EFFECTS OF RIPARIAN AND BASIN-WIDE FOREST CHANGE ON STREAM ALGAL COMMUNITIES AND HIGHER TROPHIC LEVELS IN THE SOUTHERN APPALACHIANS

by

KELSEY JILL SOLOMON

(Under the Direction of Catherine M. Pringle)

ABSTRACT

In stream ecosystems, primary producers (*i.e.,* algae) are an important food resource to consumers due to their high dietary quality relative to detritus. In headwater (or lower stream order) streams draining the southern Appalachian Mountains, forest ecosystems exert control over algal communities by regulating light availability to stream reaches, stream-water nutrient concentrations, flows, and temperatures. However, southern Appalachian forests are changing due to the massive die-off of a foundation tree species, expansion of a native invasive riparian shrub, and low-density development on once-forested mountainsides. In this dissertation, we used historical data sets, reach-scale riparian manipulations, trophic dynamic experiments, and stable isotope analyses to assess how riparian and basin-wide changes to southern Appalachian forests have affected algal communities and higher trophic levels. First, we compared algal biomass and community composition before and after the die-off of a foundation riparian tree, *Tsuga canadensis* (eastern hemlock). Thirteen years following hemlock death, we found lower algal standing crops and little change in community composition; findings suggest that this is likely due to the expansion of the native riparian shrub, *Rhododendron maximum* (rhododendron). Next, using plot-scale crayfish exclusion experiments nested within reach-scale rhododendron removal manipulations, we examined how rhododendron removal as a forest management strategy might affect algal communities while accounting for stream trophic dynamics. Short-term (2 year) response to rhododendron removal included higher total algal cell biovolume and a shift in algal community composition. Findings indicate that rhododendron removal resulted in an increase in the relative abundance of filamentous green algal taxa, with food webs potentially becoming more autochthonous. Finally, we compared algal and fish communities between forested and suburbanizing watersheds at three points in time over a ten-year period (2000, 2005, 2010). For both algae and fishes, we found evidence of biotic homogenization and taxonomic change within suburbanizing reaches, but not to the level where endemic taxa were extirpated. Combined studies show how slight changes to near-stream riparian areas or their forested watersheds can significantly change stream algal standing stocks and community composition, with changes to primary producers cascading up to higher trophic levels.

INDEX WORDS: algae, diatoms, southern Appalachians, community composition, crayfish, fish, eastern hemlock, *Rhododendron maximum,* rhododendron, headwater stream, urbanization

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DEDICATION

This dissertation is dedicated to the loving memory of
Katie Solomon Argabrite, for being my best friend and biggest supporter,
and of Damir Young-Hunt, Tiy'Rek Foxx, and Obed Garcia,
for teaching me what it means to be an educator.

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CHAPTER 1

INTRODUCTION

Understanding the abundance, distribution, and community composition of primary producers is important because changes within primary producer communities can affect one or more higher trophic levels (Fox 2004, Haddad et al. 2009, Hertzog et al. 2016). Thus, shifts in primary producer communities can also have important implications for ecosystem function (Bruno et al. 2006, McQueen et al. 2007).

Unicellular primary producers in stream ecosystems (*i.e.,* algae) are important to consumer secondary production because of their high dietary quality relative to detritus (Minshall 1978, Whitledge and Rabeni 1997, March and Pringle 2003, Crenier et al. 2017, Erdozain et al. 2019). However, algal taxa can differ in their dietary quality and palatability. Diatoms are a particularly important food source for aquatic consumers due to elevated highly unsaturated fatty acid (HUFA) content compared to other types of stream algae (*e.g.,* cyanobacteria, chlorophytes; Brett and Müller-Navarra 1997, Guo et al. 2016). In addition, because consumer feeding morphologies differ in their ability to harvest various algal sizes and growth forms, algal taxa also vary in their palatability to consumers (Steinman et al. 1992, Tall et al. 2006). Thus, changes in stream algal community composition can have consequences to stream ecosystem function through changes in primary production (Power and Cardinale 2009) and/or changes in secondary consumer structure (Ladrera et al. 2015).

Benthic algal communities in stream ecosystems are controlled by a variety of both bottom-up top-down factors. These factors often interact with one another, eliciting complex, habitat-specific responses that can be challenging to predict (Rosemond 1993, Lange et al. 2011, Piggott et al. 2015). Generally, increases in light availability favor algal taxa that have a competitive advantage in high light environments, which are typically green algal taxa (Richardson et al. 1983) and diatom taxa with "high" growth forms (Lowe et al. 1986, Steinman et al. 1992, Bixby et al. 2009). On the other hand, increases in stream flow favor taxa with low-lying, tightly adhering growth forms (Passy 2007), whereas increases in fine sediment (Yamada and Nakamura 2002, Dickman et al. 2005) or nutrient concentrations (Passy 2007, Tapolczai et al. 2016) favor motile taxa. Stream macroconsumers (*e.g.,* snails, crayfish, fish) can also exert control on algal community composition directly through consumption (Hart 1992, Creed 1994, Ranvestel et al. 2004, Schofield et al. 2008) and/or indirectly through bioturbation (Pringle et al. 1993, Parkyn et al. 1997) and food-web interactions (Creed 1994).

Benthic algal communities in forested headwater streams are connected with both their adjacent riparian forests and their forested watersheds. Algal biomass in these systems is limited by light availability and thus algal communities are often dominated by shade-adapted taxa, like *Eunotia* spp. (Lowe et al. 1986, Gardiner et al. 2009). In turn, light availability in these systems is mainly determined by riparian canopy cover (Hill et al. 2001). In addition, streams tend to integrate the properties of their watersheds. Disturbance within forested watersheds can alter stream water nutrient concentrations, flow, temperatures, and/or sediment delivery patterns (Likens et al. 1970, Burton and Likens 1973, Hornbeck et al. 1997, Swank et al. 2001), and these changes are often reflected in the algal community. Thus, both near-stream and basin-wide changes to forests could have significant impacts on algal communities via bottom-up pathways.

Over the past century, the southern Appalachians Mountains have experienced shifts in riparian vegetation due to the loss of two foundation tree species, chestnut and hemlock. The

American chestnut (*Castanea dentata*) was functionally lost to blight in the early 1900s (Anagnostakis 1987). More recently, the eastern hemlock (*Tsuga canadensis*) was lost due to an infestation by the invasive hemlock woolly adelgid (*Adelges tsugae*) in the early 2000s (Ellison et al. 2005). Since loss of hemlock, the growth of a native riparian shrub, *Rhododendron maximum,* has increased (Ford et al. 2012). Such changes in forest community structure have the potential to alter ecosystem processes in both terrestrial and stream ecosystems.

While impacts of hemlock loss have been well-documented for southern Appalachian terrestrial ecosystems (*e.g.,* Nuckolls et al. 2009, Ford et al. 2012, Brantley et al. 2013), less is known about its impacts on stream ecosystem processes (but see Webster et al. 2012b, Northington et al. 2013). Shifts in southern Appalachian riparian vegetation could change light availability in streams, thereby leading to changes in algal biomass and/or community structure. Both hemlock and rhododendron are pervasive in riparian zones of the southern Appalachians and provide year-round shade to streams (Webster et al. 2012b). Needle drop caused by hemlock death has increased both riparian canopy openness and light availability to streams (Siderhurst et al. 2010, Webster et al. 2012b), which could potentially lead to an increase in algal biomass and primary production, and a decrease in the relative abundance of shade-tolerant taxa (Lowe et al. 1986, Gardiner et al. 2009). Conversely, expansion of rhododendron could counteract some of the effects of hemlock death on algal communities by decreasing both riparian canopy openness and light availability (Ford et al. 2012, Dharmadi et al. 2019).

Moreover, some forest management strategies involve removal or reduction of rhododendron in riparian zones, which could lead to further shifts in riparian vegetation composition and changes in algal communities. Because the recruitment of harvestable overstory species (*e.g., Acer*, *Betula*, *Fagus*, and *Quercus*) is limited in areas where rhododendron dominates riparian communities (Baker and Van Lear 1998), the U.S. Forest Service has explored removing rhododendron from select stream reaches to promote the growth and regeneration of hardwoods (Elliott and Miniat 2018). Given that rhododendron is also considered to be an emerging foundation species in southern Appalachian riparian zones (Dudley et al. 2020), its removal has been shown to have cascading effects on stream ecosystem processes (Dudley et al. 2021). For example, rhododendron removal experiments have indicated that crayfish grazing mediates the response of algal biomass to rhododendron removal (Dudley et al. 2021); however, we do not know how algal community composition may be affected by rhododendron removal.

Low-density development on once-forested mountainsides also has the potential to affect algal community composition through changes in water quality. Exurbanization, a term coined by Spectorsky (1955), represents a specific kind of low-density development whereby urban migrants relocate to rural communities (Evans and Jensen-Ryan 2017). The southern Appalachian region is undergoing rapid exurban development. Exurbanization in this region has been associated with increases in stream suspended solids (Price and Leigh 2006), suspended sediment loads (Jackson et al. 2017), specific conductance (Price and Leigh 2006, Jackson et al. 2017), nitrate concentrations (Webster et al. 2012a), and summer temperature daily maxima (Jackson et al. 2017). Although deforestation and rural development within watersheds has been associated with lower occupancy and/or abundance of highland endemic fish species and higher abundance of cosmopolitan fish species (Kirsch and Peterson 2014), we know very little about how algal diatom community composition might be affected.

The subsequent chapters in this dissertation explore the effects of riparian and basin-wide forest change on stream ecosystems in the southern Appalachian Mountains, with an emphasis on algal community composition. Chapter 2 focuses on a comparison of algal communities across the Coweeta experimental basin before and after the loss of the eastern hemlock. Chapter 3 is based on a plot-scale trophic dynamics experiment nested within a reach-scale rhododendron removal manipulation to compare interactions between top-down and bottom-up drivers of algal communities before and after riparian rhododendron removal. Finally, Chapter 4 is a comparison of algal and fish communities between forested watersheds and watersheds experiencing exurbanization at three points in time over a ten-year period.

Chapter 2: How have stream algal communities changed a decade after the loss of a foundation riparian tree species in a headwater Appalachian watershed?

The eastern hemlock (*Tsuga canadensis*) was once a foundation riparian tree species in the southern Appalachian Mountains. Eastern hemlock likely played an important role structuring algal communities by providing year-round shade to stream reaches. However, introduction of the hemlock woolly adelgid (*Adelges tsugae*) to the United States from East Asia has resulted in mass mortality of eastern hemlock. In this chapter, we build upon a historical algal data set collected pre-hemlock die-off (2005-2006) to assess potential changes in stream canopy cover, algal standing crop, and algal community composition thirteen years following hemlock loss (2018-2019) over two seasons (summer and winter) in the Coweeta experimental basin.

Chapter 3: Riparian rhododendron removal significantly alters algal community composition and trophic dynamics in a southern Appalachian headwater stream

Rhododendron maximum, an evergreen shrub, is abundant in riparian zones of the southern Appalachian Mountains. Although rhododendron is native to this region, the U.S. Forest Service has explored removing rhododendron along stream banks as a management strategy to promote the growth and regeneration of hardwoods. Trophic dynamics studies that measure how both top-down and bottom-up controls change over time in response to riparian management offer insights into the mechanisms by which algal communities respond to riparian disturbance. Thus, by using plot-scale crayfish exclusion experiments nested within rhododendron removal manipulations over three years, we examined how riparian rhododendron removal might affect total algal cell biovolume, chlorophyll-*a* to cell biovolume ratios, and algal community composition in the short-term, while accounting for stream trophic dynamics. In addition, by using stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$, we also assessed how rhododendron removal management strategies affect crayfish dependence on autochthonous resources.

Chapter 4: Evidence for early stages of biotic homogenization in algal and fish communities in streams draining suburbanizing landscapes in the southern Appalachian Mountains

In the southern Appalachians, exurbanization resulting from low-density development in rural landscapes threatens stream water quality and biotic communities. Many studies that have characterized the biotic effects of urbanization have either focused on regions with high-density development or have used *space-for-time substitution,* in lieu of characterizing biota through time. Thus, there is a need to track biotic communities through time, as previously forested watersheds gradually become more urbanized. In this chapter, we assessed the potential effects of low-residency development on algal and fish communities at three points in time (2000, 2005, 2010) over a ten-year period by comparing alpha diversity, beta diversity, and community composition between reference forested and suburbanizing watersheds.

CHAPTER 2

HAVE STREAM ALGAL COMMUNITIES CHANGED A DECADE AFTER THE LOSS OF A FOUNDATION RIPARIAN TREE SPECIES IN A HEADWATER APPALACHIAN WATERSHED?

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Abstract

Given the increase of tree species loss due to non-native pests, and the interconnectedness of terrestrial and stream ecosystems, there is a need for a greater understanding of the effects of riparian tree species loss on the structural characteristics of stream ecosystems. Our objectives were to assess potential changes in stream canopy cover, algal standing crop, and algal community composition in eight southern Appalachian headwater stream reaches during both summer (September) and winter (January) thirteen years following a massive die-off of eastern hemlock (*Tsuga canadensis*). Based on the expectation of increased canopy openness and light availability following hemlock death, we predicted higher algal standing crop and changes in algal community composition. However, post-hemlock die-off in 2018/2019, we found little change in canopy cover and significantly lower algal standing crops, likely due to increased basal area of the understory shrub, *Rhododendron maximum*. Algal communities significantly differed post-hemlock die-off at summer-sampling (but not winter), due to a decrease in the relative biovolume of *Eunotia metamondon* and an increase in the relative abundance of *Eunotia rhomboidea* in 2018/2019. However, algal communities were dominated by acidophilic diatom taxa (*e.g.*, *Eunotia* spp.) across all pre- and post-die off dates. Our study highlights the relative stability of many algal taxa in rhododendron-dominated southern Appalachian streams, and the importance of long-term studies following the invasion of a non-native pest.

Introduction

Climate change (Fei et al. 2017), non-native pests (Morin and Liebhold 2015), and land-use change (Newbold et al. 2015) have impacted forest community structure across the United States. Changes in forest community structure can have important consequences for terrestrial ecosystem functions, such as carbon dynamics (Dixon et al. 1994, Fei et al. 2019) and nutrient cycling (Ellison et al. 2005). Since streams occupy the lowest elevation on the landscape, they integrate the effects of changes in their watersheds (Likens et al. 1970). Thus, changes in forest community structure can affect stream structure (Kaylor and Warren 2017) and function (Warren et al. 2016). Specifically, changes in riparian forest communities can have important implications for headwater stream primary producers, particularly benthic algae, in terms of primary production, biomass, and community composition (Hansmann and Phinney 1973, Lowe et al. 1986).

Benthic algae are an important basal resource for stream consumers, and changes in algal communities can potentially alter stream ecosystem function. In forested headwater streams, benthic algae are disproportionally important to consumer secondary production, given their high nutritive quality yet small standing biomass relative to detritus pools (Minshall 1978, Whitledge and Rabeni 1997, Crenier et al. 2017, Erdozain et al. 2019). Diatoms are a particularly important food source for aquatic consumers due to elevated highly unsaturated fatty acid (HUFA) content compared to other types of stream algae (*e.g.,* cyanobacteria, chlorophytes; (Brett and Müller-Navarra 1997) and detritus. Thus, changes in algal standing stocks and/or community composition in forested headwater streams can have important consequences to consumer biomass and production (Bilby and Bisson 1992, Wootton 2012, Bumpers et al. 2017, Kaylor and Warren 2017).

In forested headwater streams, riparian vegetation heavily influences autotrophic production by regulating light availability. Light is a key factor limiting algal biomass and production in these systems (Fisher and Likens 1973, Steinman 1992, Hill et al. 1995) and as a result, algal communities are often dominated by shade-adapted taxa (Gardiner et al. 2009).

Consequently, increases in light availability due to removal of riparian vegetation can increase algal standing stocks (Hansmann and Phinney 1973, Lowe et al. 1986, Hill and Knight 1988), primary production (Murphy and Hall 1981, Hill et al. 1995) and shift algal community structure towards taxa adapted to higher light availability (Hansmann and Phinney 1973, Lowe et al. 1986, Naymik and Pan 2005, Bixby et al. 2009). Even a subtle increase in canopy openness (*e.g.,* 0 – 25 %) can increase algal biomass and shift community composition (Majdi et al. 2015, Kaylor and Warren 2017). However, some studies have found that subtle changes in canopy cover do not necessarily correspond with changes in algal biomass or assemblages, due to the co-limitation of algal communities by multiple factors (Denicola et al. 1992, Dudley et al. 2021). Although the effects of forest clear-cutting on stream algal communities are well studied (Likens et al. 1970, Hansmann and Phinney 1973, Lowe et al. 1986), we have little understanding of how the loss of a single riparian species might affect algal communities via changes in canopy cover (but see Dudley 2018).

Introduction of the hemlock woolly adelgid (*Adelges tsugae*) to the U.S. from East Asia has resulted in mass mortality of the eastern hemlock (*Tsuga canadensis;* hereafter “hemlock”) along the eastern U.S.. In the southern Appalachians, hemlock die-off has resulted in increased light availability to streams and increased annual water temperature range (Webster et al. 2012b). Furthermore, in the southern Appalachians, eastern hemlock occupied a unique functional role as the only overstory evergreen species in riparian zones, and thus, the only overstory species shading streams in the winter months. However, we do not understand how hemlock loss has potentially affected algal standing stocks and community structure.

Here, we built upon a historical algal data set from pre-hemlock die-off (2005-2006; Bixby and Pringle, *unpublished*) to assess potential shifts in stream algal communities post-hemlock loss (2018-2019). Specific objectives of this study were to assess potential changes in stream canopy cover, algal standing crop, and algal community composition in eight headwater stream reaches between pre- and post-hemlock die-off sampling dates over two seasons (summer and winter). We also assessed potential changes in light intensity to stream reaches from 2005-2015 for these two seasons. Thirteen years following hemlock die-off, we predicted (1) significantly greater algal standing crop and (2) greater relative biovolume of "high" diatoms (*e.g.,* large *Eunotia* spp., *Gomphonema* spp., *Ulnaria* spp.) and lower relative biovolume of "low" diatoms (*e.g.,* small *Eunotia* spp., *Nupela lapidosa*). These predictions were based on our expectation of lower riparian canopy cover and greater light intensities post-hemlock die-off. Because shading by evergreen hemlock may have been particularly important in the winter, we also expected that differences in algal communities between sampling periods would be seasonally greater in winter versus summer.

Methods

Study area:

This study was conducted at the Coweeta Hydrologic Laboratory (2185 ha), a U.S. Forest Service and Long Term Ecological Research facility located in the Nantahala Mountain Range of western North Carolina, U.S.A., within the Blue Ridge Physiographic Province. Mean annual precipitation is approximately 200 cm and mean annual temperature is 12.6°C (Webster et al. 2016). Underlying bedrock consists of gneiss, schist, and metasandstone units (Hatcher 1979, 1988). Prior to invasion of the hemlock woolly adelgid (*Adelges tsugae*), dominant riparian vegetation included eastern hemlock (*Tsuga canadensis*), tulip poplar (*Liriodendron tulipifera*), red oak (*Quercus rubra*), cherry birch (*Betula lenta*), red maple (*Acer rubrum*), white oak

(*Quercus alba*), and dense understory of the shrub rhododendron (*Rhododendron maximum* L.; Webster et al. 2012). By 2005, all eastern hemlock trees in the Coweeta basin had been infected with hemlock woolly adelgid. By 2010, 50% of hemlock trees were dead and by 2014, nearly all (97%) of hemlock trees had been killed by hemlock woolly adelgid (Dharmadi et al. 2019).

Site selection and sampling:

This study was conducted at eight 100 m stream reaches (Cunningham Creek, Hugh White Creek [upper site], Hugh White Creek [lower site], Mill Branch, Reynolds Branch, Shope Fork, Upper Ball Creek, and Wykle Branch) across the Coweeta experimental basin (Table 2.1). Reach selection was based on Webster et al. (2012), which examined the effects of hemlock loss on riparian vegetation, stream temperature, light, litterfall, large wood inputs, and benthic organic matter. These reaches were selected because they were in locations that had not been logged since the area became a National Forest in late 1920s, and because streams passed through (or were adjacent to) permanent vegetation plots in which trees had been measured over time (Elliott and Swank 2008, Webster et al. 2012b). Seven of these stream reaches were located on 1st or 2nd order streams and one stream reach (Shope Fork) was located on a 4th order stream (Table 2.1). Prior to hemlock die-off, basal area of hemlocks in the riparian zones of these reaches ranged from 9.3-21.8 m^2 h^{-1} (Table 2.1). While an attempt was made to locate stream reaches with no riparian hemlock to serve as control, hemlock was ubiquitous across the Coweeta basin, meaning no stream reaches could serve as controls (Webster et al. 2012b).

Canopy cover and algal sampling events occurred twice pre-hemlock die-off (before significant crown loss; September 2005, January 2006) and four times post-hemlock die-off (after complete crown loss but with most dead hemlocks still standing; January 2018, September

2018, January 2019, September 2019) at each of the eight study reaches. We chose summer (September) and winter (January) sampling dates to account for light availability and water temperature differences between seasons.

Canopy cover and light availability:

As a proxy for light availability, percent canopy cover was estimated along each 100 m stream reach using a spherical densiometer (Forest Suppliers, Jackson, MS). Densiometer measurements were taken at one transect along each 100 m stream reach during 2005-2006 sampling events, and five transects along each stream reach during 2018-2019 sampling events.

From June 2005-September 2015, relative light intensity (lumens m^{-2}) was measured at five of the eight stream reaches (Hugh White Creek [upper], Hugh White Creek [lower], Mill Branch, Reynolds Branch, Cunningham Creek) with HOBO Pendant/light data loggers (Onset Computer Corporation, Bourne, MA). At each site, five light meters were fastened to 1 m high posts spaced at intervals of 10 m immediately adjacent to streams and light intensity was recorded every 5 min (Webster et al. 2012). Full methods for collecting light intensity are described in Webster et al. (2012). Here, we report values for January light intensities and September light intensities only, because they are the two focal months of this study.

To correct light intensity values for the variability in total solar radiation, we created a "light index" by using contemporaneous total daily solar radiation (unit: langleys) from Coweeta Weather Station 1 (Miniat et al. 2015), which is not influenced by canopy cover.

$$\text{Light index} = \frac{Daily\ average\ light\ intensity}{Daily\ total\ solar\ radiation*5}$$

While units for light intensity (lumens m^{-2}) and solar radiation (langleys) are different, the light index permits comparison of solar radiation-corrected sub-canopy light intensity from year to year. Thus, we report average monthly light index values for January and September from 2005-2015 alongside the monthly light intensity values. The multiplication of the total solar radiation by a factor of five within the light index calculation is for convenience. We dropped measurements for both light intensities and light index values from Lower Hugh White in September 2011 and January 2012 due to HOBO data logger malfunctions.

Algal standing crop:

Algal standing crop (*e.g.,* chlorophyll-*a* and ash-free dry mass [AFDM]) was assessed at the reach scale (100 m) during base flow (*i.e.,* no fewer than ten days after a high discharge event). To capture algal biomass heterogeneity at each site, three (2005-2006) and five (2018-2019) replicate samples were taken at evenly spaced intervals across the 100 m reach. For each replicate, we selected one rock from a riffle habitat with cobbles of uniform shape. We covered the rock with aluminum foil before placing the rock in a sample bag with stream water. We transported the rock samples back to the lab in the dark on ice. In the lab, periphyton was removed by brushing the rocks with a toothbrush and rinsing with deionized water, and the resulting slurry was sub-sampled and filtered through two pre-ashed, pre-weighed glass fiber filters (Sterlitech Corporation, Grade F, 47 mm) within 48 hours of sampling and then were frozen. Twenty mL subsamples from the slurry were preserved using 7% formalin solution for diatom community analysis.

One filter was used to quantify periphyton organic matter and one filter was used to determine chlorophyll-*a.* To determine organic matter, we dried the filter for one week at 60°C, weighed, ashed at 500°C for 4 hours, and re-weighed for ash-free dry mass (AFDM). We measured chlorophyll-*a* using acetone extraction methods (Method 446.0, U.S. EPA) and a UV-

Vis Spectrophotometer UV-1700 series (Shimadzu Corporation, Kyoto, Japan) to measure absorbance. We used the "aluminum foil method" (Steinman et al. 1996) to determine the rock surface area sampled. Periphyton AFDM and chlorophyll-*a* values were converted to a density by dividing the measured value by the total surface area of the sampled rock.

Algal community composition:

We focused on diatom community composition because diatoms comprise the majority of the algal communities in southern Appalachian headwater streams (Lowe et al. 1986, Greenwood and Rosemond 2005, Gardiner et al. 2009). We quantified diatom community composition using three replicate samples from each stream for two pre-hemlock die-off dates (September 2005 and January 2006) and two post-hemlock die-off dates (September 2018 and January 2019). To determine diatom densities and relative abundances, 5-10 mL aliquots were heated with 30% hydrogen peroxide for one hour and then rinsed six times with distilled water to remove oxygen by-products. We created permanent slides by evaporating processed samples onto round coverslips (Fisherbrand, Pittsburgh, PA) using a modified Battarbee chamber (Battarbee 1974). Briefly, we placed round coverslips at the bottom of 100 mL beakers (PYREX, Glendale, AZ) and evaporated processed samples within the beakers. We then mounted the coverslips to microscope slides with Naphrax mounting medium (Brunel Microscopes, Wiltshire, U.K.). We identified 500 valves per sample using a Zeiss Universal microscope (2005-2006 samples) and Leica DMRXE microscope (2018-2019 samples) under oil immersion at 1000x using brightfield optics. In samples with extremely low densities, a maximum of 180 mm of transects were counted. We used standard taxonomic references (Patrick and Reimer 1966, 1975; diatoms.org) and algal floras from the southeastern U.S. (Camburn et al. 1979, Kociolek and Kingston 1999, Thomas et al. 2009, Furey et al. 2011, Bishop et al. 2017) for species-level identification. In order to ensure taxonomic harmonization of identifications between the 2005-2006 samples and the 2018-2019 samples, we defined "species complexes" for related species that were difficult to differentiate with light microscopy through extensive consultation between the researchers who identified the pre-die off sample sets (R. Bixby) and the more recent post-die off sample sets (K. Solomon). Species complexes included *Encyonema minutum*, *Eunotia metamondon*, *Eunotia minor*, *Eunotia rhomboidea, Frustulia rhomboides*, and *Synedra rumpens* (Appendix, Table A.1), and will hereafter be referred to by the species name.

To determine cell density (cells mm^{-2}) for each taxon, we divided the number of cells of each taxon by the fractional volume of the sample (*i.e.,* volume measured / total volume of slurry), and converted to a per-area measure by dividing the measured value by the proportion of the total rock area sampled. To determine individual cell biovolumes, we measured approximately ten cells of each species and averaged the biovolumes of the ten individuals to determine a cell biovolume for each taxon (Hillebrand et al. 1999). In some cases where we were not able to measure 10 individual cells, we obtained biovolume values from the literature (diatom.ansp.org, Lowe and Pan 1996, Dye 2005). To determine cell biovolume (mm^3 m^{-2}) of each taxon, we multiplied the individual cell biovolume of each taxon by its density in the sample. To determine total cell density and total cell biovolume of each sample, we summed individual cell densities and biovolume across samples, and we considered total cell density and total cell biovolumes of each sample to be alternate measures of algal stand crop.

We assigned individual species to one of three functional groups -- low, high, motile -- based on the definitions (Appendix, Table A.2) of Rimet and Bouchez (2012), except in cases where our own or published observations of growth forms of individual species differed from that of Rimet and Bouchez (2012). Deviations from Rimet and Bouchez (2012) definitions were as follows: we classified *Eunotia* spp. with a cell biovolume less than 500 µm and a valve length to width ratio of less than eight as low instead of high, because we only observed these taxa singularly or in chains of two cells in our samples. We classified *Meridion* spp. as high because we observed these taxa erect on substrates and noted similar observations of these taxa by Lowe et al. (1986). We classified *Frustulia* spp. as motile instead of high because we only observed these taxa as single cells and not mucilaginous tubes. Finally, we classified *Brachysira* spp. as motile instead of low because of their "moderate motility" (Hamilton 2010) and classified *Chamaepinnularia* spp. as low instead of motile because of their "weak motility" (Tyree 2018).

Data analysis:

Previous research at Coweeta has suggested that while sub-canopy light transmission increased immediately following hemlock death (2006-2009), it decreased in recent years (2015-2017; Dharmadi et al. 2019). Thus, to test for trends in light intensities and light index values, we constructed both linear and quadratic mixed effects model with year as a fixed effect and stream identity as a random effect. We compared linear and quadratic models using Akaike's Information Criterion (AIC; Burnham and Anderson 2002). We considered models with ΔAIC > 2 to be significantly different (Bates et al. 2015). To assess model fit, we calculated the portion of variance explained by the fixed effects (marginal R^2) and the portion of variance explained by fixed and random effects (conditional R^2) using the *MuMIn* package in R (Barton 2020). Light intensities and light index values were log-transformed prior to analyses in order to reduce heteroscedasticity in residual variance.

To determine potential differences in mean canopy cover, AFDM, chlorophyll-*a*, total cell densities, and total cell biovolumes between seasons and pre- and post- hemlock death, we constructed linear mixed effects model with stream identity as a random effect and fixed effects of season (January or September), hemlock-death (pre [2005/2006] or post [2018/2019]), and the interaction between season and hemlock-death. We dropped the interaction term if it was not significant ($p > 0.05$). We ran the models with the *lme4* package in R (Bates et al. 2015).

We explored potential differences in diatom community composition pre/post hemlock death by season (January and September) using nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarity coefficients using the *metaMDS* function within the *vegan* package in R (Oksanen et al. 2015). The *metaMDS* function was performed with a Wisconsin double standardization and square-root transformation (Oksanen et al. 2015). To test for potential differences in diatom communities following hemlock death, we performed a Permutational Analysis of Variance (PERMANOVA; Anderson 2001) for each season (January and September) using the *adonis* function within the *vegan* package in R (Oksanen et al. 2015). For the PERMANOVAs, we identified a fixed effect of hemlock-death (pre [2005/2006] or post [2018/2019]). For both the NMDS and the PERMANOVAs, we selected the 20 most abundant taxa which represented 93.7% of the total diatom biovolume across all years and seasons. Since we were interested in potential differences in diatom communities at the reach scale, we aggregated replicate samples by summing cell biovolumes (mm^3 m^{-2}) for each of these 20 taxa across each stream reach for each sampling date. All analyses were performed in R version 3.6.2 (R Core Team 2019).

Results

Canopy cover and light availability:

Canopy cover varied as a function of season (Fig. 2.1), with greater canopy cover in September than in January after leaf-fall. There was no significant change in canopy cover post-hemlock death ($F_{1,166} = 0.61$, $p = 0.43$), despite a general trend of lower mean January canopy cover in 2018/2019 compared to 2006 (Fig. 2.1).

Light intensities and light index values were better fit by a linear than a quadratic model in all months ($\Delta AIC > 2$), except for the September light index values, which were better fit by a quadratic model ($\Delta AIC = 45.13$; Appendix, Table A.3). January and September light intensities (Fig. 2.2) and January light index values (Fig. 2.3A) showed a modest increase from 2005 to 2015. September light index values showed a humped shape response from 2005 to 2015, with a peak in light index values in 2011 (Fig. 2.3B). In all models, time only explained a small portion of light intensity or light index values (Marginal $R^2 = 0.01\text{-}0.10$).

Algal standing crop:

Ash-free dry mass was higher post hemlock die-off compared to pre hemlock die-off ($F_{1,198} = 20.04$, $p < 0.001$); however, there was no interaction between hemlock death and season ($F_{1,197} = 0.81$, $p = 0.37$; Fig. 2.4A). Conversely, chlorophyll-*a* was lower post hemlock die-off ($F_{1,194} = 9.95$, $p = 0.002$), and there was no interaction between hemlock death and season ($F_{1,195} = 0.12$, $p = 0.73$; Fig. 2.4B). Overall, there was high variability in mean chlorophyll-*a* among reaches during pre- and post- hemlock die-off sampling dates (Fig. 2.5).

Total cell density (cells mm^{-2}) was lower post hemlock die-off ($F_{1,86} = 3.10$, $p = 0.08$), but there was not a significant interaction between hemlock die-off and season ($F_{1,85} = 0.44$, $p =$

0.51; Fig. 2.4C). Total cell biovolume (mm^3 m^{-2}) was also lower post hemlock die-off ($F_{1,86}$ = 5.08, p = 0.03), and there was not a significant interaction between hemlock death and season ($F_{1,85}$ = 0.10, p = 0.75; Fig. 2.4D). Variability in total cell biovolume among stream reaches was also high (Fig. 2.6).

Algal community composition:

We identified a total of 161 diatom species or species complexes (Appendix, Table A.1). Twenty taxa represented 93.7% of the total cell biovolume across all years and seasons (Table 2.2). Diatom communities were dominated by acidophilic taxa that prefer low nutrient and low conductivity waters (*e.g., Eunotia* spp., *Meridion alansmithii, Frustulia amphipleuroides, Frustulia rhomboides*, etc.). *Eunotia minor* was the dominant taxon, making up an average of 34-56% of the relative biovolume across all years and seasons. We identified at least four species endemic to the southern Appalachians Mountains: *Eunotia bilii, Eunotia rushforthii*, *Eunotia papilioforma*, and *Meridion alansmithii* (Appendix, Table A.1).

Diatom community composition significantly differed pre/post hemlock death in September (PERMANOVA, $F_{1,14}$ = 2.51, R^2 = 0.15, p =0.004, Fig. 2.7B), but not in January ($F_{1,14}$= 1.30, R^2 = 0.09 , p = 0.22, Fig. 2.7A). The total biovolume of each growth form was lower following hemlock death in January and September (Fig. 2.8A). The relative proportions of each growth form remained similar during January (Fig. 2.8B). In September, however, the relative proportion of "high" growth forms was lower following hemlock death, while the relative proportions of "low" and "motile" growth forms were higher following hemlock death (Fig 2.8B). Diatoms with "high" growth forms comprised the majority of diatom samples, regardless of season or hemlock death (Fig. 2.8B). The relative proportions of the 20 focal taxa remained similar post-hemlock death, with the exception of *Eunotia minor* in January and *Eunotia metamondon* in September, both of which decreased (Fig. 2.9). The relative proportion of *Eunotia rhomboidea* was greater in both months post-hemlock death (Fig. 2.9). The relative proportion of *Meridion circulare* var. *constrictum* was greater in January post-hemlock death (Fig. 2.9).

Discussion

Our snapshot approach of assessing stream algae at two points in time 13 years apart allowed us to quantify algal standing crop and community composition at a relatively large spatial scale (2185 ha^2) and to examine our findings in the context of prior information on algae in the southern Appalachians. Contrary to our first prediction, stream algal standing crop was slightly, yet significantly, lower relative to pre-hemlock die-off values, both during leaf-off (January) and leaf-on (September) conditions. We had expected significantly higher algal standing crop following loss of upper-story hemlock in response to higher light penetration to the stream channel. However, our data indicate non-significant difference in canopy cover between our sampling periods (2005/2006 and 2018/2019), with light intensity increasing only slightly from 2005 to 2015 (Fig. 2.2, Appendix, Table A.3). Contrary to our second prediction, we found little difference in the relative biovolume of diatom growth forms in January, but lower relative biovolume of diatom taxa with high growth forms in September. We attribute differences in diatom community composition in September to a decrease in the relative biovolume of *Eunotia metamondon* and an increase in the relative biovolume of *Eunotia rhomboidea*. Our findings indicate that stream algal communities in southern Appalachian headwater streams exhibit stability over time, despite the loss of a major riparian tree species.

