An Investigation and Quantification of Nitrogen Sources and Sinks in

Shenandoah National Park, Virginia

Katherine Mary Coughlin Syracuse, New York

Bachelor of Science cum laude, Geoscience, William Smith College, 2016

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> Committee: Todd M. Scanlon James N. Galloway Lawrence E. Band Ami L. Riscassi

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### Abstract

Disturbances, such as invasive insects and atmospheric deposition, have the potential to significantly alter nitrogen cycling in forested watersheds. Excess nitrogen in forested watersheds can trigger nitrogen saturation, stream acidification, and it can threaten native biodiversity. This study explored two aspects of nitrogen dynamics in forested watersheds.

The first study explored the effect of the emerald ash borer in Shenandoah National Park. Stream nitrate data from 1988 to the present, at 12 watersheds, was compared to the amount of ash cover within each watershed. Seasonally sampled watersheds displayed significant results between presumed ash coverage and enhanced stream nitrate concentrations (p = 0.04). Based on the observed results, emerald ash borer activity can be detectable in heightened stream nitrate concentrations.

The second study quantified the sources and sinks of nitrogen in Shenandoah National Park. A nitrogen budget was presented for five watersheds in Shenandoah National Park. It was determined that the carbon-to-nitrogen ratio found in forest soils is an excellent indicator of how much nitrogen is stored and exported in streams each year. Additionally, it was determined that two watersheds in Shenandoah National Park are exhibiting early signs of nitrogen saturation due to heightened atmospheric deposition levels, elevated stream nitrate export, and low soil C:N ratios.

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#### **Chapter 1: Introduction**

Nitrogen is one of the most abundant elements in nature, and it is essential to life. It is arguably the most important nutrient in regulating primary productivity and species diversity in terrestrial and aquatic organisms, as it is found in all organisms' amino and nucleic acids (Vitousek et al., 2002). On Earth, the atmosphere is 78% dinitrogen gas (N<sub>2</sub>), which is a largely nonreactive and inaccessible molecule for biota. The nonreactivity of N<sub>2</sub> to organisms makes nitrogen a very scarce resource and a limited nutrient in ecosystems. Nitrogen only becomes available to organisms when it is converted from N<sub>2</sub> to a bio-reactive form (e.g., NH<sub>3</sub>).

In addition to  $N_2$  and  $NH_3$ , many forms of nitrogen exist in the environment, including organic forms, such as amino and nucleic acids, and inorganic forms like ammonium and nitrate. The nitrogen cycle utilizes a series of biogeochemical reactions to transform inert atmospheric  $N_2$  into useful molecular forms. The primary processes of the nitrogen cycle are fixation, assimilation, ammonification, nitrification, and denitrification. Each of these processes are discussed in further detail in Chapters 2 and 3.

Since the industrial revolution, and as a result of increased fossil fuel combustion, urban waste, agriculture, and livestock operations, global use of nitrogen has risen. All areas of the world, from dense cities to remote forests, have been touched by the consequences of nitrogen pollution (Vitousek et al., 2002). To date, many studies have focused on disturbances to the nitrogen cycle as a result of human activities. With respect to mountain watersheds, research has focused on the effect of heightened atmospheric

deposition levels on stream nitrate concentrations (Lawrence et al., 2000; Fakhraei et al., 2016; Porter Blett et al., 2005; Sullivan et al., 2004; Veseý et al., 2002; Wright et al., 2001), nitrogen saturation (Aber et al., 1991; Aber et al., 1998; Aber et al., 1989; Corre et al., 2003; Lovett & Goodale, 2011; Peterjohn et al., 1996), and watershed nitrogen cycling (Bonito et al., 2003; Gilliam et al., 2018; Running & Gower, 1991; Yanai et al., 2013). Additionally, studies have explored the effect of destructive, invasive insect infestations on the nitrogen cycle and forest ecosystems (Bormann et al., 1977; Dukes et al., 2009; Eshleman et al., 1998; Kosola et al., 2004; Liebhold et al., 1992; Lovett et al., 2002; Poland & McCullough, 2006; Scanlon et al., 2010; Williamson, 1996).

This thesis explores two aspects of the nitrogen cycle in Virginia's remote Shenandoah National Park (SHEN). SHEN has a long history of elevated atmospheric N deposition levels and invasive insects, which have affected forest growth, retention, and export of nitrogen (Eshleman et al., 1995; Ryan et al., 1989; Sullivan et al., 2007; Webb et al., 1995).

### **Thesis Organization**

This thesis is organized into four chapters, each exploring nitrogen cycling in select watersheds of SHEN. Chapter 2 examines the effect of the recent invasion of the emerald ash borer on nitrogen cycling and if its presence can be detected in stream nitrate concentrations. Through a combination of spatial analyses and stream nitrate analysis, it may be possible to detect if the emerald ash borer has invaded a watershed through stream nitrate peaks.

Chapter 3 quantifies the nitrogen budget of SHEN. Through this information, it was determined what components of the nitrogen budget drive the spatial variability seen

in stream nitrogen export. It was determined that the carbon-to-nitrogen (C:N) ratio found in forest soils is an excellent indicator of how much nitrogen is stored and exported into streams each year and that several watersheds in SHEN are exhibiting signs of nitrogen saturation.

Chapter 4 summarizes the work presented in each chapter of this thesis. Future research ideas based on the conclusions of this study are discussed.

# Chapter 2: Emerald Ash Borer Invasion and Elevated Stream Nitrate Levels Shenandoah National Park, Virginia

### Introduction

The effect of invasive species on native biodiversity is well known (Williamson, 1996), and the introduction of invasive species can have cascading effects on the physical landscape. There are more than 400 alien insect species in North American forests, and almost every eastern North American woody plant has an associated invasive species (Mattson et al., 1994; Mattson et al., 2007). In the eastern United States, several well-known and particularly devastating invasive insects include the gypsy moth (*Lymantria dispar*) (Liebhold et al., 1992), the hemlock woolly adelgid (*Adelges piceae*) (Orwig & Foster, 1998), and the emerald ash borer (*Agrilus planipennis*) (Poland & McCullough, 2006). Given the lack of native predators, each of these species have spread rapidly and unchecked throughout the eastern United States, and have caused widespread defoliations and mortality of select tree species. The purpose of this paper is to investigate the link between disturbances from the invasive emerald ash borer and its effect on forested watersheds within SHEN, specifically, its effect on stream nitrate concentrations.

SHEN has a long history of disturbances by invasive insects. Most recently, the gypsy moth invaded SHEN in 1984, and by 1992, had defoliated upwards of 80% of some watersheds (Eshleman et al., 1998). After the defoliations, large spikes in SHEN stream nitrate concentrations could be directly linked to the gypsy moth (Eshleman et al., 1998). Weekly samplings of stream nitrate by the Shenandoah Watershed Study (SWAS)

captured the surge of nitrate from 1992 to 2008 in the Paine Run, Piney River, and Staunton River watersheds (Figure 1).



*Figure 1*. Weekly collected stream nitrate concentrations (ueq/L) at Piney River (Panel A), Staunton River (Panel B), and Paine Run (Panel C) watersheds. Samples were collected at each watershed outlet.

Eshleman et al. (1998) and Riscassi and Scanlon (2009) noted that these increases in nitrate occurred primarily during storm events. After the gypsy moth invasion, additional nitrate in stream water was a direct result of more insect feces, dead insects, unconsumed foliage, and leaching from damaged foliage (Lovett et al., 2006). Gypsy moth defoliations can also reduce forest productivity and transpiration and increase water runoff and drainage (Kosola et al., 2004; Lovett et al., 2002; McManus & Doane,1981).

The hemlock woolly adelgid has also had severe impacts on SHEN, and it has remained a serious pest in the park since its arrival in 1951. Surveys of hemlock stands in SHEN have revealed upwards of 95% hemlock mortality in the park (Bair, 2002). Hemlocks are a late successional species with dense, shade-providing canopies. Their large canopies create a cool, dark environment that reduces the number of understory plants. Removal of hemlocks opens the canopy, allowing more light penetration and subsequent increases in forest floor temperature and moisture. Similar to the effect of the gypsy moth, sudden, wide-scale hemlock deaths increase nitrogen mineralization, nitrification, and leaching of nitrate to surface waters (Jenkins et al., 1999; Yorks et al., 2003).

