutrients and sediments.

Nutrients and fine sediment are key stressors for macroinvertebrate diversity (Wagenhoff et al. 2011). For example, excessive sedimentation can causes a decrease in EPT taxa abundance and richness due to habitat alteration (Richards et al. 1996; Yuan and Norton 2003). Nutrient enrichment also causes EPT taxa richness to decrease (Lenat 1984). Yuan (2010) found that increases in nutrient concentration are associated with decreases in invertebrate richness in large streams. Several studies have found a negative threshold relationship between macroinvertebrate diversity and stream nutrients. Evans-White et al. (2009) suggested that changes in resource quality could contribute to large-scale losses in biodiversity in nutrient-enriched lotic ecosystems and reported macroinvertebrate richness thresholds of 1.04 mg N/L and 0.05 mg P/L across streams in the Central Plains. Wang et al. (2007) reported macroinvertebrate richness threshold values of 0.86 mg N/L and 0.04 mg P/ L in wadeable streams in Wisconsin, and Weigel and Robertson (2007) reported macroinvertebrate richness threshold values of 1.92 mg N/L and 0.15 mg P/L in non-wadeable Wisconsin streams. Subsidy-stress threshold relationships, where consumers show a subsidy response (increase) at low-moderate levels of nutrient enrichment and then a stress response (decrease) at higher levels of enrichment, have been observed for aquatic macroinvertebrates (King and Richardson 2007; Wagenhoff et al. 2011).

Although macroinvertebrate indicators have been used in biological monitoring, the effects of increased levels of nutrients and sediments on communities may vary, creating difficulty in the use of these indicators as robust measures of nutrient enrichment (Smith et al. 2007). Further, the use of macroinvertebrate indicators for non-extreme, low-moderate changes in water quality has not been evaluated across large regional scales. Macroinvertebrate assemblages are subject to natural variation in addition to changes resulting from pollution or disturbance, particularly over the long term and at large spatial scales (Hodkinson and Jackson 2005). Macroinvertebrate habitat is influenced by several factors ranging from conditions of the local physical habitat to regional landscape characteristics (Lammert and Allan 1999) (Table 5).

At the local scale, reach-level properties such as channel dimensions, substrate characteristics, woody debris, water quality, water chemistry and hydraulic characteristics can affect macroinvertebrate habitat (Lenat and Crawford 1994; Richards et al. 1997). At the regional and watershed scales, geomorphology and land-use characteristics are important predictors of macroinvertebrate community impairment (Kennan 1999; Goldstein et al. 2007). Geomorphic parameters, such as surface geology, stream slope and topographic heterogeneity influence the flow regimes and physical habitat of streams and can hence affect the structure and composition of

Table 5: Landscape and stream features affecting macroinvertebrates, their scales of influence, and the factors by which they affect macroinvertebrate health.

Landscape/stream features	Scale(s) of influence	Related factors that affect macroinvertebrates	Example References
Geomorphology	Regional	Flow regime, substrate, ion concentration	Richards et al. 1996
Climate	Regional	Stream flow, temperature	Beche et al. 2006
Sediments and Substrate	Reach	Turbidity, scouring and abrasion, substrate composition, in-filling interstitial habitat, stream depth heterogeneity	Wood and Armitage 1997
Nutrients	Reach	Nutrient enrichment, dissolved oxygen, changes to assemblage composition	Wang et al. 2007
Stream flow	Reach	Flow volume, channel dynamics, erosion, floods and low flows	Statzner et al. 1988
Stream Order	Regional	Channel characteristics, food resources	Harrel and Dorris 1968

macroinvertebrate assemblages (Richards et al. 1996). The type of soil influences the substrate condition in streams and hence affects macroinvertebrate habitat. For example, clay soils are less permeable to water than are sandy soils. In areas having sandy soils, larger amounts of sand are found in the stream reaches, and the streams are generally shallow (Richards et al. 1996). Whether a stream is in a catchment with highly erodible soils or one with low erosion potential affects the vulnerability of aquatic biota to landscape alterations such as changes in riparian vegetation and urbanization. (Hawkins et al. 2000). Seasonal patterns in precipitation and temperature result in within-year changes in flow and temperature in aquatic systems (Beche et al. 2006). Similarly, year-to-year changes in precipitation produce variations in stream discharge and habitat quality and quantity (McElravy et al. 1989). These features influence the timing of emergence, reproduction, growth and development of macroinvertebrates, which, in turn, affect the seasonal replacement of organisms (Beche et al. 2006). Local richness and species turnover of benthic macroinvertebrates are controlled by both regional and local factors that influence how the assemblages respond to anthropogenic stressors (Maloney et al. 2011). Separating disturbance effects and recovery processes of macroinvertebrates from other sources of natural variability (e.g., temperature and hydrologic fluctuations) is important but often difficult (Whiles and Wallace 1995).

While various research findings and agency reports have published on the use of biological monitoring indicators (Cairns and Pratt 1993; Lenat 1993; Hodkinson and Jackson 1995), a thorough study on the use of a simple bioassessment metric as a potential water quality indicator across different ecoregions for non-extreme conditions has not been seen in the literature. The goal of this study is to evaluate if EPT taxa richness can be used as biological indicator of nutrient and sediment-based water quality in Tennessee. We chose to evaluate EPT taxa richness among the various macroinvertebrate metrics because of its ease of identification and measurement and because of its intolerance to adverse stream conditions (e.g., Lenat 1993; Feminella 1996), which makes it a potential candidate as an ecological indicator. However, an ideal ecological indicator should also respond to stress in a predictable manner, be anticipatory, predict changes that can be averted by management actions, be integrative, have a known response to disturbances, and have low variability in response (Dale and Beyeler 2001). Though the responses of EPT taxa richness to changes in stream quality have been documented for severely impacted streams (Roy et al. 2003), the sensitivity of such responses to changes in water quality under different regional settings and over different streams is unknown. We assume that EPT taxa richness is affected by regional environmental controls across ecoregions in Tennessee (Table 5). Our specific objectives are to determine: (1) whether EPT taxa richness is influenced by nutrient conditions in the stream and (2) whether changes in water quality can be detected using EPT taxa richness as an indicator under non-extreme conditions for different ecoregion settings. Our research findings can inform future water quality and bioassessment survey initiatives and help inform technical approaches for effective stream quality monitoring.

Methods

Since complex biological systems, such as aquatic systems, need to be assessed at multiple spatial scales to consider appropriate relationships between environmental effects and anthropogenic effects at each scale (Poff 1997), we used a multilevel model to specify the interactions of EPT taxa richness with other factors. A multilevel regression model is a form of regression model in which the intercepts and regression coefficients may be “fixed effects” that quantify the overall effects across the dataset, or the intercept and regression coefficients may be “random effects” that vary by hierarchical levels or groups (Gelman and Hill 2006). This regression model provides a framework for identifying the effects of regional predictors as fixed effects on individual level outcomes. The regional predictors can also vary by hierarchical level or group, and these effects are defined by random effect covariates. From a statistical perspective, mixed-effect models, such as multilevel regression, are more efficient at modeling multi-tiered effects than are fixed-effects models such as multiple linear or generalized linear regressions (Cuffney et al. 2011).

We focused our study in the state of Tennessee, which has great variation in topography, climate and geography and therefore has one of the highest freshwater species diversities of any inland state in the US (Stein 2002). Some of the major geomorphological features are the Blue Ridge Mountains (part of the Appalachian range) in the east, the Ridge and Valley province, the Cumberland Plateau, the Interior Plateau, the low-gradient Gulf Coastal Plains and the Mississippi Flood Plain at the far west end of the state (Figure 9). The diverse geomorphological and geological structures in the State provide different stream characteristics and substrates that potentially affect macroinvertebrate distributions in different ways.

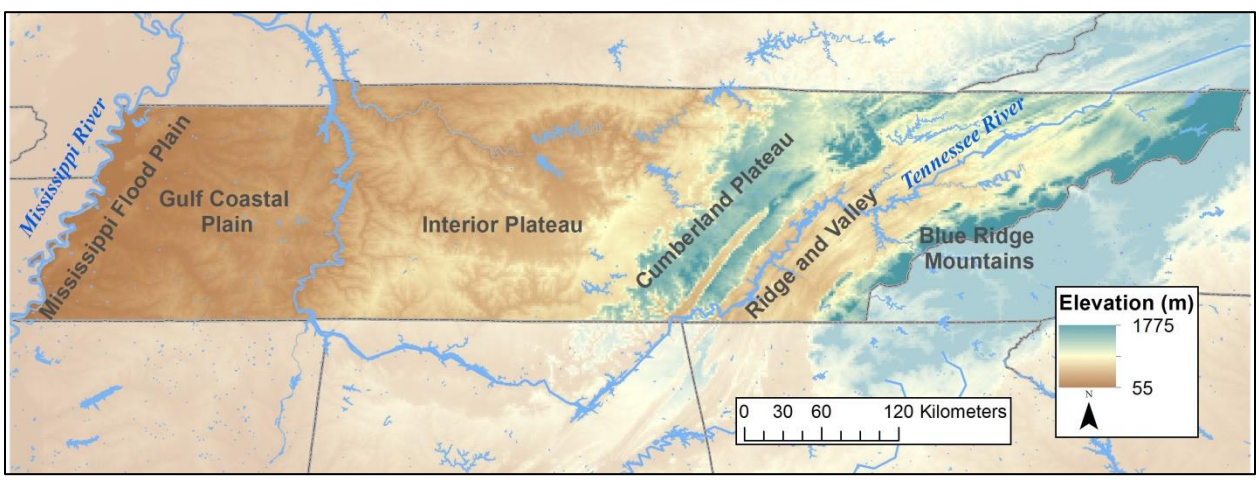

Figure 9: Physical map of Tennessee illustrating elevation gradients, major landforms and rivers.

To address the first objective, we used multilevel regression to determine the influence of regional and local stream-based factors on EPT taxa richness. We used ecoregions to account for regional differences and to identify naturally occurring variation in the

aquatic biota driven by environmental gradients, The underlying premise is that, since ecoregions are based on a combination of environmental factors including climate, land-surface form, natural vegetation, and soils (Omernik 1987), ecoregion classes provide a framework to assess spatial variation of aquatic macroinvertebrates in the midst of regional environmental effects. We partitioned the natural variation due to environmental factors from those caused by other stressors to help understand how differences between regions may influence how aquatic macroinvertebrates respond to changes in water quality. We tested the significance of local variables such as stream flow and water quality as random-effect covariates that may or may not be significant within ecoregions. We focused on total nitrogen (N), total phosphorus (P) and sediment deposition in the stream bed (sed) as water quality variables of interest. To address the second objective, we evaluated the potential of EPT taxa richness as an indicator for water quality using multilevel regression models with water quality variables as the dependent variables and EPT taxa richness and other region-dependent environmental factors as the independent variables.

Macroinvertebrate data

We used macroinvertebrate data (genus-level identification) collected by the Tennessee Department of Environmental Conservation (TDEC) from 437 streams in Tennessee over the years 2007-2010 (Figure 10). TDEC surveys and monitors several streams in Tennessee to conduct benthic macroinvertebrate assessments (TDEC 2008). These surveys are usually performed in the months of July, August, and September using standard operating protocols (TDEC 2011a). Based on semi-quantitative single habitat surveys (SQSH) in riffles or banks, taxa are identified to the genus level within a 200-organism subsample, and indices such as total taxa, EPT taxa richness, percentage of organisms in EPT order, and intolerant taxa are calculated (TDEC 2011a). The data used in our study included data collected as part of the wadeable stream assessment (WSA), reference condition assessments and other monitoring programs (Denton et al. 2010). The WSA study is a probabilistically based survey of wadeable streams in Tennessee that was built upon EPA's 2004 Wadeable Streams Assessment survey of the nation's streams (US EPA 2006). The state conducted a probabilistic monitoring of about 90 wadeable streams in 2007 and 2010 as part of a national effort (Arnwine et al. 2011). Macroinvertebrate assessments have been conducted at reference sites by TDEC using standardized numeric assessment approaches since 1996. These reference sites are the least-impacted, yet representative, streams in each ecoregion within the state. There are 162 reference stream sites in Tennessee.

Water quality data

TDEC also collects chemical samples at several macroinvertebrate sampling sites (TDEC 2011b). Nutrients collected and reported include total phosphorus (P) and total nitrogen (N) (Table 6). We summarized these data by sampling site and retained the nutrient data record closest in date to the macroinvertebrate sampling date. Since sediment data from the water quality surveys were incomplete, we used TDEC's sediment deposition rank, which is an indicator of sediment deposition determined by observation during TDEC stream habitat surveys (TDEC 2011a). Differences in sediment deposition reflect changes to the stream bed as a result of the deposition or

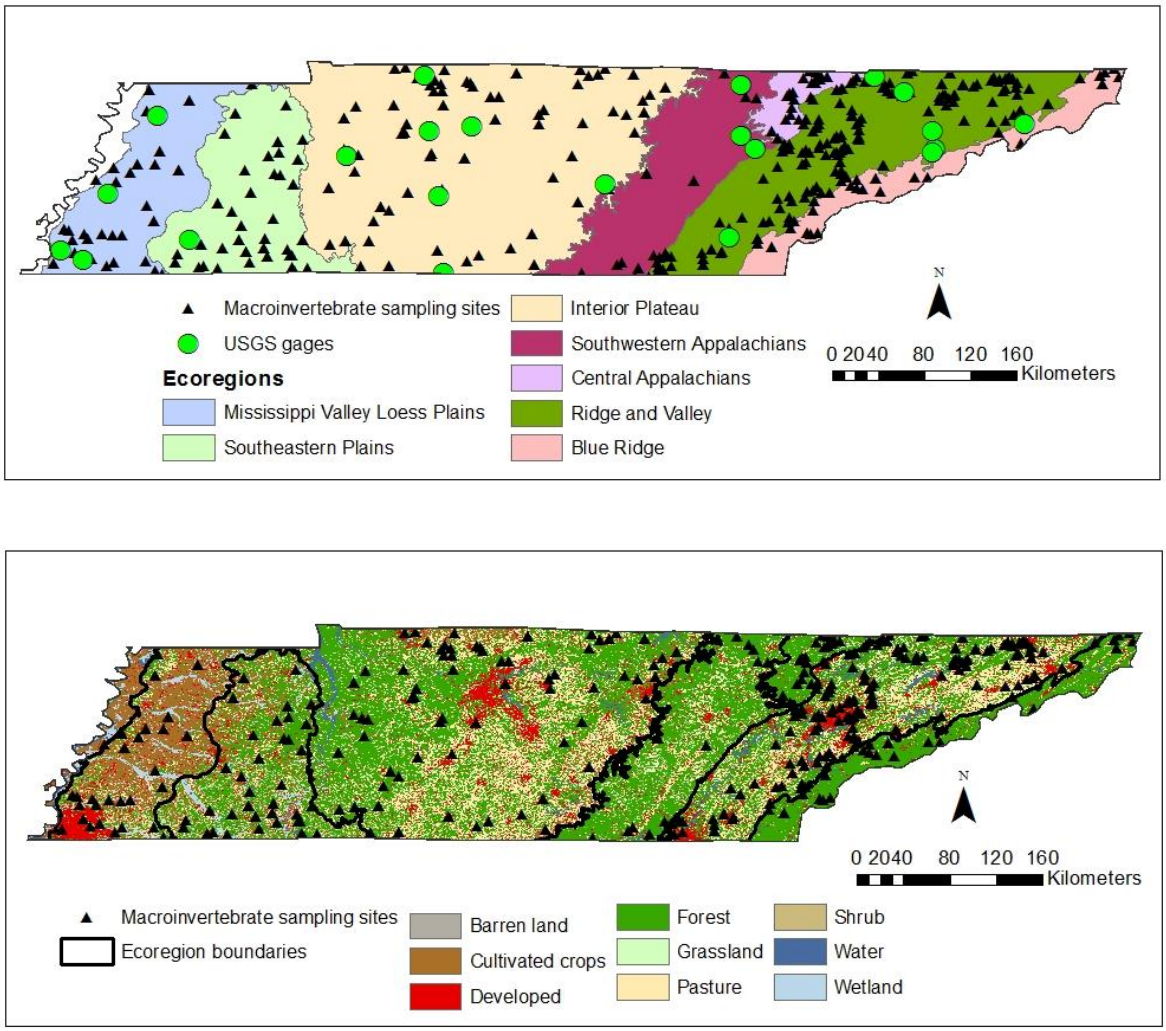

Figure 10: Upper panel: Location of the macroinvertebrate stream sampling sites and USGS gages over the ecoregions in Tennessee. Lower panel: Land cover in the study region.

Table 6: List of regional and stream variables considered to be potentially important in affecting macroinvertebrate taxa richness ([1] - McKay et al. (2012); [2]- Miller and White (1998); [3]- Soil Survey Staff (2013); [4] - TDEC (2011b); [5] - TDEC (2011a))

Category	Variable	Unit	Source	Variable code
Climate	Average temperature at the stream sampling site	°C	NHDplus[1]	Temp
	Average precipitation over several years at the stream sampling site	mm	NHDplus[1]	Precip
Geomorphology	Depth to bedrock	m	STATSGO[2]	Rockdepth
	% Sand	%	SSURGO[3]	Sand
	% Clay	%	SSURGO[3]	Clay
	%Silt	%	SSURGO[3]	Silt
	Stream Slope (change in elevation between start and end of stream segment by the stream length)	Unitless	NHDplus[1]	Stream slope
	Elevation	m	National Elevation Dataset (NED)	Elevation
Stream flow	Average stream Flow	cfs (cubic feet per second)	NHDplus[1]	AvgFlow
	Estimated daily flow	cfs	Estimated from USGS data	Flow
	Velocity	fps (feet per second)	NHDplus[1]	Velocity
Stream Order	Stream order	Categorical (nominal)	NHDplus[1]	SO
Nutrients	Total N	mg/L	TDEC - Chemical Sampling[4]	N
	Total P	mg/L	TDEC - Chemical Sampling[4]	P
	Sediment deposition	Rank (Ordinal)	TDEC - Habitat assessment[5]	Sed

erosion of small particles (gravel, sand, silt).

This qualitative variable is recorded as a rank from 1 to 20 where 1 represents poor stream conditions with heavy deposition of fine material, and 20 represents optimal conditions where sediment deposition affects less than 5% of the stream bed (TDEC 2011a). By interpreting their categorical description, we converted the qualitative nominal variable into two separate variables: a binary variable to indicate whether the sediment accumulation affected bars and islands or pools, and an interval variable describing the percentage of bottom substrate affected by sediment deposition (Table 7). This helped produce value added variables that were used to quantify the influence of sediment on EPT taxa richness. We used the variable quantifying the bottom of the substrate as an indicator of sediment to assess relationships with EPT taxa richness.

Environmental variables

Regional factors which influence aquatic macroinvertebrate communities to different extents include climate, soils, geology and terrain (Baskaran et al. in review). Spatial datasets for these variables were obtained from different sources including large regional databases (Table 6). Soil, silt, and clay texture compositions were derived from the Soil Survey Geographic (SSURGO) database (Soil Survey Staff 2013). Based on a weighted average of the soil texture fractions by component in ArcGIS® (Environmental Systems Research Institute 2012), we mapped the soil texture values to SSURGO map units for Tennessee. Depth to bedrock, representing the location of bedrock with respect to land surface, was obtained from pre-mapped State Soil Geographic Data (STATSGO) map units at a 1-km resolution (Miller and White 1998). Soil texture percentages for the SSURGO map units, and depth to bedrock from the STATSGO mapped dataset, corresponding to the macroinvertebrate stream sampling site were obtained for each macroinvertebrate sample. Long-term average precipitation and temperature (over the period 1971-2000) were obtained from the NHDplus dataset for each stream segment that contained the macroinvertebrate sampling site (McKay et al. 2012).