The small difference in canopy cover between sampling periods (separated by 13 years) is likely due to changing riparian forest dynamics following hemlock mortality. Prior to hemlock die-off, presence of mature hemlock caused light limitation for understory vegetation, primarily rhododendron in this region. However, hemlock die-off removed this light limitation, and rhododendron responded by filling in canopy gaps created by hemlock loss. Thus, expansion of rhododendron likely offset increases in light availability resulting from loss of upper-story hemlocks (Ford et al. 2012). This conclusion is supported by Dharmadi et al. (2019), who reported that light transmission increased in the Coweeta basin immediately following hemlock mortality (2006-2009), but declined to levels equivalent to pre-hemlock death by 2015-2017 due to the increase in rhododendron. We likewise found that basal area of rhododendron increased between 2004 and 2010 at six out of our eight study reaches (Table 2.1). In addition, we found that solar-corrected light intensities for September (*i.e.,* light index values) were best fit by a quadratic model (Appendix, Table A.3). We attribute this "hump-shaped" relationship to changing forest dynamics: an increase in solar-corrected light availability due to hemlock-die-off from ~2005-2011, and then a subsequent decrease in solar-corrected light availability due to the expansion of rhododendron from ~2011-2015 (Fig. 2.3). So, though canopy cover decreased and light availability increased in the years immediately following the onset of hemlock die-off in our study reaches (2006-2011; Webster et al. 2012), by the time we conducted our follow up study in 2018/2019, canopy cover had returned to pre-die off levels, likely due to increased shade provided by rhododendron.

Consistent results among multiple biomass parameters provides strong evidence of lower algal standing crop following hemlock die-off in 2018/2019. Comparing historic to recent estimates of algal standing crops can be challenging due to naturally high variability in standing crops, inconsistent methodologies, and/or use on only one biomass parameter (Stevenson 1996). However, our extensive sampling across eight stream reaches allowed us to capture the natural variability of algal standing crops in this system, and our consistent sampling and processing methods across sampling dates ensured comparability. In addition, because chlorophyll-*a* to biovolume ratios can fluctuate for primary producers (Felip and Catalan 2000), using multiple biomass parameters is the most comprehensive way to estimate algal biomass (Stevenson 1996). Thus, our finding of lower algal standing stock following hemlock die-off in terms of chlorophyll-*a*, total cell density, and total cell biovolume provides strong evidence for a change in algal standing crop between earlier and later sampling dates. It is worth noting, however, that our AFDM results were inconsistent with the rest of our algal standing crop results; we found higher AFDM following hemlock death in 2018/2019. However, AFDM is likely not a good indicator of algal biomass in southern Appalachian headwater streams, given the high autotrophic index values in this system (Greenwood and Rosemond 2005). Thus, chlorophyll-*a*, total cell biovolume, and total cell density are better indicators of algal biomass in these forested headwater streams.

Increase in the basal area of rhododendron during the timeframe of our study (2005-2019) could explain our finding of lower algal standing crop following hemlock death in 2018/2019. Light transmission to streams in 2018/2019 may have decreased compared to 2005/2006, but we were unable to document this potential change with our methods. Traditional methods for measuring canopy cover (*e.g.,* densiometers or hemispherical photos) and light availability to streams (*e.g.,* a relatively small number of HOBO sensors) often fail to accurately account for short periods of elevated light beneath canopies, or "sunflecks" (Warren et al. 2013). Sunflecks are important to in-stream autotrophy in forested streams (Heaston et al. 2017), and rhododendron has been shown to reduce the length and intensity of sunflecks to forest floors (Lei et al. 2006). Thus, it is likely that the increase of the basal area of rhododendron over the course of this study (Table 2.1) has also resulted in a decrease of the length and/or intensity of sunflecks to our streams, which could have important implications for algal communities. Nonetheless, it is important to note that all chlorophyll-*a* values reported in this study -- both pre- and post-hemlock die-off -- are close to the expected range for forested catchments (0.5-3 mg m^{-2}), and low compared to other systems (moderately enriched streams, 3-60 mg m^{-2}; enriched streams, 25-260 mg m^{-2}; Biggs 1996).

Overall, we found little change in diatom community composition over this thirteen year period, but decreases in the relative biovolumes of *Eunotia minor* (January) and *E. metamondon* (September) may be attributed to increased grazing (Passy 2007, Passy and Larson 2019), increased flow (Francoeur and Biggs 2006), and/or changes in light availability (Stenger-Kovács et al. 2013). Both *E. minor and E. metamondon* are large taxa that form ribbon-like colonies, and thus have high growth forms. Having a high growth form is likely an adaptive strategy in low light conditions (Lowe et al. 1986); however, the effect of light on diatom ecological guild remains unclear (Tapolczai et al. 2016). Contrary to Lowe et al. (1986), Stenger-Kovács et al. (2013) argue that low-lying algal taxa may be better adapted to utilize weaker irradiance better than high-profile taxa due to their vertical location in the biofilm. Thus, decreases in the relative biovolume of *E. minor* and *E. metamondon* could be attributed to multiple factors, including increased grazing or flow in addition to changes in light. Conversely, *E. rhomboidea* increased in relative biovolume post-hemlock die-off during both seasons. *Eunotia rhomboidea*'s smaller size would be advantageous under increased grazing conditions, increased flow, and/or changes in light availability (Lowe et al. 1986). However, while September diatom communities were taxonomically distinct (Fig. 2.7B), because one species (*E. metamondon*) was replaced by a functionally similar species (*E. rhomboidea*), algal communities likely function in a biologically similar manner pre- and post-hemlock death.

Our findings, in combination with previous studies conducted in rhododendron-dominated southern Appalachian headwater streams, suggest relative stability of diatom community composition, with dominance of acidophilic taxa and/or taxa that prefer low nutrient and low conductivity waters (*e.g., Eunotia* spp., *Meridion* spp., *Nupela lapidosa;* Table 2.2). Notably, the relative biovolume of most of the top 20 taxa, including *Eunotia incisa*, *Nupela lapidosa*, and *Meridion alansmithii* (an endemic taxon), remained relatively unchanged 13 years after hemlock mortality (Fig. 2.9). In addition, many taxa that were dominant in reference streams in previous studies, in and around the Coweeta experimental basin, are still dominant in this study: for example, Lowe et al. (1986) reported dominance of *Eunotia rhomboidea, Meridion* sp*., Achnanthidium minutissimum,* and *Ulnaria ulna*; Schofield et al. (2004) reported dominance of *Eunotia* spp., *Synedra* spp., *Gomphonema* spp., and *Navicula* spp.; and Greenwood and Rosemond (2005) found *Eunotia minor*, *Gomphonema parvulum* and *Meridion* sp. among the dominant taxa (Table 2.3).

Our contention that algal community composition is resistant to disturbance is consistent with past experimental work in the region. For example, Schofield et al. (2004) found little change in diatom community composition with macroconsumer exclusion and experimental sediment additions. Similarly, Dye (2005) found little change in community composition with macroconsumer exclusion and short-term nutrient additions, and Greenwood and Rosemond (2005) found little change in community composition with moderate, but long-term (2-year) nutrient additions. One exception is the Lowe et al. (1986) study, which found forest clearcutting caused a shift from diatom-dominated algal communities to a community dominated by the green algal taxon *Spirogyra* sp. Taken together, this body of work indicates that algal communities in rhododendron-dominated southern Appalachian streams are relatively stable over time and resistant to changes in single factors with the exception of light availability. In this study, changes in canopy cover (~13% relative canopy reduction in January, ~3% relative canopy increase in September) due to shifting forest dynamics were likely not large enough to have an effect on algal communities.

One limitation of our study is using time as a proxy for hemlock death; however, several lines of evidence suggest that the relatively small, but statistically significant changes within algal communities we found were due to shifting forest dynamics. For example, changes to algal communities likely cannot be attributed to changes in other bottom-up controls of algae, as there were no reported changes in annual water yield (Brantley et al. 2015), mean stream temperatures (Siderhurst et al. 2010), or stream water nutrients (Northington et al. 2013) in the years immediately following hemlock mortality between 2006-2011. We also did not find biologically significant changes in stream water nitrate, soluble reactive phosphorus (Appendix, Table A.4), temperature, or pH (Appendix, Table A.5) between algal sampling events in this study. However, we were not able to account for changes in grazing pressure on algae from the 2005/2006 samples to the 2018/2019 samples. A study in the central Appalachians found a positive correlation between abundance of grazing macroinvertebrates and hemlock mortality (Diesburg et al. 2019). It is possible that increases in grazing pressure following shifting forest dynamics could be partially responsible for decreases in algal standing stocks. Thus, we recommend that future studies attempting to quantify changes algal communities following disturbances within riparian forests also account for potential changes in grazing pressure (*e.g.,* Dudley et al. 2021).

Our study provides detailed taxonomic information across multiple stream reaches to characterize algal communities at the watershed scale at two points in time separated by thirteen years. Mortality of eastern hemlock trees due to the invasive hemlock woolly adelgid has had far-reaching effects that have crossed ecosystem boundaries (Siderhurst et al. 2010, Webster et al. 2012, Brantley et al. 2015, Diesburg et al. 2019). However, we found that canopy cover and algal communities remained very similar thirteen years after losing upper-canopy hemlocks within the Coweeta watershed, demonstrating an ecological resilience to disturbance. Our study highlights the importance long-term studies following the invasion of an non-native pest, as short-term effects can differ can drastically from long-term effects (Northington et al. 2013).

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Appendix A: Supplementary Data

Table 2.1: Characteristics of the eight study sites. *Table modified from Webster et al. 2012*

	Location UTM Zone 17S						Basal area of *Tsuga canadensis* (m^2 ha^{-1})		Basal area of *Rhododendron maximum* L. (m^2 ha^{-1})	
Stream Name	Easting	Northing	Drainage area (km^2)	Elevation (m)	Stream order	Mean channel width (m)	2004	2010	2004	2010
Cunningham Creek	276120	3881322	0.41	907	2	2.3	13.0	0.0	14.3	12.1
Hugh White Creek, lower site	278233	3881738	0.62	716	2	3.3	11.2	0.1	6.1	10.5
Hugh White Creek, upper site	278358	3881467	0.36	793	1	3.5	13.5	0.0	7.0	11.7
Mill Branch	276681	3882054	0.39	862	2	3.3	14.2	0.0	4.9	5.8
Reynolds Branch	276390	3880223	0.36	930	1	3.0	16.9	0.1	3.9	6.3
Shope Fork	277035	3882520	7.56	709	4	3.8	9.3	0.1	4.3	2.8
Ball Creek, upper site	275694	3880045	0.39	1070	2	7.7	14.4	0.2	8.9	9.6
Wykle Branch	275084	3881802	0.36	991	2	3.4	21.8	11.3	4.1	9.1

Table 2.2: List of the twenty taxa that represented 93.7% of total diatom biovolume across all years and seasons. Taxa are in descending order of abundance by biovolume across all dates (*i.e.,* most abundant at the top).

Diatom Taxon	Growth form
Eunotia minor (complex)	high
Eunotia metamondon (complex)	high
Nupela lapidosa	low
Eunotia incisa	low
Ulnaria ulna	high
Meridion alansmithii	high
Eunotia rhomboidea (complex)	low
Gomphonema parvulum	high
Achnanthidium rivulare	low
Frustulia rhomboides (complex)	high
Achnanthidium alpestre	low
Achnanthidium minutissimum	low
Gomphonema christenseni	high
Synedra rumpens (complex)	high
Navicula angusta	motile
Meridion circulare var. *constrictum*	high
Tetracyclus rupestris	low
Ulnaria acus	high
Brachysira brebissonii	motile
Brachysira microcephala	motile

Table 2.3*:* Summary of diatom taxonomy studies in rhododendron-dominated southern Appalachian headwater streams. Dominant taxa are considered to be taxa that represent >5% of total cell biovolume.

See next page

Study	Experimental factor/disturbance	Year(s) data collected	Streams used	# of diatom taxa in study	Dominant diatom taxa in reference samples	Dominant diatom taxa in experimental samples	Notes
Lowe et al. 1986	Forest clear-cut, nutrient additions (NDS)	1983	L. Hugh, Big Hurricane Branch	40	*Eunotia. rhomboidea* *Meridion circulare*	*Spirogyra sp.* *Achnanthidium lanceolata* *Achnanthidium minutissimum* *Synedra socia* *Synedra ulna*	"Experimental" reflects clear-cut and nutrient additions (Ca+N+P)
Schofield et al. 2004	Macroconsumer exclusion, sediment (bedload) addition	1997, 1999	L. Ball Creek and others^	n/a	*Achnanthes* sp. *Cymbella* sp. *Eunotia* sp. *Gomphonema* sp. *Navicula* sp. *Synedra* sp.	*Achnanthes* sp. *Eunotia* sp. *Gomphonema* sp. *Synedra* sp.	Macroconsumer exclusion had non-significant effects on community composition, "Experimental" reflects sediment additions
Greenwood and Rosemond 2005	Whole stream nutrient addition	1999, 2000, 2001, 2002	Catchment 53 and Catchment 54	35	*Eunotia minor* *Eunotia pectinalis var. rectq* *Gomphonema parvulum* *Meridion constrictum*	*Eunotia minor* *Eunotia pectinalis var. rectq* *Gomphonema parvulum* *Meridion constrictum* *Navicula tantula*	
Dye 2005	Macroconsumer exclusion, nutrient additions (NDS)	2002	Betty's Creek^	123	n/a	n/a	Detailed taxonomic information not provided – summarized in growth forms
Schofield et al. 2008	Macroconsumer exclusion, catchment development gradients	1997 and 1998	L. Ball Creek and others^	n/a	n/a	n/a	Detailed taxonomic information not provided – summarized as growth forms
Solomon 2021 (Chapter 2)	Pre- and post-hemlock death (time)	2005/2006* 2018/2019*	Shope, L. Hugh, U. Hugh, U.	161	*Eunotia minor* *Eunotia metamondon* *Nupela lapidosa*	*Eunotia minor* *Nupela lapidosa* *Eunotia incisa* *Meridion alansmithii*	"Reference" reflects pre-hemlock die-off

			Ball, Wykle, Mill, Reynolds, Cunn.			*Eunotia rhomboidea*	samples (2005) and “Experimental” reflects to post-hemlock die-off samples (2018)
Solomon 2021 (Chapter 3)	Rhododendron removal	2014, 2015, 2016	Split White Oak^, Kit Springs^	129	*Eunoita incisa* *Eunotia minor* *Eunotia* cf. *minor* *Eunotia metamondon* *Eunotia papilioforma* *Frustulia rhomboides* *Meridion alansmithii* *Pinnularia erratica*	*Eunotia papilioforma* *Eunotia minor* *Oedegonium* sp.	Macroconsumer exclusion had non-significant effects on community composition, “Experimental” reflects rhododendron removal
Solomon 2021 (Chapter 4)	Forested and suburbanizing watersheds through time	2000, 2005, 2010	Coweeta Creek and others^	170	*Achnanthidium minutissimum* *Achnanthidium rivulare* *Synedra rumpens var. fragilaroides*	*Achnanthidium minutissimum* *Achnanthidium rivulare*	“Experimental” reflects average suburbanizing watersheds over three sampling periods

Notes:
*indicates samples NOT collected during summer
^indicates stream outside of main Coweeta (CWT) basin

Figure Legends

Fig. 2.1: Mean canopy cover (%) for (A) January and (B) September by stream reach, both pre-hemlock die-off (2005 or 2006; n = 1) and post-hemlock die-off (2018 and 2019; n = 5). Bars represent standard error.

Fig. 2.2: Mean daily light intensity (lux) for (A) January and (B) September at five of the eight study sites from 2005-2015. Bars represent standard error.

Fig. 2.3: Mean light index (daily average lux/total solar radiation) for (A) January and (B) September at five of the eight study sites from 2005-2015. Bars represent standard error.

Fig. 2.4: Mean (A) ash-free dry mass (AFDM; mg m^{-2}), (B) chlorophyll-*a* (Chl-a; mg m^{-2}), (C) cell density (cells mm^{-2}), and (D) cell biovolume (mm^3 m^{-2}) for pre-hemlock die-off (2005 or 2006, n = 24) and post-hemlock die-off (for [A] and [B], 2018 and 2019 averaged, n = 80; for [C] and [D], n = 24) in January and September. Bars represent standard error.

Fig. 2.5: Mean chlorophyll-*a* (mg m^{-2}) values for (A) January and (B) September by stream reach, both pre-hemlock die-off (2005 or 2006; n = 3) and post-hemlock die-off (2018 and 2019; n = 5). Bars represent standard error.

Fig. 2.6: Mean cell biovolume (mm^3 m^{-2}) values for (A) January and (B) September by stream reach, both pre-hemlock die-off (2005 or 2006; n = 3) and post-hemlock die-off (2018 or 2019; n = 3). Bars represent standard error.

Fig. 2.7: NMDS visualizations of top 20 diatom taxa based off of total cell biovolume (μm^3 mm^{-2}) in (A) January (stress = 0.15) and (B) September (stress = 0.16). Grey circles represent stream sites pre-hemlock die-off (2005 or 2006) and black circles represent stream sites post-hemlock die-off (2018 or 2019). Polygons encompass stream sites pre-hemlock die-off ("pre", light grey hulls) and post-hemlock die-off ("post", dark grey hulls).

Fig. 2.8: (A) Total and (B) relative cell biovolume (m^3 mm^{-2}) and for all eight study reaches separated by functional group, pre-hemlock die-off ("pre", 2005 or 2006; n = 24) and post-hemlock die-off ("post"; 2018 or 2019; n = 24) in January and September.

Fig. 2.9: Change of the mean proportion of total cell biovolume for each of the 20 focal taxa following hemlock death in (A) January and (B) September. Bars represent 95% confidence intervals.

Fig. 2.1

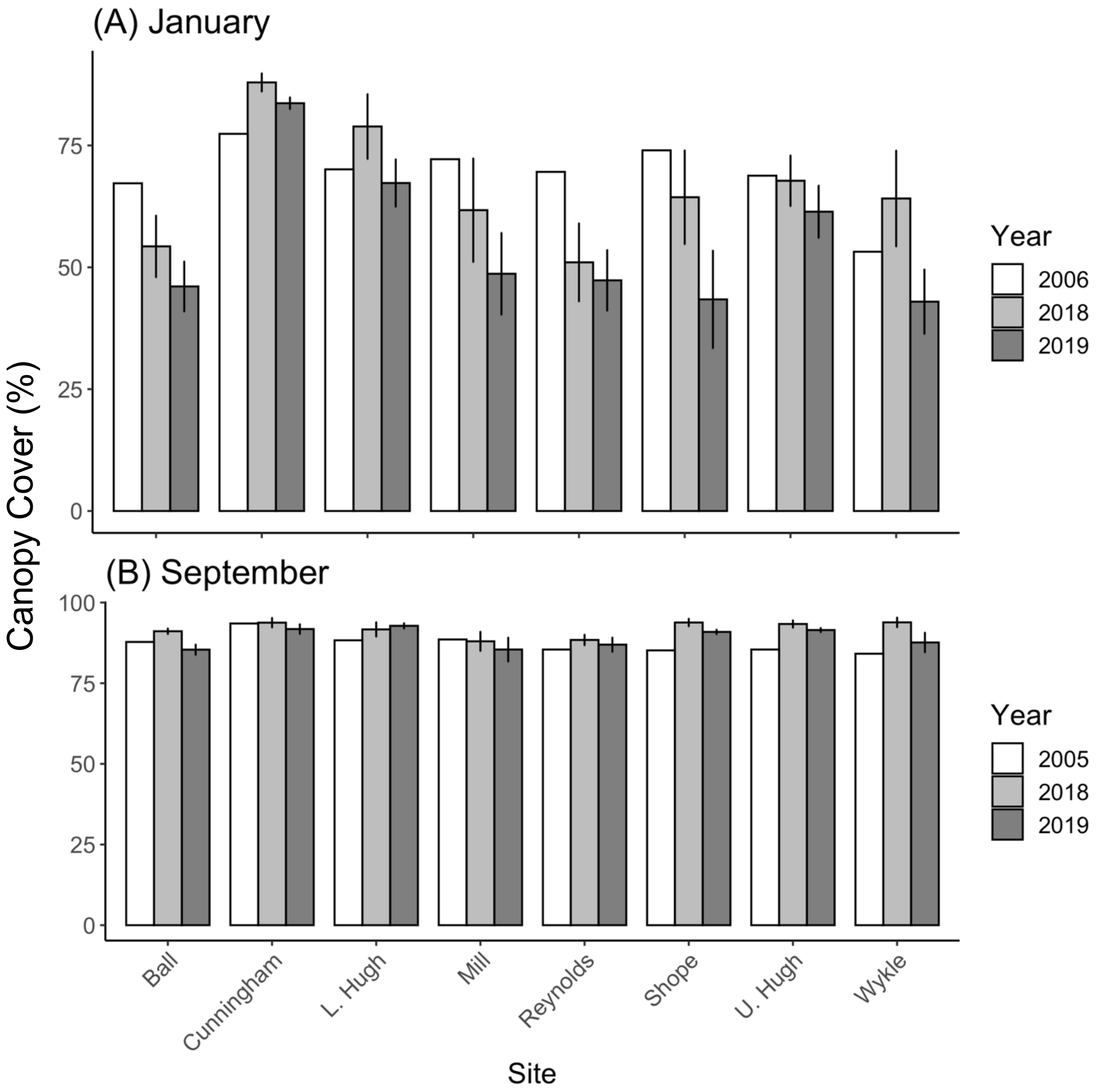

(A) January
Canopy Cover (%)
0
25
50
75
Year
2006
2018
2019
(B) September
0
25
50
75
100
Year
2005
2018
2019
Ball
Cunningham
L. Hugh
Mill
Reynolds
Shope
U. Hugh
Wykle
Site

Fig. 2.2

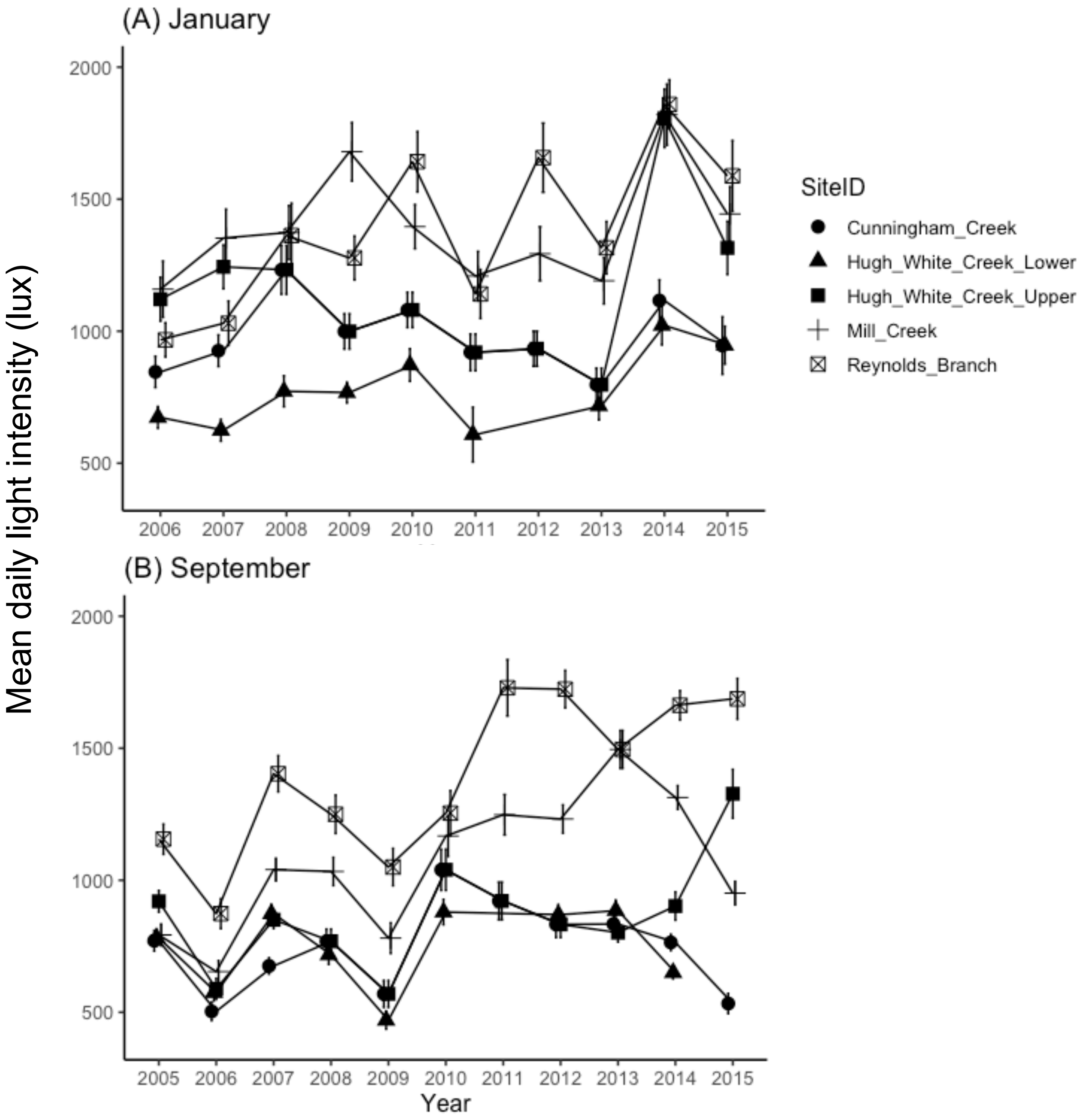
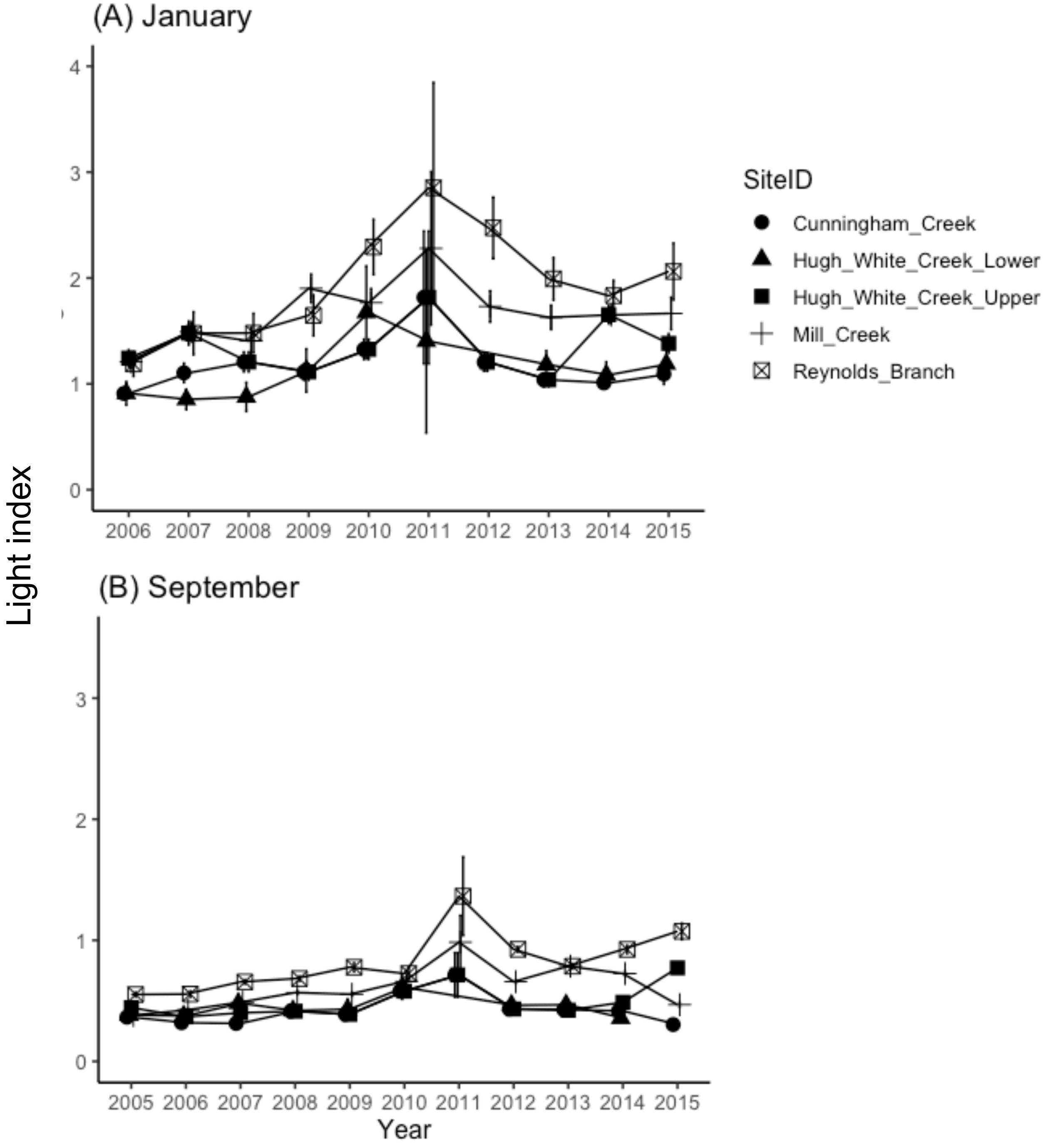

(A) January
(B) September
Mean daily light intensity (lux)
2000
1500
1000
500
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2005
Year
SiteID
Cunningham_Creek
Hugh_White_Creek_Lower
Hugh_White_Creek_Upper
Mill_Creek
Reynolds_Branch

Fig. 2.3



(A) January
(B) September
Light index
Year
0
1
2
3
4
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
SiteID
● Cunningham_Creek
▲ Hugh_White_Creek_Lower
■ Hugh_White_Creek_Upper
+ Mill_Creek
☒ Reynolds_Branch

Fig. 2.4

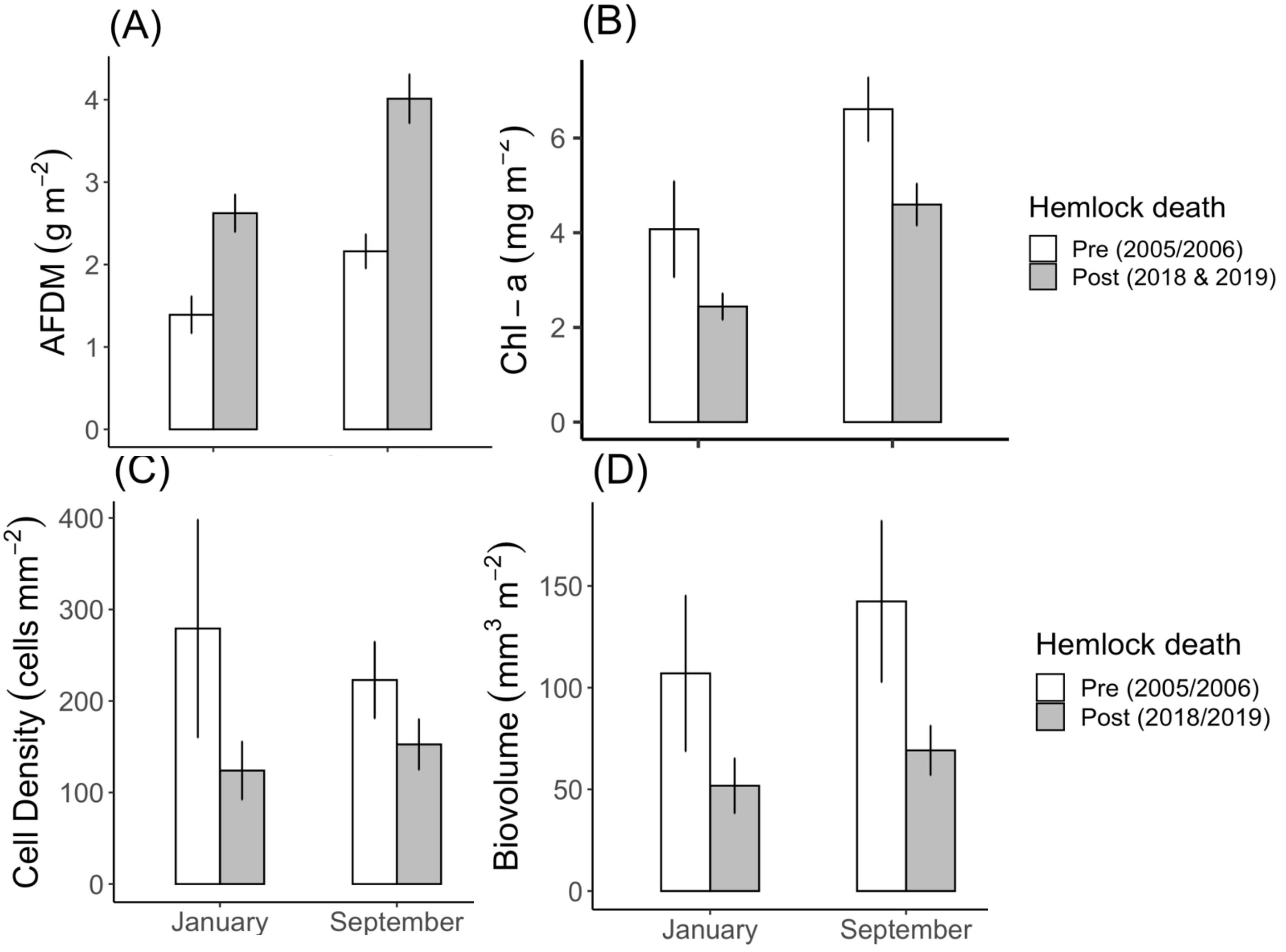
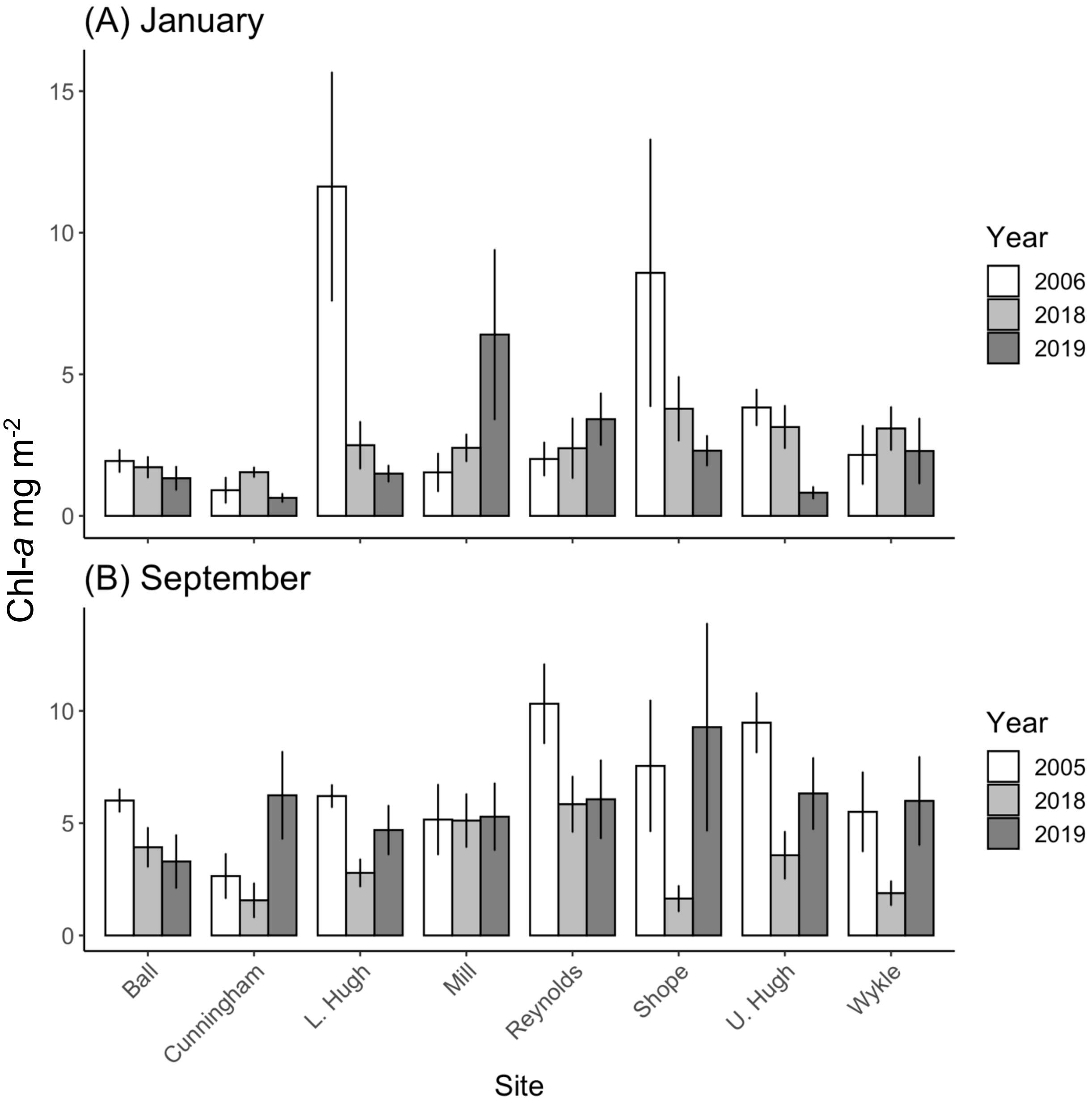