Most recently in 2013, the emerald ash borer (EAB) was detected in the northern portions of SHEN. By 2017, the EAB was found in all regions of SHEN. Since the EAB's discovery in Michigan in 2002, it has been responsible for millions of ash tree deaths (Aukema et al., 2011). The EAB has become one of the costliest insects to invade the United States (Aukema et al., 2011). Projected costs of treating ash trees in urban areas of the United States is upwards of ten billion dollars (Kovacs et al., 2010). It has been hypothesized that the EAB arrived into the United States in wood packing material shipped from Asia. In North America, the EAB preys on white, green, and black ash trees (Jendek, 1994). The blue ash is also susceptible to EAB invasion, but it is the most

resistant to infestation (Anulewicz et al., 2007; Tanis & McCullough, 2012). The average life cycle of the EAB spans from 1 to 2 years (Cappaert et al., 2005) with adults living between 3-6 weeks (Wang et al., 2010). It is the development and feeding of the EAB larvae that result in ash tree mortality. After hatching, EAB larvae bore through the outer bark and feed in the phloem and cambium. The feeding process disrupts the tree's ability to take up nutrients and water, eventually resulting in tree branch die off (Herms & McCullough, 2010). Total tree mortality typically occurs within 5 years of initial infestation. After ash mortality, ash seedlings are the most common woody seedling but remain a continued host for EAB. EAB-induced gap formations will likely facilitate the spread of invasive plants such as the amur honeysuckle (*Lonicera maackii*) and garlic mustard (*Alliaria petiolate*) (Herms et al., 2008). In North America, distribution of EAB is more likely controlled by ash presence than by climate (Sobek-Swant et al., 2012).

The ability to detect the EAB is a key component of pest management and control. To date, no detection procedure exists that can identify EAB presence before multiple generations have dispersed (Mercader et al., 2011). Poor initial identification of the EAB is likely due to the insect first colonizing the upper portions of the canopy before migrating to the main trunk (Cappaert et al., 2005; Duan et al., 2010).

This chapter investigates if stream nitrate concentrations in relatively undisturbed watersheds within SHEN can be used as an early indicator of EAB invasion. Since 2013, SWAS weekly monitoring in the Piney River watershed has seen a 30% increase in stream nitrate concentrations. The timing of these increases corresponds to the EAB's invasion just 1 year earlier. Because the EAB reduces an ash tree's ability to absorb nitrate, this study hypothesizes that the EAB is responsible for the noticeable uptick in nitrate concentrations. Specifically, of the watersheds studied, Piney River has been the most affected by the EAB invasions. This hypothesis is further fueled by the fact that nitrate in the SHEN watersheds is typically tightly cycled, so any increase is indicative of a disturbance. Thus, the objective of this study is to determine if increases in stream nitrate concentrations in SHEN watersheds is an early indication of an EAB invasion. **Methods** 

**Study area.** This study focuses on the watersheds in SHEN. Located in the Blue Ridge Mountains of western Virginia, SHEN is 777 km<sup>2</sup>, and it is primarily forested with a mix of deciduous and coniferous trees. SHEN has a mean annual average temperature of 9°C in the higher elevations and 12°C in the lower elevations (National Park Service, 2017). At the park's primary monitoring site, Big Meadows, the annual average precipitation is 132 cm. The lower areas of the park typically experience warm, humid summers and mild winters (National Park Service, 2017). The soils in SHEN are highly weathered where clay is the primary constituent in all watersheds (Rice et al., 2014). The park is underlain by three primary geologic classes: basaltic, granitic, and siliciclastic, with each class representing approximately one-third of the area in the park (Jastram et al., 2013). Figure 2 shows the 12 watersheds within SHEN that are the focus of this study.



*Figure 2*. Twelve watersheds within SHEN. Boundaries of the study watersheds are outlined in bold. The light grey line represents the park boundaries of SHEN. Watersheds with multiple sampling locations (synoptically sampled sites) are filled in with dark grey. Presumed ash coverage in SHEN is in light grey. Further detail found in *Methods: Vegetation Data*.

**Stream chemistry data.** Nitrate and discharge data were provided by the University of Virginia's SWAS. SWAS was established in 1979 to assess the effects of

acid deposition on mountain streams and their watersheds (Shaffer and Galloway, 1982).

The SWAS program sampled the 12 watersheds within SHEN, quarterly (i.e.,

seasonally), for major ions including sulfate, nitrate, chloride, calcium, magnesium,

potassium, sodium, and silica. Quarterly sampling sites include Brokenback Run, Deep

Run, Hazel River, Jeremy's Run, Madison River, Paine Run, Piney River, North Fork of

Thorton River, Rose River, Staunton River, Two Mile Run, and White Oak Canyon Run

(Figure 2; Table 1).

Table 1

Period of Record, Stream Nitrate Sampling Frequency, and Discharge Sampling Information for Quarterly and Weekly Sampled SWAS Watersheds and Synoptically

Sampled Staunton River and Piney River Watersheds

	Sampling Start Data	Sampling End Data	NO <sub>3</sub> Sampling	Discharge
Quarterly SWAS Watersheds	Start Date		Seasonally (Apr. Jul. Oct)	None
Brokenback Run (VT58)	1/31/1988	Present		
Deep Run (DR01)	1/30/1988	Present		
Hazel River (VT62)	1/31/1988	Present		
Jeremy's Run (VT51)	1/31/1988	Present		
Madison River (MAD2)	1/30/1988	Present		
Paine Run (PAIN)	1/31/1988	Present		
Piney River (PINE)	1/28/1993	Present		
North Fork of Thorton River (VT61)	1/31/1988	Present		
Rose River	1/31/1998	Present		
Staunton River (STAN)	1/31/1988	Present		
Two Mile Run (VT53)	1/31/1988	Present		
White Oak Canyon Run (VT75)	1/30/1991	Present		
Weekly SWAS Watersheds			Weekly	Daily
Paine Run (PAIN)	9/1/1992	Present		
Piney River (PINE)	9/1/1992	Present		
Staunton River (STAN)	9/1/1992	Present		
Synoptic Watersheds			Annually (Mar/Apr)	
Piney River	1/19/2006	3/30/2017		None
Staunton River	1/20/2006	4/2/2017	Annually (Mar/Apr)	None

In addition to the quarterly sites, SWAS collects weekly samples at the outlets of Paine Run, Piney River, and Staunton River (Figure 1). Samples are chemically analyzed for all major ions including nitrate. In addition to stream chemistry sampling, daily discharge is recorded at Paine Run, Piney River, Staunton River (Table 1). Within the Piney River and Staunton River watersheds, there are 29 and 18 established synoptic sampling sites, respectively (Figures 3 and 4; Table 1). Synoptic sampling sites are also established in



*Figure 3.* Piney River synoptic sampling sites and corresponding subwatersheds.

Presumed ash coverage displayed in light grey.

Paine Run; however, these watersheds were not included in the analysis because the vegetation map showed no vegetation communities with ash in the watershed. Each synoptic stream site has been sampled for nitrate at least annually from 2006 until 2017. Sampling of the synoptic sites was done by Dr. Keith Eshleman at the University of Maryland Appalachian Lab.



*Figure 4*. Staunton River synoptic sites and corresponding subwatersheds. Presumed ash coverage displayed in light grey.

All weekly and quarterly SWAS water samples were collected with grab samples. Prior to 2005, nitrate samples were processed using an atomic adsorption spectrophotometer using EPA-based methods. After 2005, nitrate was processed using ion chromatography (ASTM Method D 6919-03, ASTM 2003). Changes in processing methods created statistically significant analyte measurements, which were corrected through the use of a . Additional collection and analysis methods can be found in Robinson et al. (2013). During analysis of the SWAS data, a handful of values were identified as below the detection limit. Any value marked below the detection limit were given a value of half the detection limit.

Based on analyses of the SWAS weekly time series, peak seasonal nitrate concentrations from 2012 to 2017 were observed in late Febraury and early March. Because peak nitrate was observed in the spring, the only quarterly data used were from the spring (April) quarter. Also, only using spring quarterly data allowed for a direct comparision to the synoptically sampled data, which were also collected in the spring.