Stream flow characteristics

We obtained long-term average stream flow and velocity of the stream where the macroinvertebrate sampling site was located from NHD plus data. This average stream flow and velocity represents gage-adjusted estimates derived from flow data representing USGS gages between 1971 and 2000 (McKay et al. 2012). We estimated daily flow corresponding to the date of macroinvertebrate sampling by adjusting for a daily-flow ratio (FR) with respect to long-term flows. We obtained daily stream flow data for 21 USGS sites throughout the study region and derived the FR representing the ratio of the daily flow to the long-term average flow by date (i) for each site (Figure 10).

$$FR_i = \frac{Average\ USGS\ flow}{Daily\ USGS\ flow_i}$$

For each macroinvertebrate site, we identified the closest USGS site based on the Euclidean distance between the USGS sites and macroinvertebrate sampling sites.

Table 7: Recoding the qualitative sediment deposition variable into quantitative and binary variables based on macroinvertebrate and stream assessment protocols (Arnwine et al. 2011).

Sediment Deposition Code from TDEC	**% of bottom substrate affected by deposition**	**New sediment accumulation**	**Stream condition**
1	100	Bars and Islands	Poor
2	80	Bars and Islands	Poor
3	70	Bars and Islands	Poor
4	60	Bars and Islands	Poor
5	50	Bars and Islands	Poor
6	50	Bars and Islands; Pools	Marginal
7	50	Pools	Marginal
8	40	Bars and Islands; Pools	Marginal
9	40	Pools	Marginal
10	30	Bars and Islands; Pools	Marginal
11	30	Pools	Sub-optimal
12	22.5	Pools	Sub-optimal
13	22.5	Pools	Sub-optimal
14	10	Bars and Islands; Pools	Sub-optimal
15	10	Pools	Sub-optimal
16	5	Bars and Islands; Pools	Optimal
17	2.5	Bars and Islands	Optimal
18	2.5	Pools	Optimal
19	0		Optimal
20	0		Optimal

Using FR_i from the closest USGS site, we obtained the daily-flow ratio corresponding to the date of macroinvertebrate sampling and multiplied it by the long-term average flow to estimate the daily stream flow corresponding to the date of macroinvertebrate data collection.

Multilevel regression model

To address the first objective of analyzing aquatic macroinvertebrate responses to potential changes in water quality, we developed multilevel regression models linking EPT taxa richness to water quality and important regional environmental parameters (soil, climate, and geology). We ran separate models for N, P and sediment (EPT-N model, EPT-P model and EPT-S model) and explicitly analyzed the hierarchical structure in the data by considering both the within- and between-ecoregion variances leading to a partial pooling of data across all levels in the hierarchy (Qian et al. 2012). Since EPT taxa richness is a count of specific taxa, we fit the model with a Poisson distribution to represent count data. Using the variance inflation factors (VIF), we tested the model variables for multicollinearity. Variables with very high VIF values (>4) were identified, and those contributing to high collinearity were removed from the model. One of the assumptions of a Poisson distribution is that the mean is equal to the variance of the data. However, in some cases this relationship may not be true, and the model may be overdispersed. We tested the Poisson model for overdispersion by comparing the sum of the squared standardized residuals with a chi-square distribution expected under the model.

Estimated overdispersion ratio $= \frac{\sum_{i=1}^{n} Z_i^2}{n-k}$

Where Zi - Standardized residuals

n – number of degrees of freedom

k – number of regression coefficients

If the overdispersion value is higher than 1.5, a different model (such as negative binomial) or a standard error correction may be needed. Overdispersion values of less than 1.5 in general do not require correction (Zuur et al. 2009).

After checking for multicollinearity and Poisson distribution assumptions, we scaled the variables to z-scores and added the regional variables as fixed effects in a multilevel model (MLM) (with Poisson distribution). Fixed effects are parameter estimates that quantify the overall effects across groups, and random effects are those that vary across groups of the fixed effect parameters (Hofmann 1997). We considered the regional environmental variables as fixed effects and evaluated various covariates, including stream flow and water quality, as random effects within ecoregion groups.

Following a procedure outlined by Zuur et al. (2009), we generated a null model with the fixed effects and a random group intercept-only model that varied with ecoregion and stream order. We then allowed the slope of the parameters to vary by ecoregion and tested if the random intercept and slope model was better than the null model with a likelihood ratio test using the Akaike Information Criterion (AIC) (Akaike 1973). The likelihood ratio test determines the contribution of a single factor by comparing the fit

(measured as the deviance, i.e. 2 times the log-likelihood ratio) for models with and without the factor (Bolker et al. 2009). We then added covariates to the random effects that were at the scale of the stream and varied by ecoregion. These variables included Total N, Total P, percentage of stream substrate affected by sediments, stream velocity and stream flow. Excluding the water quality variables, we removed random effect variables where the variance explained by each random effect covariate was less than 1%. We evaluated the random effect parameters using the absolute slope coefficient of each random effect variable and using the standard errors of the random effects. The absolute slope coefficient is a sum of the fixed effect slope coefficient and the variance from the fixed effect slope caused by the random effect variable in each ecoregion. The standard errors of the coefficients were used to construct 95% confidence intervals to identify the random effects that were significant by ecoregion (Gelman and Hill 2007). Coefficients that were two standard errors away from zero were considered to be statistically significant.

Using marginal R^2 and conditional R^2 measures, we summarized the variance explained by fixed effects and fixed and random effects, respectively (Nakagawa and Schielzeth 2013). We ran the multilevel models in R using the linear mixed-effects (lme4) package (Bates et al. 2015).

To address the second objective of the study, we developed multilevel models with total N, total P and sediment as dependent variables, and EPT taxa richness and other regional variables as the independent variables. We used linear multilevel regression and added variables in the model using AIC. As in the approach described for the EPT taxa richness models, we evaluated the variance explained by fixed variables and the random effect covariates within ecoregion classes.

Results

Interpretation of sediment variable

Plotting the EPT taxa richness against the new sediment variable shows the overall negative relationship between EPT taxa richness and sediment in the stream bed (Figure 11). However, the strength and direction of the relationship differ based on the ecoregion (blue lines in Figure 11).

Multilevel EPT taxa models

The variance inflation factors (VIF) of the independent variables in the EPT taxa richness model indicated that sand, silt and clay had very high values (>4). Since the percentages of sand, silt and clay variables were correlated (they sum to 100), we removed silt from the model. The resulting model had high VIF values for temperature and elevation. Since temperature and elevation variables were correlated, we removed elevation from the model. The VIF for the variables in the final model were all below 4 and indicated no problems due to multicollinearity.

Early model results indicated warnings regarding scaling errors since the independent variables were based on different scales. For example, the precipitation values ranged

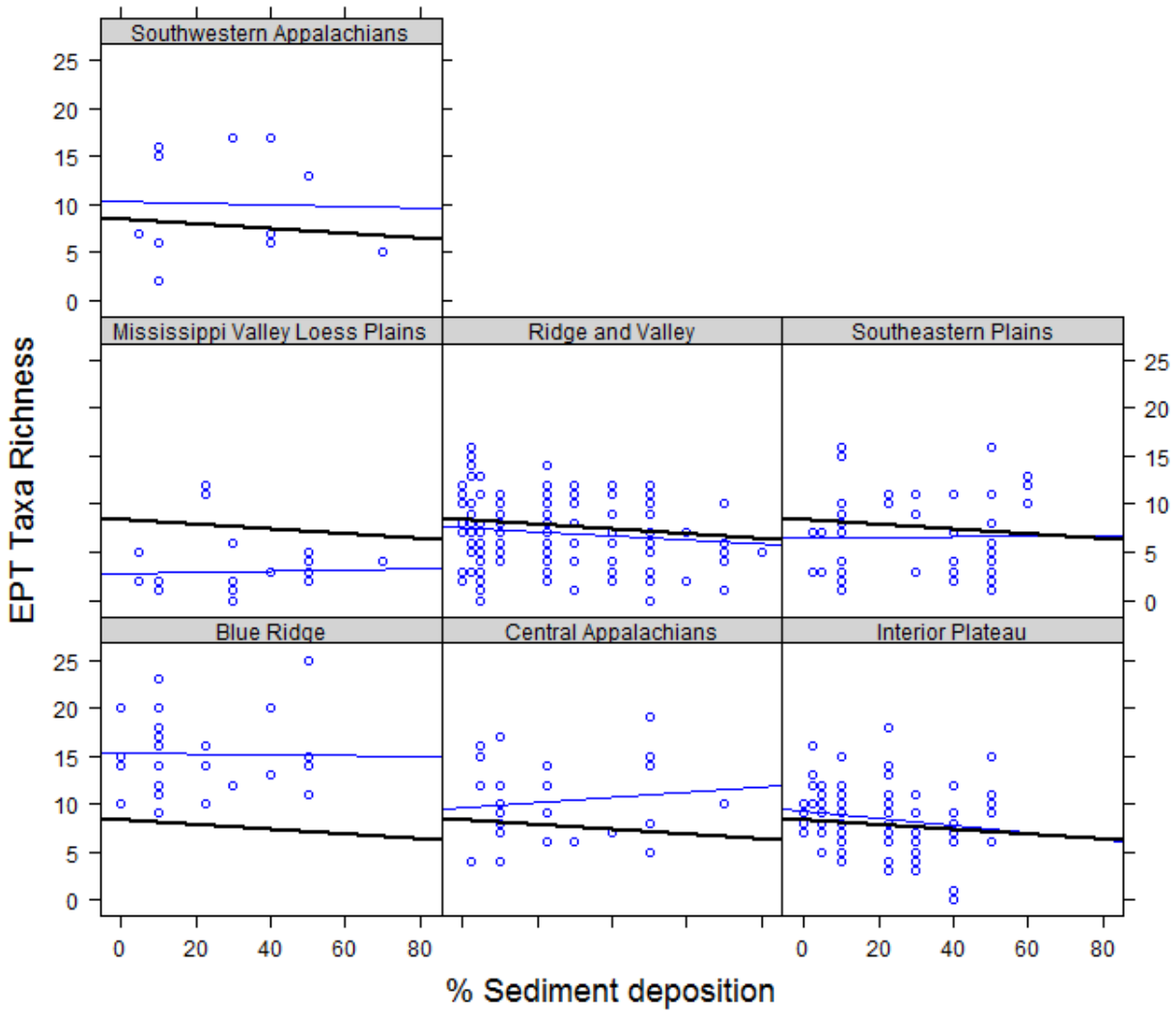

Figure 11: Relationship between EPT taxa richness and percentage of sediment deposition in stream affecting the bottom substrate by ecoregion. The black line represents the overall linear trend line of the data; the blue lines represent linear trend lines by ecoregion.

from 1052 mm to 1420 mm, whereas the stream slope ranges from 0 to 0.17. Hence, we standardized the independent variables to z-scores by subtracting the mean and dividing by the standard deviation.

We tested the Poisson model for overdispersion, and the overdispersion ratio was found to be 1.28. Since values of less than 1.5 in general do not require correction of the standard error to compensate for the overdispersion, we found the Poisson model to be appropriate to represent EPT taxa richness in this study.

We added the following regional environmental variables as fixed effects in the multilevel model (MLM) for the entire dataset - sand, clay, stream slope, rock depth, temperature and precipitation. We also included the water quality variables (N, P and sediment) and stream flow variables (average stream flow, estimated daily flow and stream velocity) as fixed effects to detect any overall significant interactions with EPT taxa richness. Ecoregions and stream order were added as groups within which the independent variable slopes and/or intercepts varied as random effects. We ran separate models for N, P and sediment (EPT-N model, EPT-P model and EPT-S model) and compared the random-slope only models with random intercept and slope models for ecoregion class and stream order as random effects (Table 8). We selected random-effect covariates based on variables that improved the model performance (based on AIC values). For the EPT-N model, we retained stream slope, velocity, daily flow and total N as random effect covariates. Stream slope, velocity and total P/Sediment were retained for the EPT-P and EPT-S models. In all three models, when the average flow was added as a random-effect covariate, the significance of average flow and daily flow as fixed effect variables was lost, and the model performance did not improve (based on AIC values).

In all three model settings, the random intercept and slope models were better than the random intercept only models based on the log-likelihood test (Table 8). In all three cases, the variance explained by the random slope and intercept models was higher (0.285 for EPT-N, 0.1 for EPT-P and 0.235 for EPT-S), indicating that they performed better at explaining the EPT taxa richness.

The final equations for the random slope and intercept models for EPT-N, EPT-P and EPT-S are:

EPT-N Model:

$$\log(E(EPT\ Taxa_{ij})) = \alpha + \beta_1 Sand_i + \beta_2 Clay_i + \beta_3 Slope_{ij} + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_{ij} + \beta_8 AvgFlow_i + \beta_9 Flow_{ij} + \beta_{10} TotN_{ij} + V_{3j} Slope_{ij} + V_{7j} Vel_{ij} + V_{9j} Flow_{ij} + V_{10j} TotN_{ij} + u_j + \epsilon_{ij}$$

EPT-P Model:

Table 8: Testing the significance of EPT taxa richness models with random slopes and intercepts compared to models with only a random intercept. The Akaike Information Criterion (AIC) of each model and the total variance explained by the random effects are listed.

Model	Fixed effects	Random effects	AIC	Variance explained by random effects
EPT-N Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, N	Random intercept only - ecoregion and stream order	2293.4	0.066
		Random intercept only - ecoregion	2298.5	0.044
		Random intercept only - stream order	2358.3	0.004
		Random slope and intercept - ecoregion and stream order with covariates (stream velocity, daily flow, stream slope and N)	2289.1	0.285
EPT-P Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, P	Random intercept only - ecoregion and stream order	2292.7	0.066
		Random intercept only - ecoregion	2297.3	0.046
		Random intercept only - stream order	2358.4	0.004
		Random slope and intercept - ecoregion and stream order with covariates (stream velocity, stream slope and P)	2285.3	0.1
EPT-S Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, sediment	Random intercept only - ecoregion and stream order	2293.3	0.066
		Random intercept only - ecoregion	2298.6	0.044
		Random intercept only - stream order	2358.5	0.005
		Random slope and intercept - ecoregion and stream order with covariates (stream velocity, stream slope and sediment)	2286.4	0.235

$$\log(E(EPT\ Taxa_{ij})) = \alpha + \beta_1 Sand_i + \beta_2 Clay_i + \beta_3 Slope_{ij} + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_{ij} + \beta_8 AvgFlow_i + \beta_9 Flow_i + \beta_{11} TotP_{ij} + V_{3j} Slope_{ij} + V_{7j} Vel_{ij} + V_{11j} TotP_{ij} + u_j + \epsilon_{ij}$$

EPT-S Model:

$$\log(E(EPT\ Taxa_{ij})) = \alpha + \beta_1 Sand_i + \beta_2 Clay_i + \beta_3 Slope_{ij} + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_{ij} + \beta_8 AvgFlow_i + \beta_9 Flow_i + \beta_{12} Sed_{ij} + V_{3j} Slope_{ij} + V_{7j} Vel_{ij} + V_{12j} Sed_{ij} + u_j + \epsilon_{ij}$$

where

$EPT\ Taxa_{ij}$ is the dependent variable indicating EPT taxa richness for observation i in ecoregion class j

α, is the intercept of the model

$\beta_{1\ to\ 12}$ corresponds to the fixed effect regression coefficient of sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term average stream flow, daily stream flow, total nitrogen, total phosphorus and sediment.

V_{3j} indicates the random effect and corresponds to the amount by which the stream slope coefficient of ecoregion j deviates from average β_3

V_{7j} indicates the random effect and corresponds to the amount by which the velocity coefficient of ecoregion j deviates from average β_7

V_{9j} indicates the random effect and corresponds to the amount by which the flow coefficient of ecoregion j deviates from average β_9

V_{10j} indicates the random effect and corresponds to the amount by which the total nitrogen coefficient of ecoregion j deviates from average β_{10}

V_{11j} indicates the random effect and corresponds to the amount by which the total phosphorus coefficient of ecoregion j deviates from average β_{11}

V_{12j} indicates the random effect and corresponds to the amount by which the sediment coefficient of ecoregion j deviates from average β_{12}

u_j is the Level-2 random effect, and

ϵ_{ij} is the Level-1 random effect.

The coefficient estimates of the fixed effects and their standard errors are summarized for the EPT-N, EPT-P and EPT-S models in Tables 9-11. The coefficient estimates of the random effects by ecoregion indicated regions where the average intercept and variance explained by random effect covariates varied by ecoregion (Tables 12-14). However not all random effect variables were significant based on a 95% confidence interval.

Multilevel water quality models

We developed three linear multilevel regression models with Total N, Total P and sediment deposition as dependent variables to test if EPT taxa richness can be an indicator of water quality (N model, P model and S model). We added the regional

Table 9: The coefficient estimates, standard error, and p-statistic of the fixed effects of the multilevel nitrogen model (EPT-N Model) with EPT taxa richness as the dependent variable and regional variables, stream characteristics and N as dependent variables.

	Fixed effect	Std. Error	Pr(>|z|)
Intercept	2.087	0.134	< 2e-16*
Sand	0.082	0.023	0.000*
Clay	0.076	0.023	0.001*
Stream slope	0.344	0.178	0.053
Rock depth	-0.064	0.023	0.004*
Temperature	-0.212	0.033	0.000*
Precipitation	0.090	0.022	0.000*
Velocity	0.129	0.079	0.101
Average stream flow	-0.177	0.047	0.000*
Daily stream flow	0.177	0.080	0.027*
Total nitrogen	-0.024	0.032	0.459

* significant at the 0.05 level

Table 10: The coefficient estimates, standard error, and p-statistic of the fixed effects of the multilevel phosphorus model (EPT-P Model) with EPT taxa richness as the dependent variable and regional variables, stream characteristics and P as dependent variables.

	Fixed effect	Std. Error	Pr(>|z|)
Intercept	2.033	0.101	< 2e-16*
Sand	0.065	0.022	0.003*
Clay	0.073	0.023	0.001*
Stream slope	0.100	0.054	0.065
Rock depth	-0.050	0.022	0.024*
Temperature	-0.159	0.033	0.000*
Precipitation	0.077	0.022	0.000*
Velocity	0.127	0.074	0.087
Average stream flow	-0.147	0.043	0.001*
Daily stream flow	0.075	0.025	0.002*
Total phosphorus	-0.023	0.022	0.300

* significant at the 0.05 level

Table 11: The coefficient estimates, standard error, and p-statistic of the fixed effects of the multilevel sediment model (EPT-S Model) with EPT taxa richness as the dependent variable and regional variables, stream characteristics and sediment as dependent variables.

	Fixed effect	Std. Error	Pr(>|z|)
Intercept	2.071	0.139	< 2e-16*
Sand	0.078	0.023	0.001*
Clay	0.077	0.024	0.001*
Stream slope	0.309	0.211	0.144
Rock depth	-0.059	0.023	0.011*
Temperature	-0.187	0.034	0.000*
Precipitation	0.082	0.022	0.000*
Velocity	0.133	0.080	0.093
Average stream flow	-0.151	0.046	0.001*
Daily stream flow	0.069	0.025	0.007*
Sediment	0.045	0.047	0.345

* significant at the 0.05 level

Table 12: Coefficient estimates of the random effects by ecoregion for the multilevel EPT- N model, with EPT taxa richness as the dependent variable and regional variables, stream characteristics and N as dependent variables. The ecoregions are listed from west to east.