(A)
AFDM (g m^{-2})
0
1
2
3
4
(B)
Chl − a (mg m^{-2})
0
2
4
6
Hemlock death
Pre (2005/2006)
Post (2018 & 2019)
(C)
Cell Density (cells mm^{-2})
0
100
200
300
400
January
September
(D)
Biovolume (mm^3 m^{-2})
0
50
100
150
January
September
Hemlock death
Pre (2005/2006)
Post (2018/2019)

Fig. 2.5



(A) January
(B) September
Chl-a mg m^{-2}
0
5
10
15
Year
2006
2018
2019
2005
Ball
Cunningham
L. Hugh
Mill
Reynolds
Shope
U. Hugh
Wykle
Site

Fig. 2.6

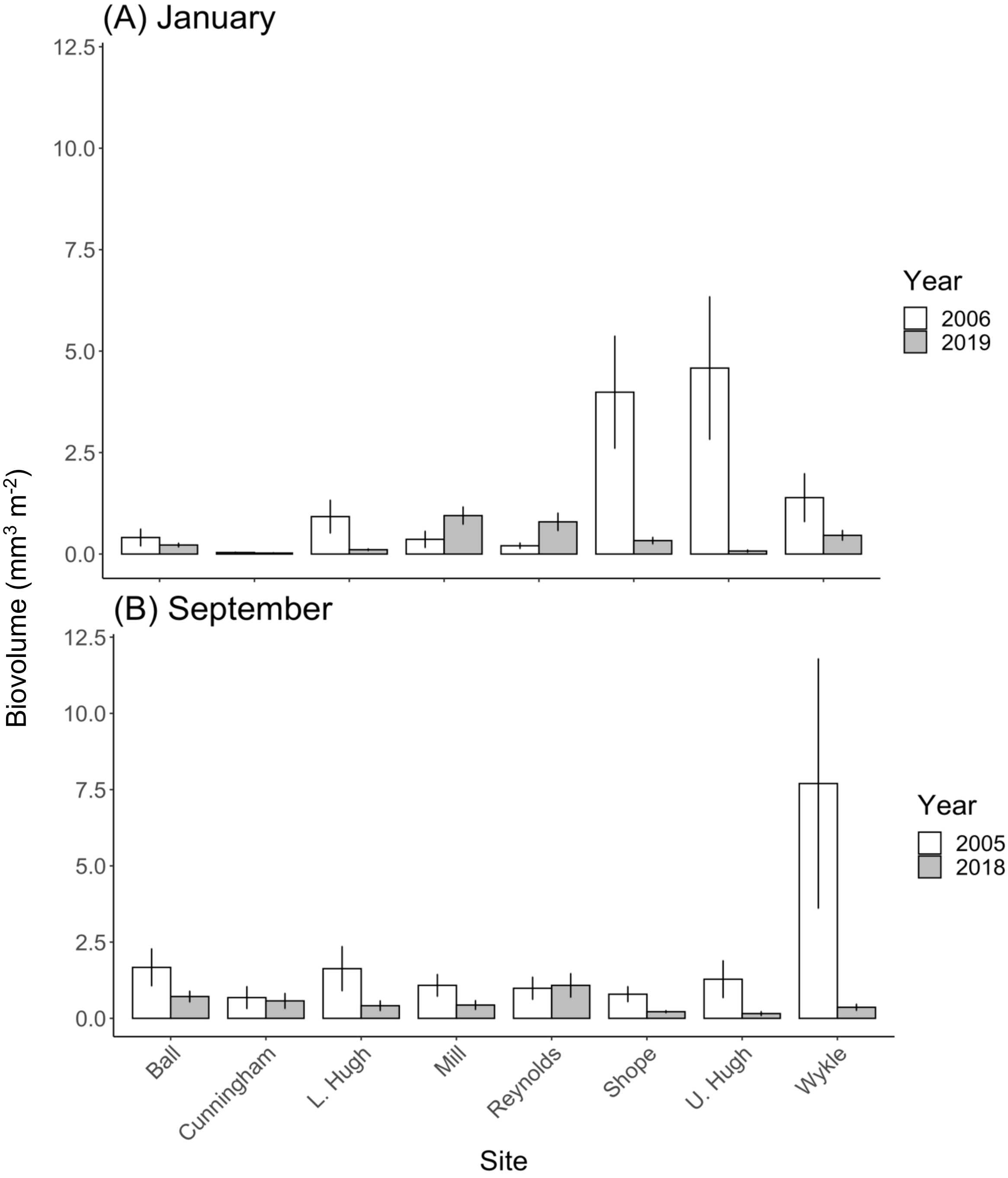
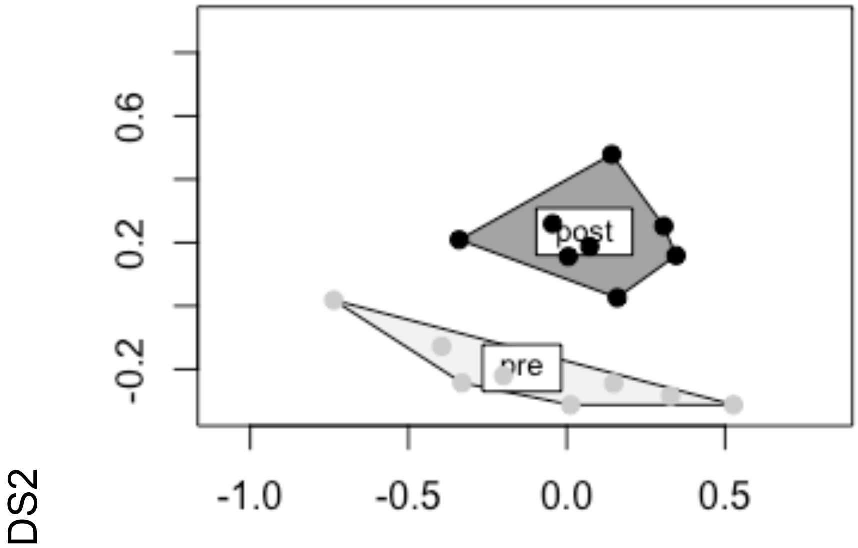

(A) January
12.5
10.0
7.5
5.0
2.5
0.0
Year
2006
2019
(B) September
12.5
10.0
7.5
5.0
2.5
0.0
Year
2005
2018
Biovolume (mm³ m⁻²)
Ball
Cunningham
L. Hugh
Mill
Reynolds
Shope
U. Hugh
Wykle
Site

Fig. 2.7

(A) January



(B) September

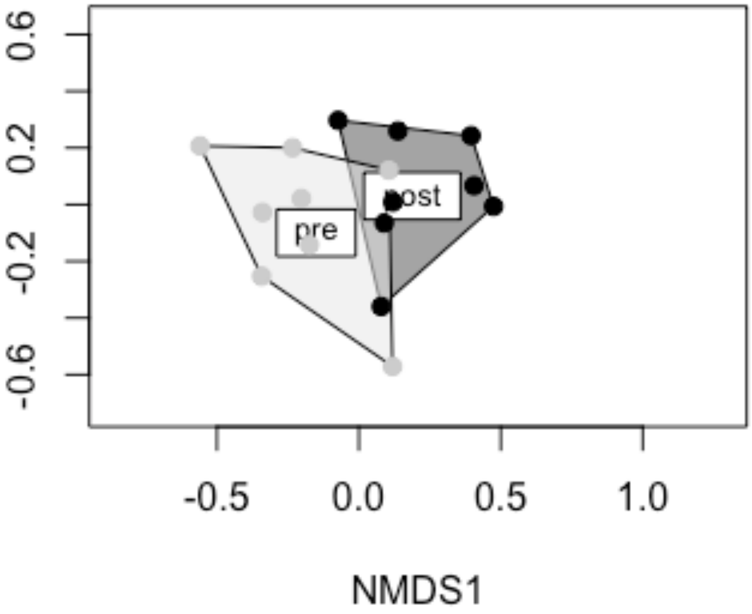
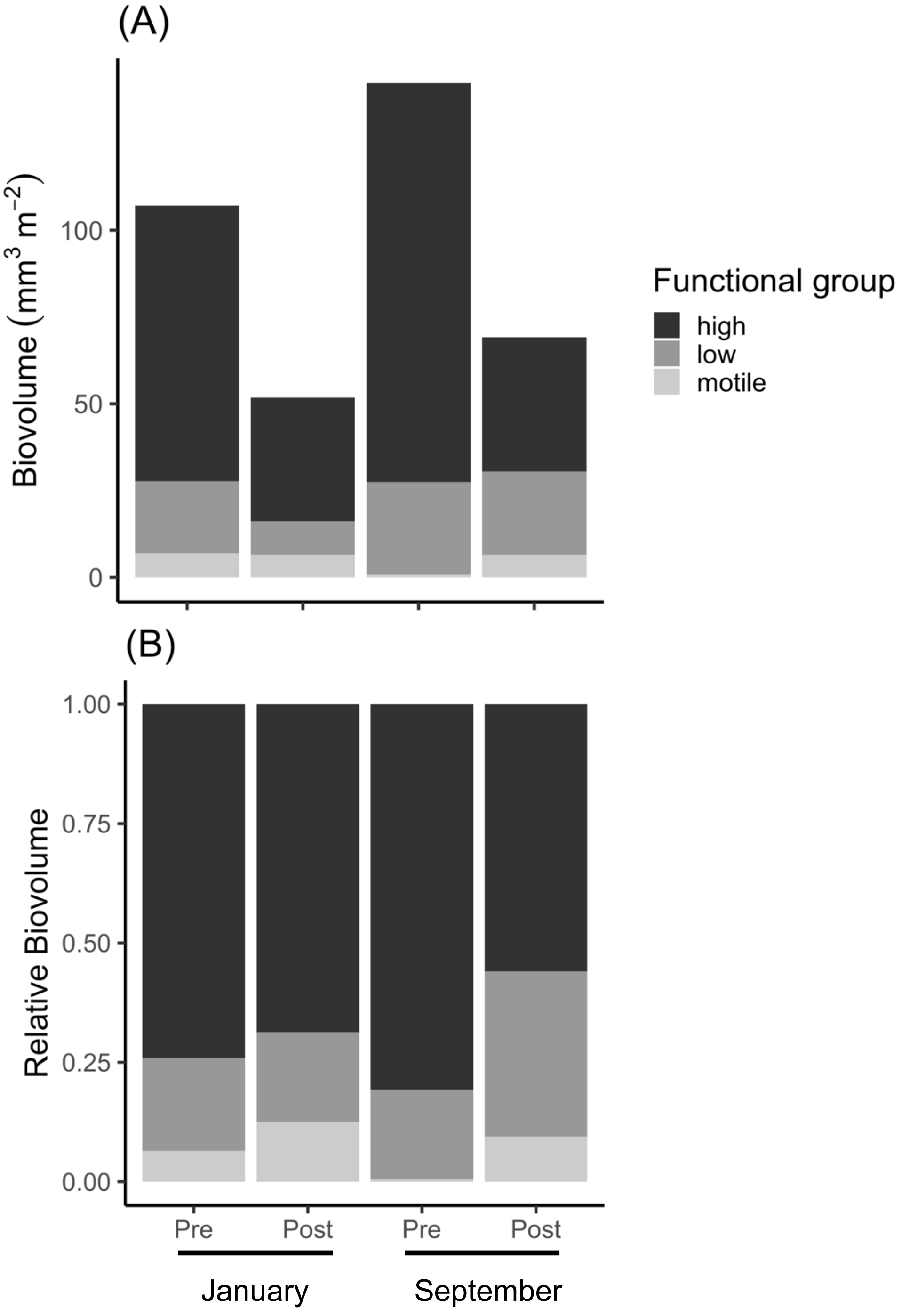
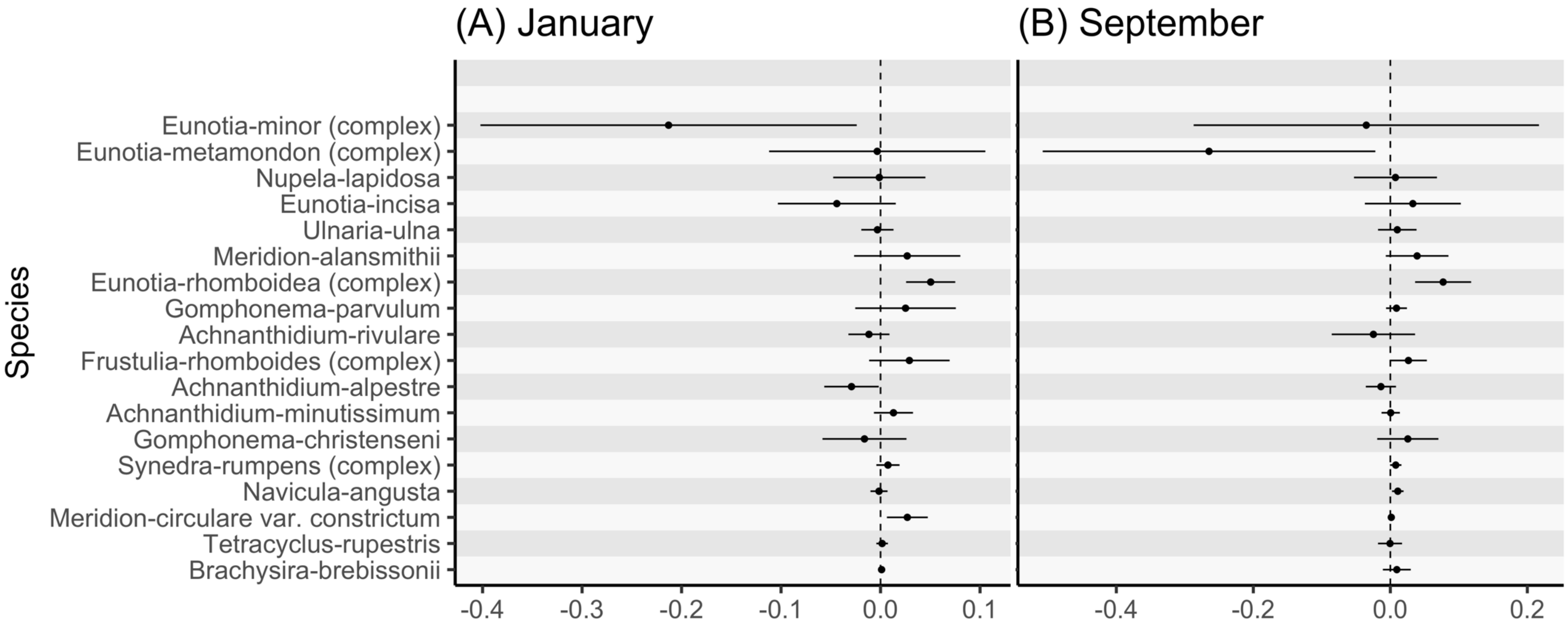


Fig. 2.8



(A)
Biovolume $(mm^3 m^{-2})$
100
50
0
Functional group
high
low
motile
(B)
Relative Biovolume
1.00
0.75
0.50
0.25
0.00
Pre
Post
Pre
Post
January
September

Fig. 2.9


(A) January
(B) September
Species
Eunotia-minor (complex)
Eunotia-metamondon (complex)
Nupela-lapidosa
Eunotia-incisa
Ulnaria-ulna
Meridion-alansmithii
Eunotia-rhomboidea (complex)
Gomphonema-parvulum
Achnanthidium-rivulare
Frustulia-rhomboides (complex)
Achnanthidium-alpestre
Achnanthidium-minutissimum
Gomphonema-christenseni
Synedra-rumpens (complex)
Navicula-angusta
Meridion-circulare var. constrictum
Tetracyclus-rupestris
Brachysira-brebissonii
-0.4
-0.3
-0.2
-0.1
0.0
0.1
-0.4
-0.2
0.0
0.2

CHAPTER 3

RIPARIAN RHODODENDRON REMOVAL SIGNIFICANTLY ALTERS ALGAL COMMUNITY COMPOSITION AND TROPHIC DYNAMICS IN A SOUTHERN APPALACHIAN HEADWATER STREAM

Kelsey J. Solomon, Maura P. Dudley, Nathan Tomczyk, Seth J. Wenger, C. Rhett Jackson, Chelsea F. Miniat, Katherine J. Elliott, Rebecca J. Bixby, and Catherine M. Pringle. To be submitted to: *Freshwater Biology*

Abstract

Multiple factors, including light availability, nutrients and grazing, interact to control stream algal communities. In the southern Appalachian Mountains*,* *Rhododendron maximum* L. (rhododendron) is an abundant, native shrub in riparian zones that provides year-round shade to stream reaches, severely limiting algal autotrophic production. The U.S. Forest Service has explored removal of rhododendron along stream riparian zones as a management strategy to promote the growth and regeneration of hardwoods. By increasing solar insolation and altering light availabilities, rhododendron removal is likely to affect algal species assemblages in ways that are mediated by crayfish, an important macroconsumer in southern Appalachian headwaters. We used a plot-scale crayfish exclusion experiment nested within a reach-scale rhododendron removal manipulation to ask: (1) How does decreased canopy cover from riparian rhododendron removal interact with top-down control by crayfish to influence total algal cell biovolume, chlorophyll-*a* to cell biovolume ratios, and algal community structure? (2) How will crayfish dependence on autochthonous resources be affected when riparian rhododendron is removed? We found that rhododendron removal increased total algal cell biovolume and shifted algal community composition from primarily singular and ribbon-forming diatoms (*e.g., Eunotia* spp.) to high-profile, stalk- and chain-forming diatoms (*e.g., Gomphonema* spp., *Encyonema minutum*) and a filamentous chlorophyte (*e.g.,, Oedogonium* sp.), and that this shift was not mediated by crayfish. However, neither rhododendron removal or crayfish exclusion had an effect on chlorophyll-*a* to cell biovolume ratios. In addition, we found evidence that rhododendron removal increased crayfish dependence on autochthonous food resources, but this effect may vary depending on watershed characteristics (*e.g.,* % rhododendron cover, stream orientation). Our results provide insight into how small decreases in total canopy cover can

change algal communities through increased number of sunflecks (*i.e.,* short periods of elevated light beneath canopies). Findings here suggest that large-scale removal of rhododendron in southern Appalachian headwater streams result in increases in the relative abundance of filamentous green algal taxa, with stream food webs potentially becoming more autochthonous.

Introduction

A fundamental goal for ecologists is to understand the factors that control the abundance and distribution of organisms, particularly primary producers. In stream ecosystems, despite their low standing biomass relative to detritus, primary producers (*e.g.,* algae) are disproportionally important to consumer secondary production because of their high dietary quality (Rosi-Marshall et al. 2016, Crenier et al. 2017, Erdozain et al. 2019). However, algal taxa can differ in their dietary quality and palatability. Diatoms are a particularly important food source for aquatic consumers due to elevated highly unsaturated fatty acid (HUFA) content compared to other types of stream algae (*e.g.,* cyanobacteria, chlorophytes; Brett and Müller-Navarra 1997). In addition, because consumer feeding morphologies differ in their ability to harvest various algal sizes and growth forms, algal taxa also vary in their palatability to consumers (Steinman et al. 1992, Tall et al. 2006). Thus, changes in algal community composition in streams can have consequences to consumer biomass and community structure (Ladrera et al. 2015).

Stream algal communities are controlled by a variety of both bottom-up and top-down factors. These factors often interact with one another, eliciting complex, habitat-specific responses that can be challenging to predict (Rosemond 1993, Lange et al. 2011, Piggott et al. 2015). Generally, increases in light availability favor algal taxa that have a competitive advantage in high light environments, which are typically green algal taxa (Richardson et al.

1983) and diatom taxa with "high" growth forms (Lowe et al. 1986, Steinman et al. 1992, Bixby et al. 2009). On the other hand, increases in stream flow favor taxa with low-lying, tightly adhering growth forms (Passy 2007). Stream macroconsumers (*e.g.,* snails, crayfish, fish) can also exert control on algal community composition directly through consumption (Hart 1992, Creed 1994, Ranvestel et al. 2004, Schofield et al. 2008) and/or indirectly through bioturbation (Pringle et al. 1993, Parkyn et al. 1997) and food-web interactions (Creed 1994). However, in forested headwater streams, algal communities are heavily controlled by light availability (Lowe et al. 1986), and light availability in these systems is mainly determined by riparian canopy cover (Hill et al. 2001). Shifts in riparian forest vegetation structure often result in changes in light availability to streams (Webster et al. 2012b, Warren et al. 2013), which can result in changes to benthic algal communities (Kaylor and Warren 2017).

Over the past century, the southern Appalachian Mountains have experienced shifts in riparian vegetation due to the loss of two foundation tree species, chestnut and hemlock, and expansion of the riparian shrub, *Rhododendron maximum* L. The American chestnut (*Castanea dentata*) was functionally lost to blight in the early 1900s (Anagnostakis 1987). Since the loss of chestnut, riparian over story species have been characterized by deciduous species (*e.g.*, *Acer rubrum* and *Liriodendron tulipifera*) and eastern hemlock (*Tsuga canadensis;* Fig. 3.1A). However, eastern hemlock experienced massive die-off due to an infestation by the invasive hemlock woolly adelgid insect (*Adelges tsugae*) in the early 2000s (Fig. 3.1B; Ellison et al. 2005). Loss of chestnut and eastern hemlock has led to expansion of the aggressive riparian shrub, *Rhododendron maximum* L. (hereafter rhododendron, Fig. 3.1B; Ford et al. 2012; Hladyz et al. 2011). Although rhododendron is native to the southern Appalachians, the U.S. Forest Service (USFS) has explored removing rhododendron from riparian areas (Fig. 3.1C) as a management strategy to promote the growth and regeneration of hardwoods (Fig. 3.1D), and thus it is important to understand how rhododendron removal might affect both terrestrial and stream ecosystem processes (Elliott and Miniat 2018, Dudley et al. 2021). Rhododendron is an evergreen species that provides year-round shade to stream reaches in the southern Appalachians, severely limiting autotrophic production in stream channels. Consequently, removal of rhododendron will tend to decrease total canopy cover (Raulerson et al. 2020, Dudley et al. 2021), slightly increase summer stream temperatures (Raulerson et al. 2020), and may increase autotrophic production via increased light availability (Dudley et al. 2021).

Changes to algal communities via riparian rhododendron removal could be difficult to detect due to top-down control on algal communities by stream macroconsumers. In the southern Appalachians, *Cambarus bartonii* (known as the common crayfish or Appalachian brook crayfish) is an important macroconsumer in headwater streams. *Cambarus bartonii* is primarily a detritivore, but also relies on macroinvertebrates and algae as food resources (Huryn and Wallace 1987, Creed and Reed 2004, Schofield et al. 2008, Dudley et al. 2021). One method to detect the effects of crayfish on benthic algal communities is through macroconsumer exclusion experiments (Pringle and Blake 1994). Macroconsumer exclusion experiments in headwater streams of the southern Appalachians have indicated that crayfish exert stronger top-down control on algal biomass and community composition under reduced canopy cover (Schofield et al. 2008) than ambient canopy cover (Schofield et al. 2004, Dye 2005). For example, Dudley et al. (2021) found that crayfish significantly affected algal standing crops only when rhododendron was removed (*i.e.,* canopy was reduced) and flows were low. This finding could potentially be explained by the ability of crayfish to more efficiently harvest filamentous algae (Evans-White et al. 2003) and algae with “high” growth forms (Passy and Larson 2019) -- algal types that typically become more abundant under increased light availability and/or low flow conditions. Thus, in order to understand how large scale riparian rhododendron removal might affect algal community composition, we must examine algal community composition under both crayfish access and exclusion conditions.

While macroconsumer exclusion experiments can provide important insight into how top-down and bottom-up controls interact to affect algal communities, alternate methods must be used to infer shifts in stream trophic dynamics following rhododendron removal, because macroconsumer presence can affect algal communities through both direct and indirect pathways. For example, while Dudley et al. (2021) found that crayfish presence decreased algal standing crop when rhododendron was removed and flows were low, it is possible crayfish presence influenced algal communities directly through dislodgement (Charlebois and Lamberti 1996) or indirectly through trophic cascades (Lodge et al. 1994, Charlebois and Lamberti 1996, Nystrom et al. 1999, Dye 2005) rather than through consumption (Whitledge and Rabeni 1997, Keller and Ruman 1998, Evans-White et al. 2001). In freshwater ecology, stable isotopes in carbon ($\delta^{13}C$) can be used to infer shifts in consumer reliance in autochthonous vs. allochthonous food sources (Finlay 2001). Thus, stable isotope analyses could be a useful tool to help understand how large-scale rhododendron removal may change trophic dynamics in southern Appalachian streams.

In this study, we present total algal biovolume and community composition data associated with research published in Dudley et al. (2021) addressing two questions: (1) How does decreased canopy cover from riparian rhododendron removal interact with top-down control by crayfish to influence total algal cell biovolume, chlorophyll-*a* to cell biovolume ratios, and community composition? (2) How will crayfish dependence on autochthonous resources be affected when riparian rhododendron is removed? First, we predict that crayfish exclusion OR rhododendron removal alone will have little effect on total algal cell biovolume and community composition, due to co-limitation of light and crayfish presence on the vertical development of algal communities. However, we predict that crayfish exclusion under rhododendron removal conditions will result in an increase in total cell biovolume and an increase in the relative biovolume of diatom taxa with high growth forms *(e.g., Gomphonema* spp., *Synedra* spp.) and non-diatom taxa (*e.g*,. chlorophytes and/or cyanobacteria). We also predict that chlorophyll-*a* to cell biovolume ratios will be lower under rhododendron removal conditions due to higher light availability. Finally, we predict that crayfish will become more dependent on autochthonous food resources with rhododendron removal, compared to crayfish from reaches with ambient rhododendron.

Methods

Study area

This study was conducted in the Nantahala Mountain Range of western North Carolina, U.S.A., within the Blue Ridge Physiographic Province, near the southern end of the Appalachian chain. Soils are deep sandy loams and are underlain by folded schist and gneiss. Two soil orders are found within both locations, immature Inceptisols and older developed Ultisols. Soil types include the Cullasaja-Tuckasegee complex along stream channels and the Edneyville-Chestnut complex and Plot fine sandy loams on the uplands (Thomas 1996).

Rhododendron removal manipulations

We conducted our experiments within the context of a larger study aimed at quantifying the effects of reach-scale riparian rhododendron removal on headwater streams. Full methods for rhododendron removal manipulations can be found in (Elliott and Miniat 2018, 2021). Briefly, in the larger study, four perennial headwater streams (2nd order) were selected within the White Oak Creek Watershed (WOC, 35°20' N latitude, 83°58' W longitude), located approximately 10 km west of Franklin, North Carolina, and approximately 21 km northwest of the Coweeta Hydrologic Laboratory. This study took place in three of the four study streams (Table 3.1). Stream study reaches were 300 m long and were selected to be similar with respect to watershed characteristics, rhododendron abundance, and percentage of dead eastern hemlock (Elliott and Miniat 2018). Within the study stream reaches, elevation ranged from 1160-1315 m (Tabl2 3.1). Channel slopes were moderate to steep (2.53 - 3.52%), relatively narrow (3.42 - 4.53 m), mostly colluvial (Table 3.1). Annual rainfall averaged 1900 mm and mean annual air temperature was 10.8°C. In the riparian zones, live overstory canopy included oak (*Quercus* spp.), birch (*Betula* spp.), American beech (*Fagus grandifolia*), and red maple (*Acer rubrum*), while rhododendron (*Rhododendron maximum*) was abundant in the understory (Dudley et al. 2021).

Different rhododendron removal manipulations were applied to each of the three study stream reaches. The *Control* reach (Kit Springs Branch) was not managed in any way for the duration of the experiment. In the *Cut* rhododendron removal treatment reach (Holloway Branch), rhododendron plants were removed by cutting stems from the stream edge to 50 m perpendicular to the stream on both sides, and moving cut branches away from stream edge. In the *Cut+Burn* rhododendron removal treatment reach (Split White Oak Branch), rhododendron plants and leaf litter were removed by cutting stems from the stream edge to 50 m perpendicular to the stream on both sides, scattering the branches on site, and burning the reach one-year post-cut. Rhododendron cutting occurred in spring (March–May) 2015 for the *Cut* and *Cut+Burn* reaches, and the prescribed fire was implemented in spring (March) 2016 for the *Cut+Burn* reach (Elliott and Miniat 2018). Rhododendron removal reduced August total canopy cover by 3.9% (CutYR) and 5.3% (BurnYR) in the *Cut* reach, and by 6.2% (CutYR) and 6.9% (BurnYR) in the *Cut+Burn* reach (Raulerson et al. 2020).

Crayfish exclusion experiments and algal sampling

Full methods for crayfish exclusion experiments are described in Dudley et al. (2021). Briefly, we quantified top-down control by crayfish on algal community composition in two study reaches (*Control, Cut+Burn*) using plot-scale crayfish exclusion experiments nested within the overall reach-scale rhododendron removal manipulations. We conducted the crayfish exclusion experiments over a 32-day period in July and August of three years: before rhododendron removal (PreYR, 2014), post-cut in the *Cut+Burn* reach (CutYR, 2015), and post-burn in the *Cut+Burn* reach (BurnYR, 2016). We placed five pairs of copper wire frames (~0.125 m^2) in five equally distributed pools along each study reach. We electrified one frame area in each pair using a 12V fence charger (Speedrite 1000 Unigizer). The electric exclusion technique excludes large-bodied macroconsumers like crayfish, but allows the entry of smaller organisms such as aquatic insects. The unelectrified frame served as a control, since crayfish were still able to access this framed area. Previous work in similar southern Appalachian streams showed that electrified treatments did not negatively affect most insect taxa colonizing substrates *(e.g.,* chironomids, hydropsychids, baetids), with the exception of heptageniid mayflies, whose abundance was lowered by electrified treatments (Schofield 2001). We placed paired frames (access, exclusion) within each pool a minimum of 7 cm apart to ensure that the electrified frame would not affect the unelectrified one.

We collected algal samples using unglazed white ceramic tiles (4.7 cm x 4.7 cm) that were attached to the inside of the wire frames using binder clips and cable ties (5 tiles in each frame). We began tile collection 3–5 days into the experiment and continued approximately weekly for the duration of each 32-day experiment (5 tiles total). To collect the tiles, we transferred the tiles to a plastic bag with a small amount of stream water, transported on ice, and processed the samples within 24 hours of collection. To process the tiles, we scraped each tile with a razor blade to remove associated periphyton, and then rinsed with deionized water to create a slurry. We raised the slurry to a known volume and preserved 20 mL of each slurry with a 7% formalin solution for algal community composition analysis.

*Algal total cell biovolume, chlorophyll-*a *to biovolume ratios, and community composition*

We quantified algal community composition using three replicate samples from each reach (*Control*, *Cut+Burn*) and crayfish treatment (access, exclusion) for all three years (PreYR, CutYR, BurnYR). Due to low algal accrual rates, we only used samples from the final collection (Week 5, Day 32) for total algal cell biovolume, chlorophyll-*a* to biovolume ratios, and algal community composition analysis. To determine community composition and cell densities of soft algae, we enumerated at least 200 diatom cells in a Palmer-Maloney nanoplankton counting chamber using an Olympus CX21LED microscope at 400x magnification. In samples with low cell densities, a maximum transect length (180 mm) was analyzed.

To determine diatom densities for samples from the *Cut+Burn* reach with associated chlorophyll-*a* values > 0.5 mg m^{-2}, we heated 10 mL aliquots with 30% hydrogen peroxide for one hour and then rinsed six times with distilled water to remove oxygen by-products. We created permanent slides by evaporating processed samples onto round coverslips (Fisherbrand, Pittsburgh, PA) using a modified Battarbee chamber (Battarbee 1974). Briefly, we placed round coverslips at the bottom of 100 mL beakers (PYREX, Glendale, AZ) and evaporated processed samples within the beakers. For all samples from the *Control* reach and samples from the *Cut+Burn* reach with chlorophyll-*a* values of <0.5 mg m^{-2}, algal cells were more diluted and required concentration for accurate enumeration. For these samples we evaporated 4-5 mL of the aliquot directly onto round coverslips. We mounted all coverslips to microscope slides with Naphrax mounting medium (Brunel Microscopes, Wiltshire, U.K.). We enumerated and identified 500 valves per sample using a Leica DMRXE microscope under oil immersion at 1000x using brightfield optics. In samples with low cell densities, a maximum transect length (180 mm) was analyzed. We used standard taxonomic references (Patrick and Reimer 1966, 1975; diatoms.org) and algal floras from the southeastern U.S. (Camburn et al. 1979, Kociolek and Kingston 1999, Thomas et al. 2009, Furey et al. 2011, Bishop et al. 2017) for diatom species identification.

To determine cell density (cells mm^{-2}) for each taxon, we divided the number of cells of each taxon by the fractional volume of the sample (*i.e.,* volume measured / total volume of slurry), and converted to a per-area measure by dividing the measured value by the proportion of the surface area of the accrual tile. To determine individual cell biovolumes, we used cell biovolume values from Solomon (2021, Chapter 2 of this dissertation), a study from the same region of the southern Appalachians with nearly identical diatom flora. For species not identified in Solomon et al. (2021, Chapter 2 of this dissertation), we measured approximately ten cells of each species and averaged the biovolumes of the ten individuals to determine a cell biovolume for each taxon (Hillebrand et al. 1999). To determine total cell biovolume ($\mu m^3\ mm^{-2}$) of each taxon, we multiplied the cell biovolume of each taxon by its density in the sample. To determine total cell biovolume ($mm^3\ m^{-2}$) of each sample, we summed individual biovolumes across samples, and we considered total cell biovolumes of each sample to be an alternate measure of algal standing crop. To determine chlorophyll-*a* to cell biovolume ratios, we divided chlorophyll-*a* concentrations (previously published in Dudley et al. 2021) by the total cell biovolume (this study) for each sample.

We assigned individual diatom taxa to one of four functional groups -- low, high, motile, planktonic -- based on the definitions of Rimet and Bouchez (2012), except in cases where our own or published observations of growth forms of individual species differed from that of Rimet and Bouchez (2012). Deviations from Rimet and Bouchez (2012) were as follows: we classified *Eunotia* spp. with a cell biovolume less than 500 µm and a valve length to width ratio of less than eight as low instead of high, because we only observed these taxa singularly or in chains of two cells. We classified *Meridion* spp. as high because we observed these taxa erect on substrate and because of similar observations of these taxa by Lowe et al. (1986). We classified *Brachysira* spp. as motile instead of low because of their "moderate motility" (Hamilton 2010) and *Chamaepinnularia* spp. as low instead of motile because of their "weak motility" (Tyree 2018). We assigned filamentous chlorophytes to a fifth functional group, "filamentous."