**Vegetation data.** Spatial vegetation data for the study watersheds were obtained from a vegetation map produced by the USGS Leetown Science Center (USGS-LCS), the Virginia National Heritage Program (VANHP), and SHEN (Young et al., 2009). SHEN vegetation communities were mapped using a combination of remote sensing, spatial modeling, field data collection, and vegetation community ordination. 10-m digital elevation models were used to map ecological gradients as a precursor to vegetation sampling and creation of vegetation community types. Vegetation community types were verified with hundreds of 400 square meter plots throughout the park (Young et al., 2009). The map has a spatial resolution of 0.5 ha with a user's accuracy of 62.3% and a producer's accuracy of 76.9%. Producer's accuracy describes how often real features on the ground are correctly shown on the classified map, whereas user's accuracy shows

how often the class on the map will actually be present on the ground. User's accuracy can also be referred to as map reliability. The Shenandoah Vegetation Map is divided into 35 natural community types, three modified/successional community types, and two disturbed/modified vegetation classes.

Of the 40 community types in the vegetation map, nine community types containing *Fraxinus Americana* (white ash) were included in the spatial analysis (Table 2). Using the Esri ArcGIS mapping analytics platform, *Fraxinus Americana*containing community types were overlain onto the study watersheds, and a presumed ash coverage (%) for each watershed was computed.

Table 2

Shenandoah Vegetation Map Ash (Fraxinus Americana) Containing Community Types Utilized in Spatial Analysis of Study of Watersheds. Community Species are Listed in Order from Highest to Lowest Species Abundance in a Specific Community

Ash Abundance	Community Type	Community Species
Primary	Central Appalachian Basic Woodland	Fraxinus Americana – Carya glabra / Muhlenbergia sobolifera – Helianthus divaricatus – Solidago ulmifolia Woodland
Primary	Central Appalachian Mafic Barren	Fraxinus Americana / Physocarpus opulifolius / Carex pensylvanica – Allium cernuum – (Phacelia dubia)
Secondary	Central Appalachian Rich Cove Forest	Acer saccharum – Fraxinus Americana – Tilia Americana – Liriodendron tulipifera / Actaea American Forest
Secondary	Central Appalachian Basic Boulderfield Forest	Tilia Americana – Fraxinus Americana / Acer pensylvanicum – Ostrya virginiana / Parthenocissus quinquefolia – Impatiens pallida Woodland
Secondary	Central Appalachian Circumneutral Barren	Juniperus virginiana – Fraxinus Americana / Carex pensylvanica – Cheilanthes lanosa
Secondary	Central Appalachian Basic Seepage Swamp	Acer rubrum – Fraxinus Americana – Fraxinus nigra – Betula alleghaniensis / Veratrum viride – Carex bromoides Forest
Tertiary	Southern Appalachian Cove Forest	Liriodendron tulipifera – Aesculus flava – (Fraxinus Americana, Tilia Americana) / Actaea American – Laportea canadensis Forest
Tertiary	Central Appalachian Montane Oak- Hickory Forest	Quercus rubra – Quercus alba – Fraxinus Americana – Carya (ovata, ovalis) / Actaea American Forest
Tertiary	Northeastern Modified Successional Forest	Prunus American – Liriodendron tulipifera – Acer rubrum – Fraxinus Americana – (Robinia pseudoacacia) Forest

**Data analysis.** It is hypothesized that watersheds with a greater amount of presumed ash coverage will also have larger increases in stream nitrate with time. Nitrate samples collected at watershed tributaries and outlets were directly compared to the presumed ash coverage within the corresponding watershed. However, because some of the Piney River and Staunton River synoptic sites were located along the stream network's main stem, the nitrate input from the stream sites' corresponding catchment areas or stream segments were required. The following mass-balance equation (1) was first used to calculate the nitrate concentration within each stream segment:

$$C_{seg} = \frac{(C_{out}Q_{out} - C_{in}Q_{in})}{Q_{out} - Q_{in}}$$
(1)

where  $C_{seg}$  was the nitrate concentration inside segment,  $C_{out}$  and  $C_{in}$ , were the NO<sub>3</sub> concentrations leaving and entering the segment, and  $Q_{out}$  and  $Q_{in}$  represented the total discharge leaving and entering the segment. Discharge for each segment was calculated using an area-weighted approach, based on the discharge at the main outlet of Paine Run and Piney River, which assumed that the discharge was linearly related to the subwatershed area.

After determining the nitrate concentration within each segment, in-stream denitrification processes occurring along the main stem needed to be taken into account. Using an adapted version of Scanlon et al. (2010) and the above mass-balance equation (1), in-stream denitrification at each site along the main stem at Piney and Staunton Rivers were recalculated using the equation (2):

$$C_{seg} = \frac{\left(C_{out}Q_{out} - C_{in}Q_{in} + \frac{L_{seg}}{L_{Total}}*exp(-\beta*mean(C_{in}Q_{in},C_{out}Q_{out}))*mean(C_{in}Q_{in},C_{out}Q_{out})\right)}{Q_{out} - Q_{in}}$$
(2)

Where  $L_{seg}$  was the length of the stream within the segment, and  $L_{total}$  was the total length of the stream in the watershed. B and  $P_{max}$  are nitrate removal terms based on work done in Scanlon et al. (2010), who showed that denitrification and biotic uptake of nitrogen can remove up to 91.9% of the nitrate in the stream network.  $P_{max}$  (91.9%) is the maximum percentage of removal of nitrogen, which is due to in-stream denitrification, and  $\beta$  (0.34) is a constant that is specific to the watershed that was presented in Scanlon et al. (2010).

To test the hypothesis, the stream nitrate data were split into two periods of record: 2009-2012 and 2013-2017. The 2009-2012 period represents a pre-EAB time frame, while 2013-2017 represents the period when the EABs were present. All nitrate data before 2009 were omitted to reduce the influence of the gypsy moth invasion (early 1990s). In all study watersheds, a ratio of mean stream nitrate concentrations was computed as seen in the equation (3):

$$\frac{Post}{Pre} NO3 \text{ Ratio} = \frac{mean NO3(2013 \text{ to } 2017)}{mean NO3(2009 \text{ to } 2012)}$$
(3)

The use of a ratio accounted for the natural spatial variability of nitrate along the stream. The ratio of the 2009-2012 to the 2013-2017 period was then compared to the percentage of presumed ash tree assemblages within each corresponding watershed. Relationships were tested using the MATLAB multiple linear regression tool. A statistically significant finding is defined when p < 0.05.

### Results

Spatially, presumed ash coverage with the quarterly watersheds greatly varies from more than 50% to 0% coverage (Figure 2). There is a tendency for the more northern watersheds to have greater presumed ash coverage than the more southern watersheds. The watersheds in the lower third of the park have very little or no presumed ash coverage; 42% of Piney River watershed is potentially covered in ash (Figure 3). Ash is not evenly distributed throughout the watershed, with more ash being found in the high elevation subwatersheds. The Staunton River watershed has a presumed ash coverage of 26% with ash being more evenly distributed throughout the watershed is more commonly found near riparian areas.

It was found that the relationship between presumed ash coverage and increased stream nitrate concentrations was most significant in the quarterly watersheds ( $r^2 = 0.29$ , p = 0.04) (Figure 5). Although the greatest increase in stream nitrate was observed in the Piney River watershed, there was no observable relationship between presumed ash coverage and stream nitrate based on the synoptic sampling ( $r^2 = 0.03$ , p = 0.51). Synoptic sampling from the Staunton River watershed also revealed a statistically insignificant relationship ( $r^2 = 0.02$ , p = 0.64).





*Figure 5*. Ratio of post-EAB invasion (2013-2017) to pre-EAB invasion (2009-2012) mean nitrate to watershed presumed ash coverage at the VTSSS quarterly sites (Panel A), Staunton River synoptic sites (Panel B), and the Piney River synoptic sites (Panel C). POST/PRE nitrate values greater than 1 indicate an increase in nitrate with time. Black circles represent tributary sites in Staunton River and Piney River.

Sites with ratios greater than 1 indicate an increase of stream nitrate with time. All quarterly watersheds, except Madison River watershed, showed increases in nitrate with a mean increase of 3.91. The Paine Run, Deep Run, and Two Mile Run watersheds all increased in nitrate despite having no presumed ash coverage. Of the 16 sites in Staunton, 10 sites were greater than 1, with the SR08 site having the highest ratio of 2.51. The Piney River watershed site, PI25, was omitted from analysis because of an extremely high ratio that was driven by the denominator's closeness to 0. Only nine of the 19 sites at Piney River increased in nitrogen, with a mean ratio increase of 1.67. The application

of the denitrification equation resulted in negative values at the PI05 and SR06 sites. A negative value means that more nitrogen is entering the subwatershed than leaving, and somewhere along the main stem, nitrogen is taken up by the instream processes not accounted for by the denitrification equation. A negative value can also be indicative of an overestimation of nitrogen loss by the denitrification equation.