Ecoregion	Intercept	Stream slope	Daily stream flow	Velocity	Total N
Mississippi Valley Loess Plains	1.960*	0.728*	0.11*	0.422*	-0.068*
Southeastern Plains	2.549*	0.963*	0.437*	0.226*	-0.079*
Interior Plateau	2.371*	0.412*	0.333*	-0.025	-0.026
Southwestern Appalachians	1.706*	0.212*	-0.035	0.278*	-0.018*
Central Appalachians	1.947*	-0.005	0.096*	-0.036	0.008
Ridge and Valley	1.911*	0.119*	0.077*	0.064*	-0.006
Blue Ridge	2.192	-0.009	0.233*	-0.029	0.021*

* significant at the 0.05 level

Table 13: Coefficient estimates of the random effects by ecoregion for the multilevel EPT-P model, with EPT taxa richness as the dependent variable and regional variables, stream characteristics and P as dependent variables. The ecoregions are listed from west to east.

Ecoregion	Intercept	Stream slope	Velocity	Total P
Mississippi Valley Loess Plains	1.720*	0.309*	0.436*	-0.054*
Southeastern Plains	2.197*	0.129*	0.238*	-0.007
Interior Plateau	2.220*	0.004	0.000	-0.005
Southwestern Appalachians	1.827*	0.168*	0.194*	-0.043*
Central Appalachians	2.040*	0.001	-0.065	-0.022*
Ridge and Valley	1.911*	0.098*	0.084*	-0.035*
Blue Ridge	2.324*	-0.012	0.002	0.006

* significant at the 0.05 level

Table 14: Coefficient estimates of the random effects by ecoregion for the multilevel EPT-S model, with EPT taxa richness as the dependent variable and regional variables, stream characteristics and sediment as dependent variables. The ecoregions are listed from west to east.

Ecoregion	Intercept	Stream slope	Velocity	Sediment
Mississippi Valley Loess Plains	1.903*	0.774*	0.461*	0.139*
Southeastern Plains	2.427*	0.766*	0.262*	0.070*
Interior Plateau	2.313*	0.280*	0.004	0.006
Southwestern Appalachians	1.732*	0.230*	0.197*	0.071*
Central Appalachians	1.980*	0.002	-0.026	0.004
Ridge and Valley	1.921*	0.121*	0.049	0.001
Blue Ridge	2.242	-0.005	-0.016	0.021

* significant at the 0.05 level

environmental variables (sand, clay, stream slope, rock depth, temperature and precipitation), stream flow variables (average stream flow, estimated daily flow and stream velocity) and EPT taxa richness as fixed effects in the MLMs. Considering ecoregion and stream order as random effects, we compared random intercept-only models with random-slope and intercept models (Table 15). We selected random effect covariates based on the variables that improved model performance. For the N and P models, we retained EPT taxa richness and clay as random effect variables. Only EPT taxa richness was retained as the random effect variable for the S model. In all three models, the variance explained by the random slope and intercept models with covariates were higher (0.318 for the N model, 0.462 for the P model and 0.03 for the S model) indicating that adding the random effect covariates improved model performance.

The coefficient estimates of the fixed effects and their standard errors are summarized for the N, P and S models in Table 16. The random effect coefficients by ecoregion indicated regions where the intercept and variance explained by the random effect covariates varied by ecoregion (Tables 17-19).

The final equations for the random slope and intercept models for N, P and S models are:

N Model:

$$TotN_{ij} = \alpha + \beta_1 Sand_i + \beta_2 Clay_{ij} + \beta_3 Slope_i + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_i + \beta_8 AvgFlow_i + \beta_9 Flow_i + \beta_{10} EPT_{ij} + V_{2j} Clay_{ij} + V_{10j} EPT_{ij} + u_j + \epsilon_{ij}$$

P Model:

$$TotP_{ij} = \alpha + \beta_1 Sand_i + \beta_2 Clay_{ij} + \beta_3 Slope_i + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_i + \beta_8 AvgFlow_i + \beta_9 Flow_i + \beta_{10} EPT_{ij} + V_{2j} Clay_{ij} + V_{10j} EPT_{ij} + u_j + \epsilon_{ij}$$

S Model:

$$Sed_{ij} = \alpha + \beta_1 Sand_i + \beta_2 Clay_i + \beta_3 Slope_i + \beta_4 Rockdepth_i + \beta_5 Temp_i + \beta_6 Precip_i + \beta_7 Vel_i + \beta_8 AvgFlow_i + \beta_9 Flow_i + \beta_{10} EPT_{ij} + V_{10j} EPT_{ij} + u_j + \epsilon_{ij}$$

where

$TotN_{ij}$ is the dependent variable indicating total N for observation i in ecoregion class j

α, is the intercept of the model

$TotP_{ij}$ is the dependent variable indicating total P for observation i in ecoregion class j

α, is the intercept of the model

Sed_{ij} is the dependent variable indicating total P for observation i in ecoregion class j

α, is the intercept of the model

$\beta_{1\ to\ 10}$ corresponds to the fixed effect regression coefficient of sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term average stream flow, daily stream flow, and EPT taxa richness

V_{2j} indicates the random effect and corresponds to the amount by which the clay

Table 15: Testing significance of water quality models with random slopes and intercepts compared to models with only a random intercept. The AIC of each model and the total variance explained by the random effects is listed. In each model category (N, P and Sed), the better model, based on a chi-square test is indicated with an asterisk.

Model	Fixed effects	Random effects	AIC	Variance explained by random effects
N Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, EPT taxa richness	Random intercept only - ecoregion and stream order	1256.1	0.0
		Random intercept only - ecoregion	1254.1	0.0
		Random intercept only - stream order	1254.1	0.0
		Random slope and intercept - ecoregion and stream order with covariates (EPT and clay)	1252.9*	0.318
P Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, EPT taxa richness	Random intercept only - ecoregion and stream order	1257.2	0.045
		Random intercept only - ecoregion	1256.9	0.02
		Random intercept only - stream order	1256.2	0.007
		Random slope and intercept - ecoregion and stream order with covariates (EPT and clay)	1243.3*	0.462
S Model	Sand, clay, stream slope, rock depth, temperature, precipitation, stream velocity, long-term stream flow, daily stream flow, EPT taxa richness	Random intercept only - ecoregion and stream order	1237.4	0.029
		Random intercept only - ecoregion	1235.4*	0.029
		Random intercept only - stream order	1239.5	0.0
		Random slope and intercept - ecoregion and stream order with covariates (EPT)	1238.7	0.03

Table 16: Coefficient estimates of fixed effects of water quality models with their standard errors.

	N Model			P Model			S Model		
	Coeff.	Std. Error	t value	Coeff.	Std. Error	t value	Coeff.	Std. Error	t value
Intercept	0.185	0.137	1.356	0.185	0.169	1.097	0.069	0.086	0.809
EPT	-0.008	0.065	-0.131	-0.029	0.072	-0.398	0.015	0.065	0.230
AvgFlow	0.019	0.052	0.368	-0.021	0.052	-0.404	0.090	0.052	1.723
Flow	-0.019	0.054	-0.357	0.022	0.053	0.419	-0.006	0.055	-0.104
Rock Depth	0.015	0.052	0.293	0.029	0.052	0.553	0.036	0.054	0.665
Velocity	0.058	0.055	1.068	-0.021	0.054	-0.384	-0.121	0.055	-2.203
Clay	0.384	0.204	1.877	0.401	0.231	1.738	-0.070	0.056	-1.253
Sand	-0.056	0.056	-1.001	-0.029	0.055	-0.524	-0.233	0.055	-4.214
Stream slope	-0.062	0.061	-1.011	-0.048	0.060	-0.798	0.004	0.061	0.069
Temp	-0.095	0.073	-1.306	-0.110	0.075	-1.472	0.004	0.079	0.054
Precip	0.054	0.058	0.935	-0.033	0.058	-0.573	-0.019	0.061	-0.321

Table 17: Coefficient estimates of random effects by ecoregion for N model. The ecoregions are listed from west to east.

	Intercept	EPT	Clay
Mississippi Valley Loess Plains	0.111	-0.023	0.267
Southeastern Plains	-0.066	-0.059*	-0.013
Interior Plateau	-0.029	-0.052*	0.046
Southwestern Appalachians	0.712*	0.098*	1.216*
Central Appalachians	0.140	-0.018	0.313
Ridge and Valley	-0.028	-0.052*	0.047
Blue Ridge	0.455*	0.046	0.811*

* significant at the 0.05 level

Table 18: Coefficient estimates of random effects by ecoregion for P model. The ecoregions are listed from west to east.

	Intercept	EPT	Clay
Mississippi Valley Loess Plains	-0.207	-0.086	-0.150
Southeastern Plains	-0.071	-0.073	0.048
Interior Plateau	0.095	-0.051	0.284*
Southwestern Appalachians	0.900*	0.046	1.437*
Central Appalachians	0.269	-0.021	0.524*
Ridge and Valley	-0.125*	-0.111	0.002
Blue Ridge	0.434*	0.094	0.664*

* significant at the 0.05 level

Table 19: Coefficient estimates of random effects by ecoregion for S model. The ecoregions are listed from west to east.

	Intercept	EPT
Mississippi Valley Loess Plains	0.030	0.003
Southeastern Plains	0.223*	0.062*
Interior Plateau	-0.168*	-0.058*
Southwestern Appalachians	0.191	0.052
Central Appalachians	0.135	0.035
Ridge and Valley	-0.051	-0.022
Blue Ridge	0.126	0.032

* significant at the 0.05 level

coefficient of ecoregion j deviates from average β_2
V_{10j} indicates the random effect and corresponds to the amount by which the EPT taxa richness coefficient of ecoregion j deviates from average β_{10}
u_j is the Level-2 random effect, and
ϵ_{ij} is the Level-1 random effect.

Discussion

Multilevel EPT taxa models

In all three types of EPT multilevel models, the incorporation of random slopes and random intercepts improved the model performance (Table 8). This signifies the importance of ecoregions in characterizing the between-ecoregion variation in the model, normally not be captured in linear regression models. In the EPT-N, EPT-P and EPT-S models, the variances explained by the random effects were 0.285, 0.1 and 0.235 respectively (Table 8). These values are higher than, or close to, most of the fixed-effect coefficients of the respective models (Tables 9-11). Hence the variance explained by each model by including the random effects is on par with the fixed effect parameter influences.

N, P, and sediment did not show statistical significance as fixed effects on EPT taxa richness (p values were 0.459 for N in the EPT-N model; 0.3 for P in the EPT-P model; 0.345 for sediment in the EPT-S model) (Tables 9-11). This indicates that any potential effect of water quality could not be detected as a first-order effect in the midst of other regional factors. However, considering water quality variables as random effects within ecoregions showed statistically significant effects (Tables 12-14). In the EPT-N model, we found that the statistically significant N coefficients were negative in the ecoregions in the West (-0.068 in the Mississippi Valley Loess Plains and -0.079 in the Southeastern Plains) and the Southwestern Appalachian ecoregion in the East (-0.018). This indicates that the negative relationship between N and EPT taxa richness is strong in the western regions and in the Southwestern Appalachian ecoregion. These areas are characterized by lands predominantly managed for agriculture or pasture (Figure 10b). This relationship, though small, is significant, and indicates that compared to other ecoregions, the changes in N in these ecoregions, can have a more significant effect on EPT taxa richness owing to the existing altered stream habitat, which is a function of the managed lands draining into these streams (Lenat 1984; Lenat and Crawford 1994; Genito et al. 2002). However, the N coefficient was positive in the predominantly forested Blue Ridge ecoregion (0.021) (Figure 10b). The Blue Ridge ecoregion has forested streams with habitat conditions that include large woody debris, effective thermal regulation, and leaf litter (Entrekin et al. 2007). In the midst of such favorable habitat conditions, changes in N in such streams may not have a significant impact on macroinvertebrates. Further, the forested streams in the Blue Ridge ecoregion have some of the highest acid deposition rates that contribute to nitrogen saturation and increased nitrate export to the streams (Cai et al. 2012). These streams are also at risk for acid and aluminum impairment, which can impact stream health and aquatic biota more significantly than nitrogen impairment (Neff et al. 2012).

In the EPT-P model, P was statistically significant in four of the seven ecoregions (Table 13). The coefficients for random effects were negative in the Mississippi Valley Loess Plains (-0.054), Southwestern Appalachians (-0.043), Central Appalachians (-0.022), and the Ridge and Valley ecoregion (-0.035). These negative relationships are in line with existing research that indicates decreasing macroinvertebrate diversity with increasing nutrient concentrations (Lenat 1984). Studies have shown that macroinvertebrate diversity is affected by increasing phosphorus concentrations because of overgrowing primary producers that create low oxygen and harmful microbial products, which are harmful to consumers such as aquatic macroinvertebrates (Wang et al. 2007).

In the EPT-S model, sediment deposition was statistically significant as a random effect in three of the seven ecoregions (Table 14). The coefficients for random effects were positive in the Mississippi Valley Loess Plains (0.139), Southeastern Plains (0.07) and the Southwestern Appalachian ecoregion (0.071). In all of these ecoregions with extensive pasture and cultivated crops, the presence of sediment deposition may have a small positive impact by providing needed sediment substrate for aquatic macroinvertebrates.

In all three EPT MLMs, sand, clay, bedrock depth, temperature and precipitation were significant as fixed effects associated with EPT taxa richness. These regional variables are the first-order regional filters that influence the stream substrate and dynamics, and hence affect macroinvertebrate habitat (Poff 1997). Studies have found that substrate composition and fine-sediment distribution are important for macroinvertebrate habitat, and this relationship is more pronounced in regions with clayey soils (Richards et al. 1993). Sandy soils also affect the stream substrate by allowing for greater runoff of sandy particles in the stream, which, depending on the particle size, may be beneficial for macroinvertebrates. The depth to bedrock shows a negative relationship with EPT taxa richness since lower depths are usually in well-drained soils with stream systems that are conducive to macroinvertebrate habitat. While checking for VIF, depth to bedrock was found to be negatively correlated with slope and positively correlated with temperature, indicating lower depth to bedrock associated with mountain environments that have higher macroinvertebrate diversity. EPT taxa richness had a negative relation with temperature and positive relation with precipitation, which is clear in the higher macroinvertebrate richness found in wetter and cooler regions such as the Ridge and Valley ecoregion (Miserendino 2001). Temperature and stream flow in aquatic systems influence the timing of emergence, reproduction, growth and development of macroinvertebrates (e.g., Beche et al. 2006).

Stream slope was not statistically significant as a fixed effect in the three models, but it was statistically significant as a random effect in many of the ecoregions of the models. The random effect of stream slope shows a positive relationship with EPT taxa richness, which indicates the higher macroinvertebrate diversity found in steeper streams. Velocity, which is correlated with stream slope, also showed similar positive relationships with EPT taxa richness at selected ecoregions Studies have shown that

sharp environmental gradients are important for thermo-regulation and for maintaining habitat for aquatic species.

Results from our regression analysis of EPT taxa richness as a function of estimated daily stream flow showed positive fixed effect coefficients (0.177 for the EPT-N model; 0.075 for the EPT-P model; 0.069 for the EPT-S model) (Tables 9-11). The relationship of macroinvertebrate richness with stream flow is well documented. Usually, lower flows result in lower stream velocity, which may cause increased fine sediment deposition that is detrimental to macroinvertebrate habitat (e.g., Dewson et al. 2007). In the EPT-N model daily stream flow was also found to be statistically significant as a random effect where the slope coefficients were positive and ranged from 0.07 in the Ridge and Valley ecoregion to 0.477 in the Southeastern Plains (Table 12). This indicates that although the effect of daily stream flow on EPT taxa richness is important through the entire study region, its effect is stronger in some ecoregions, such as the Southeastern Plains.

We found that long-term average stream flow of the stream was negatively related to EPT taxa richness. This indicates that streams with a higher average long-term flow have lower EPT taxa richness. We hypothesize that this could be because of higher macroinvertebrate diversity found in smaller headwater streams with lower flows (Clarke et al. 2008), compared to larger streams that may have higher long-term stream flow. This relationship helps evaluate the spatial patterns of macroinvertebrate interactions with stream systems, but does not capture the influence of monthly or seasonal variation in flow that may affect macroinvertebrates. However, aquatic macroinvertebrates are resilient and are known to recover within weeks after short-term variations in flow and after flood or drought events (Angradi 1997; Fritz and Dodds 2004). Hence variables that describe long-term stream flow, such as those used in this study, are useful to understand interactions over larger temporal and spatial scales.

Multilevel water quality models

The multilevel water quality models found that the effect of EPT taxa richness as a fixed effect was small with a high standard error (Table 16). However, when EPT taxa richness was considered as a random effect, it was statistically significant in some of the ecoregions. In the N model, the coefficient of EPT taxa richness (fixed effect coefficient with random effect variances added by ecoregion) was significant with a negative slope in the Southeastern Plains (-0.059), the Interior Plateau (-0.052), and the Ridge and Valley ecoregion (-0.052) and positive in the Southwestern Appalachians (0.098) (Table 17). In the P model, EPT taxa richness was not statistically significant as a random effect in any of the ecoregions (Table 18). In the sediment deposition model, EPT taxa richness was statistically significant as a random effect in the Interior Plateau (-0.058) and Southeastern Plains (0.062) (Table 19).

In the N and P models, clay was significant as a positive fixed effect with slope coefficients of 0.384 and 0.401 (Table 16). Clay was also found to be statistically significant as a random effect in specific ecoregions where the parameter slope varied from 1.216 in the Southwestern Appalachian ecoregion for the N model (Table 17) to 0.284 in the Interior Plateau ecoregion for the P-model (Table 18). Soils with high clay

content may have higher nutrient runoff due to low infiltration capacities. This positive relationship between clay and N and P was found to vary by ecoregion with higher parameter slopes in the Southwestern Appalachian ecoregion. Overall, the result from the multilevel water quality models are in line with the findings of the earlier EPT taxa models of this study where N, P and sediment deposition were not significant as a fixed effect in affecting EPT taxa richness in the midst of other environmental and regional factors. However, the relationship between N and EPT taxa richness as random effects within ecoregions was found to be statistically significant, though very small. For example, based on the N-model, we found that a unit increase in the EPT taxa richness in the Southeastern Plains, indicates an decrease in total N by a factor of 0.059 mg/L, and a unit increase in the EPT taxa richness in the Ridge and Valley ecoregion, indicates an decrease in total N by a factor of 0.052 mg/L (Table 17). These values are lower than previously published macroinvertebrate threshold levels of nitrogen, which are between 0.86 and 1.92 mg N/L (Wang et al. 2007; Weigel and Robertson 2007; Evans-White et al. 2009). Such large differences between the potential influence and the threshold levels do not satisfy the criteria needed for an effective indicator, which among other conditions, should have a predictable and low variable response to disturbances (Dale and Beyeler 2001).

This lack of a significant direct nutrient-EPT taxa richness relationship can be attributed to the following factors:

Lack of variability in data: In our study region, the N and P data were mostly on the lower range with few cases of very high nutrients such as those found in heavily impacted streams (Figure 12). This lack of variability in the data may have cause difficulties in detecting significant relationship trends. Further, studies have shown that, at low nutrient concentrations, the values of biological variables such as macroinvertebrate richness were variable due to factors other than nutrients that limit the health of biological assemblages (Wang et al. 2007).