Crayfish diets

We used stable isotope analysis to assess whether crayfish would become more dependent on autochthonous resources under rhododendron removal conditions, as well as to assess the importance of various food resources. Stable isotopes of carbon (C) are useful for tracing assimilated food sources because benthic algae are generally more $\delta^{13}C$ enriched than are terrestrial carbon, but little enrichment in $\delta^{13}C$ occurs from one trophic level to the next (Finlay 2001). Stable isotopes of nitrogen ($\delta^{15}N$) can be used in addition to $\delta^{13}C$ to enhance the discriminatory power to detect shifts in consumer reliance on autochthonous vs. allochthonous food sources (Cole and Solomon 2012, Erdozain et al. 2019). Therefore, we collected samples of crayfish and possible food resources in the *Cut* and *Cut+Burn* stream reaches (hereafter: *Cut*-treatment and *Cut+Burn*-treatment) on 6-7 August 2019, which was four years following the initial rhododendron cut in both stream reaches (CutYR, 2015) and three years following the prescribed burn in the *Cut+Burn* reach (BurnYR, 2016). Due to high in-stream variability of potential food resources for crayfish among streams in this area (Eliason 2017, Dudley 2018), we chose 100 m control reaches that were directly upstream of the *Cut* and *Cut+Burn* reaches (hereafter: *Cut*-upstream and *Cut+Burn*-upstream), and also collected samples of crayfish and possible food resources from these stream reaches. We used prior studies to determine likely food resources for *Cambarus* sp. in this area: periphyton, fine particulate organic matter, stream-conditioned leaves, and macroinvertebrates (Huryn and Wallace 1987, Creed and Reed 2004, England and Rosemond 2004, Schofield et al. 2008, Dudley 2018).

We collected macroinvertebrates by handpicking and by using dip-nets at equal intervals along each stream reach. For the remaining food resources, we collected five replicates of each resource from pool habitats at equal intervals along each stream reach. For each periphyton sample, we collected five cobbles of uniform shape, scraped each cobble with a toothbrush and rinsed with deionized water, and then transferred the slurry into a Whirl-Pak® bag. For fine particulate organic matter (FPOM), we placed a modified core sampler onto the streambed, disturbed the streambed, and collected water samples in a Whirl-Pak® bag. We collected stream-conditioned leaves from terrestrial vegetation (coarse particulate organic matter; CPOM) by hand. To collect crayfish, we set up six baited minnow traps along each stream reach on 6 August 2019. After approximately 24 hours, we checked traps for crayfish. We randomly selected eight crayfish from each reach to transport to the lab for stable isotope analyses. The remaining individuals were released.

To process periphyton samples, we poured slurries into 250 mL beakers and allowed samples to dry at 45°C until water had evaporated. To process FPOM samples, we allowed samples to settle and decanted the water. For CPOM, we washed sediment and macroinvertebrates from each sample and then allowed samples to dry at 60°C for one week. We placed living macroinvertebrates in deionized water at 10°C for 24 hours to allow them to clear their guts. We then identified macroinvertebrates to the family level before further processing (Merritt et al. 2008). We sacrificed crayfish by freezing (-10°C). We removed muscle tissues from the leg and abdomen of individuals for stable isotope analysis to ensure that body lipid content would not influence δ^{13}C measurement (Focken and Becker 1998).

All samples were stored in scintillation vials, frozen at -10°C, and then freeze dried. Samples were homogenized into a fine powder with either a glass rod (periphyton, FPOM, macroinvertebrates, crayfish) or with a ball grinder (CPOM). One to two mg of each sample was encapsulated and analyzed for δ^{13}C and δ^{15}N through isotope ratio mass spectrometry at the Center of Applied Isotope Studies (University of Georgia, Athens, GA).

Data analysis

To estimate the effects of rhododendron removal treatment (ambient or removed), crayfish access (access or exclusion) and the interaction between rhododendron removal treatment and crayfish access on total cell biovolume and chlorophyll-*a* to biovolume ratios (chlorophyll-*a:*biovolume), we constructed linear mixed effects model with stream identity as a random effect. Rhododendron ambient conditions included cell biovolume data from all years (PreYR, CutYR, BurnYR) in the *Control* reach and PreYR in the *Cut+Burn* reach. Rhododendron removal conditions included CutYR and BurnYR in the *Cut+Burn* reach. During analyses, we dropped the interaction term if it was not significant ($p > 0.05$).

We explored potential differences in algal community composition following rhododendron removal and crayfish exclusion using nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarity coefficients using the *metaMDS* function within the *vegan* package in R (Oksanen et al. 2015). The metaMDS function was performed with a Wisconsin double standardization and square-root transformation (Oksanen et al. 2015). To test for potential differences in algal communities, we performed a Permutational Analysis of Variance (PERMANOVA; Anderson 2001) using the *adonis* function within the *vegan* package in R (Oksanen et al. 2015). For the PERMANOVA, we tested for the effects of rhododendron removal treatment, crayfish access, and the interaction between rhododendron removal treatment and crayfish access. We grouped data for rhododendron removal in the same manner as for total algal cell biovolume. In order to understand which taxa may be driving an observed dissimilarity, in the case of a significant effect in the PERMANOVA, we investigated taxa that could be used as indicators for the fixed effect by performing an indicator value analysis using the *multipatt* function within the *indicspecies* package in R (De Caceres and Legendre 2009). For the NMDS, PERMANOVA, and indicator species analyses, we selected the top 20 most abundant taxa in total biovolume, which represented 87.5% of the total algal biovolume across all samples. Since we were interested in potential differences in diatom communities at the reach scale, we aggregated replicate samples by summing cell biovolumes (mm^3 m^{-2}) for each of these 20 taxa across each crayfish exclusion treatment for each stream reach for each sampling date.

To determine potential differences in crayfish δ^{13}C values, we constructed linear models with fixed effects of rhododendron removal treatment (*Cut* or *Cut+Burn*) and stream reach (treatment or upstream/control) and the interaction between rhododendron removal treatment and stream reach, and evaluated differences between crayfish groups with Tukey's HSD post-hoc analyses. We interpreted trends of crayfish δ^{13}C enrichment (increasing δ^{13}C) as indicating increasing reliance on autochthonous basal resources, as algae are typically less depleted in δ^{13}C (*i.e.,* less negative) than terrestrially-derived CPOM in southern Appalachian headwater streams (England and Rosemond 2004). We ran all linear models with the *lme4* package in R (Bates et al. 2015).

In addition to our analysis of crayfish diets based solely on the δ^{13}C values, we also estimated the portion of algae and other potential food resources in the diets of crayfish using a Bayesian stable isotope mixing models with the *IsotopeR* package in R (Ferguson and Hopkins 2016). Prior to modeling the diets of the crayfish, we adjusted the isotope values for δ^{13}C and δ^{15}N for food resources from both rhododendron removal manipulations and both stream reaches to account for fractionation during metabolism (Appendix, Table B.1). We used fractionation values from the literature which were determined using crayfish with diets similar to those of the crayfish in our study. Further, because the periphyton samples we collected were a mix of algae, bacteria, fungi, and fine benthic organic material, and previous studies have shown that pure algae have higher δ^{13}C values than periphyton (England and Rosemond 2004), we added an additional 3.00 δ^{13}C onto the values for periphyton to create "adjusted-periphyton" signatures to represent in-steam primary production. We chose 3.00 δ^{13}C to adjust the periphyton so that our algal signature was similar the pure algal signature from another stable isotope study in a similar region of the southern Appalachians (England and Rosemond 2004). Thus, adjusted-periphyton values (hereafter: algae) represented the algal component of periphyton in mixing model calculations. All analyses for this study were performed in R version 3.6.2 (R Core Team 2019).

Results

*Total algal biovolume and chlorophyll-*a *to cell biovolume ratios*

Total cell biovolume increased following rhododendron removal ($F_{1,33}$ = 8.09, p = 0.008; Fig 2A), but there was not a significant effect of crayfish exclusion on total cell biovolume ($F_{1,33}$ = 2.24, p = 0.14; Fig. 3.2A). The interaction between rhododendron removal and crayfish exclusion was not significant ($F_{1,32}$ = 0.38, p = 0.54; Fig. 3.2A).

Total cell biovolume differed among years and treatments. During PreYR, total cell biovolume was low among all reach-scale rhododendron manipulations (*Cut, Cut+Burn*) and plot-scale crayfish exclusion treatments (access, exclusion; mean 0.07-0.69 mm^3 m^{-2}; Fig. 3.3A). During CutYR and BurnYR, however, total cell biovolume was higher among all rhododendron manipulations and crayfish exclusion treatments (2.23-14.37 mm^3 m^{-2}; Fig. 3.3A) compared to PreYR biovolumes. For CutYR and BurnYR, total cell biovolumes were similar in the crayfish access plots in the *Control* reach and crayfish exclusion plots in the *Cut+Burn* reach (Fig. 3.3A). On the other hand, total cell biovolume was higher for crayfish exclusion plots in the *Control* reach in the CutYR compared to the BurnYR, and total cell biovolume was higher for crayfish access plots in the *Cut+Burn* reach in the BurnYR compared to the CutYR (Fig. 3.3A).

Neither rhododendron removal ($F_{1,30}$ = 0.30, p = 0.58), crayfish exclusion ($F_{1,30}$ = 2.15, p = 0.15), nor the interaction between rhododendron removal and crayfish exclusion ($F_{1,29}$ = 1.61, p = 0.21) had significant effects on chlorophyll-*a* to cell biovolume ratios (Fig. 3.4).

Algal community composition

We identified a total of 129 algal taxa (Appendix, Table B.2). Twenty taxa represented 87.5% of the total cell biovolume across all years and seasons (Table 3.2). All taxa identified in this study were diatoms, with the exception of *Oedogonium* sp., a filamentous chlorophyte, which was abundant in some samples. Diatom communities were dominated by acidophilic taxa that prefer low nutrient and low conductivity waters (*e.g., Eunotia* spp., *Meridion alansmithii, Frustulia rhomboides*). We identified at least four species that are thought to be endemic to the southern Appalachians Mountains: *Eunotia bilii, Eunotia rushforthii*, *Eunotia papilioforma*, and *Meridion alansmithii*.

Algal communities from control treatments (*i.e.,* rhododendron ambient, crayfish access) were dominated by diatoms with high growth forms (~60% relative abundance, Fig. 2B). When either rhododendron was removed *or* crayfish were excluded, *Oedogonium* increased in relative biovolume and all diatom growth form categories decreased in relative biovolume (Fig. 3.2B). When rhododendron was removed *and* crayfish were excluded, communities were dominated by diatoms with high growth forms (~70% relative biovolume, Fig. 3.2B). Trends in algal functional groups were similar when separated by years and treatments, but *Oedogonium* was more abundant within crayfish exclusion plots in the *Control* reach in CutYR compared to BurnYR (Fig. 3.3B).

Algal communities differed following rhododendron removal (PERMANOVA, $F_{1,9}$ = 3.22, R^2 = 0.25 , p = 0.004; Fig. 3.5A). Generally, chain- and stalk-forming diatoms with high growth forms (*Gomphonema* spp., *Encyonema minutum*) and *Oedogonium* increased in relative biovolume following rhododendron removal, while diatoms with low-lying growth forms (*Eunotia incisa, Nupela lapidosa*) decreased in relative biovolume (Table 3.2). Crayfish exclusion did not have a significant effect on algal communities (PERMANOVA, $F_{1,9}$ = 0.70, R^2 = 0.05, p = 0.70), but relative NMDS space was larger following crayfish exclusion, potentially indicating higher diversity (Fig. 3.5B). The interaction between rhododendron removal and crayfish exclusion was not significant (PERMANOVA, $F_{1,8}$ = 0.91, R^2 = 0.07 , p = 0.49). Indicator species analysis identified eight taxa that were significantly associated with rhododendron removal: *Gomphonema parvulum, Synedra rumpens* var. *fragilarioides, Fragilariforma virescens, Gomphonema gracile, Meridion circulare* var. *constrictum, Encyonema minutum, Eunotia bilunaris,* and *Gomphonema affine* var. *rhombicum.* Generally, these taxa increased in relative biovolume with rhododendron removal (Table 3.2). Notably, all seven of these are diatoms that possess high growth forms.

Crayfish diets

There was a significant interaction in crayfish δ^{13}C values between rhododendron removal treatment (*Cut*, *Cut+Burn*) and stream reach (treatment and control/upstream; $F_{1,28}$ = 20.35, p < 0.001; Table 3.3). Crayfish from the *Cut+Burn* stream were significantly less depleted in δ^{13}C than crayfish from the *Cut* stream ($F_{1,28}$ = 99.43, p < 0.001), possibly indicating a greater reliance of crayfish algal resources in the *Cut+Burn* reach compared to the *Cut* reach. The difference between reaches (treatment and control/upstream) was approaching significance ($F_{1,28}$

= 3.31, $p = 0.08$). Tukey's post hoc tests indicated that *Cut+Burn*-treatment crayfish were significantly less depleted in δ^{13}C than *Cut+Burn*-upstream crayfish ($p < 0.001$; Table 3.3); again, possibly indicating a greater reliance on algal resources of the *Cut+Burn*-treatment crayfish compared to the *Cut+Burn*-upstream crayfish. There was not a significant difference in δ^{13}C values between *Cut*-treatment and *Cut*-upstream crayfish ($p = 0.249$, Table 3.3).

Our mixing model results indicated qualitatively similar results as our analysis of crayfish δ^{13}C values. Mixing model results showed that all crayfish in this study relied heavily on algal (~30-60%) and macroinvertebrate (~20-50%) food resources, and less on CPOM (~5-10%) and FPOM (~10-20%); however, confidence intervals were high for all estimates (Fig. 3.6). *Cut+Burn*-treatment crayfish relied more heavily on algal (~45%) and less on macroinvertebrate (~25%) food resources than *Cut+Burn*-upstream crayfish (~30% and 50%, respectively; Fig. 3.6B). Crayfish from the *Cut*-treatment relied heavily on algal food resources (~55-60%; (Fig. 3.6A), and otherwise there was little difference in mixing model results between *Cut*-treatment and *Cut*-upstream crayfish.

Discussion

Our results provide insight into the mechanism by which small decreases in total canopy cover can alter algal communities, and potentially affect trophic pathways, in forested headwater streams. Contrary to our first prediction, we did not detect an interaction between riparian rhododendron removal and crayfish exclusion on total algal cell biovolume or algal community composition. However, we found that rhododendron removal increased total cell biovolume and resulted in a shift in algal community composition from primarily high-profile diatoms forming ribbon colonies (*i.e., Eunotia* spp.) to high-profile, stalk and chain-forming diatoms (*i.e.,* *Gomphonema* spp., *Encyonema* sp.) and a filamentous chlorophyte (*Oedogonium* sp.). On the other hand, crayfish had patchy, non-significant effects on total algal cell biovolume and algal community composition, indicating that crayfish feed upon algal communities indiscriminately in this region. Contrary to our second prediction, we did not find an effect of rhododendron removal on chlorophyll-*a* to cell biovolume ratios. In partial support of our third prediction, we found some evidence that crayfish were more dependent on algal resources in streams where rhododendron is removed, but this effect may depend on watershed characteristics, such as stream orientation. Findings here suggest that large-scale removal of rhododendron in the southern Appalachian headwater streams would result in increases in the relative abundance of filamentous green algal taxa and potentially in stream food webs becoming more autochthonous, atleast in the short-term.

Our results suggest that removal of riparian rhododendron, while only resulting in small decreases in total riparian cover (6.2-6.9%; Raulerson et al. 2020), had significant effects on algal communities due to increases in the number of short periods of elevated light to the stream. While total canopy loss following rhododendron removal was relatively low, canopy gaps were created in locations where removal of understory rhododendron coincided with loss of upper story hemlock (Fig. 3.1C). In turn, these canopy gaps resulted in specific locations in the stream where light and temperature were elevated (*i.e.*, "sunflecks" Raulerson et al. 2020). Sunflecks, or brief, often unpredictable periods of direct solar irradiance, are important to in-stream autotrophy in forested streams (Heaston et al. 2017), and thus increases in sunflecks created by rhododendron removal were likely responsible for our findings of increased total algal biovolumes (Fig. 3.2A) and shifts in algal community composition (Fig. 3.4A) following removal. Our findings are supported by studies in forested headwater streams in the Pacific

Northwest which have found that "patchy" light availability (rather than uniform light availability) can significantly affect periphyton accrual (Heaston et al. 2017) and alter the co-limitation of light and nutrients by algal communities (Warren et al. 2017) at the reach scale. Thus, our results provide insight into the mechanism by which small decreases in total canopy cover can significantly affect algal communities.

Our findings add to the body of research indicating that light availability is a primary control on algal communities in forested headwater streams (Hansmann and Phinney 1973, Lowe et al. 1986, Naymik and Pan 2005, Bixby et al. 2009), and identify potential indicator algal species of decreases in canopy cover in this region. Notably, a study conducted at the Coweeta Hydrologic Laboratory nearly 30 years prior to our study found remarkably similar shifts in algal community composition following a forest clear-cut (Lowe et al. 1986). Similar to our study, Lowe et al. (1986) found lower relative biovolumes of *Eunotia* spp., and higher relative biovolume of stalk- and chain-forming diatoms and a filamentous chlorophyte (*Spirogyra* sp.) following the clear-cut. In fact, three of the eight algal indicator species for rhododendron removal in this study were also found to increase in relative biovolume following the clear-cut in the Lowe et al. (1986) study (*Gomphonema parvulum, Encyonema minutum, Eunotia bilunaris).* Likewise, Sullivan (2018) found *G. parvulum* and *E. minutum* to be strongly associated with suburban sites in the Nantahala region the southern Appalachians. Thus, researchers in rhododendron-dominated areas of the southern Appalachians could potentially use the relative abundances of *G. parvulum, E. minutum, or* chlorophytes as indicators of communities affected by increased canopy openness and/or urbanization.

Rhododendron removal resulted in an increase in the relative biovolumes of stalk-forming, chain-forming and filamentous algal taxa, as well as an increase in total algal cell biovolume, and this shift in algal communities could have potential effects on stream ecosystem function. Increases in canopy gaps and thus light availability likely allowed for the vertical development of algal communities, favoring stalk- and chain- forming taxa with "high" growth forms (*Gomphonema* spp*., Encyonema minutum*, *Fragilariforma virescens*) and filamentous taxa (*Oedogonium* sp.; Lowe et al. 1986; Steinman et al. 1992; Bixby et al. 2009). *Oedegonium* is free-living and sometimes epiphytic on submerged plants (John 2015), and this taxon can be abundant along streams in this region (S. Wilde, pers. comm.). While scrapers (*e.g.,* snails) preferentially graze on diatoms with low growth forms (Steinman et al. 1992, Rosemond 1993), collector-gatherers (*e.g.,* Chironomidae, crayfish) have been shown to preferentially graze on filamentous and upright taxa (Creed 1994, Evans-White et al. 2003). Thus, shifts in algal growth forms due to rhododendron removal could have important consequences for some stream herbivores.

Indeed, our stable isotope analyses indicate that algae is likely an important food resource for crayfish, and crayfish may become more reliant on algal resources with rhododendron removal; however, this effect may depend on watershed characteristics. Our mixing models indicate that algal resources likely comprise a significant fraction of crayfish diet (~30-60%; Fig. 3.6), suggesting that effects of crayfish on algal communities are at least partially due to consumption. Our results are supported by England and Rosemond (2004), which found that consumers (crayfish and fish) relied heavily on primary producers as food resources in forested headwater streams in north Georgia, and that small decreases in canopy cover can increase crayfish reliance on autochthonous resources. Other studies outside of the southern Appalachians have also indicated that algal resources are important to consumers, even in heavily shaded streams (March and Pringle 2003, Rosi-Marshall et al. 2016, Crenier et al. 2017, Erdozain et al.

2019). In addition, we found some evidence that riparian rhododendron removal may increase crayfish dependence on algal food resources, as indicated by the significant increase in δ^{13}C in *Cut+Burn*-treatment crayfish relative to *Cut+Burn*-upstream crayfish (Table 3.3), and by the mixing model results (Fig. 3.6).

Interestingly, we did not find evidence that crayfish in the *Cut* stream became more dependent on algal resources under rhododendron removal conditions, and this lack of change may be due to different watershed characteristics between the *Cut* and *Cut+Burn* reaches. Raulerson et al. (2020) found that rhododendron removal had a greater effect on daily maximum temperatures in the *Cut+Burn* stream (2.61°C) than the *Cut* stream (0.86°C). The authors attributed this difference in effect to stream orientation, as the *Cut+Burn* stream is south-facing while the *Cut* stream is north-facing. Thus, differences in watershed orientation between the *Cut* and *Cut+Burn* reaches also may have resulted in different effects on algal communities due to differences in light availability. However, more research is needed to understand the conditions under which riparian vegetation removal could shift the relative importance of food resources to macroconsumers, and whether stream orientation can have significant effects on algal communities.

We found that crayfish did not have significant effects on algal community composition, indicating that crayfish feed on diatom-dominated algal communities indiscriminately. In streams where chlorophytes are more abundant, filamentous green algae may be more important in crayfish diets than diatoms, potentially because crayfish maxillae are more efficient at harvesting filamentous and upright algal taxa (Evans-White et al. 2003). In our study, crayfish exclusion significantly increased the total biovolume of *Oedogonium* sp. in some plots; however, this effect was patchy, and likely occurred where crayfish exclusion plots coincided with areas of high canopy loss, allowing the vertical development of algal communities. Thus, in forested headwater streams of the southern Appalachians where diatoms typically dominate the epilithic algal communities, crayfish maxillae likely indiscriminately remove algal cells (Keller and Ruman 1998). Our results are supported by two other studies in the region which found that exclusion of macroconsumers (including *Cambarus sp.*) did not have significant effects on algal communities in forested streams (Dye 2005, Schofield et al. 2008). While Schofield et al. (2008), did find that exclusion of macroconsumers increased the relative biovolumes of high taxa (*e.g., Synedra, Cymbella, Melosira*) and decreased the relative biovolumes of low taxa (*e.g., Achnanthes, Achnanthidium*), this effect was only apparent at the suburban and urban sites, and not at the forested sites. Thus, crayfish in forested headwater streams likely feed on diatom-dominated communities indiscriminately, but when algal communities have higher proportions of filamentous chlorophytes, crayfish may have significant negative effects of the biovolumes of filamentous and upright algal taxa.

Although both chlorophyll-*a* and total biovolume are often used as proxies for algal biomass, we found that rhododendron removal and crayfish exclusion had different effects on total cell biovolume than chlorophyll-*a,* which may be explained by varying chlorophyll-*a* to biovolume ratios among algal taxa. While we found that rhododendron removal increased total cell biovolume, Dudley et al. (2021) did not find significant effects of rhododendron removal on chlorophyll-*a*, but did find a significant interaction between crayfish exclusion and rhododendron removal during the BurnYR only. A potential explanation for different responses of chlorophyll-*a* and total cell biovolume to treatment effects is changing chlorophyll-*a* concentrations per cell. Other algal studies have found greater chlorophyll-*a* per cell in low light environments (Rosemond 1993, Felip and Catalan 2000), so we might expect lower chlorophyll-*a* per cell under rhododendron removal conditions. However, we did not find significant effects of rhododendron removal, crayfish exclusion, or the interaction between the two effects on chlorophyll-*a* to biovolume ratios (Fig. 3.4). We generally found higher chlorophyll-*a* to biovolume ratios when diatoms with high growth forms made up >50% of relative biovolume (Fig. 3.3B, Fig. 3.4). Indeed, Felip and Catalan (2000) found that the relationship between chlorophyll to phytoplankton biovolume in an oligotrophic lake varied depending on the phytoplankton group that was dominant. Thus, algal community composition could partially explain different responses of chlorophyll-*a* and total cell biovolume to treatment effects in this study. However, more work is needed to better understand factors controlling chlorophyll-*a* to biovolume ratios in headwater streams, and we urge researchers, when possible, to use multiple proxies for algal biomass, and not rely solely on chlorophyll-*a*.

One limitation of our study is our assumption that increased light availability (*i.e.* using canopy cover as a proxy) was the primary factor responsible for shifting algal community composition and cell biovolume following rhododendron removal. It is possible other factors associated with riparian vegetation removal *(e.g.,* changes in sediment input or nutrient concentrations) were at play. However, several lines of evidence suggest that increases in light availability following rhododendron removal was primarily responsible for changes in algal communities described in this study. First, increases in summer stream temperatures following rhododendron removal provide strong evidence that light availability increased to treatment reaches during the summer (Raulerson et al. 2020). Second, while riparian vegetation removal has been known to increase stream nutrient concentrations (Clinton 2011, Larson et al. 2019), rhododendron removal in this study actually resulted in a decrease of NO_3-N stream concentrations (Elliott and Miniat 2021). Third, Dudley et al. (2021) did not find any predictor variables potentially associated with rhododendron removal (*i.e.,* sediment, FPOM, nitrate, ammonium, phosphate) to be related to chlorophyll-*a*, providing some evidence that these factors also would not relate to algal community composition or total cell biovolume. However, increased sediment following the rhododendron cut (Dudley et al. 2021) may be partially responsible for the observed increase in motile diatom taxa (*e.g., Nitzschia* spp.) in the *Cut+Burn* reach during CutYR. Overall, these studies support our claim that increased light availability was likely the primary factor driving changes in total cell biovolume and algal community composition following rhododendron removal.

Our study highlights how small decreases in total riparian cover can affect algal abundance and community composition and potentially alter trophic pathways in forested headwater streams. We would expect large-scale removal of riparian rhododendron in the southern Appalachians to significantly increase total cell biovolume and increase the proportion of chain- and stalk-forming diatoms and filamentous chlorophytes. Increased total cell biovolume could have significant impacts on stream ecosystem function by resulting in food webs that become more autochthonous. Additionally, our findings have implications for removal of dominant understory riparian vegetation on algal communities in areas outside of the southern Appalachians.

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Appendix B: Supplementary Data

Table 3.1: Characteristics of the study reaches

Stream	Rhododendron removal treatment	Drainage area at bottom of study reach (km^2)	Elevational range of study m reach (m)	Elevational range of watershed (m)	Average bankfull channel width (m)	Mean channel slope from LiDAR	% Forest, 2006 NLCD
Holloway Branch	*Cut*	2.91	1160-1210	1024-1625	3.69	2.66%	98.2
Kit Springs Branch	*Control*	0.93	1255-1315	1170-1627	4.53	3.52%	99.6
Split Whiteoak Branch	*Cut+Burn*	1.76	1195-1265	1149-1451	3.42	2.53%	100.0

Table 3.2: Algal taxa used for NMDS, PERMANOVA, and indicator species analysis along with abbreviation used from NMDS, functional group, and mean relative biovolume (%) separated by plot-scale crayfish exclusion treatments (where A= ambient, E=exclusion) nested within reach-scale rhododendron removal manipulation (where Ambient = rhododendron ambient or Removed = rhododendron removed).These twenty taxa represent 87.5% of the relative biovolume of all taxa identified in this study (129). Taxa are in descending order of proportion of total biovolume across all dates and treatments (*i.e.,* most abundant at the top). Taxa denoted with an asterisk (*) are significantly associated with rhododendron removed treatments via indicator species analysis. N= 12 for "rhodo ambient" values; n= 6 for "rhodo removed" values.

			Mean relative abundance (%)			
			Ambient		Removed	
Algal Taxon	Abbreviation	Functional group	A	E	A	E
Oedogonium sp.	Oed-sp.	filamentous	01.40	06.00	16.97	00.31
Eunotia papilioforma	E-pap.	low	08.61	04.51	25.75	15.48
*Gomphonema parvulum**	G-par.	high	01.11	00.35	01.75	11.00
*Synedra rumpens var. fragilarioides**	S-rum.	high	00.74	00.71	00.61	03.35
*Fragilariforma virescens**	F-vir.	high	02.00	07.60	03.95	04.91
*Frustulia rhomboides**	F-rho.	high	10.61	07.16	03.23	02.71
Meridion alansmithii	M-ala.	high	05.53	06.35	02.65	03.02
Eunotia minor	E-min.	high	07.00	05.37	07.77	05.76
Eunotia cf. *minor*	E-cfm.	high	08.75	07.72	04.16	04.29
*Gomphonema gracile**	G-gra.	high	00.15	00.09	00.31	06.26
Eunotia incisa	E-inc.	low	06.63	11.79	03.52	02.66
Eunotia metamondon	E-met.	high	17.66	11.75	01.02	03.60
Odontidium mesodon	O-mes.	high	03.68	03.59	00.81	00.88
Pinnularia erratica	P-err.	motile	06.04	02.44	02.56	01.45
Meridion circulare var. *constrictum**	M-cir.	high	00.64	00.66	00.89	02.75
Gomphonema exilissimum	G-exi.	high	00.01	00.24	00.14	02.13
*Encyonema minutum**	En-min.	high	00.41	00.10	00.79	03.93
Nupela lapidosa	N-lap	low	04.25	05.34	02.28	02.98
*Eunotia bilunaris**	E-bil.	high	00.40	00.56	00.81	01.08
Gomphonema affine var. *rhombicum* *	G-aff.	high	00.00	00.00	00.02	00.87

Table 3.3: Mean δ^{13}C values for crayfish in each rhododendron removed treatment reach (*Cut*, *Cut+Burn*) and upstream (*i.e.,* control) reaches. Letters represent significant differences based on Tukey's post-hoc analyses. Values in parentheses represent standard errors. N=8.

Rhododendron removal treatment	Stream reach	δ^{13}C
Cut	Treatment	-25.32 (0.14)^{a}
	Upstream	-25.04 (0.12)^{a}
Cut+Burn	Treatment	-23.84 (0.04)^{c}
	Upstream	-24.49 (0.07)^{b}

Figure Legends

Fig. 3.1: Schematic of southern Appalachian riparian zones through time, including rhododendron removal manipulations. A) Pre-hemlock die-off, *ca*. 2000. Riparian upper story species are characterized by deciduous trees (*e.g.,* maples and tulip poplars) and eastern hemlock. Riparian understory is dominated by rhododendron. B) Post-hemlock die-off, *ca.* 2010. Eastern hemlock have lost needles but dead trunks are still standing. Rhododendron has expanded. C) Post-rhododendron removal, *ca*. 2015. Decrease in total canopy cover is low (~3-7%), but canopy gaps occur where understory rhododendron removal coincides with upper story hemlock death. D) Potential future forest scenario following rhododendron removal, *ca.* 2030. Following rhododendron removal, deciduous trees have begun to recruit and fill canopy gaps left by hemlock die-off.

Fig. 3.2: (A) Total cell biovolume (mm^3 m^{-2}) and (B) relative cell biovolume of filamentous algae and diatoms (where diatoms are categorized by functional group) for plot-scale crayfish exclusion experiments (where A = Access of crayfish and E = Exclusion of crayfish) nested within reach-scale rhododendron manipulations (where Ambient = rhododendron ambient and Removed = rhododendron removed). For boxplots, lines represent medians, boxes represent first and third quartiles, whiskers represent 1.5*IQR. For rhododendron ambient boxplots, n=12, and for rhododendron removed boxplots, n=6.

Fig. 3.3: (A) Mean total cell biovolume (mm^3 m^{-2}) and (B) relative cell biovolume of filamentous algae and diatoms (where diatoms are categorized by functional group), separated by year and reaches, for plot-scale crayfish exclusion experiments (where A = Access of crayfish

and E = Exclusion of crayfish) nested within reach-scale rhododendron manipulations (where Ambient = rhododendron ambient and Removed = rhododendron removed). Within each year, left two bars represent the *Control* reach and right two bars represent the *Cut+Burn* reach. For each bar, n=3.

Fig. 3.4: Ratio of chlorophyll-*a* (Dudley et al. 2021) to total cell biovolume (this study), separated by year and reaches, for plot-scale crayfish exclusion experiments (crayfish access [dark grey boxes] and crayfish exclusion [light grey boxes]) nested within reach-scale rhododendron manipulations (where Ambient = rhododendron ambient and Removed = rhododendron removed). Within each year, left two bars represent the *Control* reach and right two bars represent the *Cut+Burn* reach. For boxplots, lines represent medians, boxes represent first and third quartiles, whiskers represent 1.5*IQR. For each boxplot, n=3.

Fig. 3.5: Non-metrical multidimensional scaling plot (stress = 0.06) for the 20 most abundant algal taxa (red letters) based on of total cell biovolume (mm^3 m^{-2}). (See Table 1 for full taxonomic name of algal taxa associated with red letters.) Black symbols represent algal community composition for each of the four treatment types (crayfish access, rhododendron ambient; crayfish excluded, rhododendron ambient; crayfish access, rhododendron removed; crayfish excluded, rhododendron removed) by year. Polygons in (A) encompass algal samples within experimental stream reaches where riparian rhododendron was removed (*removed*) or left intact (*ambient*). Polygons in (B) encompass algal samples within plot-scale crayfish *exclusion* and *access* treatments nested within reach-scale rhododendron removal treatments.

Fig. 3.6: Mixing model results for each reach (treatment [dark grey] and upstream [light grey]) within the two streams where rhododendron was removed ([A] Rhododendron removed - *Cut* and [B] Rhododendron removed - *Cut+Burn*) for the four crayfish food resources: algae, CPOM, FPOM, and macroinvertebrates (macros). Points represent means and bars represent 25% CI.