### Discussion

At the Staunton River and Piney River watersheds, no significant relationship was found between the POST/PRE nitrate ratio and the presumed ash coverage. There was a significant relationship in the quarterly data. A significant relationship between presumed ash coverage and the POST/PRE nitrate ratio shows that sites with greater ash coverage had seen greater increases in stream nitrate concentrations during the 2013-2017 period. Watersheds including PINE, VT75, and VT66 quintupled in stream nitrate concentrations.

It is important to note that the nitrate increases seen from 2013 to 2017 are small and not of the same magnitude as the gypsy moth invasion in the early 1990s. After the EAB invades an ash tree, it takes approximately 5 years until total tree mortality, which would also contribute to the observed small increase in stream nitrate concentrations to date. Nitrate concentrations can also increase before tree mortality, which is due to impairment of nutrient uptake (Knight et al., 2013).

This study's initial hypothesis assumed that Piney River watershed would exhibit the greatest relationship between ash coverage and stream nitrate export. However, there was no significant relationship in the synoptic subwatersheds between the nitrate ratio and presumed ash coverage. This was unexpected because Piney River has one of the

largest presumed ash coverages (41.6%) and the highest stream nitrate export values. However, the increase in stream nitrate was seen in the quarterly data, where Piney River had the second-largest nitrate ratio of 5.98.

Jeremy's Run, North Fork at Thorton River, and Piney River, the three most northerly sites, exhibited some of the highest NO<sub>3</sub> ratios at 4.21, 9.92, and 5.98, respectively. While all the southerly sites from Staunton and below, except for Paine Run, had nitrate ratios below 2.12. This suggests a strong latitudinal gradient. The EAB has been moving north to south in SHEN, and the more northerly sites are most likely exhibiting infestation signs first.

A lack of significance in the synoptic data can potentially be explained by the timing of the collection of the samples in Piney River. Based on the weekly sampling at the outlet, the greatest increase in stream nitrate at Piney River was typically observed in late February and early March. However, the synoptic sampling in Piney River was not done until several weeks later in late March/early April. Based on observations from the weekly nitrate sampling, stream nitrate concentrations at the time of the synoptic sampling were more than 50% lower, and peak nitrate concentrations were missed. Additionally, the synoptic sampling was only one grab sample, which makes it difficult to get the full picture of what was happening in the subwatershed with time. Regardless of significance, 75% of all watersheds in this study increased in nitrate, and 30% of the study watersheds doubled in nitrate. Nitrate is typically tightly cycled in SHEN watersheds, so any increase is an indication of disturbance, a change in the nitrogen cycle, or random variability created by flow conditions.

Results could also be difficult to interpret owing to the complex transformations of the nitrogen cycle. Even if ash trees are dying, it is quite possible that the extra nitrogen is being stored in soils or taken up by other vegetation, effectively reducing the concentration of nitrate exported into streams. Soils are the largest pool and primary regulator of nitrogen in forested watersheds (Huntington et al., 1988; Johnson & Lindberg, 1992). The cycling of nitrogen in SHEN has also changed due to anthropogenic disturbances such as atmospheric deposition. Heightened atmospheric deposition in SHEN has added much more nitrate to the forest nitrogen cycle Sullivan et al., 2004; Webb et al., 1995).

SHEN has a long history of insect disturbance. In addition to the EAB, the woolly hemlock adelgid and, in particular, the gypsy moth, is known to have caused major devastation to the park's ecosystem (Bair, 2002; Eshleman et al., 1998). Gypsy moth outbreaks are cyclic and last on the order of 1 to 3 years in oak stands. After peak growth, gypsy moth populations remain low for 4 to 12 years until increasing again (Elkinton & Liebhold, 1990). However, natural biocontrols of the EAB have been very successful throughout the park and gypsy moth outbreaks are minimal.

This hypothesis warrants further examination as, to date, there are no other studies linking the EAB to stream geochemistry. Future work should consider the northeastern United States where the EAB has a longer known existence and impact. A more robust signal would likely be detected in sites that have experienced severe damage from the EAB. Other opportunities for future work include an improved ash coverage map. Analysis was limited by the uncertainty associated with the *presumed* ash coverage in the watersheds. In the 2009 Shenandoah Vegetation Map, what is known is the location of

community types and the proportion of ash within a community type. A map presenting only ash coverage and ash proximity to riparian areas would be immensely useful. New ash coverage maps can be created with more current remote-sensing data that produce detailed maps of species abundance and forest health across a landscape (Pontius et al., 2008). There is data available from the US Forest Service Forest Inventory and Analysis (FIA) which conducts highly accurate inventory analyses of forest types in Virginia. Results from the 2014 Annual Inventory showed that Shenandoah National Park is composed of 5% ash trees (Rose, 2016). Future studies can utilize sources such as the FIA for improved ash coverage maps.

SHEN is a tributary to the Chesapeake Bay, the largest estuary in the United States. Over the last few decades, substantial nitrogen and phosphorous loading have threatened native species and increased the frequency of eutrophication. As the Chesapeake Bay is already threatened by an intensive nutrient loading, it is essential to reduce additional input of nitrate from invasive insects such as the EAB. Unfortunately, measures to combat the EAB's spread, including state quarantines, insecticides, and biological controls, are still not effective. Yet, like any disease, early detection is key. Careful observation of stream geochemistry may reveal early indications of the EAB's presence. A detectable signal in stream nitrate concentrations in an otherwise undisturbed watershed can aid forest managers in EAB control and future eradication.

# Chapter 3: Sources and Sinks of Nitrogen in Select Watersheds Within Shenandoah National Park, Virginia

### Introduction

In many northern temperate forests, the role of nitrogen in watersheds is widely debated. Many studies believe temperate forested watersheds are nitrogen limited because nitrogen is incorporated into the vast soil nitrogen pool or assimilated into the vegetation biomass. Nitrogen oligotrophication is theorized to occur in watersheds that were once exposed to high levels of atmospheric nitrogen deposition. Current reduction in atmospheric deposition has now resulted in nitrogen limitation of forest productivity and a weakening of ecosystems response to disturbance (Groffman et al., 2018). However, another school of thought believes that this is still not the case. In 1989, Aber et al. proposed that after prolonged periods of excess nitrogen exposure, a forest becomes *saturated* with nitrogen, and the leaching of nitrogen from the watershed accelerates. Known as *nitrogen saturation*, this concept has been explored in several studies that have attempted to link enhanced nitrogen stream export to this theory. The nitrogen saturation theory suggests that not all temperate forests are nitrogen limited.

Nitrogen saturation occurs after an ecosystem is continuously exposed to nitrogen deposition (Aber et al., 1989; Agren & Bosatta, 1988). When an ecosystem becomes nitrogen saturated, it no longer retains its ability to hold onto nitrogen, and the leaching of nitrogen accelerates (Aber et al., 1998). Aber et al. (1998) proposed a series of stages of nitrogen accumulation where the final stage is defined by enhanced leaching, declines

in net primary productivity and nitrogen mineralization, and increases in foliar nitrogen. Peterjohn et al. (1996) identified seven major indicators that a temperate forest may be nitrogen saturated. These include

- 1. long-term increases in stream nitrate and base cation concentrations,
- 2. increased rates of net nitrification,
- 3. high nitrate concentrations in solution losses,
- 4. minimal seasonal variability in stream nitrate concentrations,
- 5. high nitrate discharge from a young aggrading forest,
- 6. a large increase in nitrate following fertilization of a young aggrading forest, and
- 7. a low retention of inorganic nitrogen.

It has also been predicted that reductions in the abundance of nitrogen-sensitive vegetation, suppression of microbial immobilization, and decreases in the soil organic matter decomposition rates are indicators of a nitrogen saturated forest (Emmett, 2007). In the United States, the northeastern states and California have the highest risk of nitrogen saturation due to high levels of nitrogen deposition (Driscoll & Van Dreason, 1993; Fenn et al., 1996).