Presence of other dominant stressors: Other studies have shown that compared to nutrients, changes in habitat and stream morphology are more significant stressors to macroinvertebrates (Richards et al. 1993; Wagenhoff et al. 2012). Since nutrients may interact with fine sediment and other stressors (Wagenhoff et al. 2012), their effects need to be quantified simultaneously along other stressor gradients. N and P might affect EPT through indirect pathways by affecting primary productivity and other ecosystem parameters (Richards et al. 1993). Further, the presence of other stressors such as pollutants was not considered in this study, and they may have a stronger influence on EPT taxa richness (Thorne and Williams 1997).

Temporal scale of analysis: Our current study considered data collected in the fall months. However, data from a different season or time has the potential to show different results since studies have shown that seasonality is important in structuring EPT taxa (Bispo and Oliviera 2007). Some of the variability in macroinvertebrate metrics

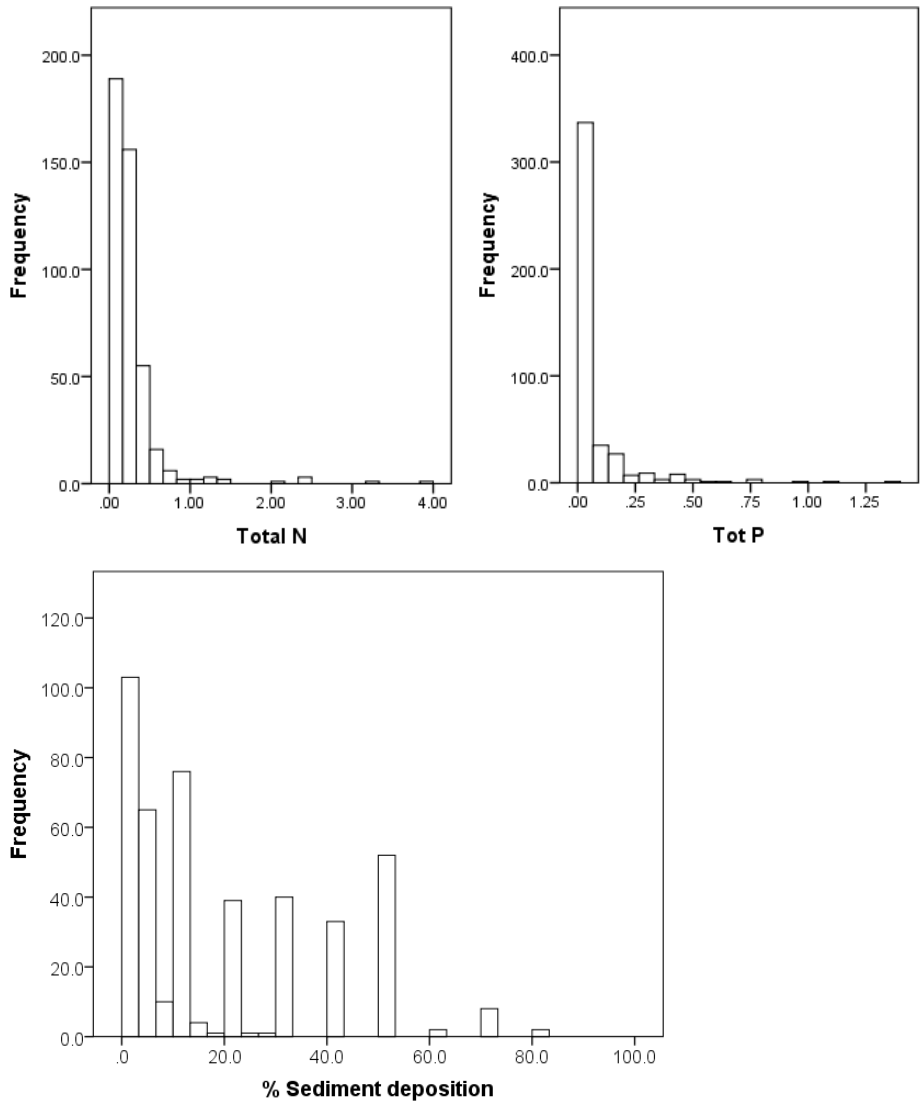

Figure 12: Histograms of water quality variables.

is related to differences in the susceptibility of species over space and time to particular stressors (Diaz et al. 2004).

The covariates and independent variables considered in this study indicated long-term temporal aggregates including long-term average stream flow, precipitation, and temperature. However, data aggregated at a different temporal scale may show varying responses and interactions with EPT taxa richness. For example, seasonal and daily precipitation disturbances can determine macroinvertebrate community structure in running waters (Robinson and Minshall 1986; Buss et al, 2004). Long-term average precipitation metrics used in this study did not capture such effects that can influence EPT taxa richness.

Spatial scale of analysis: Our current study considered hierarchical effects from regional variables using ecoregions to define the regional scale. However, a different scale of analysis, such as the catchment-scale or riparian-scale can incorporate additional effects that were not detected at the current scale of analysis (e.g., Lenat and Crawford 1994). Examples of such additional effects include physical substrate and stream bed characteristics at the local scale which are important to evaluate macroinvertebrate habitat. Owing to the absence of such detailed data at our study region, we could not address effects at the local scale.

Conclusion

Aquatic macroinvertebrates are influenced by factors at different scales. Our analysis found that regional environmental variables are primary in influencing EPT taxa richness across the state of Tennessee, but water quality variables are significant in influencing EPT taxa richness within specific ecoregions. This result helps to directly address the first objective of evaluating if EPT taxa richness is affected by water quality in the midst of regional effects. However, the magnitude and strength of the water quality effects vary by ecoregion. These differences have implications for how the macroinvertebrate community responds to water-quality changes. We found that the relationship between EPT taxa richness and water quality was weak in some ecoregions and insignificant in other ecoregions. Further, evaluating the potential of EPT taxa richness to predict water quality variables, we found that EPT taxa richness as an independent variable was statistically significant for predicting N in Southeastern Plains, Interior Plateau, Southwestern Appalachians and Ridge and Valley ecoregions. EPT taxa richness as an independent variable was statistically significant for predicting sediment in Southeastern Plains and Interior Plateau, However, the potential effects on water quality attributed by these models was much lower than water quality thresholds (Wang et al. 2007; Evans-White et al. 2009). These uncertainties in the relationship between EPT taxa richness and nutrients helped address the second objective of this study, and led to the conclusion that EPT taxa richness is not a good indicator for non-extreme changes in water quality, specifically nutrients, in Tennessee streams.

Other studies have found that, in addition to nutrients, fine sediment is a critical stressor of aquatic macroinvertebrates. Because we lacked enough sediment data for our study

region, we relied on a derived sediment deposition variable to quantify the effect of sediment. However, this variable was not significant in our analysis, which may be a reflection on the ability of the derived sediment deposition variable to quantify sediment effects. The presence of a better sediment indicator will be useful to explore the possibility of using EPT taxa richness as an indicator of changes in sediment, or combined sediment-nutrient effects.

Our result paves the way for questions on the temporal and spatial scale of analysis and other conditions under which our findings might have been different. In our analyses, we found that long-term average stream flow and daily stream flow were significant factors in influencing EPT taxa richness. Further, stream slope was a significant variable in affecting EPT taxa richness in some ecoregions. These findings bring into light the potential influence of stream hydrology and temporal stream dynamics on aquatic macroinvertebrate habitat. Seasonal and monthly changes in precipitation and solar radiation result in within-year changes in flow and temperature that can cause variations in stream flow and habitat quality of aquatic systems (McElravy et al. 1989; Beche et al. 2006). Along the same lines, local habitat characteristics such as channel dimensions, substrate characteristics, woody debris, and hydraulic characteristics can affect macroinvertebrate habitat) and are critical in influencing stream dynamics (Lenat and Crawford 1994; Richards et al. 1997).

In addition to the spatial and temporal scale of the data, data quality and how the data were processed can also potentially influence the findings of this study. For example, daily stream flow at the macroinvertebrate sampling was estimated based on a flow ratio derived from the closest USGS gage site. This calculation operates under the assumption that daily flow relationships with average flow are spatially autocorrelated owing to spatial autocorrelation of precipitation and terrain conditions. However, the spatial extent of such correlations and the presence of other local factors, were not taken into account in this study. Accounting for stream connectivity and hydrological proximity in the calculation of the closest USGS gage can provide different flow ratios, which may influence the calculation of estimate daily flow.

From the results of this study, it is clear that the large regional-scale is not the appropriate spatial scale at which to directly quantify the influence of water quality on aquatic macroinvertebrates. The within-ecoregion class scale showed some significant interactions between water quality and EPT taxa richness that needs to be explored by ecoregion. More critical is the consideration of local stream hydrology and stream habitat characteristics, which may lead to different relationships with macroinvertebrates at the local scale. Further, studies at the local scale can address these issues and field data based on experiments analyzing the response of EPT taxa richness with increasing nutrient concentrations in the midst of other stressors (such as sediment) can help study the interactions at a local scale. These research questions can have immense significance to find simple, yet effective indicators of water quality changes at the appropriate context and scale.

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CHAPTER 5 – ASSESSING EFFECTS OF SWITCHGRASS-BASED LAND-MANAGEMENT PRACTICES ON AQUATIC MACROINVERTEBRATE TAXA RICHNESS

A version of this chapter will be submitted to the Journal of the American Water Resources Association by Latha M. Baskaran, Virginia H. Dale and Liem Tran:

Latha M. Baskaran, Virginia H. Dale and Liem Tran. "Assessing effects of switchgrass-based land-management practices on aquatic macroinvertebrate taxa richness" *Journal of the American Water Resources Association*.

This article was prepared and written primarily by Latha Baskaran. Liem Tran and Virginia Dale advised on the work described here. They also reviewed early revisions of this manuscript.

Abstract

Switchgrass is an energy crop that, based on U.S. Department of Energy evaluations, is projected to be part of a future bioenergy landscape. In this study, we examine how land-management practices associated with switchgrass might affect stream flow, water quality and aquatic macroinvertebrate diversity in the Nolichucky watershed within the Tennessee River Basin. Using the hydrological model Soil and Water Assessment Tool (SWAT), we modeled current baseline conditions for stream flow and water quality and simulated a future switchgrass scenario by changing pasture-based management to switchgrass-based management. Comparing monthly estimates from the baseline scenario and switchgrass scenarios, we found increases in stream flow and decreases in nitrogen and phosphorus concentrations and loads in three subbasins located at various stages of the study area's stream network (headwater, midway, and outlet). Sediment loading and concentrations decreased in the switchgrass scenario at the headwater subbasin. To assess potential changes in aquatic macroinvertebrates, we developed regression models relating taxa in Ephemeroptera, Plecoptera and Trichoptera orders (EPT taxa richness) with stream flow and water quality from the baseline SWAT model. We applied the SWAT-simulated future stream flow and water quality over the regression model to derive estimates of potential changes in EPT taxa richness. We also computed the variations (e.g., standard deviations) of monthly data estimates to evaluate uncertainty associated with the year-to-year variations in the models. Based on the results of the EPT taxa richness projections, we found that the future switchgrass scenario increased EPT taxa richness in all three subbasins (headwater, midway and outlet) with the magnitude of improvements increasing downstream. However, the changes associated with the future scenario were smaller than the uncertainty associated with monthly variations over the years simulated. These results suggest that there are potential effects of switchgrass-based management on EPT taxa richness. However, the magnitude of those effects is region specific and depends on the spatial location of the changes and time frame considered.

Introduction

The demand for renewable energy sources, partly driven by the Renewable Fuel Standard mandates set forth by the US Government, has led to the growth of the cellulosic biofuels industry. The cellulosic biofuels industry relies on corn, grasses, dedicated bioenergy crops, crop residues and wood as biomass feedstock. Using crop

residues and bioenergy crops, the US has the potential to produce 588 to 936 million tons of biomass resources by 2040 (U. S. Department of Energy 2016). The land management changes and resulting environmental effects associated with such a cellulosic bioenergy future need to be evaluated and monitored for sustainable biomass production. Though environmental concerns of biofuels have been raised (e.g., Tilman et al. 2009; Williams et al. 2009; Gelfand et al. 2013), several studies have shown that by using appropriate feedstock and management practices, it is possible to prevent negative environmental impacts and also to restore previously degraded conditions in certain cases (Robertson et al. 2008; U.S. Department of Energy 2017).

To address the environmental sustainability of the bioenergy system, Robertson et al. (2008) identified urgent research needs, which include a systems approach to assess the full impact of bioenergy systems, a focus on ecosystem services, and an understanding of the implications of policy and management at different scales. Water quality and biodiversity are vital components of these research efforts, and there is a growing need to better our understanding of bioenergy crop production and the effects it may have on biodiversity (Dale et al. 2010). Bioenergy systems can affect the habitats of species that rely on the land used to grow bioenergy crops and the streams that drain these lands. Effects on habitat may stem from changes in land use, stream flow and water quality that result from changes in crops and their management. Such habitat alterations directly affect biodiversity at different scales and are a function of the type of bioenergy crops being considered and how they are managed on the ground (Fletcher et al. 2011). For example, dependence on grain-based biofuels such as ethanol from corn can negatively impact stream habitat and biodiversity (Williams et al. 2009).

A number of perennial grasses, crops and trees, including switchgrass (*Panicum virgatum*), corn (*Zea mays*), miscanthus (*Miscanthus x giganteus*), sugarcane (*Saccharum officinarum*), and short-rotation woody crops have been examined as potential sources of cellulosic biomass to meet energy goals (U.S. Department of Energy 2016). Currently, grain-based ethanol from corn is widely produced and is one of the major sources of biofuel in the United States. Though the environmental effects of corn production for ethanol are well documented, grain based biofuels are expected to continue to remain a significant part of the bioenergy system due to their well-established infrastructure (Robertson et al. 2008). However, with biotechnology, genetic and agronomic research, improved crop varieties and better conversion technologies, crops such as switchgrass, biomass sorghum and miscanthus have improved potential for bioenergy production (Mitchell et al. 2008; Yuan et al. 2008; Miguez et al. 2012). Among these crops, switchgrass has gained increased attention because of its vigorous growth, high yield properties, and environmental benefits as compared to annual crops, such as corn. Switchgrass is a perennial crop. Compared to corn, it has lower fertilizer requirements, improved soil conservation, improved energy gain and improved reductions in emissions of carbon dioxide (McLaughlin and Walsh 1998). In a field study of marginal lands planted with corn and switchgrass, the potential ethanol yield for switchgrass fertilized at the same rate as corn was found to be equal to or greater than the potential ethanol yield of corn grain and harvested stover (Varvel et al. 2008). Parish et al. (2012) found that spatial optimization of switchgrass plantings in a watershed

could help achieve improvements in water quality, while meeting other sustainability goals such as bioenergy production. The high yield, efficient plant uptake of N fertilizer, reduced N losses due to the deep rooted system, and drought tolerance are some key features of switchgrass in reducing environmental degradation (Wa et al. 2000; Powers et al. 2011; Eichelmann et al. 2016).

Improvements in sediment and nutrients in the stream are some of the important effects of switchgrass-based land management, which can also directly benefit aquatic biodiversity (Jager et al. 2015). These effects are also driven by stream flow, which was identified as a factor contributing to aquatic species diversity structure in Tennessee (Arnwine et al. 2011). Aquatic macroinvertebrates are considered indicators of aquatic species habitat quality since they are sensitive to water quality and habitats and have recognized responses to changes in those conditions (e.g., Johnson et al. 1993; Kerans and Karr 1994). They are affected by human-induced alterations through their food source, habitat structure and biotic interactions. Mechanisms that cause these changes to the stream ecosystems include sedimentation, nutrient enrichment, contaminant pollution, hydrologic alteration, riparian clearing and loss of large woody debris (Allan 2004). Though several studies have found significant changes in aquatic macroinvertebrates associated with land-use practices (e.g., Lenat and Crawford 1994; Sponseller et al. 2001), the effect of bioenergy based-land use on aquatic macroinvertebrates has not been studied. Since switchgrass-based land management has the potential to affect stream flow and water quality, we hypothesized that such land management also has the potential to affect aquatic macroinvertebrate diversity through changes in stream flow and water quality. We focused on EPT taxa richness, which is the number of taxa in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), as the macroinvertebrate indicator of interest. EPT taxa richness is a widely used bioindicator, owing to the intolerance of insects in EPT taxa to poor water quality (Lenat 1993). Studies have found lower EPT taxa richness in streams draining urban and agricultural watersheds than streams draining forested watersheds (Lenat and Crawford 1994).

In this study we assessed the potential effects of land managed for switchgrass on stream flow, water quality, and EPT taxa richness within a watershed in the Tennessee River Basin. We considered land management associated with switchgrass as an energy crop that has the potential to meet the Billion Ton 2016 goal (BT16) set by the U. S. Department of Energy to determine if U.S. agriculture and forest resources have the capability to potentially produce at least one billion dry tons of biomass annually, in a sustainable manner (U. S. Department of Energy 2016). By analyzing potential changes in stream flow and water quality from a baseline scenario to the switchgrass-based bioenergy scenario, we evaluated the possible effects these changes may have on an aquatic macroinvertebrate diversity indicator.

Study area and data

Our study area is the Nolichucky River watershed located in the Tennessee River basin (Figure 13). This watershed starts in the mountains in the Blue Ridge ecoregion and

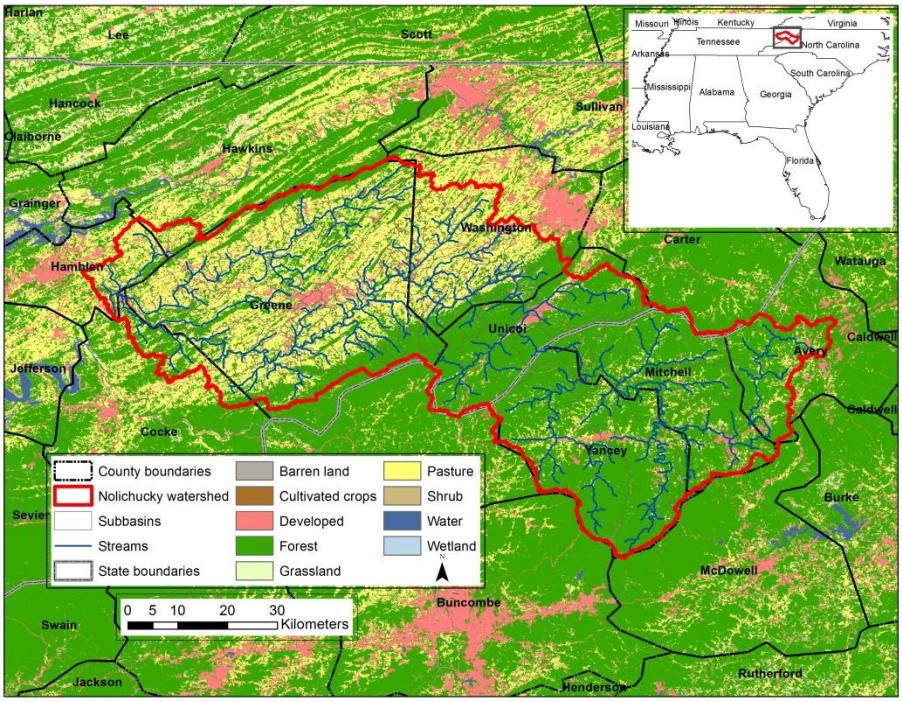

Figure 13: Land cover within and around the Nolichucky watershed. The counties in and around Nolichucky watershed have been labelled.

flows into the French Broad River in the Ridge and Valley ecoregion of Tennessee. The watershed drains an area of about 4372 square kilometers and is mostly composed of forests (59%) and pasture managed for hay (30%) (Figure 13). We assumed pasture management for hay to include fertilization using nitrogen fertilizers and harvesting three times every year (Savoy and Joines 2009).