Fig. 3.1

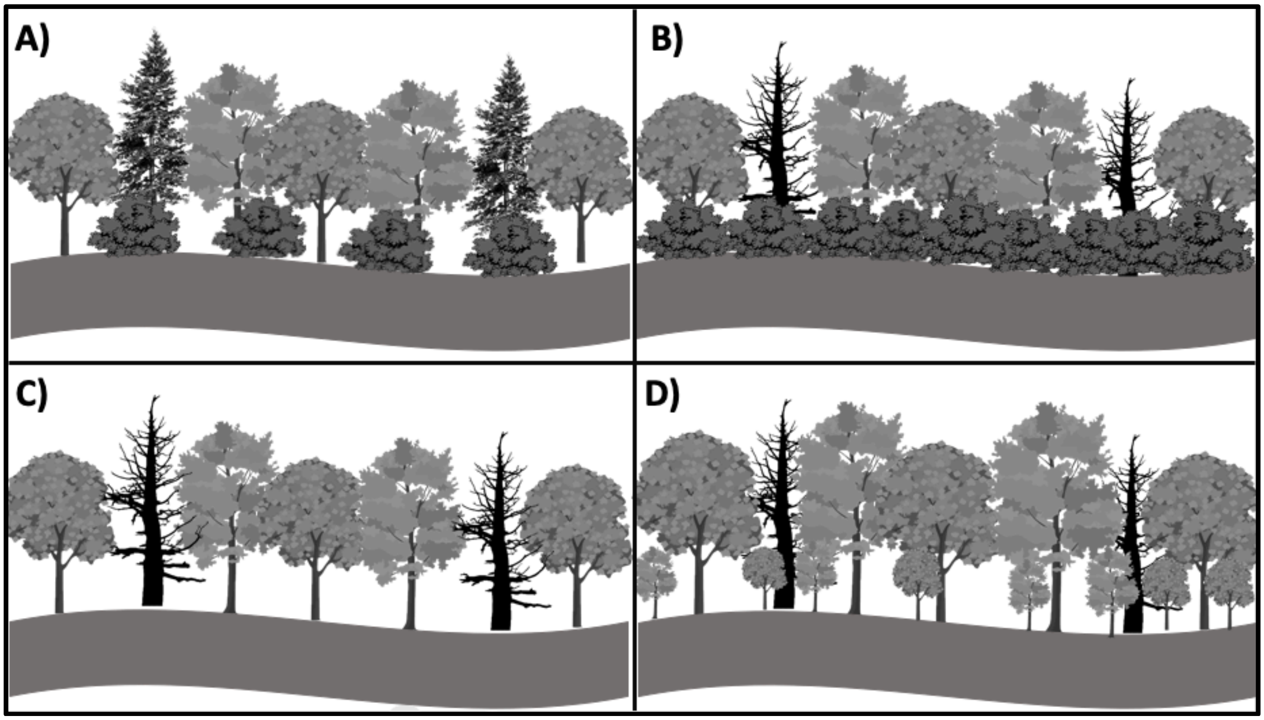


Fig. 3.2

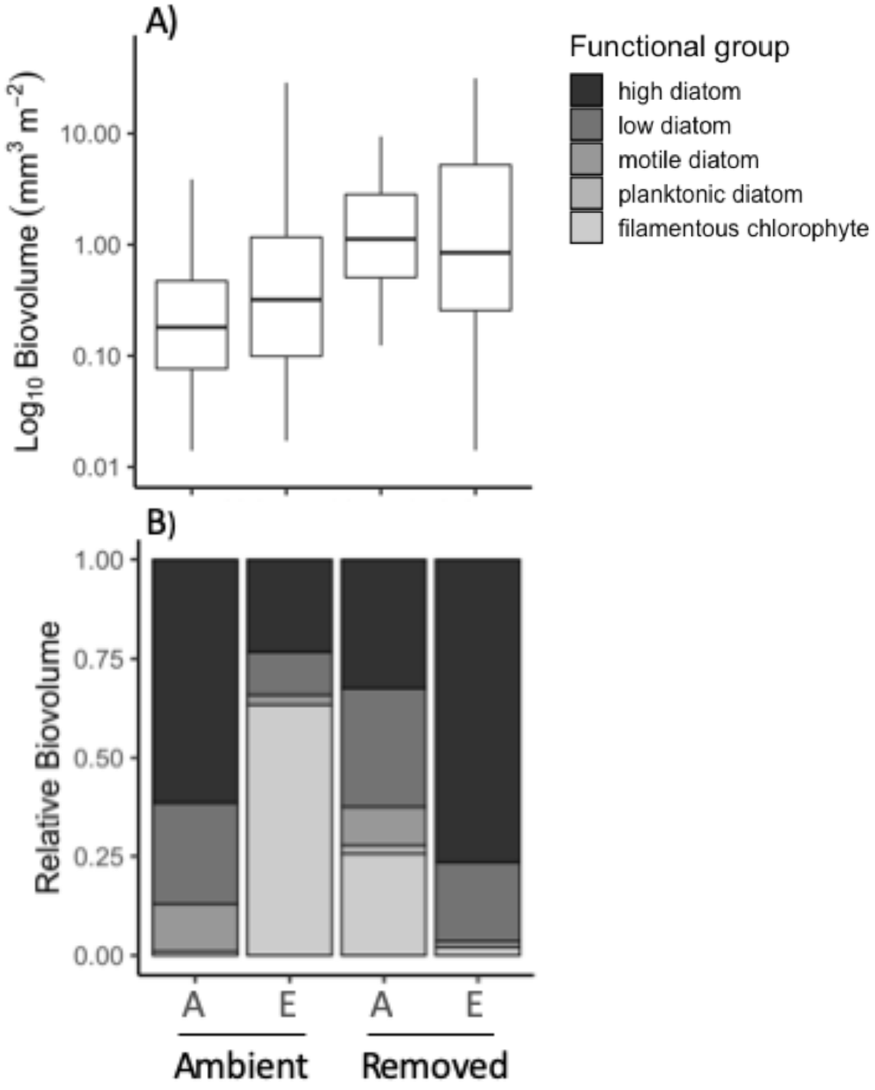

A)
Log10 Biovolume (mm3 m−2)
10.00
1.00
0.10
0.01
B)
Relative Biovolume
1.00
0.75
0.50
0.25
0.00
A
E
A
E
Ambient
Removed
Functional group
high diatom
low diatom
motile diatom
planktonic diatom
filamentous chlorophyte

Fig. 3.3

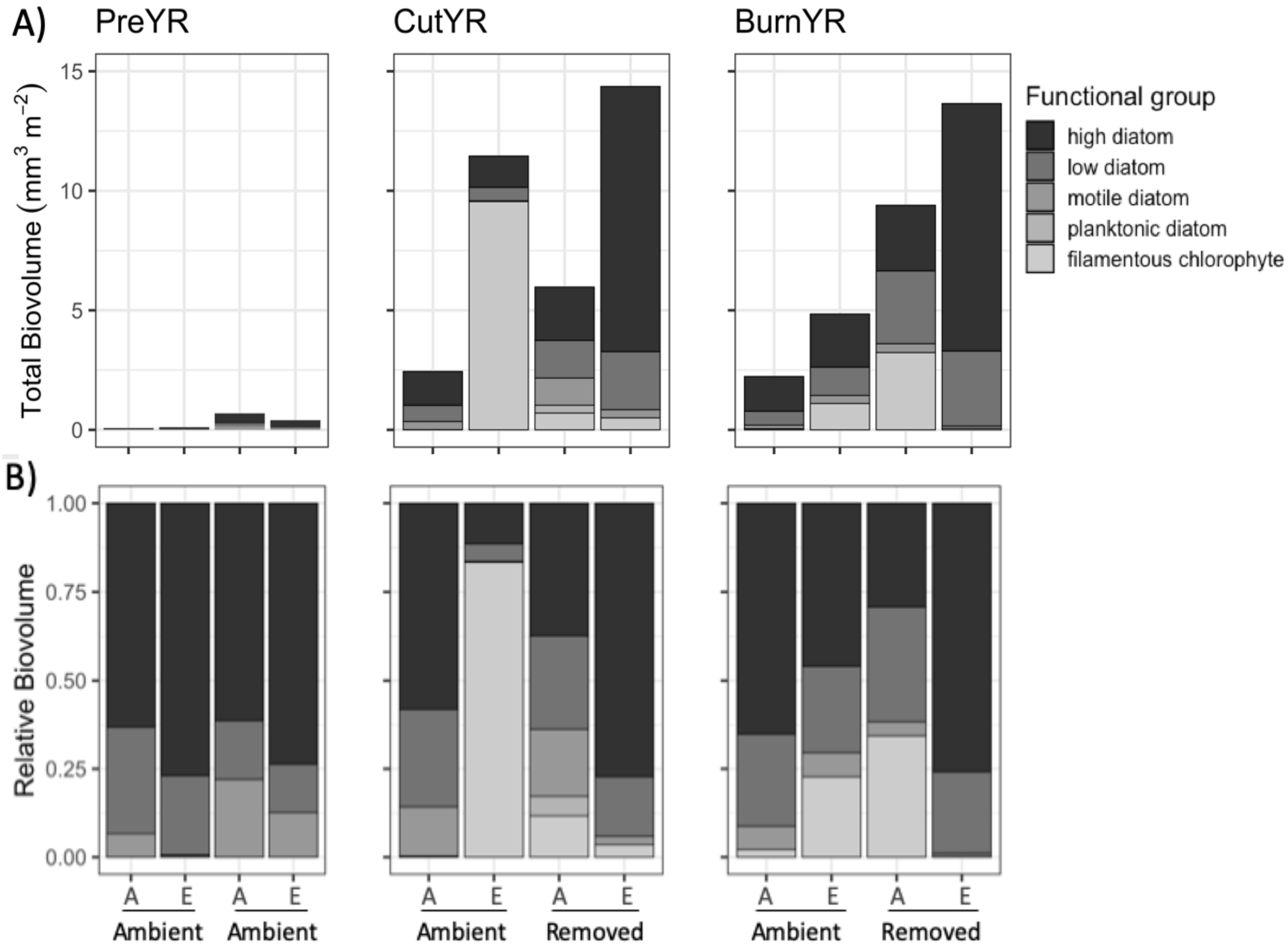

A)
PreYR
CutYR
BurnYR
Total Biovolume (mm^3 m^{-2})
15
10
5
0
Functional group
high diatom
low diatom
motile diatom
planktonic diatom
filamentous chlorophyte
B)
Relative Biovolume
1.00
0.75
0.50
0.25
0.00
A
E
A
E
Ambient
Ambient
A
E
A
E
Ambient
Removed
A
E
A
E
Ambient
Removed

Fig. 3.4

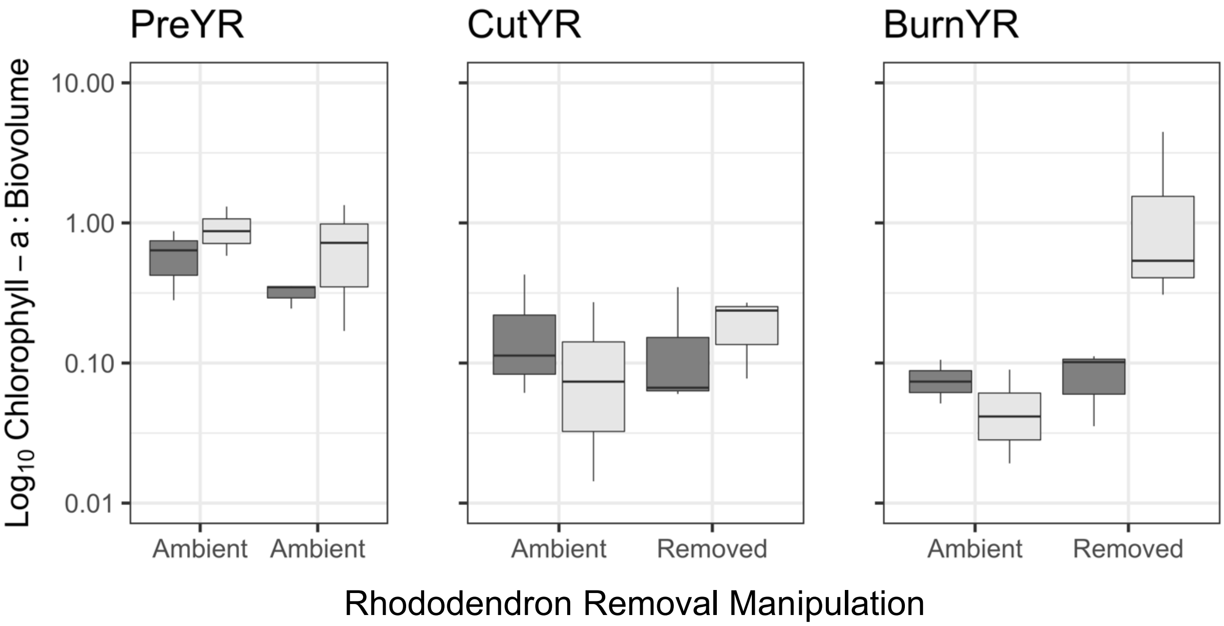

PreYR
CutYR
BurnYR
Log10 Chlorophyll – a : Biovolume
10.00
1.00
0.10
0.01
Ambient
Ambient
Ambient
Removed
Ambient
Removed
Rhododendron Removal Manipulation

Fig. 3.5

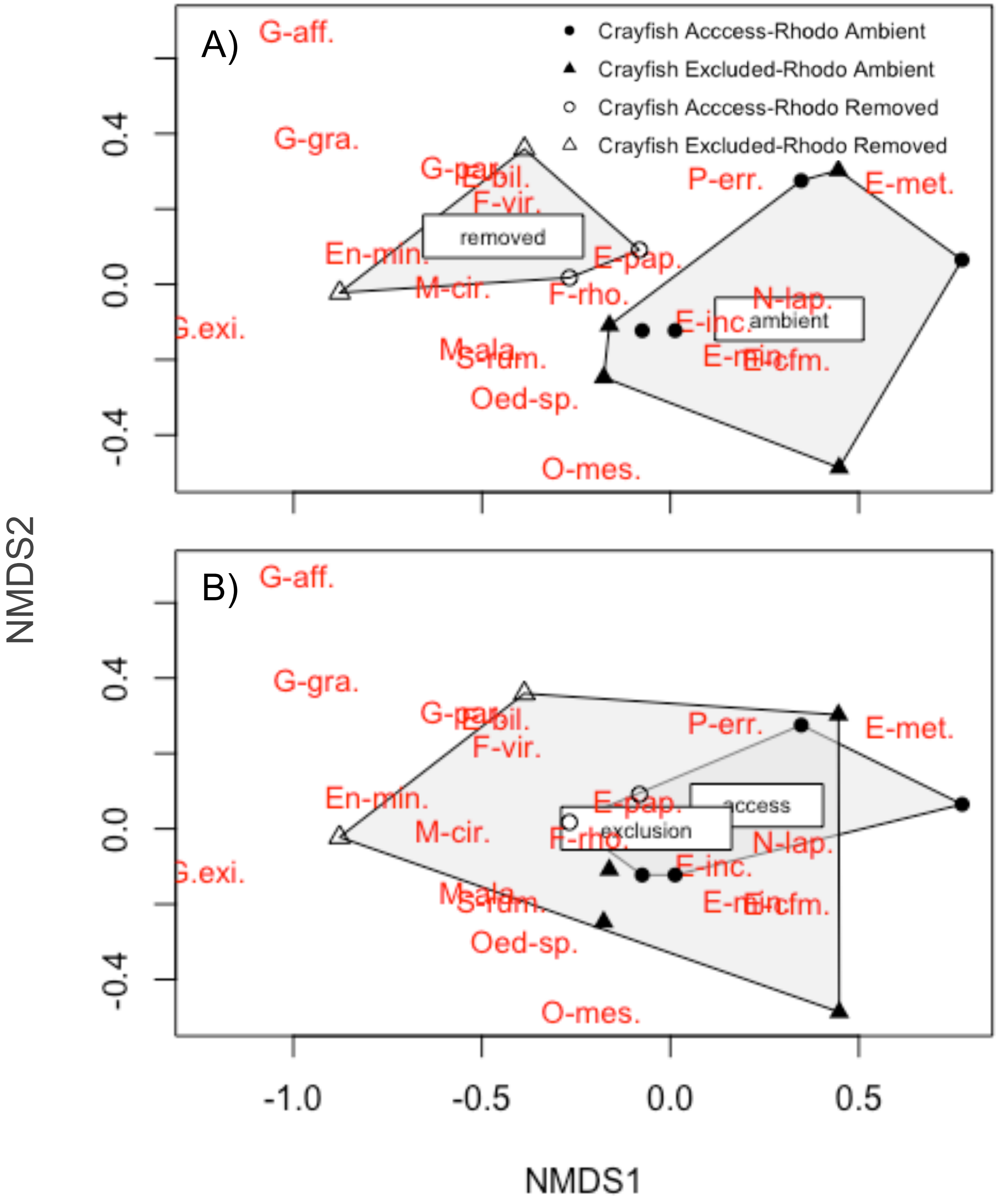
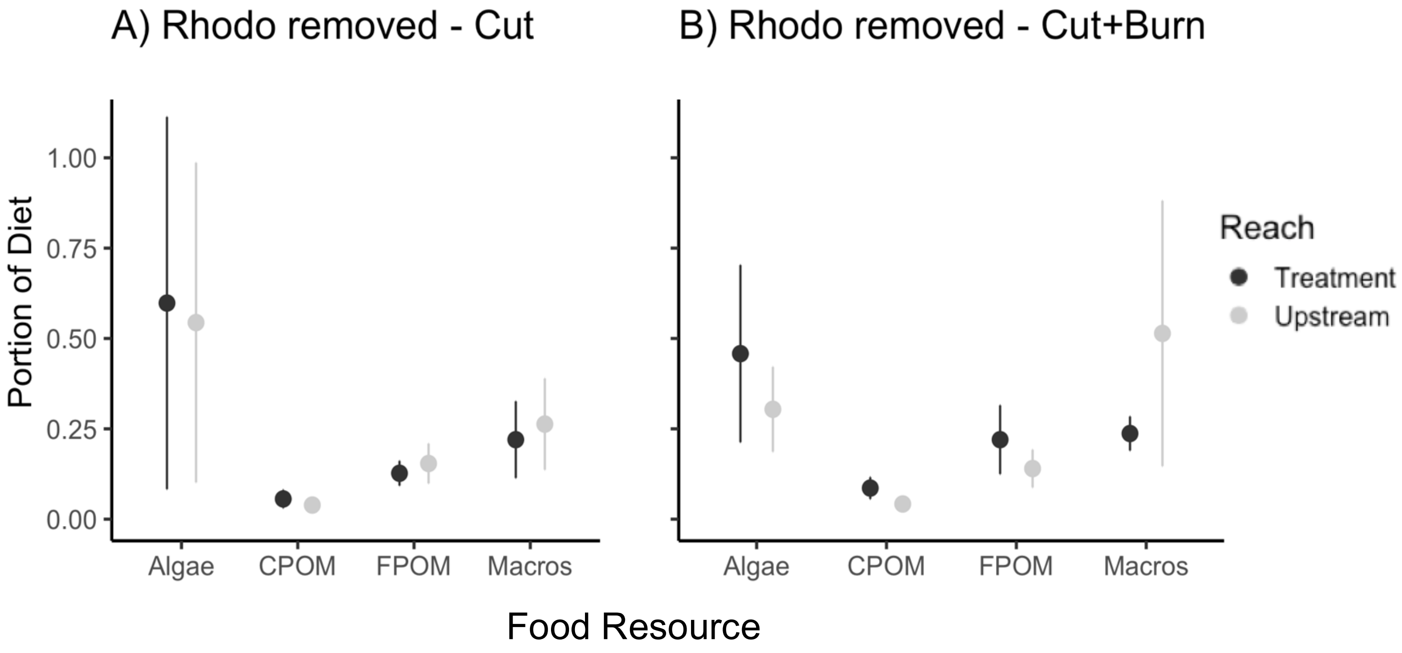

A)
B)
● Crayfish Acccess-Rhodo Ambient
▲ Crayfish Excluded-Rhodo Ambient
○ Crayfish Acccess-Rhodo Removed
△ Crayfish Excluded-Rhodo Removed
removed
ambient
access
exclusion
G-aff.
G-gra.
G-par.
E-bil.
F-vir.
En-min.
M-cir.
E-pap.
F-rho.
P-err.
E-met.
N-lap.
E-inc.
E-min.
E-cfm.
M-ala.
S-rum.
Oed-sp.
O-mes.
.exi.
0.4
0.0
-0.4
-1.0
-0.5
0.0
0.5
NMDS2
NMDS1

Fig. 3.6


A) Rhodo removed - Cut
B) Rhodo removed - Cut+Burn
Portion of Diet
1.00
0.75
0.50
0.25
0.00
Algae
CPOM
FPOM
Macros
Food Resource
Reach
Treatment
Upstream

CHAPTER 4

EVIDENCE FOR EARLY STAGES OF BIOTIC HOMOGENIZATION IN ALGAL AND FISH COMMUNITIES IN STREAMS DRAINING SUBURBANIZING LANDSCAPES IN THE SOUTHERN APPALACHIAN MOUNTAINS

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Abstract

In aquatic ecosystems, urbanization is driving both community change and biotic homogenization, a process characterized by distinct biotic communities becoming more similar to one another over time. Two limitations of studies that have characterized effects of urbanization on stream biota are focusing only on regions with high-density development, rather than low-density development, and/or using *space-for-time substitution*, rather than characterizing biota through time. Here, we ask the questions: 1) how do stream biotic communities in reference forested watersheds differ from biotic communities within suburbanizing watersheds that are experiencing increasing low-density development in rural landscapes? and (2) how have these biotic communities changed over time within forested watersheds and within suburbanizing watersheds? To address these questions, we examined stream algal and fish communities in terms of alpha diversity, beta diversity, and taxonomic composition using a *repeated snapshot* approach at three points in time over a ten-year period (2000, 2005, 2010) in forested (n = 4) and suburbanizing (n = 4) watersheds within the southern Appalachian landscape. We found higher alpha diversity and different taxonomic composition within both algal and fish communities in suburbanizing watersheds compared to forested watersheds. Algal communities in suburbanizing watersheds were characterized by a higher relative abundance of *Achnanthidium rivulare* and motile taxa (*e.g., Nitzschia* spp. and *Navicula* spp.), compared to forested watersheds. Fish communities within suburbanizing watersheds were characterized by a lower relative abundance of *Cottus bairdi* (mottled sculpin), an endemic taxon, and higher relative abundances of two cosmopolitan taxa (*e.g., Rhinichthys atratulus* [blacknose dace] and *Semotilus atromaculatus* [creek chub]), compared to forested watersheds. For algal communities, we found lower beta diversity within suburbanizing reaches in 2005 and

2010 compared to 2000, and significant differences in taxonomic composition among years within both forested and suburbanizing watersheds. However, we found little evidence of change over time within fish communities. Our findings suggest that both algal and fish communities in streams within suburbanizing watersheds in southern Appalachia are in the early stages of biotic homogenization, but have not yet reached the damaging level of urbanization that would extirpate endemic taxa.

Introduction

The rapid growth of cities is pervasive across the globe and is accelerating in response to increasing human population and migration to urban areas (Chen et al. 2014, Scheuer et al. 2016). Urbanization tends to homogenize the physical conditions of terrestrial and aquatic environments, as cities are built to meet the relatively narrow needs of human societies (McKinney 2006). Habitat homogenization resulting from urbanization often drives biotic homogenization, a process characterized by the gradual replacement of regionally distinct, native species with cosmopolitan, non-native species (Olden 2006). Biotic homogenization resulting from urbanization has been documented for a variety of organisms, including birds (Marzluff 2001), terrestrial plants (Decandido et al. 2004, Bertin 2013, Palma et al. 2017), insects (Knop 2016), and freshwater fishes (Scott 2006). In stream ecosystems, watershed urbanization often results in lower alpha diversity and lower relative abundances of sensitive taxa for both unicellular (*e.g.,* benthic algae) and multicellular (*e.g.,* fish) organisms (Walsh et al. 2005). While biotic homogenization as a result of watershed urbanization has been documented for stream fish communities (Scott 2006, Erős et al. 2020), evidence for homogenization of species in stream benthic algal communities is limited (Dunck et al. 2019). As urbanization becomes more widespread, we need to understand how to best detect, quantify, and mitigate the effects of biotic homogenization and community change before native species are lost.

A major limitation of studies that have characterized the biotic effects of urbanization is their dependence on *space-for-time substitution*, where data from watersheds in different stages of urbanization are used to infer temporal trends (Godwin et al. 2009, O'Brien and Wehr 2010), rather than to characterize changes in biota through time. In general, characterizing biota through time has proven difficult since: (1) spatial and temporal scales of data collection are usually not designed to detect long term trajectories of community change in urbanizing areas (Gido et al. 2010); (2) differentiating between natural and anthropogenically imposed community shifts is difficult due to a lack of pre-disturbance data on community variability in urban landscapes; and (3) in many cases, long-term studies have not used standardized sampling methods which would allow for comparative studies (Geheber and Piller 2012). Many studies that have demonstrated biotic homogenization have either compared historical to present-day assemblages *(e.g.*, Marchetti et al. 2006) or compared community assemblages along a gradient of land uses (*e.g.,* Walters et al. 2003, Scott 2006). The *space-for-time substitution* method has widely recognized limitations in stream systems due to the high spatiotemporal variability of biotic assemblages (Sundermann et al. 2008, Kappes et al. 2010). Few studies have attempted to track local community composition through time, as watersheds urbanize. Such studies can provide a valuable complementary approach to the more commonly used *space-for-time substitution* technique (Carter et al. 2009, Wenger et al. 2009) and can shed light on the importance of place-specific analyses and management strategies.

Furthermore, generalizations about the effect of watershed urbanization on stream communities are typically derived from studies that focus on high-density development, rather than low-density development. Less is known about the effects of low-density development on aquatic communities, even though rural landscapes cover 15 times more land area compared to the more intensely studied urban landscape in the U.S. (Brown et al. 2005, Jackson et al. 2021). Exurbanization, a term coined by Spectorsky (1955), represents a specific kind of low-density development whereby urban migrants relocate to rural communities (Evans and Jensen-Ryan 2017). There is growing concern over the impact of exurbanization on environmental resources, particularly water resources (Hansen et al. 2005, Compas 2007, Evans and Jensen-Ryan 2017, Jackson et al. 2017).

The Blue Ridge region of the southern Appalachian Mountains is a hotspot for freshwater biodiversity (Elkins et al. 2019), but exurbanization from surrounding metropolitan areas has resulted in low-density development on once-forested mountainsides, which threatens relatively undisturbed mountain streams. Low-density development in this region has been associated with increases in stream suspended solids (Price and Leigh 2006), suspended sediment loads (Jackson et al. 2017), specific conductance (Price and Leigh 2006, Jackson et al. 2017), nitrate concentrations (Webster et al. 2012a), and summer temperature daily maxima (Jackson et al. 2017). While the southeastern United States has close to 90 fish species that are found nowhere else in the world (Mayden 1987), much of the endemic fish biodiversity in this region is now considered imperiled (Elkins et al. 2019). Deforestation and rural development within watersheds has been associated with reduced occupancy and/or abundance of highland endemic fish species and increased abundance of cosmopolitan fish species (Kirsch and Peterson 2014). However, there is mixed empirical evidence for an increase in fish community homogenization, in terms of beta diversity, in the region (Petersen et al. 2021). The southern Appalachian region is also home to multiple endemic benthic algal diatom taxa (*e.g., Eunotia bilii* and *Meridion* *alansmithii).* While increased light availability and nutrient concentrations associated with low-density development would likely increase stream algal biomass (Hagen et al. 2010), we know very little about how algal community composition might be affected by low-density development. Thus, we need a better understanding of how low-density development is potentially affecting the diverse aquatic communities of the southern Appalachian mountains.

In the 1990s, researchers associated with the Coweeta Long Term Ecological Research (LTER) Program designed a sampling strategy to document changes in southern Appalachian streams whose catchment land uses were predicted to change between 2000-2030. At the onset of this study in 2000, Gardiner et al. (2009) characterized diatom and fish communities at forested reference sites and sites where a socioeconomic model suggested new building construction would influence stream ecosystems in the future. Gardiner et al. (2009) found that diatom communities were species-poor at reference sites, where riparian canopy cover was significantly higher than all other sites. In addition, sites with < 85% forested watersheds had fewer highland endemic fish taxa and more cosmopolitan fish taxa compared sites with > 85% forested watersheds. The authors predicted homogenization within algal and fish communities at sites where building activities occurred over the next decade.

Here, we took a *repeated snapshot* approach and resampled biotic communities at the eight study sites characterized by Gardiner et. al (2009) to ask: In southern Appalachia, (1) how do biotic communities in reference forested watersheds differ from biotic communities within suburbanizing watersheds experiencing increasing low-density development in rural landscapes? (2) How have biotic communities changed over time within forested and suburbanizing watersheds? To address these questions, we then examined algal and fish communities in terms of alpha diversity, beta diversity, and taxonomic composition at three points in time over a ten-year period (2000, 2005, 2010) in four forested and four suburbanizing watersheds. Building on the initial findings in Gardiner et al. (2009), we predicted that biotic communities within suburbanizing watersheds would have higher alpha diversity, lower beta diversity, and significantly different taxonomic composition compared to forested watersheds. We also predicted that alpha diversity would increase, beta diversity would decrease, and taxonomic composition would differ over time for biotic communities within suburbanizing watersheds, but that there would be relatively little change among sampling years within forested watersheds.

Methods

Study sites

The study area is in the Blue Ridge ecoregion of western North Carolina and northeastern Georgia within the Little Tennessee (LT) and French Broad (FB) watersheds (Fig. 4.1). The LT and FB watersheds are characterized by different land use histories (Wear and Bolstad 1998). In the LT, the most common land cover change between 1950 and 1990 was the conversion of non-forested to forested land cover, and from forested without buildings to forested with buildings (Gardiner et al. 2009). Low density residential development substantially increased over this time period, particularly in the form of vacation homes built on previously forested hill slopes. The FB, in comparison to the LT, had higher proportions of agricultural land uses in both 1950 and 1990, as well as higher housing densities. At the onset of this study in summer 2000, rural second home development was evident in the LT, and agricultural lands were being converted to residential and commercial land uses in the FB (Gardiner et al. 2009). Asheville, North Carolina, the largest city in the study area, is in the north-central portion of the FB watershed, and has experienced rapid population growth between 2000 and 2019 (2000 population: 72,606; 2010 population: 83,420; 2019 population: 92,870; U.S. Census Bureau).

At the onset of this study, eight study watersheds were selected. These eight study sites were either forested reference watersheds or watersheds that were predicted to undergo low-density development over the next three decades (Table 4.1, Figure 4.1; Gardiner et al. 2009). Using the decision tree in Gardiner et al. (2009), we assigned each of the eight study watersheds to one of two categories, “forested” or “suburbanizing”, based upon their land use in the final year of sampling for this study (2010). The first land use category, “forested,” was comprised of two reference watersheds located on protected National Forest lands (Coweeta and Avery), and two predominantly forested watersheds (Darnell and Wayah), all of which had greater or equal to 98% forested and less than or equal to 1% developed land covers in 2010 (Fig. 4.2). Coweeta is located in the LT watershed and contains the United States Forest Service Coweeta Hydrologic Laboratory which is within the Nantahala National Forest, while Avery is located in the FB watershed within the Pisgah National Forest (Fig. 4.1). Darnell and Wayah are both located in the LT within the Nantahala National Forest (Fig. 4.1). The second land class category, “suburbanizing,” was comprised of four watersheds (Watauga, Hoopers, Gap, and Robinson) which had less than 85% forested and greater than 10% developed land cover in 2010 (Fig. 4.2). Watauga is located in the LT, while Hoopers, Gap, and Robinson are located in the FB (Fig. 4.1). All watersheds were restricted to sizes between 10 and 40 km^2 in area and between 550 and 720 m in elevation in order to avoid differences in fish assemblage structure due to watershed size or elevation (Table 4.1; Gardiner et al. 2009).

Generally, suburbanizing watersheds had higher percent developed land cover (Fig. 4.2) and building densities (Table 4.2) than forested watersheds. During the time period of this study

(2000-2010), suburbanizing watersheds experienced increases in percent developed land cover (Fig. 4.2) and building densities (Table 4.2), and decreases in percent forested land cover (Fig. 4.2), while forested watersheds experienced few changes in land cover (Fig. 4.2) or building densities (Table 4.2). In addition, bed particle sizes were generally lower, while ion concentrations were generally higher, in suburbanizing reaches compared to forested reaches (Table 4.2).

Biological sampling

We sampled algal communities at each of the eight study sites in July or August of 2000, 2005, and 2010 following procedures of Gardiner et al. (2009). Ten replicates samples were collected with a modified Loeb sampler (Loeb 1981) from submerged wood or rock substrates along a 100 m longitudinal transect under base flow conditions (*i.e.,* at least 10 days after a high discharge event). Samples were preserved in 10% formalin solution. We then combined 2 mL from each of the replicate samples at each site to create a single composite sample for each site for each year.

To quantify algal community composition, we created a permanent slide for each composite sample by evaporating processed samples onto round coverslips (Fisherbrand, Pittsburgh, PA) using a modified Battarbee chamber (Battarbee 1974). We then mounted the coverslips to microscope slides with Naphrax mounting medium (Brunel Microscopes, Wiltshire, U.K.). We only quantified diatom community composition because diatoms have been found to comprise the majority of the algal communities in southern Appalachian streams (Lowe et al. 1986, Greenwood and Rosemond 2005, Gardiner et al. 2009). We identified 500 valves per sample using a Zeiss Universal microscope under oil immersion at 1000x using brightfield optics. We used standard taxonomic references (Patrick and Reimer 1966, 1975) and algal floras from the southeastern U.S. (Camburn et al. 1979, Kociolek and Kingston 1999) for species-level identification. To determine cell density (cells mL^{-1}) for species, we divided the number of cells of each species by the fractional volume of the sample analyzed (*i.e.,* volume of sample used for permanent mount divided by the total volume of composite sample). We calculated cells per unit volume (cells mL^{-1}), in lieu of cells per unit substrate area (cells mm^{-2}), because the diatom data set from study year 2000 did not include surface area estimates (leaving us unable to calculate cell densities as cells mm^{-2}).

We assigned individual diatom taxa to one of four functional groups -- low, high, motile, or planktonic -- based on the definitions of Rimet and Bouchez (2012), except in cases where our own or published observations of growth forms of individual species differed from that of Rimet and Bouchez (2012). Deviations from Rimet and Bouchez (2012) definitions were as follows: we classified *Eunotia* spp. with a cell biovolume less than 500 µm and a valve length to width ratio of less than eight as low instead of high, because we only observed these taxa singularly or in chains of two cells in unprocessed samples. We classified *Meridion* spp. as high because we observed these taxa erect on substrate in unprocessed samples and because of similar observations of these taxa by Lowe et al. (1986). Since we were only interested in benthic algal communities for this study, we dropped planktonic taxa from all analyses.

We quantified fish communities at each of the eight study sites between April and early November in 2000, 2005, and 2010 following the methods described in Gardiner et al. (2009). At each site, we collected quantitative samples using backpack electro-shockers, seines, and dip nets over a representative 50 m reach. To ensure comparable catch per unit effort, we attempted to equalize electroshocking time per area sampled. We identified fishes to species level and enumerated them in the field. We preserved one voucher specimen of each species for the Georgia Museum of Natural History for future reference and returned the rest of the specimens to the stream.

We assigned fish taxa to one of three groups – highland endemic, cosmopolitan, or other -- following procedures described by Scott (2006). Four species were categorized as "other" (*i.e.,* neither highland endemic nor cosmopolitan): *Oncorhynchus mykiss* (rainbow trout) and *Salmo trutta* (brown trout), two non-native and widely distributed coldwater species, and *Rhinichthys cataractae* (longnose dace) and *Notropis telescopus* (telescope shiner), which are native to the southern Appalachian Mountains but found elsewhere. *Cottus bairdi* sp. (mottled sculpin) was classified as highland endemic species based on designations made by Scott and Helfman (2001), Scott and Bettinger (2005), and Kirsch and Peterson (2014), and because it share benthic habitat and prey requirements with many highland endemic darter species (Walters et al. 2003).

Data analyses

To understand how biotic communities differ between forested and suburbanizing watersheds, and how biotic communities may change among sampling year (2000, 2005, 2010) in both types of watersheds, we characterized biological communities in terms of alpha diversity *(i.e.,* species richness), beta diversity (*i.e.,* the ratio between regional and local species diversity), functional group (low/high/motile for algae; endemic/cosmopolitan/other for fishes), and taxonomic composition. For all analyses, we treated sampling year as a categorical variable.

We estimated beta diversity for both algae and fish using the Sørensen dissimilarity coefficient, which is a common metric based upon presence/absence data (Legendre and Legendre 2012). We made these estimates separately for forested and suburbanizing reaches for each sampling year using the *beta.pair* function within the *betapart* package in R (Baselga et al. 2021). To determine potential differences in beta diversity between land class and among sampling year, we constructed a linear model with the fixed effects of land class (forested and suburbanizing), sampling year (2000, 2005, 2010), and the interaction between land class and sampling year.

We also tested for differences in the relative abundances of functional groups (low/high/motile for algae; endemic/cosmopolitan/other for fishes) by constructing a linear model with the fixed effects of land class (forested and suburbanizing), sampling year (2000, 2005, 2010), and the interaction between land class and sampling year. Due to our small sample size for site (n = 4 forested watersheds and n = 4 for suburbanizing watersheds), we did not include site as a random effect because less than five groups in multilevel models typically add little over classical models (Gelman and Hill 2006). For all linear models, we only reported the interaction term if it was significant $p > 0.05$), and dropped the interaction term from the model if it was not significant. We ran all linear models with the *lme4* package in R (Bates et al. 2015).

We explored potential differences in both algal and fish taxonomic composition based on land class (forested and suburbanizing) among sampling year (2000, 2005, 2010) using nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarity coefficients using the *metaMDS* function within the *vegan* package in R (Oksanen et al. 2015). The metaMDS function was performed with a Wisconsin double standardization and square-root transformation (Oksanen et al. 2015). To test for potential differences in both algal and fish communities, we performed a Permutational Analysis of Variance (PERMANOVA; Anderson 2001) using the *adonis* function within the *vegan* package in R (Oksanen et al. 2015). For the PERMANOVA, we tested for the effects of land class (forested or suburbanizing) and sampling year (2000, 2005, 2010), and the interaction between land class and sampling year. We dropped the interaction term if it was not significant. For algae, due to the large number of taxa (186) and many rare species, we only included taxa with >2% relative abundance in at least one site during at least one year for both the NMDS and the PERMANOVA analyses. For fishes, we included all taxa for the NMDS and the PERMANOVA analyses.