In SHEN, large variability exists in stream nitrate export among watersheds. The objective of this study was to evaluate all of the sources and sinks in SHEN watersheds through the use of a nitrogen budget. This nitrogen budget was then used to determine if: (a) any of the watersheds studied could be considered nitrogen saturated, and (b) what elements of the nitrogen budget are driving the most stream nitrate variability among the watersheds. Nitrogen budgets. In order to determine if nutrient loading is resulting in nitrogen saturation in the SHEN watersheds, a fundamental understanding of all of the nitrogen-cycling components and how they change with time is required. However, tracking the effects of nitrogen deposition is extremely challenging due to the complex biogeochemical cycle, which absorbs, transforms, and releases nitrogen. To this day, nitrogen cycling in forest ecosystems is one of the least understood nutrient cycles (Johnson & Turner, 2014). For example, many nitrogen cycling studies in forested watersheds have found nitrogen accumulation rates far greater than what can be accounted for by measured nitrogen inputs (Bormann et al., 1993; Jenkinson & Powlson, 1970; Johnson & Todd, 1998). Studies by Eshleman et al. (2013) and Yanai et al. (2013) also created nitrogen budgets, and they found it difficult to quantify if excess nitrogen was lost through gaseous losses, soil uptake, or a combination of both.

In this current study, nitrogen cycling in a forested watershed was examined using Lovett and Goodale's (2011) simplified mass balance equation:

$$D - V - S = Y + G \tag{4}$$

In this equation, D is nitrogen deposition, V is uptake of nitrogen by vegetation, S is the incorporation of nitrogen into soils, Y is the export of nitrogen via streams, and G is the gaseous loss of nitrogen. This mass balance equation is expressed in terms of rates (kg N ha<sup>-1</sup> yr<sup>-1</sup>) and not in terms of gross accumulation (kg N ha<sup>-1</sup>).

**Sources and sinks of nitrogen.** To further demonstrate the sources and sinks of nitrogen, its main pathways as it travels through a forested ecosystem, are shown in Figure 6. As included in the Lovett and Goodale (2011) mass balance equation, nitrogen can be deposited onto the Earth's surface by either wet deposition or dry deposition. Wet

deposition occurs when nitrogen combines with atmospheric water and is deposited onto the Earth's surface in precipitation. The most abundant species deposited by wet deposition are  $NO_3^-$  and  $NH_4^+$ .



Figure 6. Generalized nitrogen cycle in a forested ecosystem.

Dry deposition is the transport of gaseous and particulate species from the atmosphere to the land surface without the assistance of precipitation. Unlike wet deposition, dry deposition is influenced by surface cover type, atmospheric turbulence and velocity, and the chemical properties of the depositing species (Seinfeld & Pandis, 2016). Species of nitrogen that are deposited by dry deposition include  $N_20$ ,  $NO_2$ ,  $HNO_3$ ,  $NO_3^-$ ,  $NH_4^+$ , and NO (Hauglustaine et al.,1994). N<sub>2</sub>O, NH<sub>3</sub>, and HNO<sub>3</sub> comprise the majority of dry deposition (Kharol et al., 2018). Available dry deposition data are limited, which is due to high spatial variability and poorly established measurement techniques (Nieder & Benbi, 2008).
Several studies of total nitrogen deposition have shown that during the  $20^{\text{th}}$  century, the amount of nitrogen deposition increased globally from 31.6 Tg N yr<sup>-1</sup> to 103 Tg N yr<sup>-1</sup> (Galloway et al., 2004). Large increases in nitrogen deposition in the  $20^{\text{th}}$  century have been reduced in the US in recent decades, thanks to the Clean Air Act in the 1990s.

Nitrogen can also enter an ecosystem via biological nitrogen fixation (BNF). BNF is carried out by a large array of microbes and bacteria that break the triple bond in unreactive N<sub>2</sub> to create reactive ammonia (NH<sub>3</sub>). Nitrogen fixation of N<sub>2</sub> gas is a fundamental reaction for introducing nitrogen from the atmosphere into the pedosphere. Before the start of the industrial era, estimates suggest that BNF in unmanaged terrestrial ecosystems contributed 128 Tg N yr<sup>-1</sup> globally (Galloway et al., 2004). However, BNF is extremely challenging to measure, and recent estimates have pushed pre-industrial global BNF production to as low as 58 Tg N yr<sup>-1</sup> with a potential range of 40 to 100 Tg N yr<sup>-1</sup> (Vitousek, Menge, Reed, & Cleveland, 2013). BNF in temperate forests is typically low, relative to tropical forests, which is due to the lack of nitrogen-fixing species. It is estimated that during peak rates of an early to intermediate temperate forest, BNF rates can range between 10 and 30 kg N  $ha^{-1}yr^{-1}$  (Cleveland et al., 1999). Although BNF is not included in Lovett and Goodale's (2011) mass balance equation, it is added for our purposes because it is a significant input. The following equation (5) is the Lovett and Goodale (2011) mass balance equation that is modified to incorporate BNF (*B*) as a source of nitrogen.

$$B + D - V - S = Y + G \tag{5}$$

After nitrogen enters a forested ecosystem, either by BNF or deposition, it can undergo nitrogen assimilation by vegetation. After water, nitrogen is considered one of the main factors limiting plant growth (DeVries et al., 2013; Fernández-Martínez et al., 2014; Wang et al., 2002). Plants absorb nitrogen from the soil as either  $NO_3^-$  or  $NH_4^+$  and then convert these into organic molecules such as DNA and amino acids. Nitrogen availability for plant uptake is made possible by genetic microbial depolymerization capacity, chemical recalcitrance of soil organic matter (SOM) compounds, and climatedriven enzyme activity (Rennenberg & Dannenmann, 2015). Mature temperate forests typically require 100 kg N ha<sup>-1</sup>yr<sup>-1</sup> to maintain maximal growth, which, interestingly, is similar to the amount of N needed on agricultural plots (Butterbach-Bahl & Gundersen, 2011; Kreutzer et al., 2009; Rennenberg & Dannenmann, 2015).

Nitrogen can also be made available for plant uptake in soils where it can undergo processes such as mineralization and nitrification. Nitrogen mineralization is the conversion of organic nutrients (detritus) by microorganisms into soluble forms that can be absorbed by plants and microbes. If the soil is rich in nitrogen, then microbial needs are met and mineralization occurs. If detritus is low in nitrogen, microorganisms will scavenge and immobilize nitrogen in their biomass (i.e., immobilization). Mineralization and immobilization occur simultaneously in the soil. However, the net increase or decrease of mineral nitrogen depends on the ratio of carbon to nitrogen in the organic decomposing layer of soil. Nitrification is the microbial oxidation of  $NH_4^+$  to  $NO_2^-$  or  $NO_3^-$ . Most nitrate in forested ecosystems is primarily formed in situ via nitrification. This process can also act as a sink of nitrogen as some nitrifiers release  $NO_x$  and  $N_2O$  into the atmosphere. Nitrate is primarily controlled by the amount of

available  $NH_4^+$ . Ecosystem disturbances, such as fire or clear cutting that increase  $NH_4^+$  availability in soils, will likely have higher nitrification rates.

Denitrification can be considered one of the final fates of nitrogen after it has traveled through a forested ecosystem. Denitrification is the reduction of nitrate to N<sub>2</sub> or  $N_2O$  gas. This is an essential piece of the nitrogen cycle as it is the only point where  $N_2$ reenters the atmosphere. This process is dependent upon soil O<sub>2</sub> and carbon concentrations. Carbon is important because most denitrifiers are heterotrophs that require carbon as the electron donor. Studies have also shown that warmer soil temperatures are strongly correlated with larger denitrification rates (Li et al., 1992; Maag & Vinther, 1996; Stanford et al., 1975). Of the sources and sinks in the nitrogen cycle, denitrification can also be considered one of the most difficult to measure. Available methods are problematic because they lack sensitivity, they are expensive to run, or they disturb the soils in the process of measuring nitrogen (Groffman et al., 2006). Additionally, it is inherently difficult to measure N2 emissions because of the high background atmospheric concentration. Denitrification also poses a challenge because of its high spatial and temporal variability (Groffman & Tiedje, 1989). Mass balance, or the difference of inputs and outputs, is a commonly used method to estimate denitrification fluxes at field and watershed scales (David & Gentry, 2000; Goolsby et al., 1999; Pribyl et al., 2005).

Nitrate, ammonium, and dissolved and particulate N can also be lost by leaching, runoff, and erosion. Streams and groundwater are considered a major sink as they transport nitrogen out of the watershed. In Shenandoah National Park, nitrate and ammonium represent 85% of the total nitrogen exported via streams (Buffam, 1999).