We obtained daily stream flow data from 2001 to 2015 at a United States Geological Survey (USGS) stream gage located at the outlet of Nolichucky watershed (USGS site id 03467609) (U.S. Geological Survey 2016). From the Tennessee Department of Environmental Conservation (TDEC), we obtained macroinvertebrate data collected between 2007 and 2010 at 104 sampling sites within the Tennessee section of the Nolichucky watershed. These data include biological samples collected by wadeable stream assessments, routine watershed monitoring, 303(d) monitoring, antidegradation monitoring and permit compliance/complaint investigation (Denton et al. 2010).

We obtained water quality data from TDEC in the form of total nitrogen (N), total Phosphorus (P) and suspended sediment concentrations at selected sampling sites throughout the watershed (TDEC 2011). These data are based on chemical sampling protocols and are collected once in 5 years at any one site. Depending on data availability, we used water quality data collected during 2005, 2006 or 2010.

Potential energy crops

Based on future energy scenarios from the BT16 study, pasture and agricultural lands can be converted to energy crops each year. Though pasture and agriculture can be replaced simultaneously, in this study we considered conversion from only pasture since agricultural land occupied less than 1% of the Nolichucky watershed. BT16-produced scenarios of bioenergy futures are based on economic criteria and are at the county-scale. We obtained BT16 scenarios projected to 2040 for Greene County, which lies within the Ridge and Valley ecoregion section of the Nolichucky watershed (Figure 13). At a farmgate price of $60 per dry ton of biomass cost, the future scenarios included bioenergy feedstocks consisting of switchgrass, miscanthus, poplar and willow as future energy crops that could replace pasture within Greene County (U. S. Department of Energy 2016).

Methods

The first step towards addressing the objectives of this study is to derive changes in stream flow and water quality between a baseline scenario representing current land cover, and a bioenergy future scenario. Using the baseline and future stream flow/water quality, we derived relationships between stream conditions and EPT taxa richness and analyzed potential changes under the future scenario (Figure 14). Each step of this process, as indicated in Figure 14, is described below.

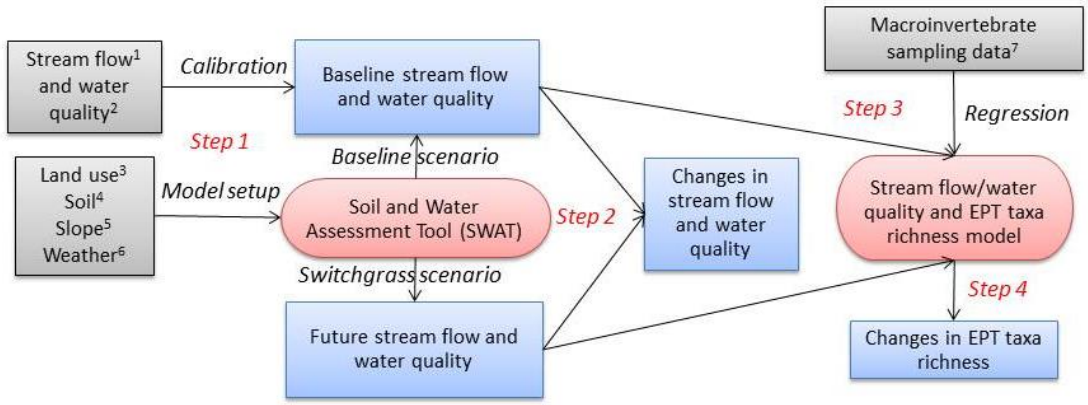

Figure 14: Steps describing the study approach. Grey boxes indicate the input data (1- U.S. Geological Survey 2016), 2 – TDEC 2011, 3 – Homer et al. 2015, 4 - Wolock 1997, 5 - Jarvis et al. 2008, 6 - Girvetz et al. 2013, 7 – Denton et al. 2010). Blue boxes indicate model output and the oval red boxes represent the models.

Step 1: Baseline model setup and calibration

To understand the potential effects of energy-crop-related land-management changes, the hydrologic foundation needs to be established using a hydrologic model that can capture the land use-water relationships in the region. We used the Soil and Water Assessment Tool (SWAT) to simulate baseline conditions and future land management associated with energy crops. Several previous studies have used SWAT to simulate bioenergy future scenarios with different bioenergy feedstock in the landscape (e.g., Cibin et al. 2012; Baskaran et al. 2013; Yasarer et al 2016). SWAT is a river-basin or watershed-scale model developed to predict the impact of management practices on water, sediment, and agricultural chemical yields in watersheds with varying soils, land use, and management conditions over long periods of time (Gassman et al. 2007). The model, which is physically based and computationally efficient, uses readily available inputs and enables users to study long-term impacts. Input parameters for modeling perennial switchgrass growth include chemical applications, existing land cover, elevation data, soils, hydrology, and climate. In SWAT, a watershed is divided into a number of subbasins based on the topography of the watershed or based on user defined subbasin boundaries. For each subbasin within the study area, SWAT generates hydrologic response units (HRUs) based on a unique combination of land use, soil type, and slope category. Output from SWAT includes stream flow, total sediment, nitrate, total nitrogen, total phosphorous, and other water quality parameters.

We delineated the stream network and sub-watersheds in the study region using a 90-m resolution digital elevation model obtained from Shuttle Radar Topographic Mission (SRTM) data (Jarvis et al. 2008). We designated the macroinvertebrate stream sampling sites as subbasin outlets to be able to obtain SWAT output for those sites. We obtained baseline land-cover information from 30-m resolution National Land Cover dataset (NLCD) available for the year 2011 (Homer et al. 2015), and soil information from the State Soil Geographic dataset (STATSGO) (Wolock 1997). Rainfall, temperature, surface radiation and wind speed data were obtained from nearby weather stations summarized by the global climate model simulations (CMIP3) (Girvetz et al. 2013). We simulated the baseline SWAT model from 2000 to 2011 with a spin-up period of 3 years for model conditioning. We calibrated daily and monthly stream flow of the baseline SWAT model with the 2003-2010 data collected at the USGS stream gage located at the watershed outlet (Figure 15) (Appendix). Water quality output was calibrated for total nitrogen and total phosphorus loadings using N and P observations at the outlet of the watershed (Figure 15). The Nash-Sutcliffe efficiency (NSE, Nash and Sutcliffe 1970) and R^2 metrics were used to evaluate model performance. The R^2 statistic provides an estimate of how well the variances of observed values are replicated by the model predictions. R^2 can range from 0 to 1, where 0 indicates no correlation and 1 represents perfect correlation (Krause et al. 2005). Typically, R^2 values of 0.5 or greater are considered acceptable in a SWAT calibration framework (Santhi et al. 2001). NSE can range from $-\infty$ to 1, where an NSE of 1 corresponds to a perfect match between modeled and observed data. An NSE of 0 indicates that the model predictions are as accurate as the mean of the observed data. For evaluating monthly SWAT simulations, NSE values greater than 0.5 are considered satisfactory (Moriasi et al. 2007). We also evaluated the model by comparing the monthly residuals

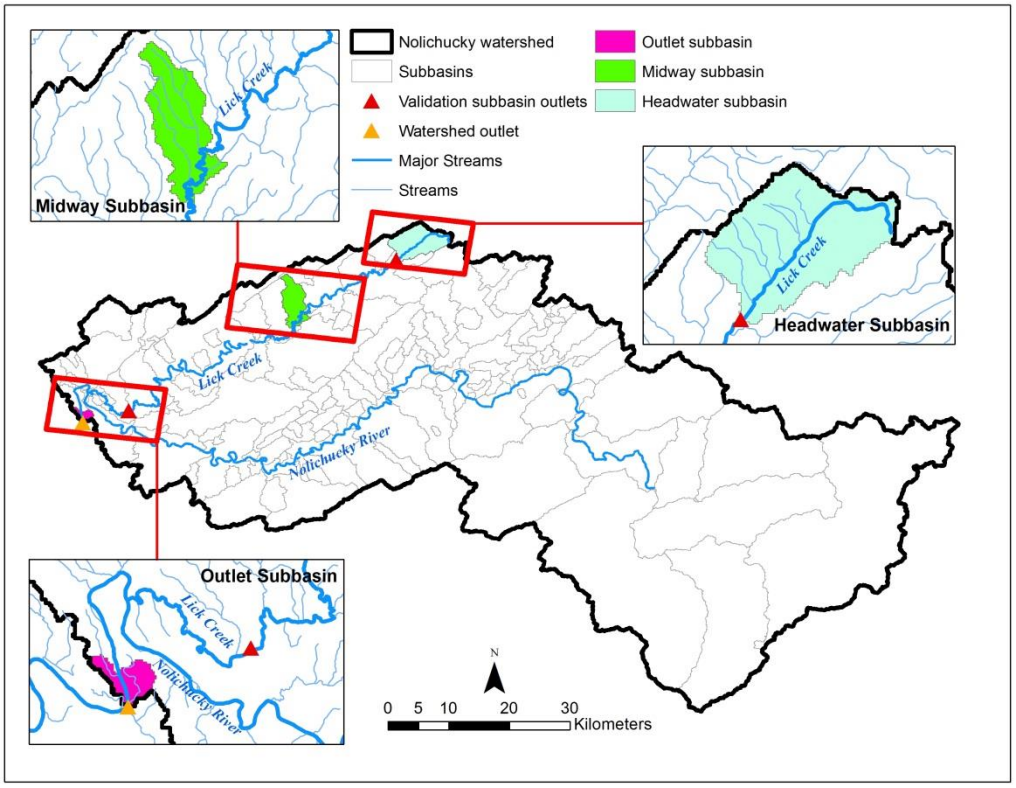

Figure 15: Headwater and midway subbasins along Lick Creek, and the outlet subbasin along the Nolichucky River, in which model results are summarized. Subbasin outlets that were used to calibrate and validate the model are indicated as orange and red triangles respectively.

between observed and predicted data.

We used daily stream flow data for 2011 at the watershed outlet to validate the model. Since we did not have extensive water quality observations at the same outlet, we used a split-location calibration and validation approach (Arnold et al. 2012) and validated N and P observations from two subbasins within the Nolichucky watershed (Figure 15). We did not have enough data to calibrate sediment output from SWAT. However, we obtained limited sediment data at the watershed outlet (8 observations) and used the data to verify that sediment outputs from SWAT are reasonable (Arnold et al. 2012). As with the calibration evaluation metrics, we used NSE and R^2 values to evaluate the model performance. In cases where the data were skewed, we applied a log transformation to the observed and simulated data before calculating the evaluation metrics.

Step 2: Simulating bioenergy scenario

The future land-use scenario involved the conversion of pasture to switchgrass. We changed the management from pasture for hay to simulate management based on growing switchgrass. We simulated switchgrass as a perennial crop with a 10-year rotation. The default SWAT crop module parameters for switchgrass were updated based on regional yield observations, and management recommendations (Baskaran et al. 2010). For example, in the harvesting practices of switchgrass, some stubble is left behind (Parrish and Fike 2005). We assumed a harvest efficiency of 0.8, such that 20% of the above-ground biomass will be left on the ground after harvest. Management guidelines and fertilizer amounts for switchgrass were derived from State/County fertilizer recommendations (e.g., UT Extension 2011). In the case of Tennessee, the crop budgets for 2008 recommended applying 44.8 kg/ha/year of phosphorus starting from the establishment year. From the third year, 65 kg/ha/year are recommended (UT Extension 2009). The first two years do not receive nitrogen, to discourage the growth of weeds from the applied nitrogen.

Analyzing changes in stream flow and water quality

We obtained the SWAT projections for monthly average stream flow (m^3/s), nitrogen loading (kg), phosphorus loading (kg), sediment loading (tons) and sediment concentration (mg/L) for each subbasin outlet for the baseline and switchgrass scenarios for 2003 and 2011. We derived the nitrogen (N) and phosphorus (P) concentrations in mg/L using the stream flow and loadings information. To obtain potential changes in stream flow and water quality between the baseline and switchgrass scenarios, we compared the monthly stream flow, N and P loading, N and P concentration, sediment loading, and sediment concentration results from the two scenarios.

The results were summarized for three subbasins of the Nolichucky watershed to represent a headwater subbasin, a subbasin in the middle portion of the stream, and a subbasin at the outlet of the watershed (Figure 15). Relative positions of streams in flow space provide a conceptual framework for evaluating the relative importance of various factors in regulating macroinvertebrate population and community processes (Poff and Ward 1989). The headwater and midway subbasins were selected along Lick Creek, a

stream draining the northern parts of the Nolichucky watershed. The area drained by Lick Creek is dominated by pasture, and accounts for about 15% of the Nolichucky watershed. Results were also summarized at the subbasin at the outlet of the Nolichucky watershed. This outlet subbasin drains the entire Nolichucky watershed, which includes regions draining the Lick Creek and the Nolichucky River (Figure 15). The Nolichucky River drains the predominantly forested section of the Nolichucky watershed (Figure 13). The headwater, midway and outlet subbasins represent sections along a stream that drain increasing amounts of land in switchgrass under the switchgrass scenario. Though the outlet subbasin drains the entire watershed, it includes contributions from pastures that account for about 30% of the watershed. We quantified model uncertainty at the monthly scale by observing the standard deviation of the monthly values across the years simulated.

Step 3: Evaluating EPT taxa richness as a function of stream flow and water quality

We used EPT taxa richness values for the macroinvertebrate sampling sites in the Nolichucky watershed along with SWAT-generated stream flow and water quality data to derive EPT taxa richness and water quality relationships. Owing to the absence of water quality data at all the macroinvertebrate sites, we relied on SWAT-generated data, which were calibrated for stream flow, N and P loadings at the Nolichucky watershed outlet. From the baseline calibrated SWAT setup, we obtained stream flow and water quality parameters for the macroinvertebrate sampling sites on the corresponding dates of data collection. Using EPT taxa richness as the dependent variable, we ran a series of Poisson regression models with stream flow, N load, P load, N concentration and P concentration as independent variables. We used the Akaike Information Criterion (AIC) to identify the model better suited to represent EPT taxa richness. We identified variables that were significant based on the magnitude of their coefficient and the p value (significance tested at a 0.05 level).

Step 4: Potential changes in EPT taxa richness under a bioenergy landscape

We selected the regression models with significant coefficients derived in the previous step and applied the stream flow and water quality estimates from the switchgrass scenarios to obtain estimates of predicted EPT taxa richness under a bioenergy landscape. As in step 2, the results were summarized at three subbasins along different sections of the stream (headwater, middle and outlet). We compared the predicted EPT taxa richness values for the baseline scenario to that for the switchgrass scenario to estimate potential changes in EPT taxa richness under a bioenergy landscape.

Results

The stream flow calibration and validation results from the baseline SWAT model indicated satisfactory performance at the monthly scale (NSE > 0.5) (Appendix). The R^2 and NSE for the predicted stream flow were 0.71 and 0.58 for calibration, and 0.82 and 0.73 for validation. The R^2 and NSE values for monthly flow residuals were also acceptable: R^2 and NSE values of 0.6 and 0.55 for calibration, and 0.55 and 0.54 for validation. The reasonable monthly residual results indicate that the model also

captures monthly variations and not just overall monthly stream flow trends. The monthly predictions however, missed peak flows at several stages of the calibration (Appendix). Many of the peaks not captured by the model were in the spring months. The peaks near months 28, 40 and 100 are in the months of March and April. This may indicate the lack of appropriate snow melt effects simulated by the model. A large part of the Nolichucky watershed is in the Appalachian mountain range, with high peaks that contribute to a significant amount of snowmelt. Previous studies have noted difficulties in representing spatial and temporal variations in precipitation from snowmelt (Grusson et al. 2015). Further, in a watershed as large as the Nolichucky, all precipitation events may not be represented by the precipitation gages. Localized storm events can contribute to sudden increases in stream flow, but precipitation gages may not have captured those events.

The daily calibration results for stream flow were better than random (R^2 of 0.46 and NSE of 0.29), but the model evaluation statistics were low and not considered satisfactory (R^2 and NSE less than 0.5). The validation results were slightly better, with R^2 and NSE values of 0.53 and 0.46. With large temporal variations in precipitation, it is difficult to accurately calibrate SWAT at the daily scale. The daily model predictions capture the overall monthly and seasonal trends in stream flow but miss daily peaks (Appendix).

Analyzing changes in stream flow and water quality

We simulated switchgrass-based land management on the pasture lands in the Nolichucky watershed. We compared the differences in monthly stream flow, N load, P load, N concentration and P concentration at three subbasins located at different stages of the stream segment (Figures 16-18). We only considered monthly results for this analysis owing to the large uncertainty in the daily SWAT results (calibration results with an R^2 of 0.46 and NSE of 0.29). Using the monthly variation across the years simulated, we plotted one standard deviation above and below the mean to estimate the 68% confidence interval in the simulated data.

EPT taxa richness as a function of stream flow, N and P

EPT taxa richness values, collected between 2007 and 2010, from 104 macroinvertebrate sampling sites in Nolichucky watershed range from 0 to 12. We estimated the relationship between EPT taxa richness and the SWAT generated variables (monthly stream flow, N and P loads, N and P concentrations, sediment load and sediment concentration) using Poisson regression models (Table 20). We did not use daily SWAT results for this analysis since model calibration and validation results showed large uncertainty and unreliability of daily estimates. We developed an initial multivariate model with monthly stream flow, N and P loads, N and P concentrations, sediment load and sediment concentration as independent variables. We also developed univariate models with flow and each water quality variable tested independently. Based on the AIC values, the multivariate model performed better with N and P as significant variables.

However, the univariate models also identified monthly stream flow as a significant

Figure 16: Charts with monthly stream flow, N and P load, N and P concentration, sediment load and sediment concentration at the headwater subbasin for the baseline and switchgrass scenarios. Values for one standard deviation from the mean values are also plotted as dotted lines on the charts. The location of the headwater subbasin is indicated in pink on the watershed map at the upper right panel.