Finally, we explored changes in the mean relative abundances of the most abundant algae and fish taxa among sampling year in both forested sites and suburbanizing sites. We selected the twenty most abundant algae and fish taxa based on total cell density and fish counts, respectively, across all sampling dates within forested reaches and all sampling dates within suburbanizing reaches. All analyses were performed in R version 3.6.2 (R Core Team 2019).

Results

Algae

We identified a total of 170 diatom species across all stream reaches and sampling years, with at least two species endemic to the region, *Meridion alansmithii* and *Gomphonema mehleri* (Appendix*,* Table C.1)*. Achnanthidium minutissium* and *Achnanthidium rivulare* (both "low" diatoms) were the two most abundant taxa in both forested and suburbanizing reaches across all sampling years (Appendix*,* Table C.1).

Algal communities differed between forested and suburbanizing reaches in terms of both diversity and functional metrics. Mean alpha diversity was higher in suburbanizing reaches compared to forested reaches during all years (Table 4.3). Beta diversity was lower in suburbanizing reaches compared to forested reaches ($F_{1,30} = 7.49$; $p = 0.01$; Fig. 4.3). The relative density of "high" diatoms was lower in suburbanizing reaches compared to forested reaches

($F_{1,20} = 7.06$, $p = 0.02$), while the relative density of “motile” diatoms was higher, and this difference was approaching significance ($F_{1,20} = 3.1$, $p = 0.09$; Fig. 4.4). The relative density of “low” diatoms did not differ between forested and suburbanizing reaches ($F_{1,20} = 0.76$, $p = 0.40$; Fig. 4.4).

Algal community composition also differed between forested and suburbanizing reaches (PERMANOVA, $F_{1,18} = 5.03$, $R^2 = 0.17$, $p = 0.001$; Fig. 4.5). In forested reaches, *Achnanthidium minutissimum* and *Achnanthidium rivulare* were co-dominant taxa, each comprising 20-35% of the relative cell densities across all sampling years (Fig. 4.6A). In suburbanizing reaches, however, *Achnanthidium rivulare* was the dominant taxon, comprising 30-50% of the relative cell densities across all sampling years, whereas *Achnanthidium minutissimum* only comprised 10-11% of the relative cell densities (Fig. 4.6B). Additionally, *Eunotia* spp. and *Nupela lapidosa* were generally more abundant in forested reaches compared to suburbanizing reaches, whereas *Nitzschia* spp. and *Navicula* spp. were more abundant in suburbanizing reaches compared to forested reaches (Fig. 4.6).

Algal communities differed among years in diversity metrics but not with functional metrics. Mean alpha diversity decreased between 2000 and 2005 and increased between 2005 and 2010 in both forested and suburbanizing reaches (Table 4.3). There was a significant difference in beta diversity among sampling years ($F_{2,30} = 6.59$, $p = 0.004$) and a significant interaction between land class and sampling year on beta diversity ($F_{2,30} = 9.95$, $p < 0.001$; Fig. 4.3). Beta diversity in suburbanizing reaches was lower in 2005 than 2000 ($p < 0.001$) and lower 2010 than 2000 ($p=0.006$; Fig. 4.3). However, beta diversity in forested reaches was not significantly different among sampling years (Fig. 4.3). The relative densities of functional groups did not differ significantly among sampling year ("low", $F_{2,20} = 0.59$, $p = 0.56$; "high", $F_{2,20} = 2.12$, $p = 0.16$; "motile", $F_{2,20} = 0.76$, $p = 0.51$; Fig.4. 4).

Algal community composition also differed among sampling year (PERMANOVA, $F_{2,18} = 1.86$, $R^2 = 0.13$, $p = 0.04$; Fig. 4.5). In forested reaches, the relative densities *of Synedra rumpens* var. *familaris* and *Synedra rumpens* var. *fragilarioides* were higher in 2005 (mean 6.3% and 10.9%, respectively) and 2010 (mean: 5.0% and 6.2%, respectively) compared to 2000 (mean: 0.2% and 0.4%, respectively; Fig. 4.6A). In suburbanizing reaches, relative densities of the dominant taxon *Achnanthidium rivulare* were higher in 2005 (mean 48.0%) and 2010 (mean 43.9%) compared to 2000 (mean 29.5%; Fig. 4.6B).

Fishes

We identified a total of 32 fish species from seven families across all stream reaches and sampling years (Appendix*,* Table C.2). Twelve species were endemic to the region. One taxon was federally listed as "threatened" (*Erimonax monacha* [spotfin chub]), and one taxon was listed as of "North Carolina Special Concern" in the Little Tennessee River and its tributaries (*Clinostomus* sp. cf. *funduloides* [smoky dace]; N.C. Wildlife Resources Commission 2017). *Cottus bairdi* (mottled sculpin) was the dominant taxon across all sampling years in both forested and suburbanizing reaches (Appendix*,* Table C.2).

Fish communities differed between forested and suburbanizing in some diversity and functional metrics, but not others. Mean alpha diversity was higher in suburbanizing reaches compared to forested reaches for all years (Table 4.4), but beta diversity did not differ between forested and suburbanizing reaches ($F_{1,30} = 1.61$, $p = 0.21$; Fig.4. 7). There was a higher relative abundance of cosmopolitan taxa ($F_{1,20} = 16.57$, $p < 0.001$) and lower relative abundance of “other” taxa ($F_{1,20} = 12.47$, $p = 0.002$) in suburbanizing reaches compared to forested reaches (Fig. 4.8). The relative abundance of highland endemic taxa did not differ between forested and suburbanizing reaches ($F_{1,20} = 2.37$, $p = 0.14$; Fig. 4.8).

Fish community composition differed between forested reaches and suburbanizing reaches (PERMANOVA, $F_{1,20} = 7.46$, $R^2 = 0.25$, $p = 0.001$; Fig. 4.9). *Cottus bairdi* (mottled sculpin) and *Oncorhynchus mykiss* (rainbow trout) had higher mean relative abundances in forested reaches (49.5-72.0% and 3.8-11.4%, respectively; Fig. 4.10A), compared to suburbanizing reaches (19.6-46.0% and 0.0-0.1%, respectively; Fig. 4.10B). Conversely, *Notropis rubicroceus* (saffron shiner) and *Semotilus atromaculatus* (creek chub) had higher mean relative abundances in suburbanizing reaches (2.7-19.2% and 6.6-14%, respectively; Fig. 4.10B) compared to forested reaches (0.0-0.3% and 0.0-0.4%, respectively; Fig. 4.10A)

Fish communities varied little among year within both suburbanizing and forested reaches. Mean alpha diversity increased slightly over time in suburbanizing reaches (Table 4.4). Mean alpha diversity decreased between 2000 and 2005 and increased between 2005 and 2010 in forested reaches (Table 4.4). Beta diversity ($F_{2,30} = 0.10$, $p = 0.90$; Fig. 4.7) did not differ among sampling year. While relative abundance of cosmopolitan taxa differed among sampling year ($F_{2,20} = 5.26$, $p = 0.01$), there were no significant differences between sampling years within forested reaches or within suburbanizing reaches (Fig. 4.8). The relative abundances of highland endemic taxa ($F_{2,20} = 2.60$, $p = 0.1$) and “other” taxa ($F_{2,20} = 2.60$, $p = 0.1$) did not differ among sampling year (Fig. 4.8). Taxonomic composition also did not differ among sampling year (PERMANOVA, $F_{1,18} = 1.29$, $R^2 = 0.09$, $p = 0.22$; Fig. 4.9).

Discussion

Our temporal analysis revealed that relatively small amounts of watershed development can affect stream biological communities, but that algal communities may respond more rapidly to urbanization than fish communities. In support of our first prediction, we found consistent differences in both algal and fish communities between forested and suburbanizing watersheds. We found higher alpha diversity and significant differences in functional and taxonomic composition in suburbanizing watersheds compared to forested watersheds for both algal and fish communities, indicating biotic communities in suburbanizing watersheds are in early stages of biotic homogenization. Contrary to our second prediction, while we found some evidence of change over time within algal communities in terms of beta diversity and taxonomic composition, we found little evidence of change over time within fish communities. Our findings add the body of literature indicating that single-celled organisms respond more rapidly to changes in water quality than multicellular organisms.

Our finding of higher alpha diversity within suburbanizing watersheds compared to forested watersheds for both algal (Table 4.3) and fish (Table 4.4) communities indicates that suburbanizing streams in southern Appalachia are in the early stages of biotic homogenization. While biotic homogenization typically leads to decreases in alpha diversity within fish communities, alpha diversity may actually increase in the early stages of homogenization (Scott and Helfman 2001). This is because stream conditions may change or degrade enough to allow the establishment of cosmopolitan taxa, while still remaining hospitable to highland endemics, but supporting them in reduced numbers (Scott and Helfman 2001). Similarly, while urbanization has been found to correlate with lower species richness in stream algal communities (Sonneman et al. 2001, Teittinen et al. 2015), increases in light and nutrient availability may increase algal productivity and species richness in forested streams in early stages of urbanization (Lowe et al. 1986, Gardiner et al. 2009). Thus, if development continued to increase in our suburbanizing watersheds, we might eventually observe decreases in alpha diversity within both algal and fish communities.

Taxonomic differences between forested and suburbanizing watersheds also support the notion that fish communities within suburbanizing reaches have undergone some biotic homogenization, but urbanization has not reached a level that would extirpate highland endemic taxa. Although we did not find significant differences in highland endemic fish taxa between forested and suburbanizing reaches, we did find higher relative abundances of cosmopolitan taxa in suburbanizing reaches, indicating homogenization in these reaches (Scott 2006; Fig. 4.8). Several other studies have shown that biotic homogenization is initially driven by increases in abundance of cosmopolitan fish species, as opposed to decreases in abundance of endemic species (Walters et al. 2003, Taylor 2004). Furthermore, taxonomic differences between forested and suburbanizing watersheds in were mainly driven by lower relative abundances of a highland endemic taxon, *Cottus bairdi* (mottled sculpin), and higher relative abundance of several cosmopolitan, “weedy” taxa (*e.g., Rhinichthys atratulus* [blacknose dace] and *Semotilus atromaculatus* [Creek Chub]; Fig. 4.10) in suburbanizing watersheds. Interestingly, while some highland endemic taxa were completely absent from suburbanizing reaches (*e.g., Salmo trutta* [brown trout] and *Clinostomus* sp. [smokey dace]), others were present in higher abundances in suburbanizing reaches compared to forested reaches (*e.g., Erimonax monacha* [spotfin chub] and *Notropis rubicroceus* [saffron shiner]; Fig. 4.10). Urbanization has likely affected fish communities within suburbanizing reaches by altering processes that create and maintain diverse chemical and physical stream characteristics, which generally benefit cosmopolitan taxa, but may also initially benefit some endemic taxa (Kirsch and Peterson 2014).

Several lines of evidence suggest that higher nutrient concentrations and lower bed particle sizes in suburbanizing reaches compared to forested reaches led to distinct algal communities between the two watershed types. First, higher ion concentrations in suburbanizing reaches (Table 4.2) likely explain higher relative abundances of *Achnanthidium rivulare* and lower relative abundances of *Achnanthidium minutissimum* found in suburbanizing reaches compared to forested reaches (Fig. 4.6). While both of these taxa are widespread throughout the Appalachian mountains, they differ in their ecology (Ponader and Potapova 2007). *Achnanthidium rivulare* has been found to have a greater tolerance for elevated nutrients compared to other *Achnanthidium* species in the Appalachian Mountains. On the other hand, *Achnanthidium minutissimum* has been found to be associated with rivers with low nutrient and ionic concentrations, and to be absent or in low abundance in rivers with elevated nutrients in the Appalachians (Ponader and Potapova 2007). Furthermore, the positive correlation between the relative abundance of motile diatoms and nutrient levels is well documented (Passy 2007, Tapolczai et al. 2016). Even relatively low levels of watershed urbanization (*e.g.,* 2.7 and 4.4% impervious surface cover) have been found to correlate with increases in the relative abundance of motile diatoms in the northeastern United States (Smucker et al. 2013). Thus, in our study, higher nutrient concentrations and smaller bed particle sizes in suburbanizing reaches (Table 4.2) likely led to higher relative abundances of motile diatoms (Fig. 4.4; *e.g., Nitzschia* spp. and *Navicula* spp., Fig. 4.6B). Overall, our study adds to the body of research indicating that relatively low levels of urbanization can have significant effects on algal community composition.

While a decade may be a relatively short time span in the context of biotic homogenization for fishes, algae are typically more responsive to changes in water chemistry than multicellular organisms (Lowe and Pan 1996, Brown et al. 2009). Thus, our finding of lower algal beta diversity in later sampling years within suburbanizing watersheds, but no change in fish beta diversity among years, may be explained by our relatively short sampling time span (10 years). Although building density increased between 2000 and 2010 in all four of the suburbanizing watersheds, changes in water quality over this time period were potentially not large enough in magnitude to result in fish beta diversity change. Consistent with our findings, (Petersen et al. 2021) did not find strong evidence taxonomic homogenization (in terms of beta diversity) in a comparison of southern Appalachian fish communities at two times periods 13 years apart. However, beta diversity is just one factor to consider in management decision making, and other factors, like changes in the abundance and richness of endemic taxa, should also be considered (Petersen et al. 2021).

While patterns associated with beta diversity within fish communities have been well studied in both reference and urbanizing streams (Olden and Poff 2004, Marchetti et al. 2006, Campbell and Mandrak 2020, Erős et al. 2020, Petersen et al. 2021), much less is understood about benthic algal communities (Dunck et al. 2019). Still, recent studies indicate that decreases in beta diversity within benthic algal communities can occur with nutrient enrichment (Leboucher et al. 2019) and substratum simplification (Petsch et al. 2017). Thus, higher ion concentrations and substratum simplification associated with suburbanizing streams in this study (Table 4.2) may have driven documented decreases in diatom beta diversity. While we found some evidence for homogenization of algal communities over time within suburbanizing reaches, we encourage additional studies with more frequent sampling to disentangle the effects of urbanization on diatom beta diversity and natural variability in diatom beta diversity.

It is possible that the differences between biological communities between forested and suburbanizing watersheds found in this study partly reflect legacy effects of land use, in addition to current land use practices. For example, there is evidence that nutrient concentrations and suspended solids are higher in streams within Appalachian watersheds that have recovered from past agriculture compared to forested reference watersheds (McTammany et al. 2007). In addition, historical (1950s) land use in Appalachian watersheds and riparian corridors has been found to be a good or better predictor of fish diversity and richness than current land use practices (Harding et al. 1998, Burcher et al. 2008). While little is known about legacy effects of land use on algal communities in this area, there is evidence that streams within Appalachian watersheds with a history of agriculture, once reforested, have comparable algal biomass to those in forested reference watersheds (McTammany et al. 2007). This is likely because elevated nutrients have little effect on algal communities when light is limiting (Lowe et al. 1986, Greenwood and Rosemond 2005).

Finally, the temporal variability in algal community composition within both forested and suburbanizing watersheds (Fig. 4.5) could be partially attributed to changes in the riparian zone during this time period. The eastern hemlock (*Tsuga canadensis*) was once abundant in the riparian zones of southern Appalachian streams. However, the hemlock wooly adelgid (*Adelges tsugae*), an invasive hemipteran, reached the southern Appalachians in the early 2000s and caused a massive die-off of eastern hemlocks. By 2005, all eastern hemlock trees in the Coweeta experimental basin had been infected with hemlock woolly adelgid and by 2010, 50% of hemlock trees were dead (Dharmadi et al. 2019). Hemlock death initially resulted in increased canopy openness and light availability to Appalachian streams (Webster et al. 2012b), which may have affected algal communities in the short-term within both forested and suburbanizing reaches. Such changes could affect algal community structure and diversity in ways not detected in our study. Suburbanizing sites had less canopy cover than forested sites in 2000, but canopy cover and light availability were not directly measured at diatom sampling locations through time (Gardiner et al. 2009). While there is evidence that diatom communities in southern Appalachian headwater streams display relative stability through time (Solomon 2021, Chapter 2 of this dissertation) we need a better understanding of how algal communities vary naturally through time in forested reference streams.

Conclusions and Recommendations

The consistency of observed differences between forested and suburbanizing sites, despite the project's broad temporal sampling schedule, suggest that relatively small amounts of watershed development can impact both algal and fish communities. Our findings from 2000, 2005, and 2010 demonstrate how ecological monitoring at a broad temporal scale (every five years) can be effective in characterizing differences in biological, physical, and chemical conditions between forested reference and suburbanizing stream reaches. We realize the limitations of suggesting trends or characterizing community dynamics over time from only three data points, taken five years apart. While this project was originally intended to span from 2000-2030, it was cut short due to funding challenges, a common problem in long-term monitoring efforts (Likens and Lindenmayer 2018). Continued study of these rapidly changing landscapes over the next few decades will be necessary to tease apart the influence of land use legacies and contemporary development on stream ecosystems.

Understanding effects of urbanization on stream ecosystems is increasingly important as urbanization and exurbanization become more widespread. Thus, we offer suggestions for future monitoring efforts attempting to characterize biotic communities through time in urbanizing streams. In addition to the sampling framework presented in this study, we recommend the addition of canopy cover surveys, large woody debris counts, monthly stream temperature measurements, and estimations of stream slopes, which could allow future researchers to test more mechanistic hypotheses regarding how urbanization affect stream habitats and biota. Furthermore, we recommend that algal and fish surveys be conducted at least bi-annually to capture the degree of community variability at a finer temporal resolution. Our findings indicate that long-term monitoring of stream reaches in early stages of urbanization has strong potential to reveal additional insights regarding the complex interrelationship between low-density land development and stream ecosystems.

Acknowledgements

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Appendix C: Supplementary Data

Table 4.1: Characteristics of the eight study reaches

	Location UTM Zone 17 S				
Site	Easting	Northing	Elevation (m)	Drainage Area (km^2)	Mean bankfull width (m)
Forested					
Avery	341921	3907410	700	15.8	8.72
Coweeta	278818	3882457	677	16.6	6.58
Darnell	283588	3871167	660	14.7	6.40
Wayah	272822	3893401	664	34.9	7.87
Suburbanizing					
Watauga	285390	3900773	618	16.8	4.38
Hoopers	366047	3921937	645	39.4	7.64
Gap	370762	3929143	661	20.3	5.14
Robinson	365782	3924142	648	14.8	5.34

Table 4.2: Building densities, (bldgs. km^{-2}), bed D_{90} particle sizes (mm) through time, and stream ion concentrations (mg L^{-1}) for the eight study watersheds. Ion concentrations represent mean ± SE for each stream reach for all sampling years combined (n = 9; n = 3 for each sampling date in 2000, 2005, and 2010).

	Building Density (bldgs km^{-2})			Bed D_{90} Particle Size (mm)			Ion concentrations (mg L^{-1})					
Site	2000	2005	2010	2000	2005	2010	NO_3-N	NH_4-N	PO_4-P	K	Na	Ca
Forested												
Avery	0.00	0.00	0.00	265.0	202.1	237.3	0.06 ± 0.002	0.02 ± 0.001	0.01 ± 0.003	0.64 ± 0.03	2.24 ± 0.42	1.22 ± 0.10
Coweeta	0.90	0.90	0.84	149.0	270.4	197.3	0.05 ± 0.002	0.01 ± 0.002	0.02 ± 0.005	0.56 ± 0.04	2.22 ± 0.41	1.18 ± 0.13
Darnell	1.43	1.29	1.22	193.0	238.3	261.0	0.02 ± 0.004	0.01 ± 0.002	0.02 ± 0.003	0.53 ± 0.03	2.41 ± 0.46	1.10 ± 0.13
Wayah	2.86	3.64	4.35	200.0	272.0	270.2	0.08 ± 0.008	0.01 ± 0.001	0.02 ± 0.004	0.61 ± 0.03	2.91 ± 0.48	1.42 ± 0.14
Suburbanizing												
Watauga	36.46	38.91	44.10	112.0	140.1	120.0	0.18 ± 0.008	0.03 ± 0.005	0.02 ± 0.005	1.65 ± 0.10	4.34 ± 0.58	3.11 ± 0.15
Hoopers	18.71	35.05	47.42	21.6	27.0	31.0	0.32 ± 0.007	0.03 ± 0.002	0.04 ± 0.009	1.79 ± 0.08	5.62 ± 0.56	5.12 ± 0.24
Gap	35.93	41.15	50.71	400.0	222.1	182.0	0.33 ± 0.029	0.02 ± 0.003	0.02 ± 0.006	1.84 ± 0.06	6.09 ± 0.55	5.35 ± 0.18
Robinson	60.14	71.70	105.75	70.4	78.1	100.0	0.51 ± 0.022	0.02 ± 0.002	0.03 ± 0.007	1.68 ± 0.04	5.04 ± 0.52	6.28 ± 0.13

Table 4.3: Alpha diversity as species richness for algal taxa among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010. Means ± SE are included for each land use classification (forested or suburbanizing) in each year.

	Year		
	2000	2005	2010
Forested			
Avery	43	33	33
Coweeta	24	10	41
Darnell	51	16	19
Wayah	51	33	35
Mean	**42.3 ± 6.4**	**23.0 ± 5.9**	**32.0 ± 4.7**
Suburbanizing			
Watauga	51	31	35
Hoopers	57	47	64
Gap	39	32	50
Robinson	56	39	49
Mean	**50.8 ± 4.1**	**37.3 ± 3.7**	**49.5 ± 5.9**

Table 4.4: Alpha diversity as species richness for fish taxa among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010. Means ± SE are included for each land use classification (forested or suburbanizing) in each year.

	Year		
	2000	2005	2010
Forested			
Avery	6	6	9
Coweeta	9	5	8
Darnell	6	7	13
Wayah	10	9	12
Mean	**7.8 ± 1.0**	**6.8 ± 0.9**	**10.5 ± 0.2**
Suburbanizing			
Watauga	14	11	14
Hoopers	11	17	17
Gap	13	13	14
Robinson	15	14	12
Mean	**13.3 ± 0.9**	**13.8 ± 1.3**	**14.3 ± 1.0**

Figure Legends

Fig. 4.1: Map depicting the eight study watersheds: Avery, Coweeta, Darnell, Gap, Hoopers, Robinson, Watauga, and Wayah. Study watersheds are located within the larger Little Tennessee and French Broad watersheds of the Blue Ridge ecoregion. Inset (upper left) depicts location of the two larger watersheds (Little Tennessee and French Broad) in relation to state borders.

Fig. 4.2: Changes in percent land cover (forested, agriculture, developed, and other) through time in the eight study watersheds. Watersheds are grouped by land use category: forested (left column) and suburbanizing (right column).

Fig. 4.3: Algal beta diversity (based on Sørensen dissimilarity) among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010. The bold line within each box represents the median, and the lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively. The lines represent minimum and maximum values, except in the case of outliers, which are defined as values more distant than 1.5x the length of the box away from the box, and are represented by points.

Fig. 4.4: Relative densities of diatom species separated by functional group (low, high, and motile) among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010.

Fig. 4.5: Non-metrical multidimensional scaling plot (stress = 0.12) for algal taxa within forested (light grey symbols, light grey hulls; n = 4) and suburbanizing (black symbols, dark grey hulls; n

= 4) watersheds through time. Symbols represent algal communities within one stream reach during one sampling year (circles = 2000; triangles = 2005; squares = 2010). Polygons encompass algal communities within same land use classification (forested or suburbanizing) during the same year (2000, 2005, 2010). Red arrows represent movement of the community centroid for land use classification (forested or suburbanizing) through time.

Fig. 4.6: Mean relative density of twenty most abundant algal taxa (based on total cell density) among stream reaches within (A) forested (n = 4) and (B) suburbanizing (n = 4) watersheds by sampling year (2000, 2005, 2010).

Fig. 4.7: Fish beta diversity (based on Sørensen dissimilarity) among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010. The bold line within each box represents the median, and the lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively. The lines represent minimum and maximum values, except in the case of outliers, which are defined as values more distant than 1.5x the length of the box away from the box, and are represented by points.

Fig. 4.8: Relative abundances of endemic, cosmopolitan, and other fish taxa among stream reaches within forested (n = 4) and suburbanizing (n = 4) watersheds in 2000, 2005, and 2010.

Fig. 4.9: Non-metrical multidimensional scaling plot (stress = 0.12) for fish taxa within forested (light grey symbols, light grey hulls; n = 4) and suburbanizing (black symbols, dark grey hulls; n = 4) watersheds through time. Symbols represent fish communities within one stream reach during one sampling year (circles = 2000; triangles = 2005; squares = 2010). Polygons encompass fish communities within same land use classification (forested or suburbanizing) during the same sampling year (2000, 2005, 2010). Red arrows represent movement of the community centroid for land use classification (forested or suburbanizing) through time.

Fig. 4.10: Mean relative abundance of the twenty most abundant fish taxa (based on total counts) among stream reaches within (A) forested (n = 4) and (B) suburbanizing (n = 4) watersheds by sampling year (2000, 2005, 2010).

Fig. 4.1

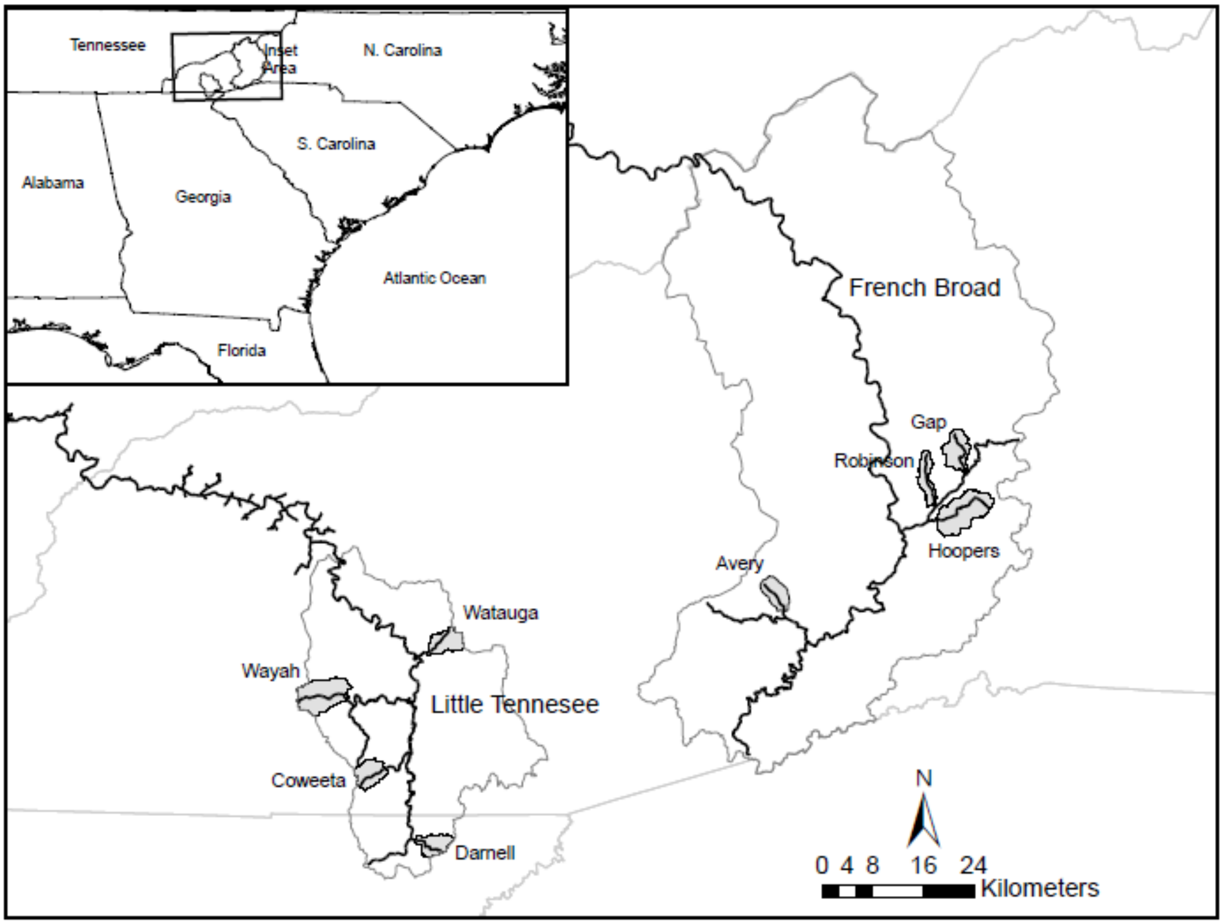

Tennessee
Inset Area
N. Carolina
S. Carolina
Alabama
Georgia
Atlantic Ocean
Florida
French Broad
Gap
Robinson
Hoopers
Avery
Watauga
Wayah
Little Tennesee
Coweeta
Darnell
N
0 4 8 16 24
Kilometers

Fig. 4.2

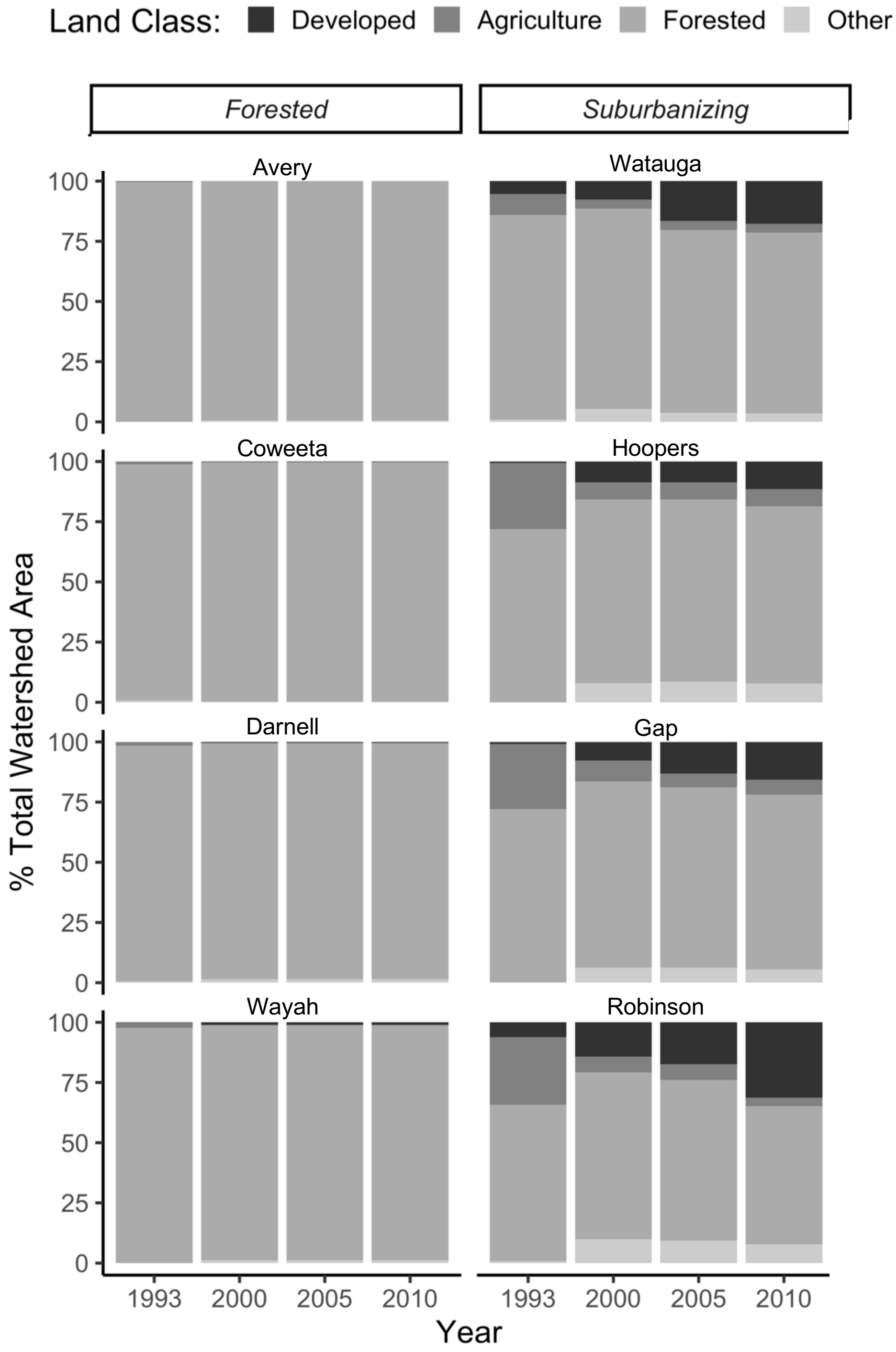
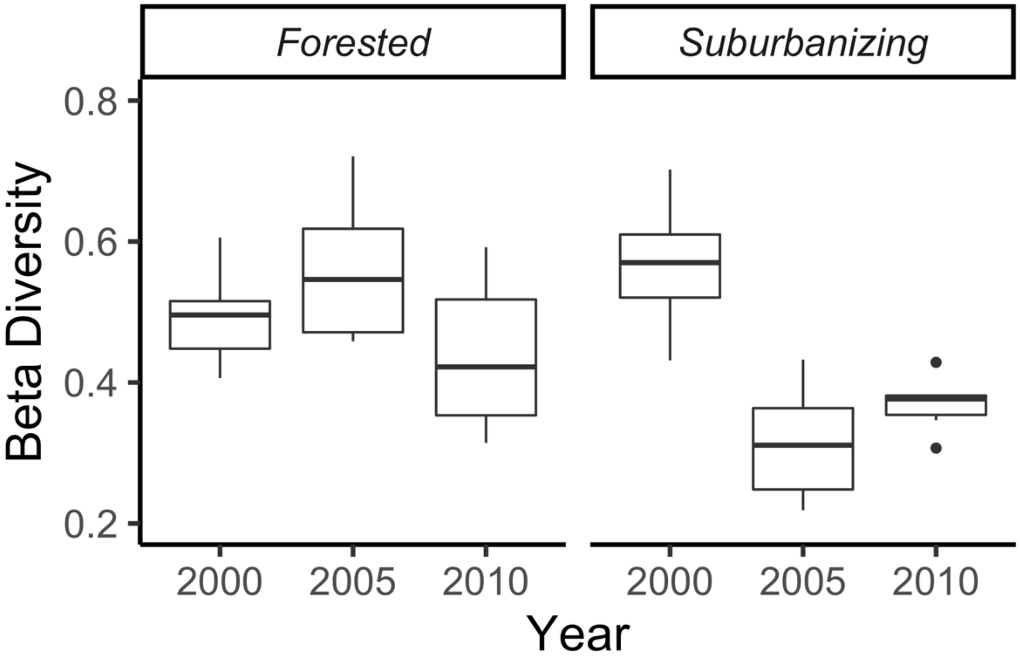

Land Class:
Developed
Agriculture
Forested
Other
Forested
Suburbanizing
Avery
Watauga
Coweeta
Hoopers
Darnell
Gap
Wayah
Robinson
% Total Watershed Area
0
25
50
75
100
1993
2000
2005
2010
Year