Dissolved organic nitrogen comprise the other 15%. Studies of forest nitrogen dynamics have used stream nitrate concentrations to assess the effects of nitrogen deposition (Aber et al., 1991; Lovett et al., 2000; Stoddard, 1994). Large nitrate transport can also be indicative of insect defoliations (Eshleman et al., 1998, Lovett et al., 2002; Riscassi & Scanlon, 2009). However, one must take into account processes near or within streams, as they can change in-stream nitrate concentrations. In-stream processes, such as biological assimilation and denitrification, can alter the flux of nitrate exported via streams (Peterson et al., 2001). Additionally, a dilution effect can cause significant in-stream variability of nitrate concentrations with headwaters having higher concentration values than downstream sites (Scanlon et al., 2010). It is important to note that in-stream denitrification is a gaseous process, which would fall into the gaseous losses part of the nitrogen budget.

In this present study, five SHEN watersheds (Figure 7) were analyzed using a combination of field work data, models, and literature to create a nitrogen budget. The objectives of the paper were to: (a) use the nitrogen budget to determine what watersheds were nitrogen saturated, and (b) determine what mechanisms within the nitrogen budget are driving the variability among SHEN watersheds' stream nitrate export.



Figure 7. Five watersheds (grey) in SHEN, Virginia used for analysis in this study.

## **Materials and Methods**

**Estimates of nitrogen deposition (D).** Total nitrogen deposition was estimated for each watershed, annually, for 1988-2017, using a combination of more recently available modeled data as well as measured data from the SHEN Big Meadows (VA28) station (Table 3, Figure 7). Wet deposition data from the National Atmospheric Deposition Program (NADP)/National Trends Network (NTN) and dry deposition data from the Clean Air Status and Trends Network (CASTNET) were available at the VA28 site from 1981 to the present.

Table 3

	Dry Deposition Species Measured		Wet Deposition Species Measured	
Watershed	2000-2015	1989-2000	2000-2015	1989-2000
Big Meadows (VA28)	NO3-, HNO3, NH4+, NH3, and NOM determined using TDEP Maps.	NO3-, HNO3, and NH4+ from CASTNET.	NH4+ determined using TDEP Maps.	NO3- and NH4+ from NTN.
North Fork at Dry Run (NFDR)		NO3-, HNO3, and NH4+ estimated with the linear regression between TDEP and CASTNET for 2000-2015 period. NH3 and NOM estimated with trend lines of NH3 and NOM from TDEP 2000-2015 period.		NO3- and NH4+ estimated using precipitation weighted ratios based on precipitation data from PRISM and deposition data from NTN.
Paine Run (PAIN)				
Piney River (PINE)				
Staunton River (STAN)				
White Oak Run (WOR)				

Determine Deposition Rates at Each Study Watershed

Note. The methods used to measure deposition differ in the 1989-2000 period at Big Meadows (VA28).

For the 2000-2017 period, the NADP Total Deposition Maps (TDEP) data sets were used to estimate annual wet and dry nitrogen deposition for individual watersheds. The TDEP annual wet deposition grids were calculated from annual precipitation, weighted concentrations, measured at NADP/NTN sites and Oregon State's parameter elevation regression on independent slopes model (PRISM) precipitation estimates (Oregon State, 2009). The TDEP annual dry deposition grids are calculated from measured air concentrations and modeled deposition velocities using the community multiscale air quality (CMAQ) model (Schwede & Lear, 2014). Wet and dry deposition grids were available at 4 and 12 km<sup>2</sup> spatial resolution, respectively. Dry deposition data, when TDEP grids were not available (pre-2000), were estimated by calculating the linear relationship between TDEP estimates at the individual watershed and CASTNET data at VA28 for the concurrent period of record (2000-2017). This relationship was then used to estimate the TDEP deposition for each watershed based on CASTNET data during the 1981-1999 period. Wet deposition data, when TDEP grids were not available (pre-2000), was estimated by multiplying the VA28 NADP/NTN nitrogen deposition flux by the mean annual precipitation ratio of each watershed to VA28. Precipitation data were available at a 4-km resolution from the PRISM data set.

Nitrogen flux via streams (Y). Flux of nitrogen leaving streams was calculated using the RStudio-based package for Exploration and Graphics for River Trends (EGRET), similar to techniques used in Hirsch et al. (2010) and Sprague et al. (2011). EGRET uses weighted regressions on time, discharge, and season (WRTDS) to describe long-term changes in stream flux and concentration (Hirsch & De Cicco, 2015). EGRET was designed to accept data from the U.S. Environmental Protection Agency (EPA) STORET data, U.S. Geological Survey hydrologic data, and, in our case, user-supplied flat files. The EGRET package requires a continuous record of daily stream discharge for the study period, an analyte concentration time-series, and metadata about the site and the analyte being evaluated.

Daily stream discharge and weekly nitrate concentration data for the five watersheds of this study were provided by the University of Virginia's SWAS Laboratory. SWAS was established in 1979 to assess the effects of acid deposition on mountain streams and their watersheds. In addition to daily discharge measurements, SWAS has been responsible for the collection and interpretation of water-quality

parameters including pH, specific conductance, acid neutralizing capacity (ANC), and major ions. Collection and analysis methods can be found in Robinson et al. (2013). Analyte concentrations used in this analysis were based on weekly base-flow grabsample measurements. High-flow discharge samples were not utilized in this analysis. Studies in SHEN have shown the dissolved nitrogen annual flux would be underestimated by approximately 30% if only baseflow concentration values are used (Buffam, 1999). The period of record for daily discharge data and nitrate concentration data varied between watersheds. In order to run the WRTDS model, nitrate concentration data required the same period of record as the daily discharge data. This condition resulted in pieces of available data being cut from the analysis. Table 4 lists the period of record used in the EGRET and WRTDS analysis. Using WRTDS and the listed period of records, a mean annual nitrate flux (kg NO<sub>3</sub><sup>-</sup> ha<sup>-1</sup> yr<sup>-1</sup>) for each watershed is reported. Table 4

Watershed	Period of	Period of Analysis	
	Start Date	End Date	
North Fork at Dry Run	8/1/1987	9/26/2011	
Paine Run	10/1/1992	12/29/2016	
Piney River	10/1/1992	12/31/2016	
Staunton River	9/8/1992	12/31/2016	
White Oak Run	11/1/1979	12/31/2016	

Start and End Dates of EGRET Nitrate Flux Analysis for the Watersheds of Interest

*Note*. Starting and ending dates are dependent upon the available stream nitrate concentration data and daily discharge data over the study period.

**Biological nitrogen fixation.** Estimates of BNF were based on previous global and local literature. Globally, estimates of BNF range from 40-100 Tg N yr<sup>-1</sup> (Paul & Clark, 1989; Schlesinger, 1991); however, rates of BNF change dramatically based on the biome (Cleveland et al., 1999). For example, a boreal forest, on average, fixes 2 kg N ha<sup>-1</sup> yr<sup>-1</sup>, whereas tropical forests fix 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Cleveland et al., 1999). At local scales, symbiotic nitrogen fixation by trees can be as large as 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Binkley et al., 1994). In temperate forests, symbiotic nitrogen fixation is one of the major nitrogen inputs (Liao & Menge, 2016). Over the years, many hypotheses have emerged to explain the global variability of BNF. Energy wise, BNF is a relatively expensive process (Vitousek & Field, 1999; Vitousek & Howarth, 1991), so the transformation would be most abundant in regions with high amounts of available carbon. This would include areas with high solar radiation, precipitation, and net primary productivity (Cleveland et al., 1999).

As temperate forests proceed through succession, nitrogen limitation increases. (Thomas et al., 2010; Vadeboncoeur, 2010). Mature temperate forests experience nitrogen limitation because nitrogen-fixing species abundance declines with successional stage (Menge et al., 2010; Richardson et al., 2004). Nitrogen fixers decline with succession, which is due to their low growth rates, high mortality rates, and low recruitment rates (Liao & Menge, 2016). Symbiotic nitrogen-fixing trees in the United States are rare to start with, as they comprise less than 1% of the basal area (Menge et al., 2010). The rarity of nitrogen-fixing trees is driven by a latitudinal gradient where the more nitrogen-fixing trees are found in lower-latitude American forests than higherlatitude American forests (Jenny, 1950; Rundel, 1989; Vitousek & Howarth, 1991).