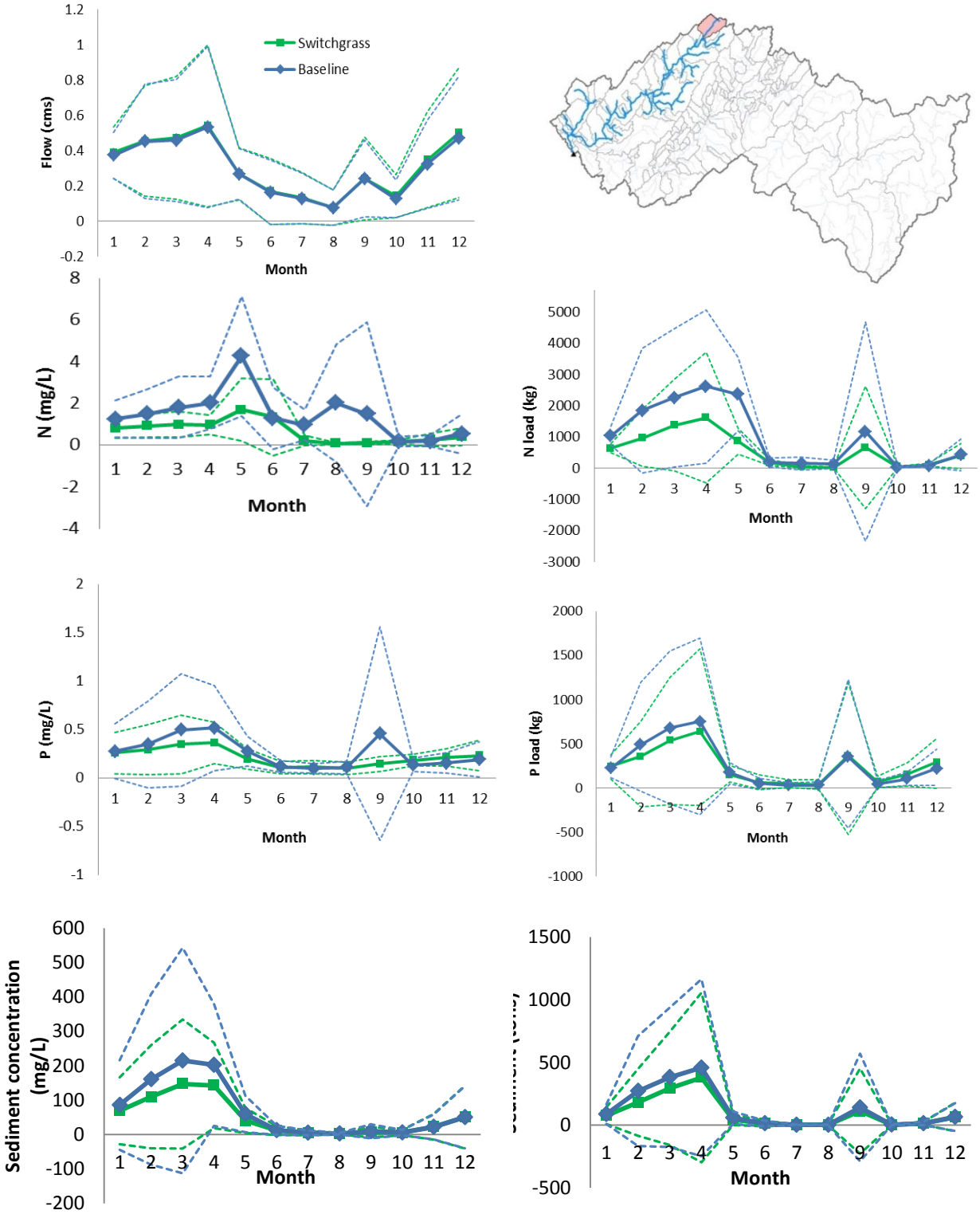

Figure 16: Continued

Figure 17: Charts with monthly stream flow, N and P load, N and P concentration, sediment load and sediment concentration at a midway subbasin for the baseline and switchgrass scenarios. Values for one standard deviation from the mean values are also plotted as dotted lines on the charts. The location of the midway subbasin is indicated in pink on the watershed map at the upper right panel.

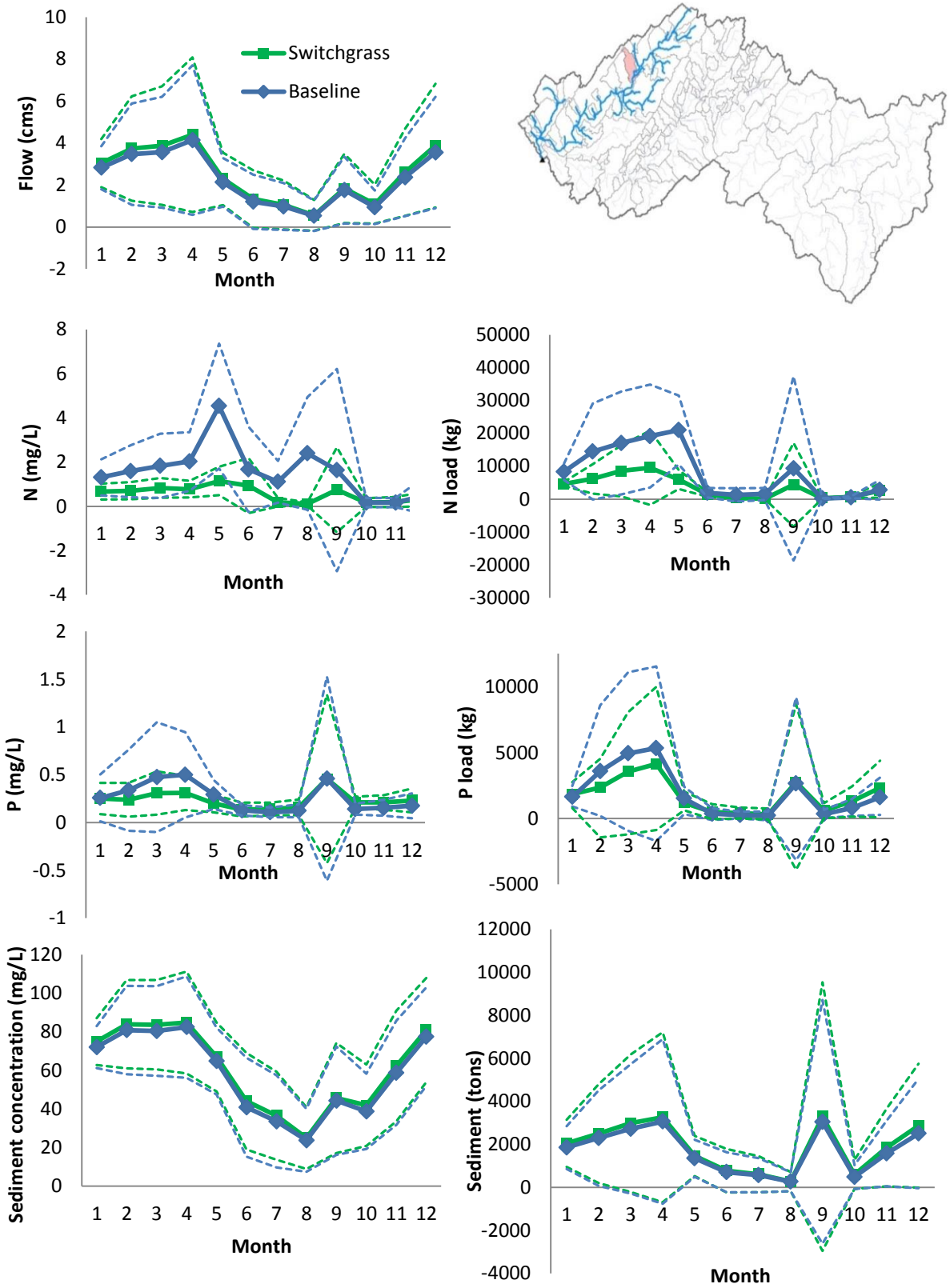

Figure 17: Continued

Figure 18: Charts with monthly stream flow, N and P load, N and P concentration, sediment load and sediment concentration at the outlet subbasin for the baseline and switchgrass scenarios. Values for one standard deviation from the mean values are also plotted as dotted lines on the charts. The location of the midway subbasin is indicated in pink on the watershed map at the upper right panel.

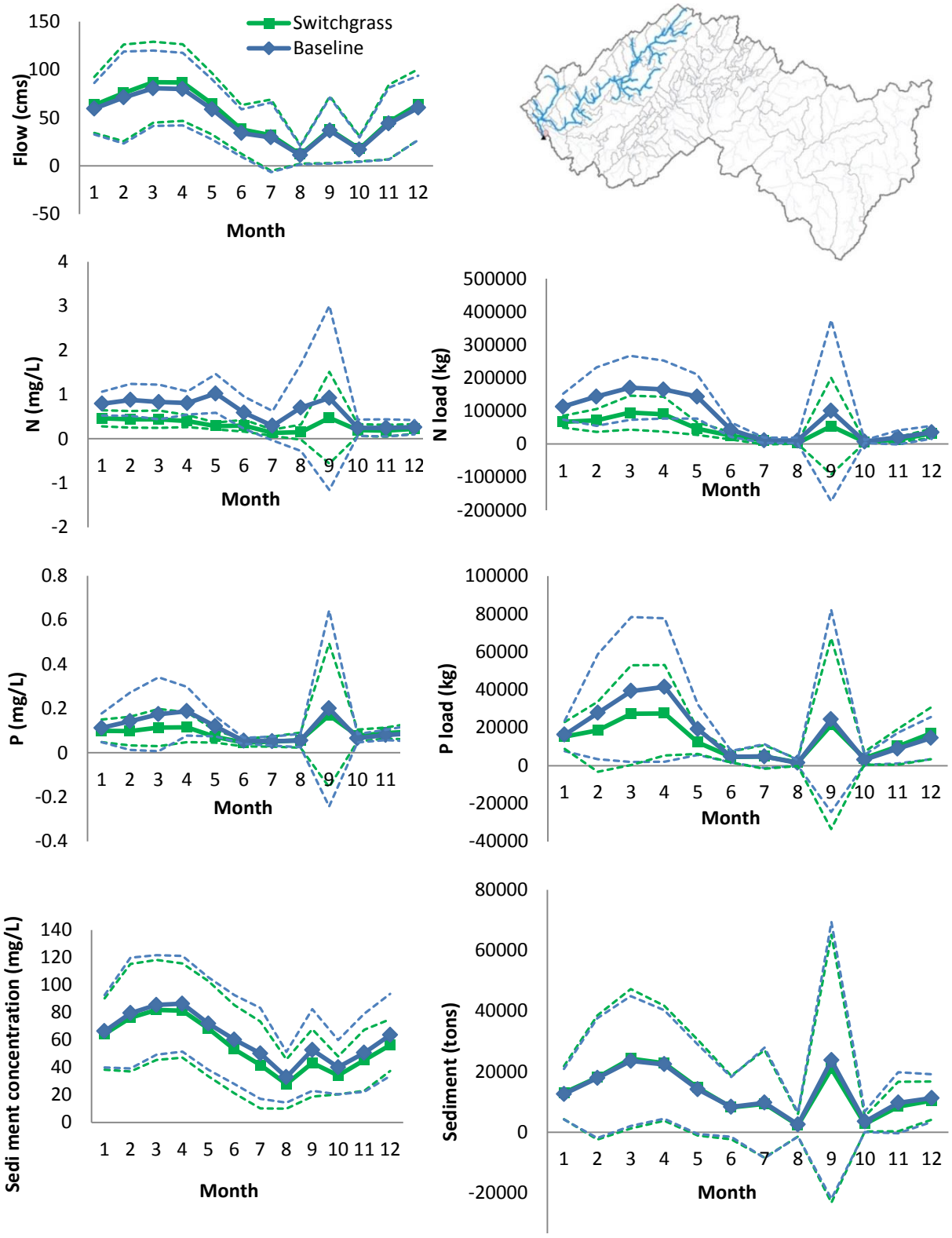

Figure 18: Continued

Table 20: Results from Poisson regression models with EPT taxa richness as the dependent variable and monthly stream flow, monthly N concentration, monthly P concentration, monthly N loadings, monthly P loadings, monthly sediment concentration, and monthly sediment loadings as independent variables. Model AIC values and regression coefficient and p-values of significant variables (at a 0.05 level) are presented.

Independent Variables	AIC	Significant variables	Regression coefficient	P value
Monthly stream flow, monthly N concentration, monthly P concentration, monthly N loadings, monthly P loadings, monthly sediment concentration, monthly sediment loadings	508.26	Monthly N Monthly P	0.0448 -1.68	0.0339 0.0005
Monthly stream flow	515.32	Monthly stream flow	0.03346	0.00312
Monthly N concentration	522.96	-		
Monthly N loadings	513.09	Monthly N loadings	9.567e-05	0.00086
Monthly P concentration	514.6	Monthly P	-1.05395	0.00458
Monthly P loadings	514.79	Monthly P loadings	0.0003088	0.00232
Monthly sediment concentration	523.09	-		
Monthly sediment loadings	521.79	-		

variable in influencing EPT taxa richness. Since the multivariate model may have masked the effect of some variables, we further analyzed the variables identified as significant in the univariate models.

Potential changes in EPT taxa richness due to switchgrass-related land management

We applied output from the baseline and switchgrass scenarios on the regression model relating EPT taxa richness with the monthly stream flow and monthly P concentration to obtain estimates of predicted EPT taxa richness. The regression coefficients for monthly N concentration, sediment load and sediment concentration were not significant; therefore no clear relationship of those metrics with EPT taxa richness was apparent in the data used in this study. The regression coefficients for monthly N and P loads were significant, but very small (0.00009 and 0.0003), and hence not considered to be significant for this analysis of potential changes in EPT taxa richness.

The average of changes in predicted EPT taxa richness between baseline and switchgrass scenarios based on monthly flows in the three subbasins of the Nolichucky watershed (headwater, middle and subbasin outlet), showed an increase in EPT taxa richness (6.5, 0.04 and 0.002) (Figure 19). Similarly, the average of changes based on monthly P concentration models for the outlet subbasin, middle subbasin and headwater subbasin were 0.13, 0.1 and 0.24 respectively (Figure 20).

Discussion

The change in stream flow from a baseline scenario to a switchgrass scenario indicated slight increases in flow in all three subbasins (Figures 16-18). The average monthly increases in stream flow in the headwater, midway and outlet subbasins were 3.5%, 8.2% and 6%. The magnitudes of these changes were very low in the headwater and midway subbasins (average increases of 0.009 and 0.18 m^3/s). Since the outlet subbasin drains the entire Nolichucky watershed, including the forested regions, the magnitude of changes at the outlet is relatively larger, with an average increase of 3.5 m^3/s. However, an examination of one standard deviation from the mean shows the uncertainty associated with these values. In all three subbasins the areas within the dotted bands representing the monthly standard deviation are much larger than the differences between the scenario results (solid lines in Figures 16-18). The magnitude of the average standard deviation is 0.229 m^3/s for the headwater subbasin, 1.736 m^3/s for the midway watershed and 30.85 m^3/s for the downstream subbasin near the outlet. Such downstream increase in uncertainty in SWAT-derived stream flow is a function of the increasing percentage of water and has been demonstrated in other studies (Baskaran et al. 2010). However, the outlet subbasin also receives flow from the entire watershed, which includes forested area in the Blue Ridge ecoregion. The forested streams in the Blue Ridge ecoregion have high acid deposition rates, which contribute to nitrogen saturation and increased nitrate export to the streams (Cai et al. 2012). The contribution of stream flow and water quality from the entire Nolichucky River watershed into the outlet subbasin influences the magnitude of stream flow at the outlet, and can

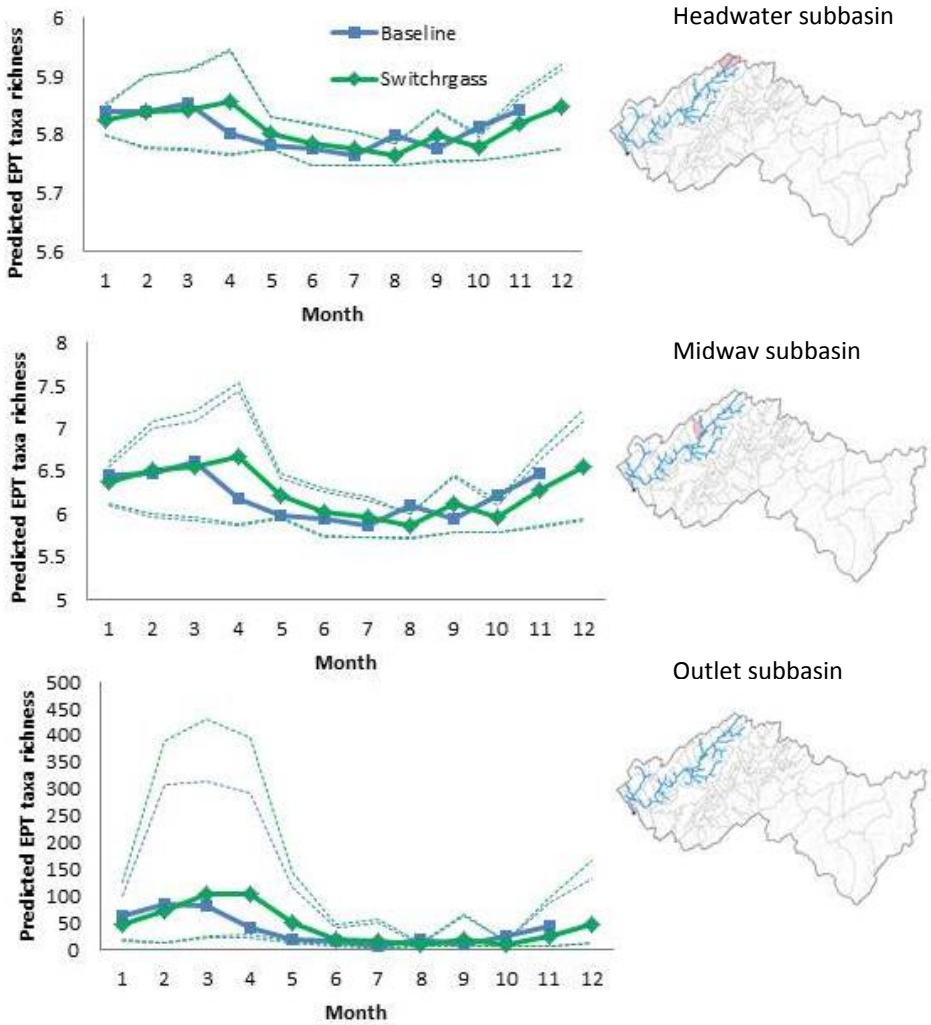

Figure 19: Charts with monthly predicted EPT taxa richness based on the monthly flow regression model for the baseline and switchgrass scenarios. Average monthly results for the headwater, midway and outlet subbasin are presented (location of the subbasins are shown in the right side panels). One standard deviation from the mean values are also plotted as dotted lines on the charts.