Fig. 4.3


Forested
Suburbanizing
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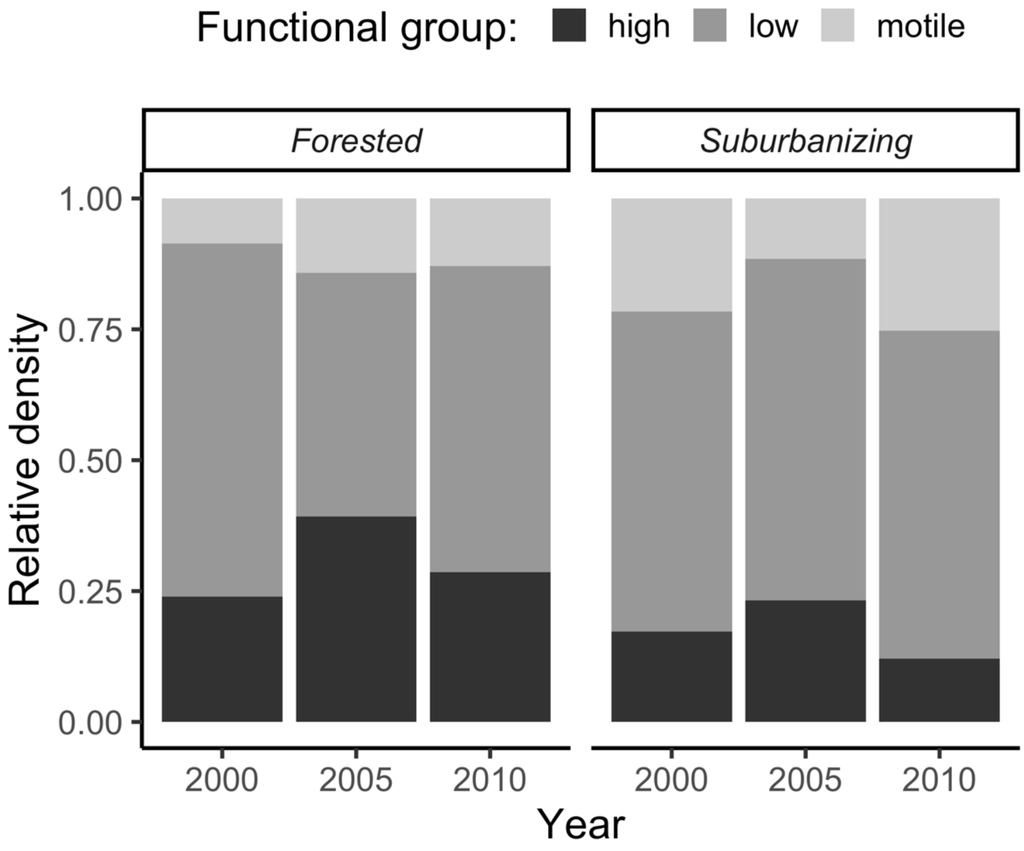
0.6
0.4
0.2
Beta Diversity
2000
2005
2010
2000
2005
2010
Year

Fig. 4.4


Functional group:
high
low
motile
Forested
Suburbanizing
Relative density
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0.75
0.50
0.25
0.00
2000
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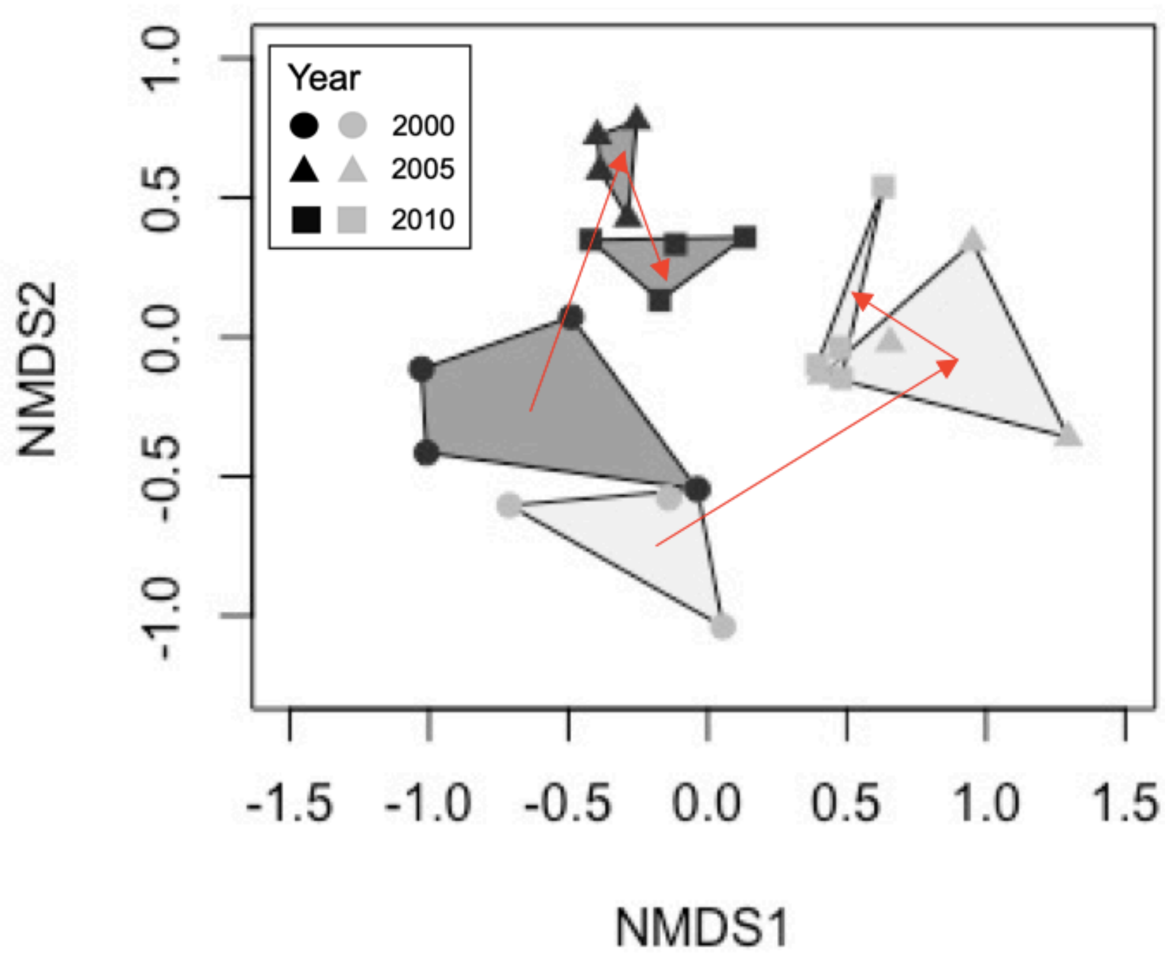
2010
2000
2005
2010
Year

Fig. 4.5


Year
2000
2005
2010
NMDS2
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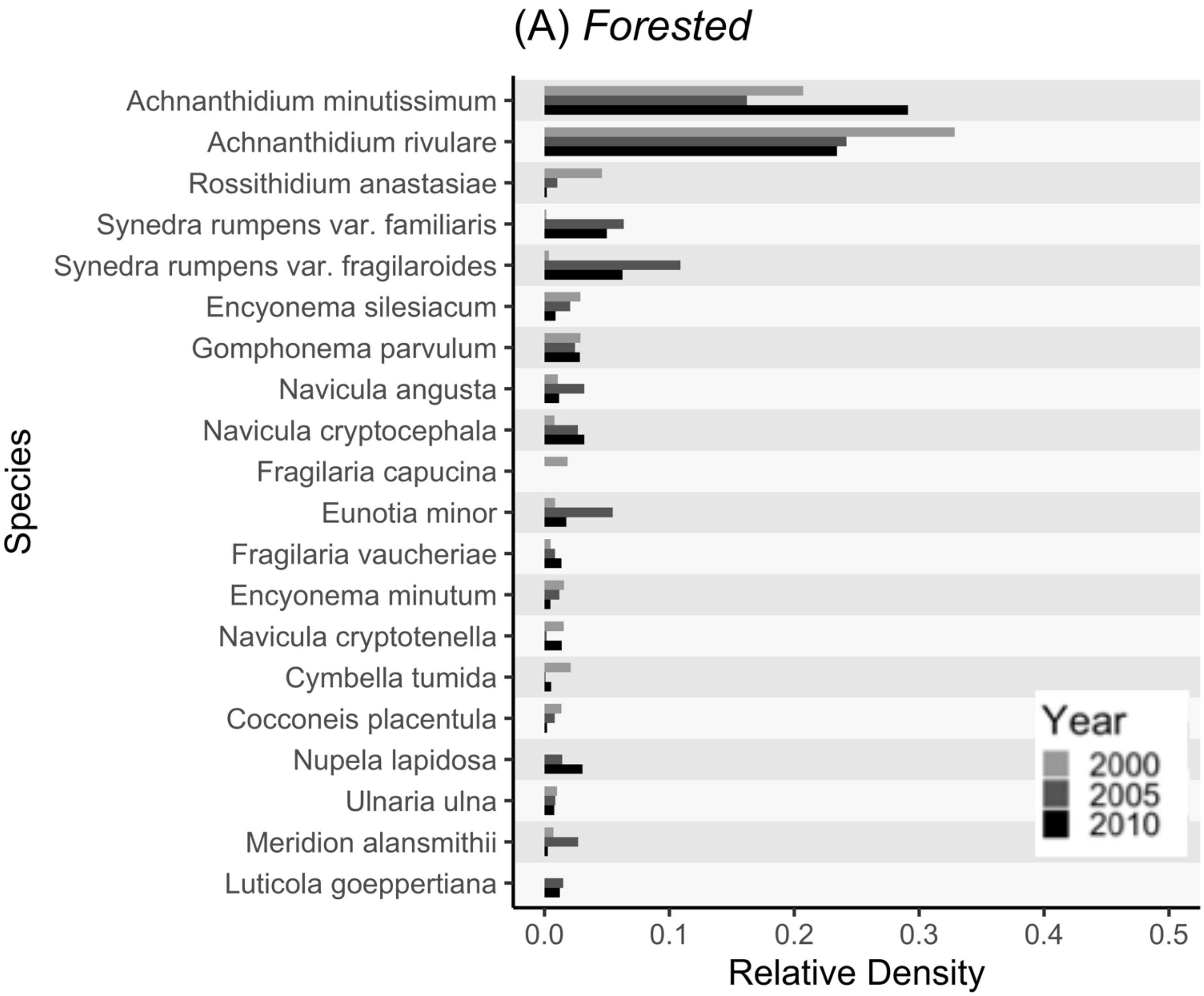
0.5
0.0
-0.5
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
NMDS1

Fig. 4.6


(A) Forested
Species
Achnanthidium minutissimum
Achnanthidium rivulare
Rossithidium anastasiae
Synedra rumpens var. familiaris
Synedra rumpens var. fragilaroides
Encyonema silesiacum
Gomphonema parvulum
Navicula angusta
Navicula cryptocephala
Fragilaria capucina
Eunotia minor
Fragilaria vaucheriae
Encyonema minutum
Navicula cryptotenella
Cymbella tumida
Cocconeis placentula
Nupela lapidosa
Ulnaria ulna
Meridion alansmithii
Luticola goeppertiana
0.0
0.1
0.2
0.3
0.4
0.5
Relative Density
Year
2000
2005
2010

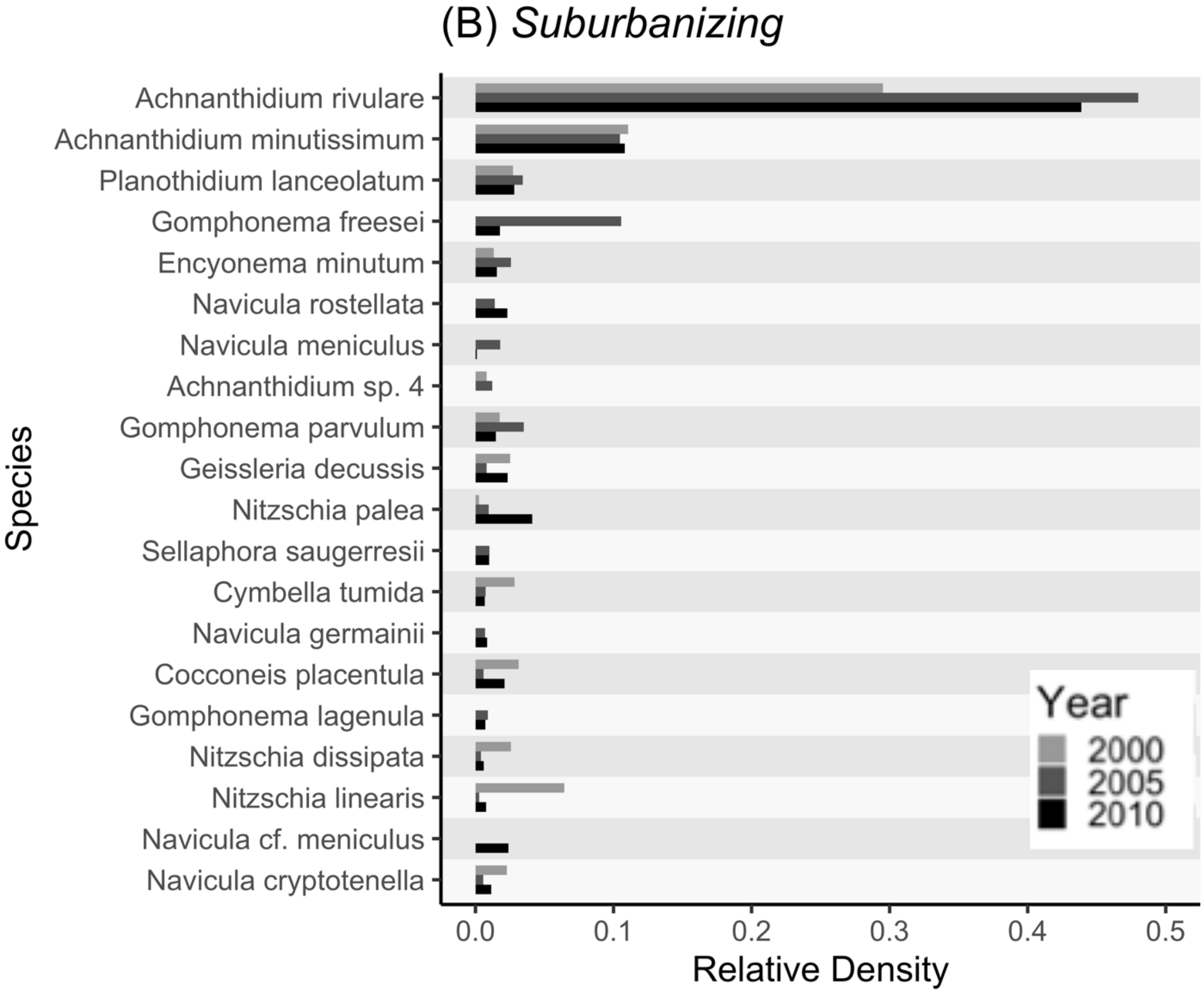

(B) Suburbanizing
Species
Achnanthidium rivulare
Achnanthidium minutissimum
Planothidium lanceolatum
Gomphonema freesei
Encyonema minutum
Navicula rostellata
Navicula meniculus
Achnanthidium sp. 4
Gomphonema parvulum
Geissleria decussis
Nitzschia palea
Sellaphora saugerresii
Cymbella tumida
Navicula germainii
Cocconeis placentula
Gomphonema lagenula
Nitzschia dissipata
Nitzschia linearis
Navicula cf. meniculus
Navicula cryptotenella
0.0
0.1
0.2
0.3
0.4
0.5
Relative Density
Year
2000
2005
2010

Fig. 4.7

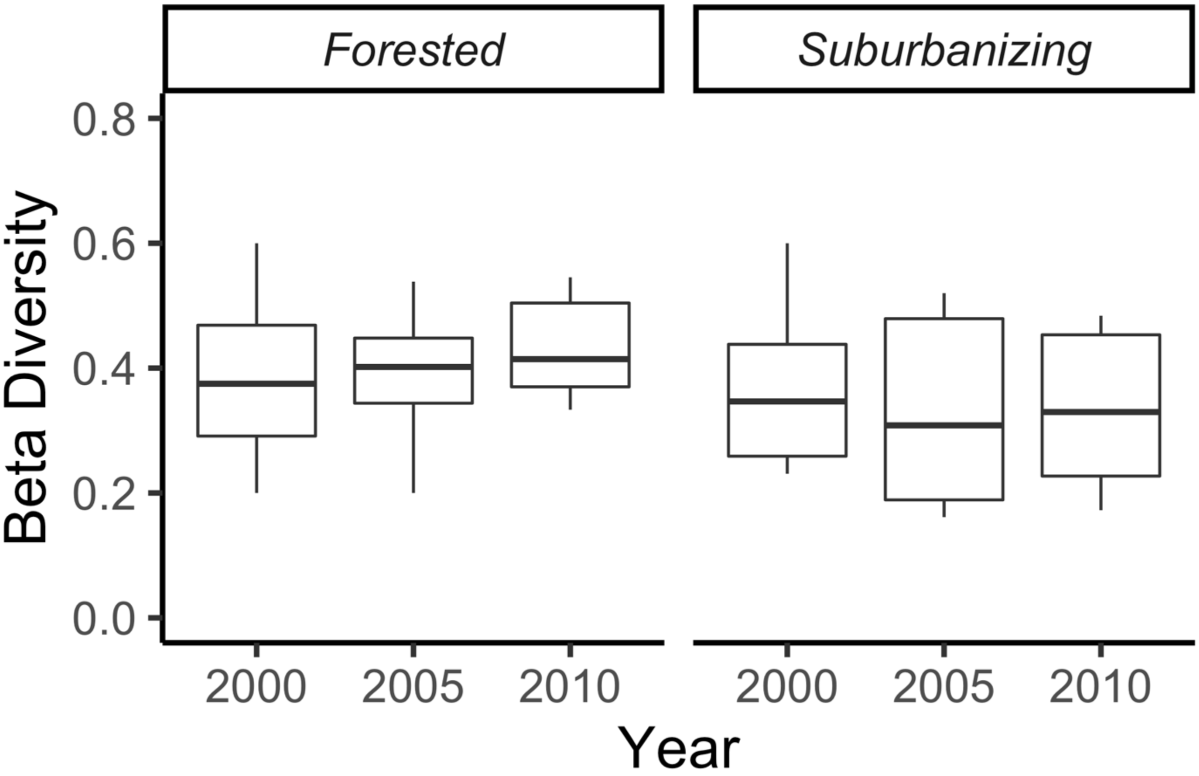

Forested
Suburbanizing
Beta Diversity
0.8
0.6
0.4
0.2
0.0
2000
2005
2010
2000
2005
2010
Year

Fig. 4.8

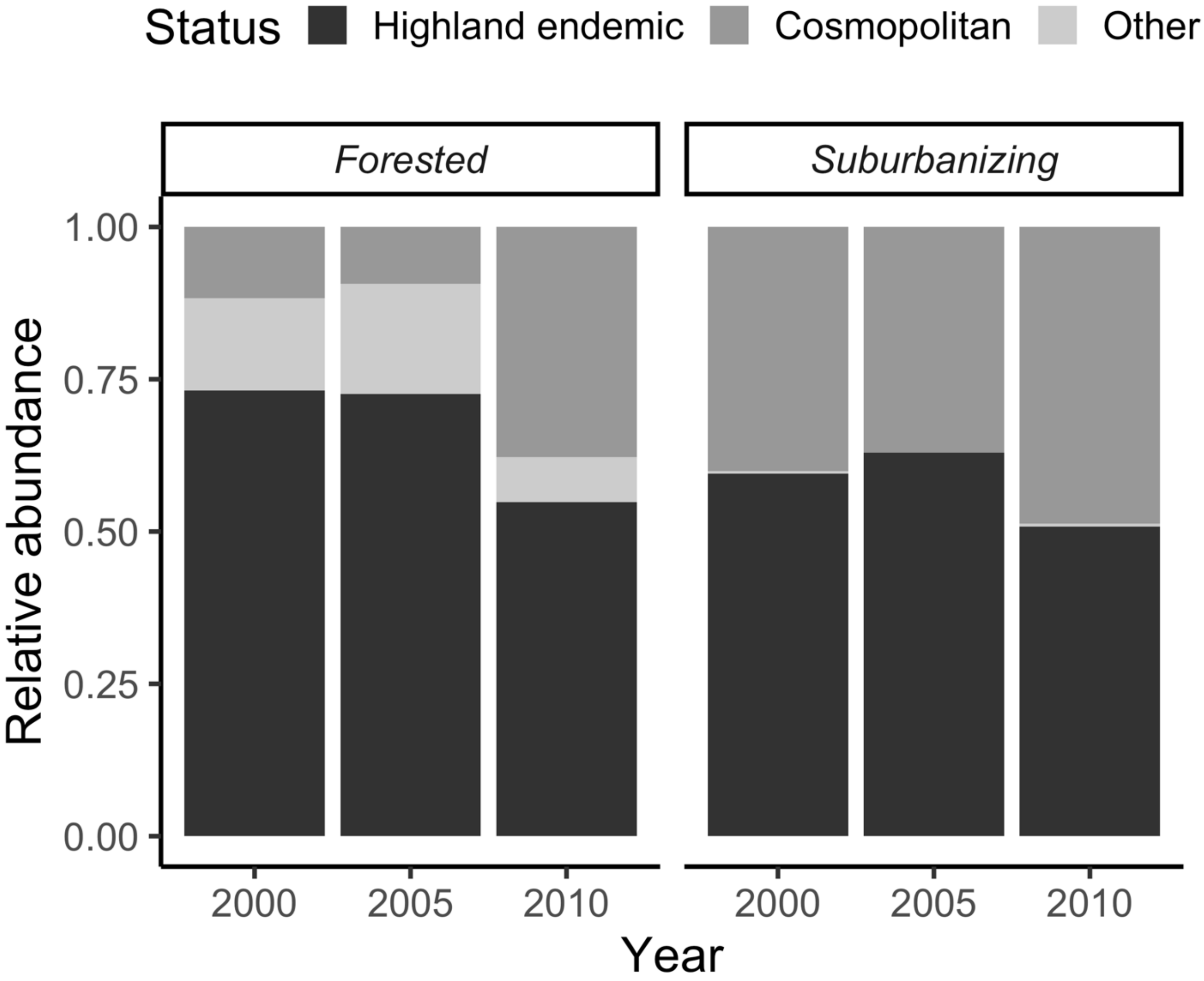

Status
Highland endemic
Cosmopolitan
Other
Forested
Suburbanizing
Relative abundance
1.00
0.75
0.50
0.25
0.00
2000
2005
2010
2000
2005
2010
Year

Fig. 4.9

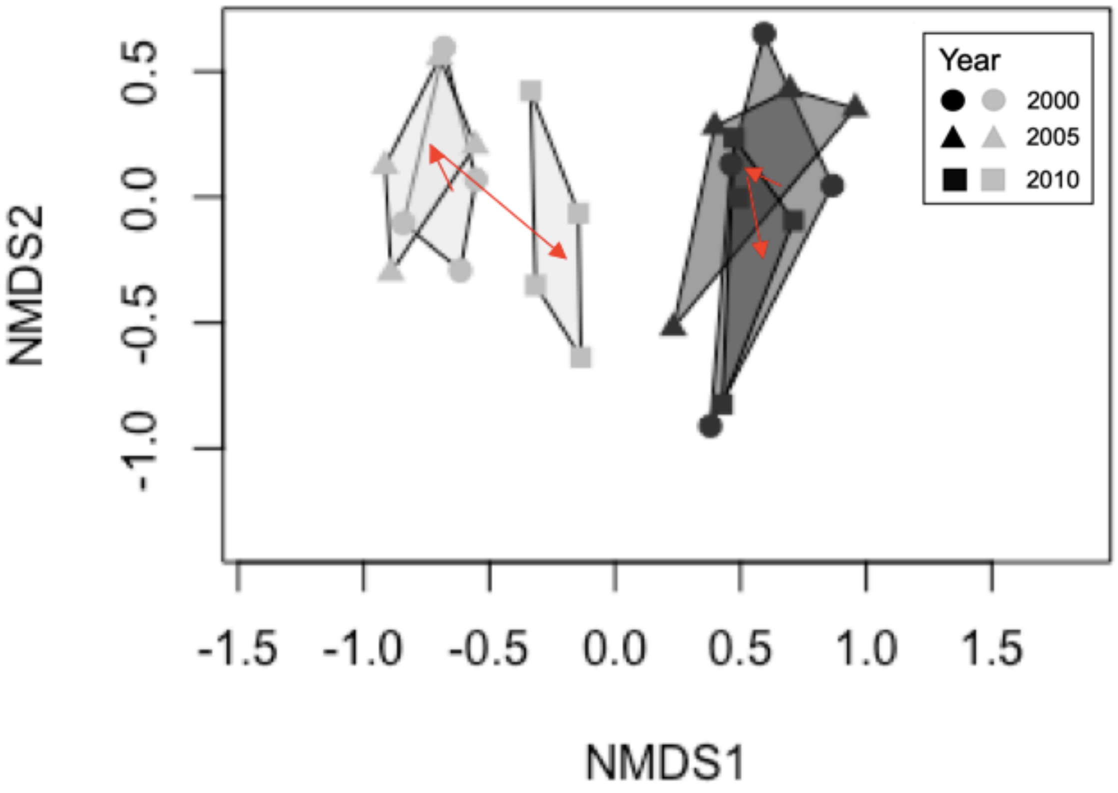
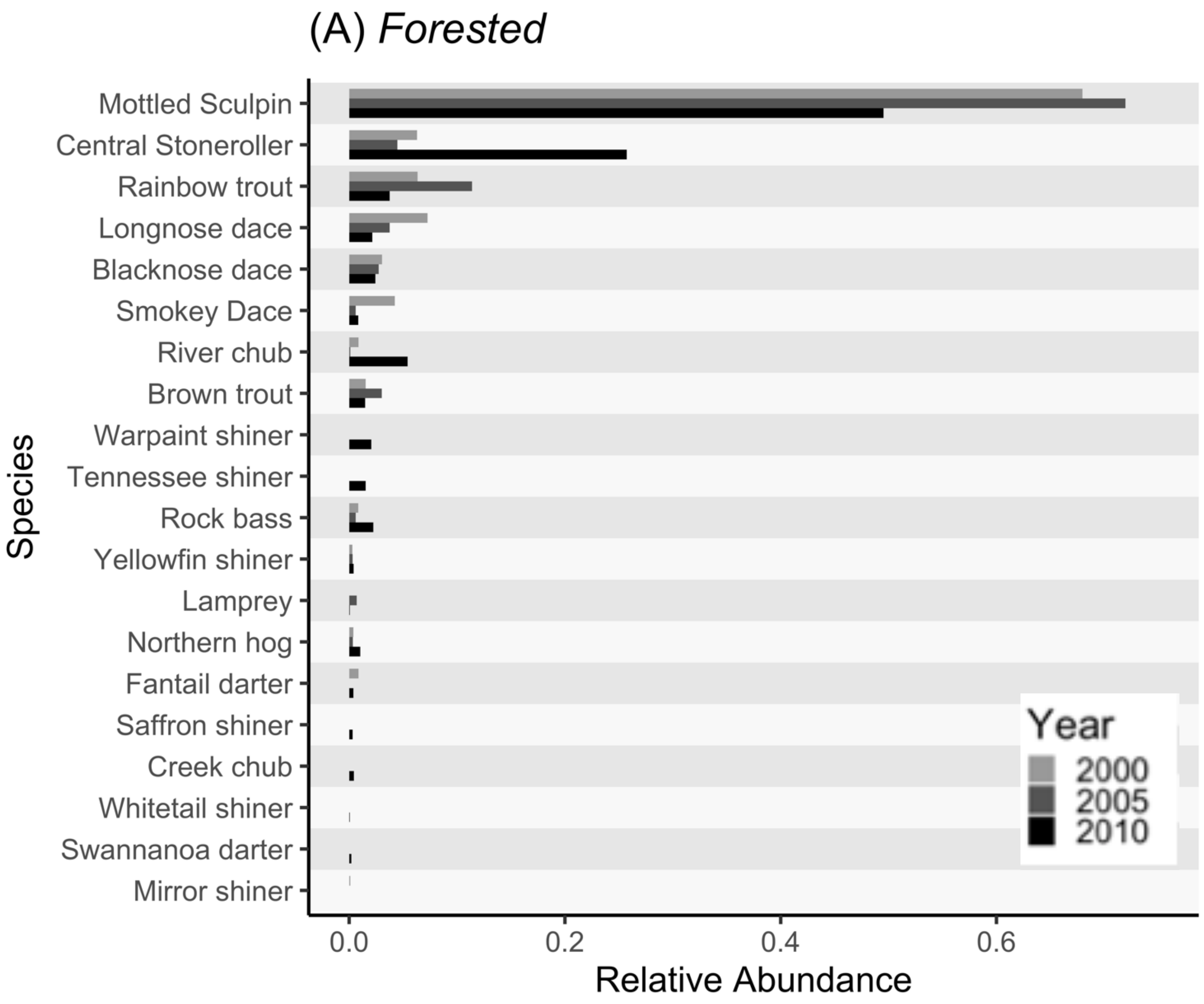
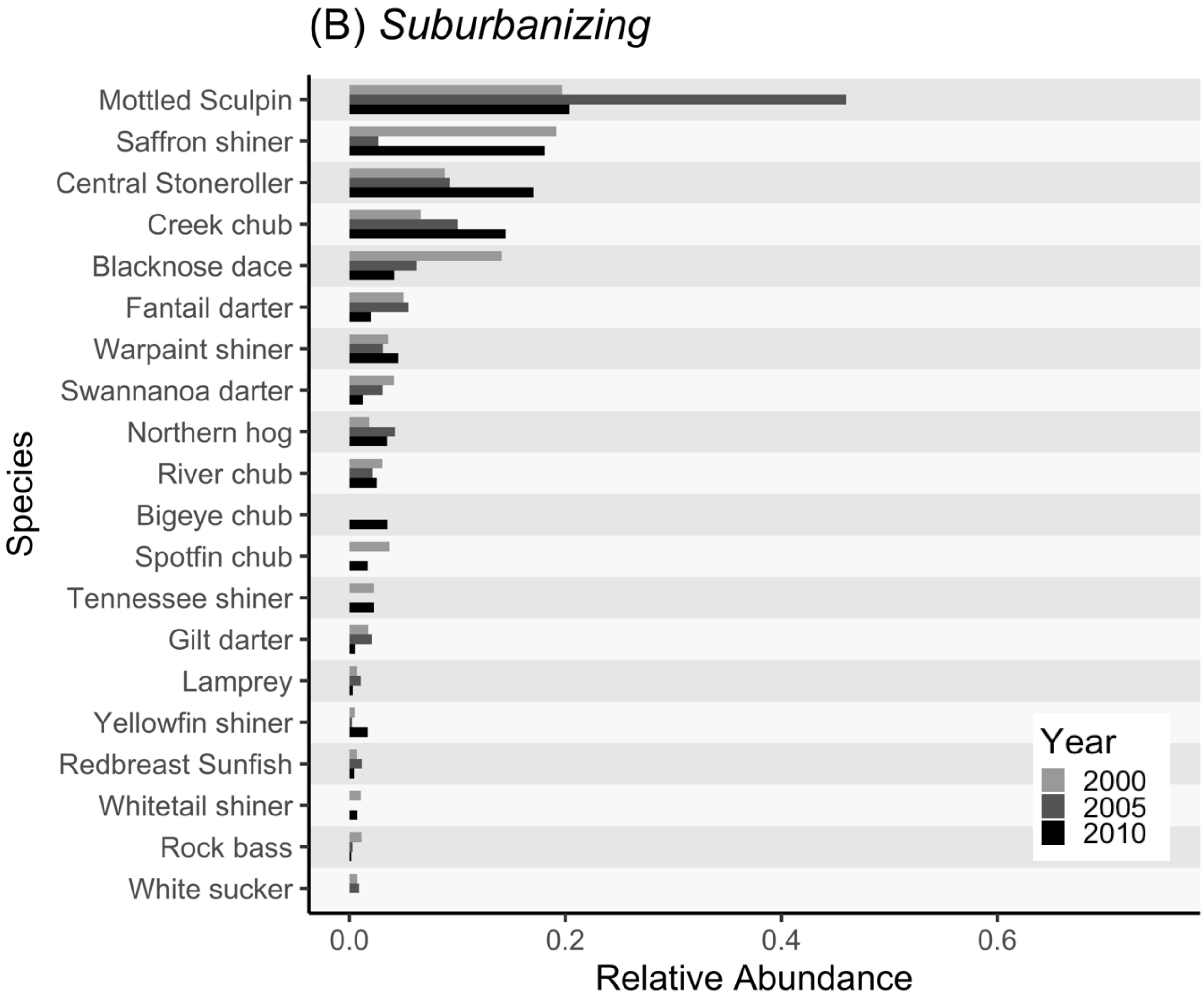


Fig. 4.10


(A) Forested
Species
Mottled Sculpin
Central Stoneroller
Rainbow trout
Longnose dace
Blacknose dace
Smokey Dace
River chub
Brown trout
Warpaint shiner
Tennessee shiner
Rock bass
Yellowfin shiner
Lamprey
Northern hog
Fantail darter
Saffron shiner
Creek chub
Whitetail shiner
Swannanoa darter
Mirror shiner
0.0
0.2
0.4
0.6
Relative Abundance
Year
2000
2005
2010


(B) Suburbanizing
Species
Mottled Sculpin
Saffron shiner
Central Stoneroller
Creek chub
Blacknose dace
Fantail darter
Warpaint shiner
Swannanoa darter
Northern hog
River chub
Bigeye chub
Spotfin chub
Tennessee shiner
Gilt darter
Lamprey
Yellowfin shiner
Redbreast Sunfish
Whitetail shiner
Rock bass
White sucker
0.0
0.2
0.4
0.6
Relative Abundance
Year
2000
2005
2010

CHAPTER 5

SUMMARY/CONCLUSIONS

In this dissertation, we used historical data sets, *snapshot* and *repeated-snapshot* sampling approaches, reach-scale riparian manipulations, trophic dynamics experiments, and stable isotope analyses to further our understanding of how changes to southern Appalachian forests have affected stream ecosystem processes. This approach allowed us to assess how both near-stream and basin-wide shifts in forest ecosystems have affected stream algae, crayfish, and fish. Our findings contribute to 40+ years of ecological research within the Coweeta Long Term Ecological Research Program.

In Chapter 2, we compared algal standing crop, algal community composition, and stream canopy cover before and after the loss of eastern hemlock during January and September on a decadal time-scale. We found significantly lower algal standing crop values post-hemlock die-off, both during leaf-off (January) and leaf-on (September) conditions. Lower algal standing crop values following hemlock death can likely be explained by the expansion of the riparian shrub, *Rhododendron maximum*. We also found significant differences in diatom community composition in September, but not January. Differences in September community composition were caused by a decrease in the relative biovolume of *Eunotia metamondon* and increase in the relative biovolume of *Eunotia rhomboidea* following hemlock death. Thus, while September diatom communities were taxonomically distinct, because one species was replaced by a similar species, diatom communities before and after hemlock die-off likely function in a similar manner. Findings in this second chapter support the contention that many algal taxa in southern

Appalachian headwater streams exhibit relative stability over time (*i.e.*, 13 years following the loss of a major riparian tree species).

In Chapter 3, we used a reach-scale experimental removal of rhododendron to quantify the short-term (2 year) response of algal cell biovolume, algal community composition, and crayfish dependence on autochthonous resources to the loss of this riparian shrub. Further, we used a plot-scale crayfish exclusion experiment nested within the rhododendron removal manipulations to account for trophic dynamics. We found that rhododendron removal increased total cell biovolume and resulted in a shift in algal community composition from primarily high-profile diatoms forming ribbon colonies (*i.e., Eunotia* spp.) to high-profile, stalk and chain-forming diatoms (*i.e., Gomphonema* spp., *Encyonema* sp.) and a filamentous chlorophyte (*Oedogonium* sp.), and that this effect was not mediated by crayfish. In addition, we found some evidence that crayfish will become more dependent on algal resources in streams where rhododendron is removed, but this effect may depend on watershed characteristics, like stream orientation. Findings in this chapter offer insight into the mechanism by which relatively small decreases in total riparian canopy cover (3.9-6.9% relative decreases) can alter algal communities and potentially affect trophic pathways by increasing the occurrence and/or intensity of sunflecks. Our findings suggest that large-scale removal of rhododendron in southern Appalachian headwater streams would likely result in increases in the relative abundance of filamentous green algal taxa, with food webs potentially becoming more autochthonous.