Spatial analyses have observed a gradient where nitrogen-fixing tree abundance significantly changes at the 35° latitude (Menge et al., 2017). At latitudes below 35°, nitrogen-fixing trees comprise 12.7% of the basal area. This number drops to 1.2% in forests above 35° (Menge et al., 2017).

Estimates of biological nitrogen fixation in temperate forests ranges from 0.07 to 26 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Becker, 1980; Boring & Swank, 1984; Cleveland et al., 1999; Hardy & Havelka, 1975; Jenkinson et al., 1990; Todd et al., 1978). Coweeta Hydrologic Laboratory in North Carolina reported BNF rates of 12 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Todd et al., 1978). Of all the watersheds with reported BNF values, Coweeta is the closest, geographically, to SHEN, which makes it the most appropriate for the application of a similar value of nitrogen fixation. However, BNF rates in SHEN are likely lower than the more southern Coweeta, because SHEN lies above the 35° latitude divide, at 38°, where nitrogen-fixing species are significantly less abundant. Using information from Coweeta and the 35° nitrogen-fixing tree abundance threshold, a range nitrogen-fixation values from 0.5 to 4 kg N ha<sup>-1</sup> yr<sup>-1</sup> was applied to the SHEN watersheds.

Soil nitrogen storage. Like biological nitrogen fixation, soil accumulation rates of nitrogen in SHEN were estimated based on the literature. Typically, soil nitrogen content increases from the beginning stages of succession until steady state (Olson, 1963). However, large amounts of variability exist in soil nitrogen accumulation rates across the world. Numerous studies have attempted to determine how both carbon and nitrogen change during forest stand development, yet each of these revealed considerably variable results (Gough et al., 2007; Knops & Tilman, 2000; Law et al., 2001; Zak et al., 1990). In a recent effort to determine global patterns of carbon and nitrogen storage by Yang et al. (2011), 124 studies of soil pool accumulation were synthesized. Yang et al. (2011) found that carbon and nitrogen accumulation increased during succession and then eventually approached an equilibrium state (carbon and nitrogen flux near zero) during the later stage of forest development (approximately 70 years). Yang et al. (2011) also found that soil carbon and nitrogen pools in the same watershed accumulated nitrogen at similar rates with time.

However, the degree at which nitrogen accumulates in successional mature forests fluctuates globally (Johnson et al., 2007; Johnson & Turner, 2014). Yang et al. (2011) determined mean soil nitrogen accumulation varies between 1 and 11 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Rates of soil nitrogen accumulation can vary based on vegetation type (Bocock, 1963), soil mineral content (Berg & Staff, 1981), climate (Berg & Staff, 1981), and elevation (Shedayi et al., 2016). Thus, in this present study, estimates of soil nitrogen accumulation rates were based on forests with similar vegetation, climate, and mineral soil.

There are several studies that examined soil nitrogen uptake in watersheds similar to SHEN. For example, Yanai et al. (2013) conducted an analysis of nitrogen budgets at Hubbard Brook, a northern hardwood forest in New Hampshire. The researchers found no evidence that soil was a substantial sink, and they determined the forest floor to accumulate nitrogen at 2 kg N ha<sup>-1</sup> yr<sup>-1</sup>. A chronosequence of a Rhode Island forest, led by Hooker and Compton (2003), also determined that soil accumulated nitrogen at -0.8 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This was the average rate of accumulation 100 years after agricultural abandonment. The study watershed was dominated by mixed oak, and it was contained in a temperate climate.

In another nitrogen budget study at Coweeta Hydrologic Laboratory in North Carolina, researchers determined a low-elevation (702 m) watershed dominated with oak and pine to accumulate 2.61 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Knoepp & Swank, 1998). A high-elevation (1347 m) watershed dominated by hardwood trees accumulated nitrogen at 12.7 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Large soil nitrogen uptake rates at the higher elevation soils in Coweeta were due to a larger soil nitrogen pool and greater soil moisture (Knoepp & Swank, 1998). It was also found by (Knoepp & Swank, 1998) that colder temperatures at the higher elevation sites resulted in greater detritus and organic matter, which provided a larger and more steady flow of nitrogen with time.

Vegetation and soil wise, SHEN watersheds most closely resemble the forests in Coweeta and Rhode Island. SHEN is primarily dominated by clay-rich utisols (McNab, 1994). Utisols are formed under moist, warm climates, and they are acidic and low in plant nutrients. SHEN forests are principally composed of mixed oak forest types. The highly weathered soils in Coweeta are also closest to the utisols found in SHEN. Because of this, a soil uptake range between 1 to 7 kg N ha<sup>-1</sup> yr<sup>-1</sup> was applied to the SHEN watersheds. Based on results from Yang et al (2011), it can also be concluded that SHEN watershed soils have likely approached, or are approaching, a state of equilibrium in terms of nitrogen accumulation. This is because SHEN was established as a park over 84 years ago in 1935—14 years longer than the 70 years required for the soil equilibrium accumulation rates established by Yang et al (2011).

**Soil C:N ratio.** To further explore SHEN soil storage, available soil C:N ratio data from two UVA-led soil surveys were analyzed. C:N ratios in the North Fork of Dry Run (NFDR), Paine Run (PAIN), Piney River (PINE), Staunton River (STAN), and

White Oak Run (WOR) were based on two SHEN soil surveys in 2000 and 2017 (Table 5). During the 2000 soil survey, samples were collected from an approximately 20-centimeter deep pit. Soil samples were air dried until obtaining a stable mass. Total nitrogen and carbon were performed at the Agricultural Analytical Services Lab at Penn State University by combustion on a Fisons NA1500 Elemental Analyzer, following methods of Nelson and Sommers (1996). Additional field and laboratory methods for the 2000 soil survey can be found in Cosby et al. (2001).

During the 2017 soil survey, two samples per plot were taken with a 1-inch diameter corer to a depth of approximately 15 cm. Soils from the field were frozen for subsequent nutrient analysis. Afterwards in the laboratory, frozen soil samples were pushed through a sieve to separate them from their rocky constituents. They were then dried in an oven for approximately 48 hours; 5-gram subsamples of dry soils were ground with a mortar and pestle. Ground samples of 20 to 30 mg were tinned and analyzed for carbon and nitrogen using a Carlo Erba Elemental Analyzer with a 1020°C combustion tube and a 650°C reduction tube, with helium as a carrier gas (Dinh et al., 2017). C:N ratios among the SHEN watersheds in the 2000 and 2017 surveys were tested statistically with an ANOVA. A paired *t* test was used to determine if the C:N ratio changes were significant at each watershed from 2000 to 2017.

Table 5

Total Number of Soil Borings Collected in the 2000 and 2017 Soil Surveys for the Five Study Watersheds

Watershed	Number of Sites Investigated		
	2000 Soil Survey	2017 Soil Survey	
NFDR	5	N/A	

PAIN	7	19
PINE	6	16
STAN	6	15
WOR	6	N/A

**Biomass assimilation of nitrogen.** Like soil storage, nitrogen uptake by vegetation remains an important retention pathway in forests (Edmonds et al., 1995; Emmett et al., 1993; Johnson, 1992). Most of the nitrogen that biomass takes up annually goes to the fine roots and foliage. However, most fine-litter nitrogen is returned annually to the forest floor. The accumulation of nitrogen in the biomass wood represents the actual long-term sink of nitrogen each year (Goodale et al., 2002). Vegetation uptake generally depends on forest age and successional stage (Fenn et al., 1998). An early successional forest has a smaller soil nitrogen pool, it depends more on soil for nitrogen, and it retains nitrogen in plant biomass better than older trees (Fenn et al., 1998).

Many studies have documented net changes in vegetation uptake based on successional age in temperate forests. Studies have shown that forests in the early stage of succession accumulate the greatest amount of nitrogen. Borman et al. (1993) found early-stage forests accumulate between 70 and 81 kg N ha<sup>-1</sup> yr<sup>-1</sup> in New Hampshire, USA. Developing stage forests in temperate climates were found to accumulate anywhere between 11 and 21.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Guo et al., 2008; Johnson et al., 2003; Turner & Lambert, 2011). Mature temperate forests were found to have the least variability and the smallest annual accumulation rates, between –0.7 and 4.3 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Jenkinson & Powlson, 1970; Johnson, 1995; Johnson et al., 2007; Morrison & Foster, 2001, Trettin et al., 1999). Yanai et al. (2013) conducted a nitrogen budget study in a mature northern hardwood forest in the United Stated, and they also concluded that biomass accumulation remains constant each year at 4 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Before its establishment in 1936, areas of SHEN were cultivated. However, recent spatial analyses have shown that no watershed in this study had more than 36% open area. Since only a small portion of SHEN watersheds were logged, this study's watersheds are most likely mature temperate forests.