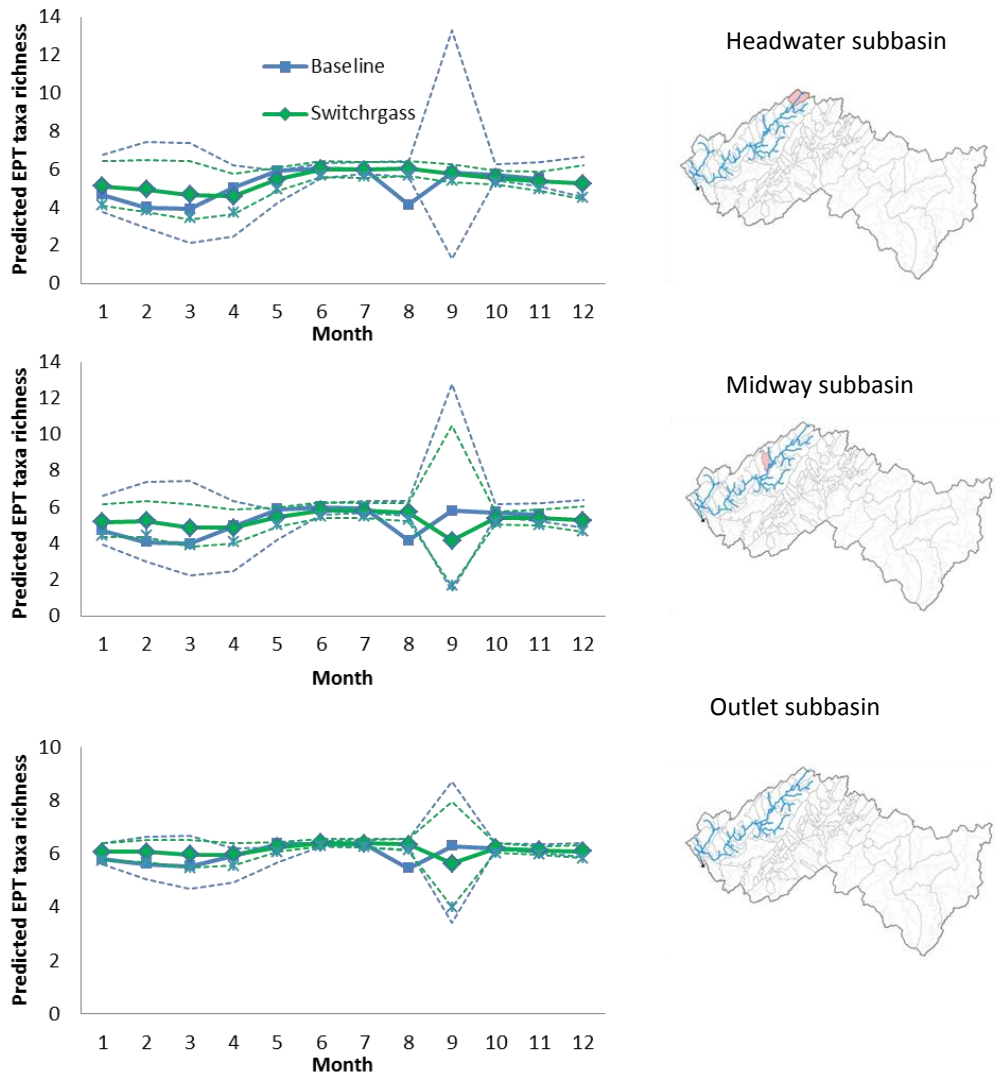

Figure 20: Charts with monthly predicted EPT taxa richness based on the monthly P concentration regression model for the baseline and switchgrass scenarios. Average monthly results for the headwater, midway and outlet subbasin are presented (location of the subbasins are shown in the right side panels). One standard deviation from the mean values are also plotted as dotted lines on the charts.

affect the magnitude of the potential changes and associated standard deviations.

The average monthly N and P loads and concentrations decreased from the baseline to the switchgrass scenario. Sediment loading and concentration decreased under the switchgrass scenario for the headwater subbasin. However, for the midway and outlet subbasins, sediment concentration from the switchgrass scenario was very close to, and in some cases, slightly higher than the sediment from the baseline scenario. Moving downstream, the greater uncertainty associated with predictions from larger streams causes large variations in the sediment estimates too. Similar to the stream flow results, although the average change is significant, the uncertainties surrounding these water quality variables is large as indicated by the area included within the dotted lines (Figures 16-18). These results indicate that although the changes in stream flow and water quality may be significant, within the timeframe considered, they are within the magnitude of monthly variation of the 9-year time period considered (2003 to 2011). Considering a longer time frame may provide different results depending on the land-use changes considered. If additional land-use changes cause changes in stream flow and water quality, the effects can cumulatively increase over the years and affect long-term trends. However, modeling such long-term trends is beyond the scope of this study.

The baseline calibrated SWAT model enabled us to develop relationships between EPT taxa richness and stream flow and water quality. Macroinvertebrate sampling data are useful bioindicators; however, it has been difficult to identify clear relationships between EPT taxa richness and stream flow/water quality (Smith et al. 2007). One of the roadblocks to identifying such relationships is the lack of stream flow and water quality data corresponding to the same site and date of collection as the macroinvertebrate data. With output from the calibrated SWAT model, we were able to describe relationships between monthly stream flow and water quality with EPT taxa richness.

The monthly stream flows were positively correlated with EPT taxa richness. Studies have shown that lower flows result in lower stream velocity, which may cause increased sedimentation that is detrimental to macroinvertebrate habitat (e.g., Dewson et al. 2007). Likewise, based on the size of the stream, increase in stream flow can remove fine sediment and provide favorable macroinvertebrate habitat (Poff and Ward 1989).

We did not find a significant relationship between monthly N concentrations and EPT taxa richness. This result could have been influenced by the presence of large forested land in the Nolichucky watershed that contributes to the total nitrogen concentrations in the stream through organic nitrogen and nitrate input from forests. Large N inputs from the forested streams could have masked the effects from N draining agricultural lands in the Nolichucky watershed, especially in streams along the Nolichucky river.

Monthly P concentration effects were significant and negatively related to EPT taxa richness. Studies have shown that an increase in P in streams affects leaf decomposition and primary production, which can cause oxygen depletion and cause

changes to the macroinvertebrate structure (Elwood et al. 1981). Monthly N and P loads were significantly related to EPT taxa richness with positive coefficients.

The monthly sediment loadings and concentrations were not significantly related to EPT taxa richness. Though sediment is expected to influence EPT taxa habitats, the lack of significant interactions can be attributed to the data quality and the difference in the type of suspended sediment considered. Our validation based on limited sediment data did not provide acceptable results (NSE < 0.5), and hence the sediment estimates may be unreliable. Further, sediment values from SWAT refer to the sediment concentration from runoff, which flows through the stream system. This suspended sediment is different from the sediments deposited in the stream bed, which are of greater importance to macroinvertebrates. One of the biggest impacts of sedimentation on macroinvertebrates is associated with the fines (silt and clay sized particles) eroded from agricultural land (Walling et al. 1990). Compared to rocky substrates, sandy substrates are poor habitats because the shifting nature of the bed provides unsuitable attachment and poor food conditions (Merritt and Cummins 1996). Such fine sediment and sandy substrates are not captured by SWAT in the time frame considered. Longer simulations covering decades may have the potential to alter the stream substrate and affect macroinvertebrate habitat (Beche et al. 2006).

Applying the monthly stream flow and monthly P concentration regression relationships to the output of the SWAT switchgrass scenario, we found an increase in EPT taxa richness under the switchgrass scenario (Figures 19 and 20). Though switchgrass requires N and P fertilizers, the amounts are less than those for conventional hay management (Baskaran et al. 2013). Switchgrass is more efficient in water uptake and use and hence requires less water, leading to greater water routed as groundwater (VanLoocke et al. 2012).

The uncertainty associated with these results was high based on monthly standard deviations of the predicted EPT taxa richness values (Figures 19 and 20). For the stream flow based model (Figure 19), the standard deviation was very high in the spring months. This result is a function of the variation in stream flow because of temporal variability in precipitation events and snowmelt in the spring months (Strauch et al. 2012). Also, the onset of the growing season (e.g., leaf-out) creates substantial change in water use by deciduous plants/trees, which rapidly changes soil moisture conditions. The EPT taxa richness predictions for the outlet watershed were very large (average of 38) and beyond the range of EPT taxa richness currently observed in the Nolichucky watershed (0-12). We attribute this to the size of the stream at the Nolichucky watershed outlet, where the average stream flow is 38 m^3/s. The macroinvertebrate data used to develop EPT taxa richness regression models were based on small, lower order streams throughout the Nolichucky watershed and did not include data from the larger, higher order streams and hence did not capture taxa richness relationships associated with those streams. Other studies have shown varying macroinvertebrate assemblage structure based on stream size and possible non-linear responses to changes in stream flow (Heino et al. 2005; Poff and Zimmerman 2010). The relationship

between water quality and EPT taxa richness may not be linear beyond the conditions defined by the small, low order streams.

Conclusion

In this study we found that changes to a stream after pasture managed for hay in the watershed were converted to a switchgrass-based landscape showed increases in stream flow and improvements in water quality. Though these results have uncertainties associated with them, other studies have shown similar results (e.g., Lee et al. 2003). Using EPT taxa richness as a bioindicator of stream health, our predictions of EPT taxa richness under a switchgrass scenario showed potential for slight increases. However the magnitudes of these changes are very small in comparison to the monthly variations of EPT taxa richness. We conclude that there is potential for changes in EPT taxa richness associated with switchgrass-based management, but the magnitude of those changes cannot be reliably proven under the context of the current study region and its baseline conditions.

Data availability is a problem for many environmental modeling studies, especially since spatially and temporally extensive data are not always available. A fundamental assumption of this study is that SWAT model output can be used to derive EPT taxa richness-water quality relationships for a different model. When different ecological models are coupled by one model feeding into another, the propagation, dispersion and potential magnification of uncertainty can influence the apparent results of the models. In this study we addressed uncertainty by considering the standard deviation of monthly estimates. In spite of the uncertainty issues, the usefulness of models is un-debatable and has been accepted by the decision-making and modeling community (Dale and Van Winkle 1998).

The results from this study are also constrained by the temporal scale of the data and simulations. We calibrated SWAT using daily stream flow data from 2003 to 2010; however, we calibrated water quality using daily samples available for 10 to 15 dates in each subbasin. Such a calibration approach helps capture overall water quality ranges, but may miss daily water quality trends. We analyzed monthly output to avoid interpretations at the daily scale that may not be accurate.

We considered changes in the stream system over a period of about 9 years. The magnitude of the water-quality changes associated with growing switchgrass within this time frame was not found to be significant under the overall water quality trends characterized by high variability within a year (as evidenced by the month to month variations seen in Figures 16-18). However a longer-term analysis may provide different results owing to increasing effects with time that can change stream substrate, thermal regimes and hydraulics, which in turn can change macroinvertebrate community structure. For example, Durance and Ormerod (2007) found that over a 25-year period, climate change had the potential to impact macroinvertebrate composition and abundance through changes to the streams thermal structure, particularly in headwater streams.

In our analysis we found that the conversion to switchgrass management improved water quality and aquatic biodiversity, and the effects increased from the headwaters to the outlet of the watershed. Such effects illustrate the need to address the spatial location of a study area while estimating the effects of land-use change on a stream system. These effects would be expected to differ and possibly grow if the study were extended from a small watershed (at the scale of HUC8) to a larger region, such as river systems draining into the Gulf of Mexico. The ways in which these effects scale up is an important research area that needs to be addressed in future efforts.

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Appendix

We used SWAT-CUP, which is a program to calibrate SWAT model runs, to perform the calibration using the Sequential Uncertainty Fitting Procedure referred to as SUFI-2 (Abbaspour 2011). Latin hypercube sampling was used to sample from the possible parameter space in a stratified sampling approach (McKay et al. 1979). 400 to 1000 parameter combinations were generated and used to run the model to analyze the corresponding changes in the model output (Abbaspour et al. 2007). To find a solution of the parameters and the confidence limits to the model output, the SUFI-2 calibration approach, which considers the percentage of data captured (bracketed) by the prediction uncertainty, was used in this study.

We calibrated daily SWAT-simulated stream flow against observed stream flow from 2003 to 2010 at the Nolichucky watershed outlet. Initial calibration output indicated a time lag of about a day between observed and predicted flow. This delay occurred because of lag between how rainfall measurements and USGS flow measurements are observed. Rainfall from 6 AM in the previous day to 6 AM in the current day is considered as the current day rainfall; whereas stream flow from 7 AM in the previous day to 7 AM in the current day is considered as stream flow measurement for the previous day (personal communication R. Srinivasan). This difference caused the predicted stream flow to be off by a day when compared to the observed stream flow. To correct for this issue, we shifted observed precipitation by a day and calibrated the model.

Initial differences between observed and predicted stream flow indicated low baseflow and low peak flows simulated by the model. We selected parameters to calibrate and adjust for such differences in baseflow and peak flows, which included curve number, baseflow alpha factor, surface runoff lag time and threshold for return flow to groundwater to occur (Abbaspour 2015) (Table 21). We increased the Manning's "n" roughness value for overland flow to better simulate the varying slope and terrain in the Nolichucky watershed. We also decreased the average HRU slope steepness to correct for the time lag in observed and predicted flows. We ran a series of calibration runs until the R^2 and NSE values were considered reasonable for the monthly stream flow calibration (greater than 0.5). The final R^2 and NSE for the monthly stream flow were 0.71 and 0.58 respectively (Table 22). The best parameter values provided by SUFI-2 were used to update the Nolichucky watershed SWAT setup (Table 21). The differences between observed and simulated stream flow based on these parameter values are shown in Figures 21 and 22.

We also calculated the correlation between observed and predicted monthly stream flow residuals to evaluate model performance beyond the average stream flow trends (Figure 23 and Table 22). Using stream flow data from 2011, we validated the calibrated SWAT setup and obtained R^2 and NSE values for the validation period (Table 22).

For water quality calibration, we used total nitrogen and total phosphorus concentrations (in mg/L) collected at the watershed outlet for calibration, and data from two other

Table 21: SWAT calibrated parameter values.

Parameter code	Parameter description (units)	Range	Calibrated value
CN2	Curve number (CN)	35-98	CN*0.2796
Alpha_bf	Baseflow alpha factor (1/days)	0-1	0.0473
EPCO	Plant uptake compensation factor	0-1	0.348
Surlag	Surface runoff lag time	0.05-24	2.043
GWQMN	Threshold depth of water in the shallow aquifer required for return flow to occur (mm)	1-5000	15.45
OV_N	Manning's "n" value for overland flow	0.01-30	30
HRU_SLP	Average slope steepness [m/m]	0-1	0.05

Table 22: SWAT calibration and validation results for daily stream flow, monthly stream flow, and residuals of monthly stream flow. Calibration was based on data over the period 2005 to 2010. Validation was based on data from 2011.

	Calibration		**Validation**	
Variable	R^2	NSE	R^2	NSE
Daily stream flow	0.46	0.29	0.53	0.46
Monthly stream flow	0.71	0.58	0.82	0.73
Residuals of monthly stream flow	0.6	0.55	0.55	0.54

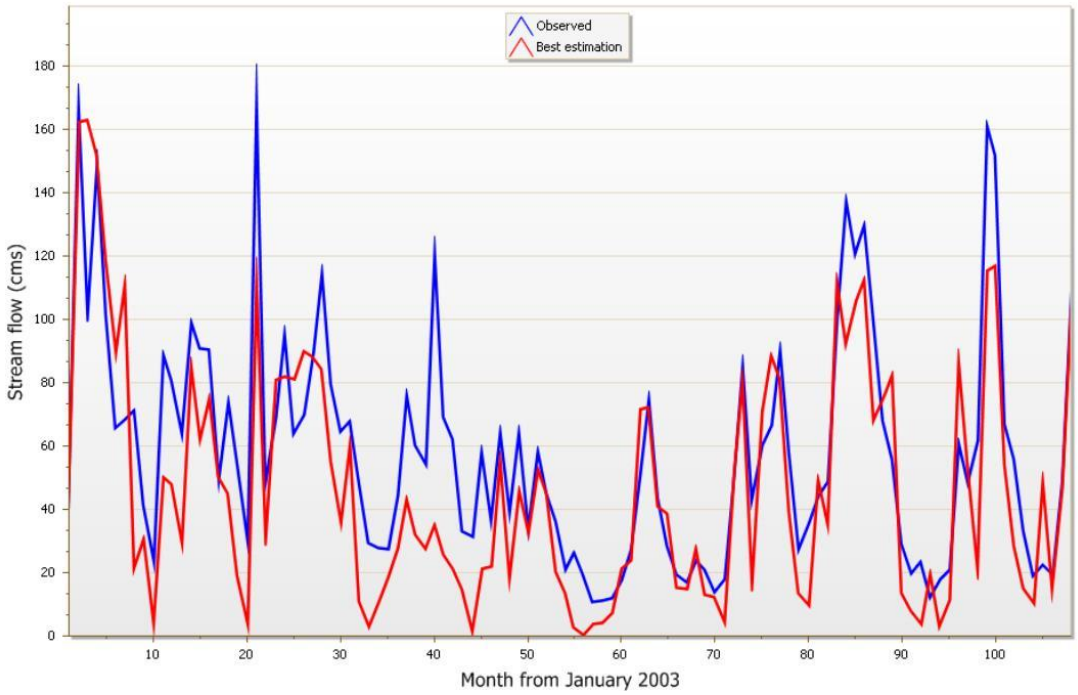

Figure 21: Observed and predicted stream flow for calibration between January 2003 to December 2011.

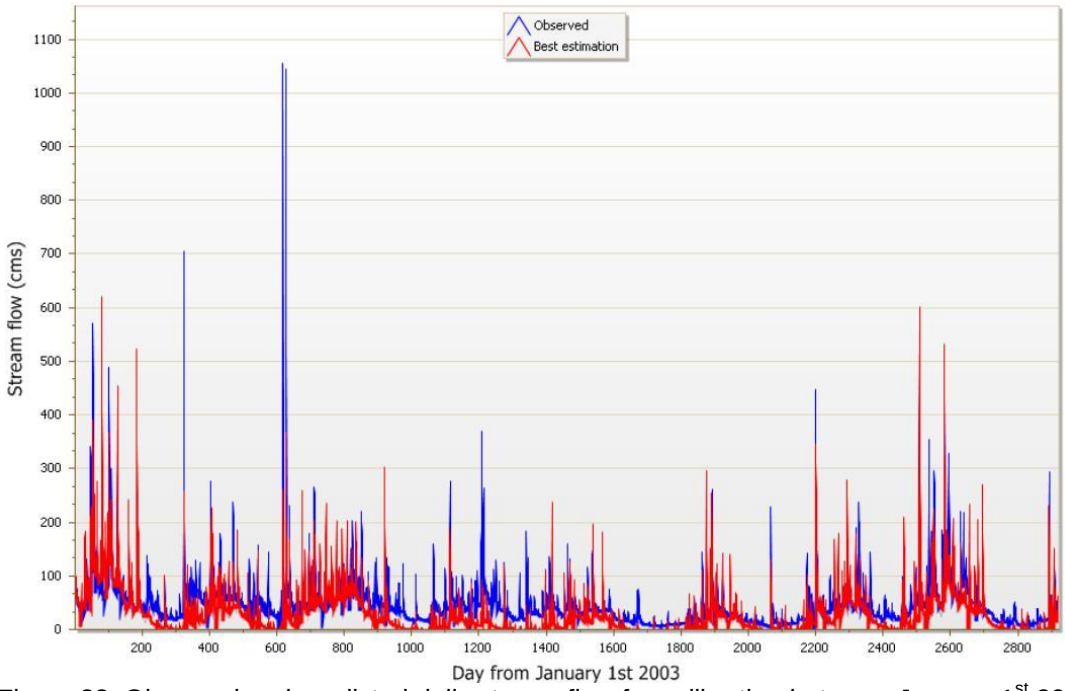

Figure 22: Observed and predicted daily stream flow for calibration between January 1st 2003 to December 31st 2011.