In Chapter 4, we compared algal and fish communities within forested and suburbanizing watersheds over ten years to examine how low-density development on forested mountainsides may be affecting biotic communities in southern Appalachian streams. We found higher alpha diversity and significant differences in functional and taxonomic composition in suburbanizing watersheds compared to forested watersheds for both diatom and fish communities, indicating that algal and fish communities in suburbanizing watersheds are in early stages of biotic homogenization. Although we found some evidence of change over time within diatom communities in terms of beta diversity and taxonomic composition, we found little evidence of change over time within fish communities. Findings in Chapter 4 suggest that relatively small amounts of watershed development can impact stream communities, but that diatoms may respond more rapidly to low-density development than fish.

Together, our findings from Chapter 2 and Chapter 3 indicate that expansion of rhododendron following hemlock death has maintained low-light availabilities, thus contributing to the persistence of dominant shade-tolerant algal taxa (*e.g.*, *Eunotia* spp., *Nupela lapidosa*). Removal of rhododendron from riparian zones as a management strategy has the potential to shift algal communities to favor diatoms with high growth forms and filamentous soft algae, and to increase consumer reliance on autochthonous resources. Although removal of rhododendron could be a necessary management strategy in some watersheds, maintaining rhododendron in streams draining suburbanizing watersheds may be one method to help mitigate potential changes in algal communities (and thus higher trophic levels) due to increasing urbanization.

Our research highlights both the utility and the limitations of taking a *snapshot* approach in examining algal communities. *Snapshot* sampling approaches can be useful when monitoring algal communities over a long-term period (*e.g.*, 10+ years), particularly when sample replication is high. For example, findings from Chapter 2 indicate stability in diatom communities following hemlock death on a decadal time scale. However, if we had only quantified algal communities in the years immediately following hemlock death, when rhododendron had not yet filled in the canopy gaps left by hemlock, we may have reached a different (and potentially misleading) conclusion. On the other hand, *snapshot* sampling approaches can be problematic when there is not a strong understanding of temporal variation in communities. For example, results from Chapter 4 indicated a shift in algal communities in forested reference streams through time from 2000-2010. This shift could be attributed to shifts in light availability in the riparian zone immediately following hemlock death; however, it could also be attributed to natural temporal variation in algal communities. Thus, our findings suggest that we need specific studies that track variation in algal community composition in forested reference streams through time, which would allow us to better understand conclusions derived from *snapshot* sampling events.

Through these studies, we provide new synthetic information on how changes in southern Appalachian forests are affecting stream algal communities. Moving forward, we recommend that, in addition to studies that track temporal variation in algal communities in forested reference streams, future research on algal communities of southern Appalachians streams should include: (1) creation of a voucher flora for the region to ensure taxonomic harmonization among algal studies; (2) incorporation of soft algal taxa into algal enumeration; (3) identification of growth forms for specific diatom species; and (4) measurements of primary production. In addition, we recommend further exploration into the importance of sunflecks to stream algal communities, which might include: (1) plot-scale *in-situ* experiments using shade-cloth and, (2) use of PAR sensors and photo-degrading fluorescein dye techniques to gain a better understand of the timing and intensity of sunflecks.

In summary, this dissertation provides comprehensive evidence that stream algal communities of the southern Appalachian Mountains are intricately connected with their adjacent forests. We found that slight changes to near-stream riparian areas or their forested watersheds can have significant impacts on stream algal standing stocks and community composition, and that these changes can cascade to higher trophic levels. This body of research highlights that tracking changes in the abundance, distribution, and community composition of primary producers is an essential step to understanding stream ecosystems in a changing world.

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APPENDIX A

CHAPTER 2

Table A.1: List of all diatom species and species complexes identified in this study with functional group, mean cell biovolumes (µm^3 mm^{-2}), and source of biovolume measurement: 2005-2006, samples from this study; 2018-2019, samples from this study; ANS, www.diatom.acnatsci.org; Lowe & Pan, Lowe and Pan (1996); Dye, Dye (2005).

Taxon	Functional group	Cell Biovolume (µm^3 mm^{-2})	Biovolume Source
Achnanthidium alpestre	low	90.51	2005-2006
Achnanthidium deflexum	low	88.77	ANS
Achnanthidium minutissimum	low	66.83	2005-2006
Achnanthidium rivulare	low	54.81	2005-2006
Achnanthidium sp. 1	low	38.02	2018-2019
Achnanthidium sp. 2	low	77.10	2018-2019
Achnanthidium sp. 3	low	49.62	2018-2019
Achnanthidium sp. 4	low	90.78	2018-2019
Achnanthidium sp. 5	low	140.02	2018-2019
Achnanthidium sp. 6	low	64.86	2005-2006
Achnanthidium subahudsonis	low	82.70	2005-2006
Adlafia detenta	motile	524.56	2018-2019
Adlafia sp. 1	motile	221.56	2018-2019
Asterinoella formosa	planktonic	495.83	2005-2006
Aulocoseira sp. 1	planktonic	144.64	2018-2019
Brachysira brebissonii	motile	172.48	ANS
Brachysira microcephala	motile	311.78	2018-2019
Brachysira serians	motile	212.56	2005-2006
Brachysira sp. 1	motile	169.96	2018-2019
Caloneis bacilum	motile	2665.86	2018-2019
Chamaepinnularia hassiaca	low	44.06	2005-2006
Chamaepinnularia sp. 2	low	77.56	2018-2019
Chamaepinnularia sp. 3	low	56.02	2018-2019
Cocconeis placentula	low	750.61	2005-2006
Decussata placenta	motile	1016.87	2018-2019
Diatoma moniliformis	low	171.48	2018-2019
Encyonema minutum (complex)	high	226.87	2005-2006
Includes*: Eunotia minutum* and *Eunotia silesiacum*			
Encyonema minutum var. pseudogracilis	high	1769.03	2018-2019
Encyonema neogracile	high	600.00	2005-2006
Encyonema silesiacum	high	473.33	2005-2006
Cymbella turgida	high	2000.00	2005-2006
Encyonema sp. 1	high	2026.71	2018-2019

Eunotia bilii	high	7573.07	2005-2006
Eunotia bilunaris	low	341.34	2005-2006
Eunotia braendlei	high	7414.60	2005-2006
Eunotia canicula	low	1213.20	2018-2019
Eunotia cataractarum	low	155.17	2018-2019
Eunotia curvata var. subarctuata	low	161.36	2005-2006
Eunotia exigua	low	111.64	2005-2006
Eunotia incisa	low	331.21	2005-2006
Eunotia kociolekii	low	277.29	2018-2019
Eunotia metamondon (complex)	high	4376.74	ANS
Includes: *Eunotia metamondon, Eunotia braendlei,* and *Eunotia maior*			
Eunotia minor (complex)	low	1223.04	2005-2006
Includes: *Eunotia minor, Eunotia c.f. minor,* and *Eunotia implicata*			
Eunotia mucophila	low	537.87	2005-2006
Eunotia muscicola var. tridentula	low	107.06	2005-2006
Eunotia mydohaimasiae	low	485.29	2018-2019
Eunotia naegelii	low	508.86	2005-2006
Eunotia nymanniana	low	218.55	2018-2019
Eunotia orthohedra	low	96.90	2018-2019
Eunotia paludosa	low	107.06	2005-2006
Eunotia parallela	high	2388.47	2018-2019
Eunotia rhomboidea (complex)	low	418.75	2005-2006
Includes: *Euntoia rhomboidea* and *Eunotia papilioforma*			
Eunotia richbuttensis	low	953.96	2018-2019
Eunotia rushforthii	low	218.71	2018-2019
Eunotia sp. 2	low	659.19	2018-2019
Eunotia sp. 25 Furey	low	115.45	2018-2019
Eunotia sp. 3	low	983.47	2018-2019
Eunotia sp. 4	low	627.96	2018-2019
Eunotia sp. 5	low	848.65	2018-2019
Eunotia sp. 6	low	415.38	2018-2019
Eunotia veneris	high	2094.30	2018-2019
Fallacia insociabilis	motile	98.27	2005-2006
Fragilaria sp. 1	high	141.39	2018-2019
Fragilaria tenera	high	993.00	2005-2006
Fragilaria vaucheriae	high	170.42	2005-2006
Fragilariforma virescens	high	292.01	ANS
Frustulia amphipleuroides	motile	3808.71	2005-2006
Frustulia crassnervia	motile	592.44	ANS
Frustulia rhomboides (complex)	motile	3476.45	2005-2006

Includes: *Frustulia rhomboides* and *Frustulia saxonica*

Frustulia vulgaris	motile	3330.68	2005-2006
Geissleria kriegeri	motile	420.36	2018-2019
Gomphonema affine	high	1254.41	ANS
Gomphonema affine var. rhombicum	high	307.40	2018-2019
Gomphonema angustatum	high	300.22	ANS
Gomphonema brasilense	high	763.47	2005-2006
Gomphonema c.f. angustum	high	428.92	2018-2019
Gomphonema christenseni	high	1116.34	2005-2006
Gomphonema clevei	high	613.51	2005-2006
Gomphonema exilissimum	high	259.15	2018-2019
Gomphonema gracile	high	434.03	2005-2006
Gomphonema innocens	high	244.29	2018-2019
Gomphonema louisiananum	high	254.27	2018-2019
Gomphonema parvulum	high	283.39	2005-2006
Gomphonema pusilium	high	500.00	2005-2006
Gomphonema sierrianum	high	376.25	2018-2019
Gomphonema sp. 1	high	138.91	2018-2019
Gomphonema sp. 2	high	220.33	2018-2019
Gomphonema sp. 28 SESQA	high	237.15	2018-2019
Gomphonema sp. 3	high	258.79	2018-2019
Gomphonema sp. 4	high	296.83	2018-2019
Gomphonema sp. 5	high	351.25	2018-2019
Gomphonema sp. 6	high	433.17	2018-2019
Gomphonema sp. 7	high	682.48	2018-2019
Gomphonema sp. 8	high	126.88	2018-2019
Gomphosphenia sp. 1	high	170.99	2018-2019
Gyrosigma acuminatum	motile	7020.99	ANS
Humidophila arcuata	low	46.03	2005-2006
Humidophila contenta	low	50.18	2005-2006
Humidophila contenta var. biceps	low	89.99	2005-2006
Humidophila schmassmanni	low	64.86	2005-2006
Karayevia clevei	low	108.61	2005-2006
Lindavia sp. 1	planktonic	104.78	2018-2019
Luticola goeppertiana	motile	538.24	2005-2006
Luticola mutica	motile	478.21	ANS
Meridion alansmithii	high	822.26	2005-2006
Meridion circulare	high	635.08	ANS
Meridion circulare var. constrictum	high	374.44	2005-2006
Microcostatus krasskei	motile	40.75	2005-2006

Navicula angusta	motile	742.16	2005-2006
Navicula cryptocephala	motile	293.25	2005-2006
Navicula cryptotenella	motile	396.68	2005-2006
Placoneis placentula	motile	754.06	2005-2006
Navicula sp. 2	motile	302.75	2018-2019
Navicula sp. 3	motile	171.71	2005-2006
Navicula symmetrica	motile	590.58	ANS
Navicula tripunctata	motile	380.13	2005-2006
Nitzschia dissipata	motile	356.40	2005-2006
Nitzschia sp. 1	motile	106.82	2018-2019
Nitzschia sp. 2	motile	145.39	2018-2019
Nitzschia sp. 6	motile	126.93	2018-2019
Nupela carolina	low	194.17	2018-2019
Nupela elegantula	low	116.17	2018-2019
Nupela lapidosa	low	313.68	2005-2006
Nupela sp. 1	low	191.32	2018-2019
Nupela sp. 2	low	440.27	2018-2019
Nupela wellneri	motile	95.47	2018-2019
Odontidium mesodon	low	934.87	ANS
Orthoseira roeseana	planktonic	250.00	2005-2006
Pinnularia erratica	motile	7095.81	2005-2006
Pinnularia mesolepta	motile	530.77	Dye
Pinnularia obscura	motile	500.00	Lowe & Pan
Pinnularia sp. 1	motile	202.37	2018-2019
Pinnularia sp. 4	motile	3793.79	2018-2019
Pinnularia sp. 5	motile	6559.22	2018-2019
Pinnularia subcapitata var. paucistriata	motile	595.66	2005-2006
Pinnularia termitina	motile	713.36	2005-2006
Planothidium lanceolatum	low	195.11	2005-2006
Planothidium peragalli	low	495.41	2018-2019
Platessa sp. 1	low	87.11	2018-2019
Platessa stewartii	low	156.43	2005-2006
Psammothidium harveyi	low	272.30	2005-2006
Psammothidium helveticum	low	244.92	ANS
Psammothidium subatomoides	low	33.31	2005-2006
Sellaphora atomoides	low	11.71	2005-2006
Sellaphora laevissima	motile	511.69	2018-2019
Sellaphora saugerresii	motile	21.46	2005-2006
Stauroneis gracilis	motile	9431.92	2018-2019
Stenopterobia delicatissima	motile	480.82	2018-2019

Synedra famelica	high	190.32	2005-2006
Synedra goulardii	high	1159.32	Dye
Synedra minuscula	high	248.25	2005-2006
Synedra rumpens var. familiaris	high	190.32	2005-2006
Synedra rumpens var. fragilarioides	high	328.93	2005-2006
Synedra rumpens var. meneghiniana	high	314.62	2005-2006
Synedra sp. 1	high	105.50	2018-2019
Synedra sp. 2	high	162.43	2018-2019
Tetracyclus rupestris	low	723.13	2005-2006
Ulnaria acus	high	1420.11	ANS
Ulnaria ramesi	high	2472.08	2018-2019
Ulnaria sp. 1	high	5025.76	2018-2019
Ulnaria ulna	high	4071.81	2005-2006

Table A.2: Definitions of diatom ecological guilds/functional groups from Rimet and Bouchez (2012)

Functional group	Definition
low	species of short stature, including prostrate (adhering to the substrate with the entire valve surface), adnate (apically attached but parallel to the substrate), erect (apically attached but perpendicular to the substrate), slow moving species
high	species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, stalked, and colonial centrics
motile	fast moving species

Table A.3: AIC table associated with light intensities and total solar-corrected light intensities (light index). Asterisk (*) indicates significant term ($p < 0.05$).

Response	Month	Slope	Quadratic term	AIC	Marginal R^2	Conditional R^2
Light intensity	January	2.42×10^{-2}*	*n/a*	0	0.01	0.17
		-6.04×10^{-4}	2.26×10^{-3}	11.29	0.02	0.17
	September	3.55×10^{-2}*	*n/a*	0	0.04	0.28
		3.60×10^{-2}*	-4.62×10^{-5}	13.39	0.04	0.28
Light index	January	2.51×10^{-2}*	*n/a*	0	0.01	0.17
		9.23×10^{-2}*	-6.13×10^{-3}*	3.24	0.02	0.17
	September	0.11*	-6.79×10^{-3}*	0	0.10	0.51
		3.86×10^{-2}*	*n/a*	45.13	0.08	0.49

Table A.4: Stream water nitrate (NO_3-N, μg L^{-1}) and soluble reactive phosphorus (PO_4-P, μg L^{-1}) concentrations during algal sampling events at the eight stream reaches. White columns represent pre-hemlock die-off (2005 and 2006), grey bars represent post-hemlock die-off (2018 and 2019). N=2 for each sample. Standard error in parentheses.

	NO_3 (*μg* L^{-1})						**SRP (*μg* L^{-1})**					
	Jan. '06	Jan. '18	Jan. '19	Sep. '05	Sep. '18	Sep. '19	Jan. '06	Jan. '18	Jan. '19	Sep. '05	Sep. '18	Sep. '19
Ball	46.5 (2.5)	168.8 (3.6)	62.3 (2.8)	44.0 (0.2)	90.9 (11.4)	108.6 (5.3)	0.0 (0.0)	0.0 (0.0)	2.7 (0.6)	0.4 (0.4)	0.1 (0.1)	4.7 (0.5)
Cunningham	13.0 (7.0)	1.65 (0.7)	3.2 (0.1)	10.2 (1.1)	12.7 (1.1)	23.0 (3.1)	0.0 (0.0)	0.0 (0.0)	1.4 (0.3)	0.0 (0.0)	1.3 (0.8)	5.2 (1.2)
L. Hugh	13.5 (1.5)	9.8 (0.1)	10.1 (2.6)	37.9 (0.0)	23.2 (1.7)	52.3 (1.1)	0.0 (0.0)	0.0 (0.0)	4.0 (0.1)	0.0 (0.0)	2.8 (1.0)	3.4 (0.7)
Mill	6.5 (1.5)	5.3 (0.7)	11.4 (2.7)	11.2 (1.0)	5.3 (2.7)	29.2 (7.6)	0.0 (0.0)	2.0 (2.0)	3.1 (0.8)	0.0 (0.0)	4.2 (1.9)	3.8 (0.2)
Reynolds	145.5 (2.5)	16.75 (0.3)	28.9 (0.7)	48.6 (0.6)	17.4 (1.9)	22.6 (3.0)	0.0 (0.0)	0.0 (0.0)	4.9 (0.2)	0.0 (0.0)	2.3 (0.0)	4.2 (1.0)
Shope	65.0 (2.0)	23.95 (0.2)	11.3 (0.9)	58.5 (0.3)	28.2 (6.2)	39.7 (18.3)	0.0 (0.0)	0.0 (0.0)	2.3 (0.0)	0.0 (0.0)	0.6 (0.6)	1.4 (0.4)
U. Hugh	9.5 (0.5)	3.25 (0.2)	7.6 (1.7)	17.7 (1.6)	16.1 (8.1)	20.3 (1.1)	0.0 (0.0)	0.0 (0.0)	2.4 (0.0)	0.6 (0.6)	2.3 (2.3)	1.0 (0.3)
Wykle	64.0 (22.0)	16.45 (2.2)	21.0 (10.9)	70.1 (3.4)	20.9 (3.6)	37.6 (3.0)	0.0 (0.0)	0.0 (0.0)	4.1 (2.9)	0.0 (0.0)	1.1 (1.1)	3.3 (0.3)

Table A.5: Stream water temperature (°C) and pH during algal sampling events at the eight stream reaches. White columns represent pre-hemlock die-off (2005 and 2006), grey bars represent post-hemlock die-off (2018 and 2019).

	Temperature (°C)						**pH**					
	Jan. '06	Jan. '18	Jan. '19	Sep. '05	Sep. '18	Sep. '19	Jan. '06	Jan. '18	Jan. '19	Sep. '05	Sep. '18	Sep. '19
Ball	5.9	4.6	5.4	16.8	16.7	17.0	6.70	6.02	6.00	6.50	5.82	6.08
Cunningham	7.4	7.2	7.6	16.3	16.5	17.4	6.70	6.40	6.53	6.20	5.94	6.22
L. Hugh	6.6	5.7	7.1	17.1	18.4	18.4	6.70	6.42	6.84	6.50	6.56	6.32
Mill	8.3	8.0	8.4	17.0	16.5	17.3	6.80	6.22	6.75	6.80	5.84	6.39
Reynolds	7.4	7.2	7.1	15.8	15.7	15.2	6.60	6.33	6.40	6.40	5.87	5.96
Shope	6.7	6.9	6.6	17.5	17.6	17.4	6.80	6.54	6.75	6.40	6.14	6.47
U. Hugh	7.5	6.9	8.1	16.5	17.5	17.8	6.80	6.88	6.48	6.50	6.01	6.07
Wykle	6.8	6.3	6.1	15.6	16.8	17.0	6.50	6.02	6.48	6.90	5.41	6.39

APPENDIX B

CHAPTER 3

Table B.1: Fractionation factors used to adjust isotope values of crayfish food sources.

Food Source	Discrimination factor δ^{13}C	Discrimination factor δ^{15}N	Source of estimate
Adjusted-periphyton (Algae)	1.57+3.00	3.35	Glon et al. (2016) England and Rosemond (2004)
CPOM	0.40	2.40	Ercoli et al. (2014) McCutchan et al. (2003)
FPOM	0.40	2.40	Ercoli et al. (2014) McCutchan et al. (2003)
Macroinvertebrates	2.00	1.00	Glon et al. (2016)

Table B.2: List of algal species identified in this study with functional group and mean cell biovolumes (μm^3 mm^{-2}) used for calculations. All cell biovolumes are from Solomon (2021, Chapter 2 of this dissertation), except for those taxa marked with an asterisk (*), which were calculated in this study.

Taxon	Functional Group	Cell Biovolume (μm^3 mm^{-2})
Achnanthidium alpestre	low	90.51
Achnanthidium deflexum	low	88.77
Achnanthidium minutissimum	low	66.83
Achnanthidium rivulare	low	54.81
Achnanthidium sp. 1	low	38.02
Achnanthidium sp. 2	low	77.1
Achnanthidium sp. 3	low	49.62
Achnanthidium sp. 4	low	90.78
Achnanthidium subahudsonis	low	82.7
Adlafia sp. 1	motile	221.56
Brachysira brebissonii	motile	172.48
Brachysira microcephala	motile	311.78
Caloneis bacillum	motile	2665.86
Chamaepinnularia hassiaca	low	44.06
Chamaepinnularia sp. 2	low	77.56
Chamaepinnularia sp. 3	low	56.02
Cocconeis placentula	low	750.61
Decussata placenta	motile	1016.87
Diatoma moniliformis	low	171.48
Encyonema minutum	high	226.87
Encyonema minutum var. *pseudogracilis*	high	1769.03
Encyonema neogracile	high	600
Encyonema sp. 1	high	2026.71
Eunotia bilii	high	7573.07
Eunotia bilunaris	high	341.34
Eunotia cf. *minor*	high	1223.04
Eunotia canicula	high	1213.2
Eunotia cataractarum	low	155.17
Eunotia exigua	low	111.64
Eunotia incisa	low	331.21
Eunotia jemtlandica	high	4878.1
Eunotia kociolekii	low	277.29
Eunotia metamondon	high	4376.74
Eunotia minor	high	1223.04

Eunotia mucophila	high	537.87
Eunotia muscicola var. *tridentula*	low	107.06
Eunotia mydohaimasiae	high	485.29
Eunotia nymanniana	low	218.55
Eunotia orthohedra	low	96.9
Eunotia paludosa	high	107.06
Eunotia papilioforma	low	696.61
Eunotia parallela	high	2388.47
Eunotia rhomboidea	low	418.75
Eunotia richbuttensis	high	953.96
Eunotia rushforthii	low	218.71
Eunotia sp. 1	low	137.92
Eunotia sp. 25 Furey	low	115.45
Eunotia sp. 4	high	627.96
Eunotia sp. 5	high	848.65
Eunotia sp. 6	low	415.38
*Eunotia variodentula**	low	159.48
Eunotia veneris	high	2094.3
Fragilaria vaucheriae	high	170.42
Fragilariforma sp. 2	high	757.4
Fragilariforma virescens	high	292.01
Frustulia crassnervia	high	592.44
Frustulia rhomboides	high	3476.45
Frustulia vulgaris	high	3330.68
Geissleria kriegeri	motile	420.36
Gomphonema affine	high	1254.41
Gomphonema affine var. rhombicum	high	307.4
Gomphonema angustatum	high	300.22
Gomphonema c.f. *angustum*	high	428.92
Gomphonema christenseni	high	1116.34
Gomphonema exilissimum	high	259.15
Gomphonema gracile	high	434.03
Gomphonema innocens	high	244.29
Gomphonema parvulum	high	283.39
Gomphonema sp. 1	high	138.91
Gomphonema sp. 2	high	220.33
Gomphonema sp. 28 SESQA	high	237.15
Gomphonema sp. 3	high	258.79
Gomphonema sp. 4	high	296.83
Gomphonema sp. 40 SESQA	high	773.14

Gomphonema sp. 5	high	351.25
Gomphonema sp. 6	high	433.17
Gomphonema sp. 7	high	682.48
Gomphonema sp. 8	high	126.88
Humidophila schmassmanni	low	64.86
Lindavia sp. 1	planktonic	104.78
Luticola goeppertiana	motile	538.24
Meridion alansmithii	high	822.26
Meridion circulare	high	635.08
Meridion circulare var. *constrictum*	high	374.44
Microcostatus krasskei	motile	40.75
Navicula angusta	motile	742.16
Navicula cryptocephala	motile	293.25
Navicula germainii	motile	570.37
Navicula perminuta	motile	71.92
Navicula sp. 1	motile	308.92
Navicula tripunctata	motile	380.13
Navicula veneta	motile	153.74
Neidium densestriatum	motile	570.43
Nitzschia amphibia	motile	188.15
Nitzschia dissipata	motile	356.4
Nitzschia sp. 1	motile	106.82
Nitzschia sp. 2	motile	145.39
Nitzschia sp. 3	motile	201.99
Nupela carolina	low	194.17
Nupela elegantula	low	116.17
Nupela lapidosa	low	313.68
Nupela sp. 1	low	191.32
Nupela sp. 2	low	440.27
Nupela wellneri	motile	95.47
Odontidium mesodon	low	934.87
Oedogonium		
life form 1*	filamentous	714.375242
life form 2*	filamentous	131.767497
life form 3*	filamentous	288.793096
Pinnularia erratica	motile	7095.81
Pinnularia mesolepta	motile	530.77
Pinnularia obscura	motile	500
Pinnularia sp. 2	motile	297.22
Pinnularia sp. 4	motile	3793.79

Pinnularia sp. 6*	motile	9494.12
Pinnularia sp. 7*	motile	8517.58
Planothidium lanceolatum	low	195.11
Platessa hustedtii	low	97.47
Platessa sp. 1	low	87.11
Platessa stewartii	low	156.43
Psammothidium harveyi	low	272.3
Psammothidium helveticum	low	244.92
Psammothidium subatomoides	low	33.31
Sellaphora atomoides	low	11.71
Stenopterobia delicatissima	motile	480.82
Synedra famelica	high	190.32
Synedra rumpens var. *fragilarioides*	high	328.93
Synedra sp. 2	high	162.43
Tetracyclus rupestris	low	723.13
Ulnaria acus	high	1420.11
Ulnaria ulna	high	4071.81

Table B.3: Mean $\delta^{13}C$ values for crayfish food resources by rhododendron removal treatment (*Cut, Cut+Burn*) and stream reach (treatment and upstream). N=5 for periphyton, CPOM, and FPOM; 5<N<9 for macroinvertebrates. Values in parentheses are standard errors.

Rhododendron removal treatment	Stream reach	Periphyton	CPOM	FPOM	Macroinvertebrates
Cut	Treatment	-27.83 (0.27)	-28.97 (0.30)	-27.18 (0.06)	-27.73 (0.86)
	Upstream	-28.36 (0.10)	-30.64 (0.07)	-27.19 (0.12)	-28.09 (0.73)
Cut+Burn	Treatment	-27.56 (0.20)	-29.11 (0.42)	-27.55 (0.03)	-25.88 (0.67)
	Upstream	-28.18 (0.05)	-30.35 (0.15)	-27.61 (0.06)	-26.07 (0.46)
	Mean	**-27.98** (0.18)	**-29.77** (0.42)	**-27.38** (0.12)	**-26.95** (0.56)

Table B.4: Mean δ^{15}N values for crayfish food resources by rhododendron removal treatment (*Cut, Cut+Burn*) and stream reach (treatment and upstream). N=5 for periphyton, CPOM, and FPOM; 5<N<9 for macroinvertebrates. Values in parentheses are standard errors.

Rhododendron removal treatment	Stream reach	Periphyton	CPOM	FPOM	Macroinvertebrates
Cut	Treatment	2.87 (NA)	-1.64 (0.21)	NA (NA)	2.79 (0.42)
	Upstream	3.24 (0.03)	-2.87 (0.35)	NA (NA)	2.40 (0.67)
Cut+Burn	Treatment	1.06 (0.50)	-3.10 (0.30)	0.96 (0.09)	1.17 (0.50)
	Upstream	2.26 (1.14)	-4.30 (0.34)	NA (NA)	2.26 (1.30)
	Mean	**2.355** (0.46)	**-2.98** (0.03)	**0.96** (NA)	**2.15** (0.35)

APPENDIX C

CHAPTER 4

Table C.1: Diatom taxa identified in this study with functional group.

Species	Functional Group
Achnanthidium exiguum	low
Achnanthidium minutissimum	low
Achnanthidium minutissimum var. *jackii*	low
Achnanthidium rivulare	low
Achnanthidium sp. 1	low
Achnanthidium sp. 10	low
Achnanthidium sp. 11	low
Achnanthidium sp. 12	low
Achnanthidium sp. 13	low
Achnanthidium sp. 14	low
Achnanthidium sp. 15	low
Achnanthidium sp. 16	low
Achnanthidium sp. 17	low
Achnanthidium sp. 18	low
Achnanthidium sp. 2	low
Achnanthidium sp. 3	low
Achnanthidium sp. 4	low
Achnanthidium sp. 5	low
Achnanthidium sp. 6	low
Achnanthidium sp. 7	low
Achnanthidium subhudsonis	low
Achnanthidium sp. 8	low
Amphora libyca	low
Amphora sp. 1	low
Anoemoeneis sphaerophora var. *costata*	motile
Anomoeoneis sphaerophora	motile
Bacillaria paradoxa	motile
Brachysira vitrea	low
Caloneis bacillum	motile
Caloneis sp. 1	motile
Cocconeis placentula	low
Craticula cuspidata	motile
Craticula halophila	motile
Cymbella affinis	low
Cymbella cf. *cistula*	high
Cymbella naviculiformis	low

Cymbella tumida	high
Diatoma mesodon	high
Encyonema minutum	high
Encyonema minutum var. *pseudogracilis*	high
Encyonema silesiacum	high
Eolimna minima	motile
Eunotia bilunaris	high
Eunotia exigua	low
Eunotia implicata	high
Eunotia incisa	low
Eunotia minor	high
Eunotia pectinalis	high
Eunotia rushforthii	low
Eunotia sp. 1	high
Eunotia sudetica	high
Fallacia insociabilis	motile
Fragilaria capucina	high
Fragilaria capucina var. *gracilis*	high
Fragilaria vaucheriae	high
Fragilariforma virescens var. *exigua*	high
Frustulia amphipleuroides	motile
Frustulia crassinervia	motile
Frustulia rhomboides	motile
Frustulia saxonica	motile
Frustulia vulgaris	motile
Geissleria decussis	motile
Geissleria punctifera	motile
Gomphonema angustatum	high
Gomphonema cf. subclavatum	high
Gomphonema clavatum	high
Gomphonema freesei	high
Gomphonema gracile	high
Gomphonema lagenula	high
Gomphonema mehleri	high
Gomphonema minutum	high
Gomphonema parvulum	high
Gomphonema pumilum	high
Gomphonema rhombicum	high
Gomphonema subclavatum	high
Gomphosphaenia grovei	high

Gyrosigma attenuatum	motile
Halamphora veneta	motile
Hannaea arcus	low
Hantzschia amphioxys	motile
Humidophila contenta	high
Lemnicola hungarica	low
Luticola goeppertiana	motile
Melosira varians	high
Meridion alansmithii	high
Meridion circulare var. *constrictum*	high
Navicula angusta	motile
Navicula cf. *cryptotenella*	motile
Navicula cf. *meniculus*	motile
Navicula cf. *salinarum*	motile
Navicula cf. *schroeteri*	motile
Navicula cf. *tripunctata*	motile
Navicula cf. *viridula/rostellata*	motile
Navicula cocconeiformis	motile
Navicula cryptocephala	motile
Navicula cryptotenella	motile
Navicula difficultissima	motile
Navicula germainii	motile
Navicula lanceolata	motile
Navicula lateropunctata	motile
Navicula meniculus	motile
Navicula radiosa	motile
Navicula rhynchocephala	motile
Navicula rostellata	motile
Navicula salinarum	motile
Navicula schroeteri	motile
Navicula sp. 1	motile
Navicula sp. 2	motile
Navicula sp. 3	motile
Navicula sp. 4	motile
Navicula trivialis	motile
Navicula viridula	motile
Nedium productum	motile
Neidium ampliatum	motile
Nitzschia amphibia	motile
Nitzschia cf. *capitellata*	motile

Nitzschia dissipata	motile
Nitzschia frustulum	motile
Nitzschia gracilis	motile
Nitzschia inconspicua	motile
Nitzschia linearis	motile
Nitzschia obtusa var. nana	motile
Nitzschia palea	motile
Nitzschia perminuta	motile
Nitzschia recta	motile
Nitzschia sp. 1	motile
Nupela lapidosa	low
Pinnularia cf. *legumen*	motile
Pinnularia gibba var. *linearis*	motile
Pinnularia lundii	motile
Pinnularia maior	motile
Pinnularia mesogonglya	motile
Pinnularia obscura	motile
Pinnularia sp. 1	motile
Pinnularia sp. 2	motile
Pinnularia sp. 3	motile
Pinnularia subcapitata	motile
Pinnularia viridis	motile
Placoneis cf. *gastrum*	motile
Planothidium delicatulum	low
Planothidium dubium	low
Planothidium frequentissimum	low
Planothidium lanceolatum	low
Planothidium peragalli	low
Platessa lutheri	low
Platessa stewartiii	low
Psammothidium marginulatum	low
Psammothidium semiapertum	low
Psammothidium subatomoides	low
Reimeria sinuata	low
Rhoicosphenia abbreviata	low
Rhopalodia gibberula var. *vanheurckii*	motile
Rossithidium anastasiae	low
Sellaphora pupula	motile
Sellaphora pupula var. capitata	motile
Sellaphora saugerresii	motile

Stauroneis anceps	motile
Surirella angusta	motile
Surirella linearis	motile
Surirella minuta	motile
Surirella sp. 1	motile
Surirella sp. 2	motile
Surirella tenera	motile
Synedra rumpens	high
Synedra rumpens var. *familiaris*	high
Synedra rumpens var. *fragilarioides*	high
Ulnaria acus	high
Ulnaria ramesii	high
Ulnaria ulna	high

Table C.2: Fish taxa identified in this study with common name and functional group.

Species	Common name	Functional group
Ambloplites rupestris	Rock bass	cosmopolitan
Campostoma anomalum	Central stoneroller	cosmopolitan
Catostomus commersoni	White sucker	cosmopolitan
Clinostomus sp.	Smokey dace	highland endemic
Cottus bairdi	Mottled sculpin	highland endemic
Cyprinella galactura	Whitetail shiner	cosmopolitan
Erimonax monacha	Spotfin chub	highland endemic
Etheostoma blennioides	Greenside darter	cosmopolitan
Etheostoma flabellare	Fantail darter	highland endemic
Etheostoma swannanoa	Swannanoa darter	highland endemic
Hybopsis amblops	Bigeye chub	cosmopolitan
Hybrid Lepomis sp.	Hybrid	cosmopolitan
Hypentelium nigricans	Northern hog	cosmopolitan
Ichthyomyzon greeleyi	Lamprey	cosmopolitan
Lepomis auritus	Redbreast sunfish	cosmopolitan
Lepomis cyanellus	Green sunfish	cosmopolitan
Lepomis macrochirus	Bluegill	cosmopolitan
Luxilus coccogenis	Warpaint shiner	highland endemic
Micropterus salmoides	Largemouth bass	cosmopolitan
Nocomis micropogon	River chub	cosmopolitan
Notropis leuciodus	Tennessee shiner	highland endemic
Notropis lutipinnis	Yellowfin shiner	cosmopolitan
Notropis rubicroceus	Saffron shiner	highland endemic
Notropis spectrunculus	Mirror shiner	highland endemic
Notropis telescopus	Telecope shiner	other
Oncorhynchus mykiss	Rainbow trout	other
Percina evides	Gilt darter	highland endemic
Phenacobius crassilabrum	Fatlips minnow	highland endemic
Rhinichthys atratulus	Blacknose dace	cosmopolitan
Rhinichthys cataractae	Longnose dace	other
Salmo trutta	Brown trout	other
Salvelinus fontinalis	Brook trout	highland endemic
Semotilus atromaculatus	Creek chub	cosmopolitan