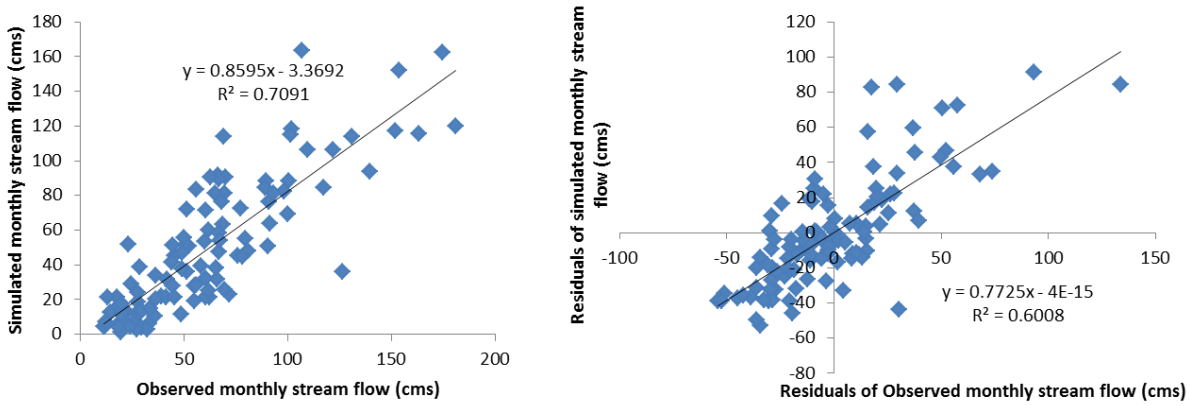

Figure 23: Plot between observed and predicted monthly stream flow and monthly residuals of observed and predicted monthly stream flow. The linear regression equations relating the variables and their R^2 values are also indicated in the charts.

subbasins (subbasin 1 and subbasin 105) for validation (Figure 15). These datasets were concentration values based on grab samples collected during selected dates in 2010 for N calibration, 2005 and 2006 for P calibration, 2005, 2006 and 2010 for N validation and years 2005 and 2006 for P validation. The concentration values were converted to daily loadings (kg) using stream flow observed at the watershed outlet for calibration and using stream flow simulated by SWAT for the validation subbasins. We removed observations collected during periods when the differences in observed and predicted stream flow were very high, causing unreliable loading estimates. The loadings data were skewed in many cases owing to outliers as a function of the variation in daily stream flow. To correct for such skewed data, we log transformed the observed and simulated data and estimated R^2 and NSE values for the calibration and validation subbasins (Table 23 and Figure 24).

Table 23: SWAT calibration and validation results for daily N and P loadings at selected subbasins.

Subbasin	**Total N**		**Total P**	
	R^2	NSE	R^2	NSE
Calibration – subasin 116	0.324	0.308	0.7	0.874
Validation – subasin 1	0.792	0.715	0.8	0.312
Validation – subasin 105	0.64	0.684	0.47	0.482

To verify sediment output from SWAT, we compared the sediment concentration from SWAT with sediment concentration obtained at the outlet of the watershed. We removed observations collected during periods where the differences in observed and predicted stream flow were very high, causing unreliable sediment estimates. The NSE for the sediment concentration was found to be 0.347. R^2 was found to be 0.67 for the log-transformed observed and simulated data.

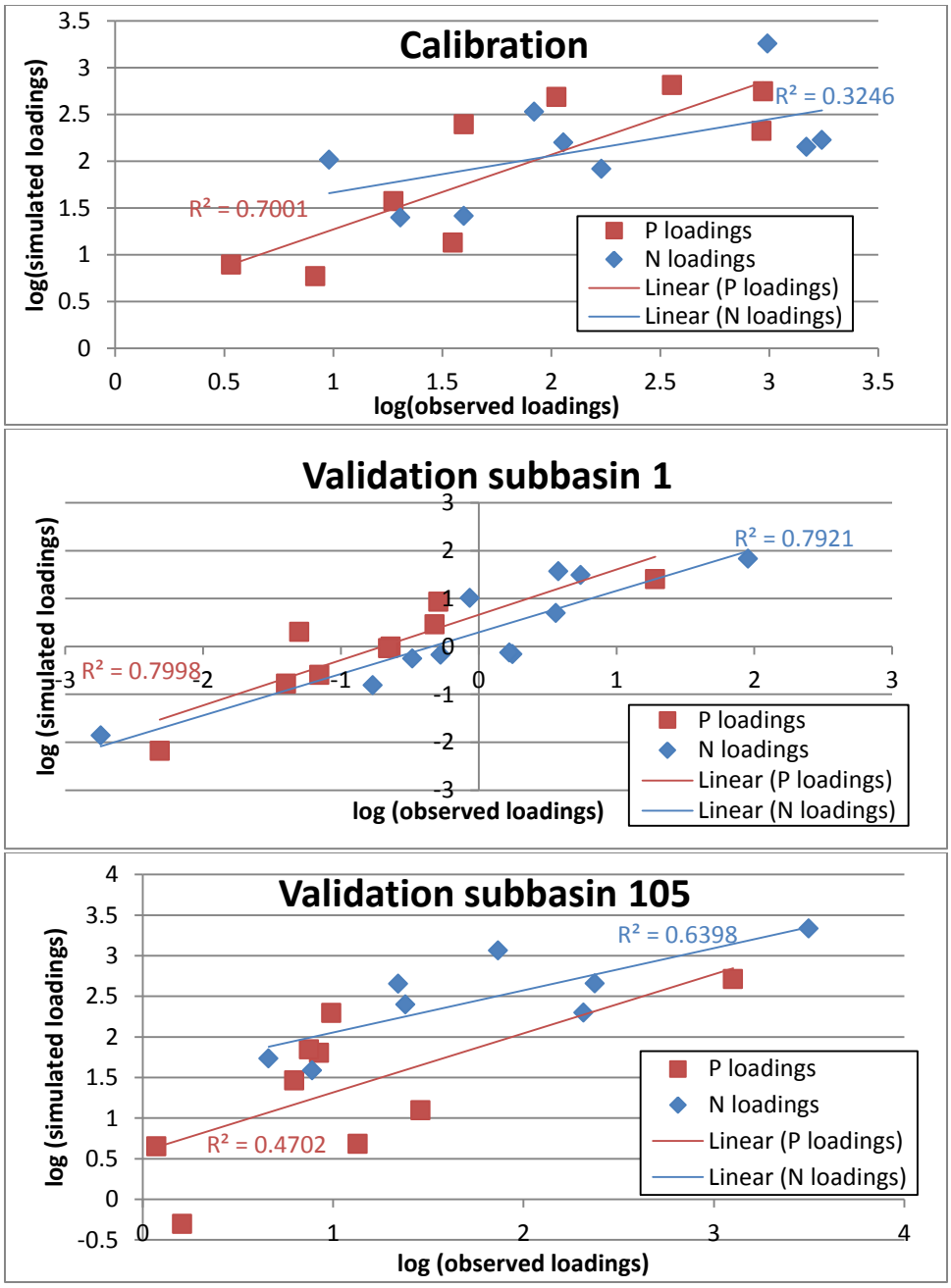

Figure 24: Calibration and validation results for water quality at subbasins in the Nolichucky watershed. The blue R^2 values indicate the fit between observed and simulated N loadings. The red R^2 values indicate the fit between observed and simulated P loadings.

CHAPTER 6 – CONCLUSION

The environmental implications of bioenergy choices are extensive, complex, intertwined, and dependent on factors both endogenous and exogenous to a region. There is a strong need to understand how bioenergy crops can be grown more sustainably (Dale et al. 2011). This research assessed the effects of stream flow and water quality on EPT taxa richness in the midst of regional environmental factors in Tennessee. Understanding these effects helped set the context to analyze the influence of switchgrass-based land management on EPT taxa richness through changes in stream flow and water quality. Based on the results of this study, and under the spatial context considered, the effects of switchgrass-based land management on EPT taxa richness could not be demonstrated in the short term (1-9 years).

Summary of results by objectives of study

In addressing the first objective of the study to determine the key natural factors at different scales that affect EPT taxa richness, we found that the regional factors and stream flow characteristics influencing EPT taxa richness varied spatially by ecoregion classes (Chapter 3). Three ecoregion classification schemes were evaluated to stratify the regional constraints and Omernik's ecoregion classification (Omernik 1987), which captured the soil and precipitation spatial gradients, was found to be the better classification scheme for evaluating EPT taxa richness. For example, slope was positively correlated to EPT taxa richness in the Interior Plateau, Ridge and Valley and Blue Ridge ecoregions. Temperature was found to be negatively correlated to EPT taxa richness in three of the eastern ecoregions (Central Appalachians, Ridge and Valley and Blue Ridge ecoregions), where the undulating terrain has the potential to influence temperature and stream conditions. Long-term average stream flow was correlated to EPT taxa richness in the Mississippi Valley Loess Plains, Interior Plateau and Blue Ridge ecoregions, and stream velocity was correlated to EPT taxa richness in all ecoregions other than Central Appalachians and the Blue Ridge ecoregion. These results highlight the need to consider location and geomorphological context while studying aquatic macroinvertebrates. The results also bring to light the importance and of need for a multi-scale analysis, which takes into consideration regional effects across ecoregions before considering other local-scale variables such as water quality and sediment.

Chapter 4 evaluated the potential of EPT taxa richness as an indicator of water quality in Tennessee using a multilevel modeling approach. The use of the multilevel regression model helped quantify the influence of regional variables in affecting EPT taxa richness by ecoregion (objective 2). Temperature and average stream flow had the largest fixed effect regression coefficients and were some of the strongest factors affecting EPT taxa richness across ecoregions. Daily stream flow was found to be significant as a random variable in some of the ecoregions. Total N, total P and sediment were found to be significant random variables in some ecoregions, but the magnitudes of their influence were small based on their slope coefficients (<0.08 for EPT-N model, <0.05 for EPT-P model and <0.14 for EPT-S model). To evaluate if EPT

taxa richness has the potential to be an indicator of water quality in the study area, multilevel regression models with water quality variables as dependent variables and EPT taxa richness as the independent variable were assessed. The results from these models indicated that EPT taxa richness was significant as a random effect within some ecoregions, but the magnitudes of these effects based on their slope coefficients were small (<0.1 for the N model and <0.06 for the S model). Based on the results from the two different multilevel regression models (EPT taxa richness as dependent variable in the first set, and EPT taxa richness as the independent variable in the second set), the relationship between N, P and sediment and EPT-taxa richness was found to be significant in some ecoregions. This result directly addresses the second objective of this study – to identify the influence of water quality on EPT taxa richness in the midst of regional environmental variables in Tennessee. However, owing to the low strength of the interactions and uneven responses across regions, EPT taxa richness was not found to be a useful indicator of water quality within the context of this study.

Chapter 5 directly addressed the third objective of evaluating if switchgrass-based land-management changes influence EPT taxa richness. This study found that, based on the study region and baseline conditions, the influence on EPT taxa richness of converting from pasture to switchgrass-based land management was very small and within the standard deviations of monthly variations of the system. Hence the influence of switchgrass-based land-management changes on EPT taxa richness cannot be proven within the regional context and baseline conditions considered for this study.

Though the three manuscripts presented in this research addressed different issues and used different approaches, the results of each manuscript were useful for the next analysis and the three also share common themes. All three parts of this research highlighted the influence of stream flow with respect to EPT taxa richness. In chapter 3, stream flow and stream velocity were found to be correlated with EPT taxa richness across several ecoregions (Table 4). These correlations were among the highest when correlations with regional variables were also considered. In chapter 4, the results from the multilevel models indicated the statistically significant influence of stream flow as a fixed effect across the study region. In chapter 5, the relationship between EPT taxa richness and monthly stream flow was significant and used to study potential impacts of switchgrass-related land-management changes on EPT taxa richness through changes in stream flow.

The research reported in chapter 4 found that the effect of changes in N loads in streams following conversion from pasture to switchgrass were not statistically significant on EPT taxa richness in the Ridge and Valley ecoregion, whereas changes in P loads were statistically significant as a random effect in the Ridge and Valley ecoregion. These results are in concordance with those of the modeling approach in chapter 5. The EPT taxa richness models for Nolichucky watershed (Chapter 5), were based on subwatersheds draining the Ridge and Valley ecoregion. Model results indicate that monthly P models were significant predictors of EPT taxa richness in the Nolichucky watershed. However, monthly N models were not significant in predicting EPT taxa richness.

When studying ecosystem processes, the context is set by processes with long turnover times, while the mechanisms are derived from processes with a shorter turnover time (Carpenter and Turner 2000). In the context of this study, broad-scale environmental factors set the regional context and affect the degree to which local stream-based variables influence aquatic habitat.

It is important to note that the results of this study reflect observations and data aggregated and analyzed at the particular spatial and temporal scale. The environmental constraints and stream-based processes focused in this study are based on associations of EPT taxa richness measurements at a point in time with long-term metrics such as average stream flow, precipitation, temperature and static metrics such as soil texture and geology. For example, the response of EPT taxa richness to variations in long-term average stream flow describes the processes operating at that temporal scale, such as the processes influencing stream flow relationships with the physical habitat. Long-term average stream flow affects physical stream characteristics such as channel dimension, distribution of riffles and pools, and substrate composition, which are largely determined by the interaction between the flow regime and geomorphology (Frissell et al. 1986; Poff and Zimmerman 2010). Such physical stream characteristics set the context for macroinvertebrate habitat and diversity (Bunn and Arthington 2002). However, these relationships of EPT taxa richness with long-term stream flow do not explain associations at a finer temporal scale such as seasonal variations, flooding, or droughts. More data at a finer temporal scale (hourly or daily flow estimates before time of EPT taxa richness data collection) are needed to explore such associations.

The large, regional spatial scale considered in this research is useful for assessing potential indicators in the midst of regional constraints. However, the results may not be transferable to a different spatial scale. The mechanisms by which ecological processes affects species diversity can change considerably over time and space in hierarchical landscapes, and in some cases even reverse depending on the scale at which the relationships are observed (Cadotte and Fukami 2005; MacMahon and Diez 2007). Further, interactions across hierarchical landscapes operate at different spatial and temporal scales and can sometimes produce nonlinear patterns and dynamics (Soranno et al. 2014). Aquatic macroinvertebrates are known to be affected by processes at different spatial scales, and discerning effects by scale is critical for stream management efforts (Li et al. 2001). The analysis in this study provided some insight into the relative influences of different environmental factors and stream-based factors on EPT taxa richness. This study also emphasized the need to focus on within-ecoregion spatial scales to be able to discern water quality and aquatic macroinvertebrate associations.

Lessons learned and future research directions

The lessons learned from this study can help improve and address future research involving the environmental contexts of aquatic macroinvertebrates. Among existing ecoregion classification schemes, Omernik’s ecoregion classification was found to be

the better ecoregion classification to characterize the macroinvertebrate habitat in the context of broad-scale regional variations. However, a different ecoregion classification, derived from clustering EPT taxa richness and related environmental variables, might partition the regional characteristics more effectively (Hargrove and Hoffman 2004). Even so, such statistically generated ecoregions may not be appropriate from a management or conservation standpoint since it will be difficult to identify and understand the spatial extent of clusters.

The multilevel models described by chapter 4 of this research provide a method to account for regional effects at a large scale and then observe the effects at a smaller scale. This method can help quantify the relative influence of flow and physical habitat changes on aquatic species (Dunabar et al. 2010). Though multilevel models have been used in some aquatic studies (e.g., Wagner et al. 2006, Qian et al. 2011, Cheruveli et al. 2013), the potential for further applications is large since the issue of scale is a constant factor influencing aquatic species and aquatic phenomenon. The same multilevel modeling framework can be applied for other aquatic species such as fish and mussels to evaluate whether they may be a better indicator of changes in water quality in Tennessee.

Efforts to design optimal landscapes of bioenergy cropping systems have been carried out using economic and environmental targets, including farmer profit, production goals, and water quality (Parish et al. 2012). The results from the SWAT model and EPT taxa richness regression models (chapter 5) indicated an increase in magnitude of effects going downstream in a watershed, as a result of more land being converted to switchgrass. This result has implications for designing bioenergy landscapes, since changes were largest near the outlet of the watershed. Further evaluation is needed to understand the effect of this phenomenon when there are other crops in the landscape. In the case of improvements in environmental quality, such as seen with aquatic macroinvertebrates, this makes a strong case for designing landscapes where a larger part a watershed can be converted to maximize downstream benefits. In cases of worsening water quality or other negative environmental impacts, it would be better to design landscapes such that only parts of a larger watershed are converted to avoid cascading effects and amplifications of negative impacts. The results of this study also help alleviate some of the environmental concerns related to bioenergy futures. Changes from pasture land to switchgrass management may not have a marked impact on aquatic biodiversity, as found by this study. Based on BT16 estimates, such land-management conversions to energy crops from pasture (managed for hay) are expected to be about 2.5 million acres or about 4% of the total energy crops by 2040 (U. S. Department of Energy 2016).

This study found that it is unlikely that EPT taxa richness will be affected by switchgrass-based land management under the conditions currently considered, which are defined by the regional context, the study area considered (watershed-scale), and baseline assumptions. This result is also constrained by the assumptions of this study, which included assumptions about the temporal scale of the data and analysis. The study was conducted over the years 2003 to 2011, and the aquatic macroinvertebrate

changes predicted for these 9 years were largely within the standard deviations of monthly estimates. Nieme et al. (1990) found that most aquatic systems recovered within 3 years following heavy chemical stresses and non-chemical stresses including logging, flooding, dredging and drought. Aquatic macroinvertebrates are resilient and can recover within weeks after short-term flood or drought events (Angradi 1997; Fritz and Dodds 2004). The hyporheic zone in the stream sediment bed provides refuge for aquatic macroinvertebrates during such hydrologic changes (William and Hynes 1974). The only conditions under which recovery has not been seen was when the physical habitat was altered, pollutants remained in the system or the stream was isolated (Nieme et al. 1990). Focused stream restoration on a severely impacted stream in Finland showed recovery in 4-8 years (Muotka et al. 2002). The stressors considered in this current study are not such extreme cases that would cause such extensive changes. On the other hand, observing prolonged land-use changes over a longer time period may have produced different results. For example, Durance and Ormerod (2007) found that over a 25-year period, climate change had to potential to impact macroinvertebrate composition and abundance through gradual changes to the thermal profile of the streams.

In addition to nutrients and stream flow, aquatic macroinvertebrates are also impacted by chemical toxicity, changes in pH and changes in dissolved oxygen in the stream (e.g., Merritt and Cummins 1996; Connolly et al. 2004). These factors have not been directly addressed by the current study and may have been present as confounding factors not accounted for. Only a complete analysis of all stressors can help decisively address reasons for changes in aquatic macroinvertebrate structure.

Thy hypothesis of this research was that, EPT taxa richness as an aquatic macroinvertebrate index has potential to be a bioindicator of water quality in Tennessee since it is sensitive to environmental changes, widely used and easy to measure (e.g., Lenat 1993). However, this metric does not capture potential changes to individual organisms or species. It is usually very difficult to identify aquatic macro-organisms at the species level. Recent advances in DNA bar coding techniques show promising ways to better identify macroinvertebrate species (Ball et al. 2005; Hajibabaei et al. 2011). These techniques have biomonitoring implications since the ability to distinguish larvae at the species level through barcoding makes biodiversity assessments for aquatic communities comparable to those used for terrestrial ecosystems where estimates of biodiversity for plants and animals are never quantified at the level of genus or family (Sweeney et al. 2011). Next-Generation Sequencing (NGS) technologies that are a non-destructive and inexpensive source of DNA for biodiversity analysis of benthic macroinvertebrates are rapidly changing the landscape of biodiversity analysis by targeting various habitats and a wide array of organisms (Hajibabaei et al.2012). The DNA barcodes of stream macroinvertebrates is expected to improve descriptions of community structure and water quality for both ecological and bioassessment purposes (Sweeney et al. 2011). With advances in better technology for DNA barcoding, it will be useful to revisit the current research question to assess whether better estimates of EPT taxa richness or specific species obtained using NGS technologies can provide

insight into the potential effects of land-management changes on aquatic macroinvertebrates.

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