Several methods exist to measure biomass nitrogen accumulation rates. To directly measure forest net primary productivity (NPP) and biomass, via field measurements, is disadvantageous due to high cost, short time periods of measurement, and overall large time consumption (Houghton, 2005; Tang et al., 2010). This is why, in this study, remote-sensing products and vegetation models were used to estimate forest NPP and biomass. The remote sensing NASA Earth Observations TERRA/MODIS NPP (1 Month) product was used to estimate the annual accumulation rate of nitrogen in biomass. Additional information about the TERRA/MODIS NPP product can be found in Running and Gower (1999). NPP data was available from 2000 to 2016 at a 1-kilometer spatial resolution. Average annual NPP was calculated for each watershed, based off the 1-kilometer resolution data. NPP data were presented in grams of carbon m<sup>-2</sup> day<sup>-1</sup>, which was converted to kg N ha<sup>-1</sup> yr<sup>-1</sup> using a fixed C:N ratio. The fixed C:N ratio (165) was selected based on a synthesis of literature examining C:N ratios for the wood part of trees (Dickson, 1989; Martin et al., 2014; Millard & Grelet, 2010; Vitousek, Fahey, Yazaki et al., 2016). It was also taken into account that less than 10% of the NPP is actually accumulated in the standing biomass each year. The remaining 90% of nitrogen is recycled to the forest floor or exported out of the watershed via denitrification or stream and groundwater (Perakis & Sinkhorn, 2011). Biomass accumulation was calculated using the equation (6):

$$\mathbf{V} = \mathbf{NPP} * \mathbf{C} : \mathbf{N} * \mathbf{0.1} \tag{6}$$

Where V is the amount of N accumulated by biomass (kg N ha<sup>-1</sup> yr<sup>-1</sup>), NPP is the net primary productivity determined by remote sensing data, C:N is the fixed carbon to nitrogen ratio for the woody part or trees, and 0.1 represents that amount nitrogen that is accumulated in the standing biomass each year.

## Results

**WRTDS stream nitrate flux.** Each studied watershed exported the greatest amount of nitrate between 1988 and 1995 (Figure 8). This period of time corresponds to the gypsy moth defoliation, which removed upwards of 80% of the canopy in parts of SHEN. Between 1988 and 1995, the greatest amounts of nitrogen were removed from PINE and NFDR at mean rates of 4.29 and 4.68 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. The least amount of nitrate was removed from PAIN, STAN, and WOR at mean rates of 1.67, 0.65, and 0.86 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. By 2005, the watersheds returned to a pre-gypsy moth nitrate export levels. From 2005 onward, NFDR and PINE still exported the most nitrate, at 1.15 and 0.91 kg N ha<sup>-1</sup> yr<sup>-1</sup>, while PAIN, STAN, and WOR exported the least at 0.19, 0.18, and 0.05, respectively. NFDR displayed the highest variability in stream nitrate export. In recent years, PINE has been the only watershed to have exhibited an upward trend in nitrate flux. From 2002 to 2016, PINE nitrate increased at a rate of 0.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>.



*Figure 8*. Annual estimates of nitrate fluxes and flow-normalized fluxes in SHEN watersheds. Estimates are based off the weighted regressions that consider time, discharge, and season.

**Nitrogen deposition.** In SHEN, chemical species are not uniformly deposited (Figure 9). TDEP maps at Big Meadows (primary air quality measuring site) reveal that dry deposition comprises a significantly larger proportion of the total nitrogen budget.



*Figure 9*. Wet and dry species composition at Big Meadows (VA28) in SHEN. Species information based off of TDEP maps.

From 2000 to 2015, dry deposition has been, on average, 1.9 times greater than wet deposition. Of the dry deposition species,  $NO_3^-$  and  $HNO_3$  had the largest rates of deposition, and reductions of these species have driven the most change in total nitrogen deposition rates. Over the period of record, dry  $NO_3^-$  and  $HNO_3$  have declined the most at a rate 0.43 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Dry deposition of  $NH_4^+$  and NOM, and wet deposition of  $NO_3^-$  also declined but at much smaller rates of 0.03, 0.07, and 0.05 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Wet deposition rates of  $NH_4^+$  have remained constant and, interestingly, dry deposition of  $NH_3$  has increased at 0.04 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

Despite the changing proportions of deposition of chemical species to one another, a decline in total nitrogen deposition (Figure 10) can be observed within each watershed. On average, total deposition at SHEN watersheds has declined from 18.1 to 9.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>, a 49% change in deposition rates. Throughout the time period, NFDR, PINE, and STAN received the greatest amount of deposition at 434, 372, and 360 kg N ha<sup>-1</sup>, respectively. Total nitrogen deposition was the least at PAIN (328 kg N ha<sup>-1</sup>) and WOR (323 kg N ha<sup>-1</sup>).



*Figure 10*. Total nitrogen deposition at NFDR, PAIN, PINE, STAN, and WOR. All values before the year 2000, based on linear extrapolations from NTN and CASTNET data.

**Nitrogen biomass assimilation rates.** Over the period of record, nitrogen biomass accumulation did not vary significantly between the SHEN watersheds (Figure 11). On average, the SHEN watersheds accumulated 4.7 kg N ha<sup>-1</sup> yr<sup>-1</sup>, with an average standard deviation of 1.94 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Average accumulation among the watersheds varied from 4.1 to 5 kg N ha<sup>-1</sup> yr<sup>-1</sup>.



*Figure 11*. Biomass accumulation of nitrogen at NFDR, PAIN, PINE, STAN, and WOR. Estimates of biomass accumulation from 2000 to 2016 are based off of the TERRA MODIS NPP remote-sensing product.

**Nitrogen budget.** On average, nitrogen deposition was the largest source, comprising 78% of the inputs (Figure 12). Nitrogen fixation only accounted for 22% of the inputs in the SHEN watersheds. Despite total nitrogen deposition declining with time, deposition still composed the largest source of nitrogen to any SHEN watershed. Stream nitrate, perhaps the most well-studied element of the nitrogen budget in the SHEN watersheds, only accounted for 6% of the nitrogen sinks in SHEN. Vegetation accumulation and soil storage accounted for 26% and 18% of the nitrogen sinks, respectively.

Based on Lovett and Goodale's (2011) modified mass balance equation, 50% of the nitrogen leaving the SHEN watersheds is unaccounted for. Most of this unaccountedfor nitrogen is likely exiting the watersheds via denitrification. However, it possible that other nitrogen accumulation in vegetation and soil is larger than what is actually shown in Figure 13.

It should also be noted that NFDR and PINE had the greatest total deposition rates and stream export rates throughout the period of record. Besides the pattern noted between deposition and stream export at NFDR and PINE, no other notable patterns were observed. No statistical testing was done to compare the sources and sinks to one another because of the large amount of uncertainty and estimates made in the data.

Nitrogen pools, sources, and sinks in WOR and NFDR further compared to each other in Figure 13. The soil nitrogen pool is significantly larger (75%) than the biomass and groundwater pools in both watersheds. In both watersheds groundwater comprises only a very small portion of total nitrogen stored (<1%).



*Figure 12.* Estimates of nitrogen sources and sinks in select SHEN watersheds. Nitrogen shown as kg N ha<sup>-1</sup> yr<sup>-1</sup>. Soil storage and fixation rates are based off the fixed estimated values; the deposition, stream export, and biomass uptakes are based on actual measurements. Note that fixation, soil storage, and the residual term are based off of ranges of values.



*Figure 13*. Estimates of nitrogen pools, sources, and sinks in White Oak Run (left panel) and North Fork of Dry Run (right panel) watershed. Nitrogen fluxes (red) shown as kg N ha<sup>-1</sup> yr<sup>-1</sup> and nitrogen pools (green) shown as kg N ha<sup>-1</sup>.

**Soil C:N ratio.** From 2000 to 2017, the C:N ratio decreased in PINE (p = 0.026), and it increased at STAN (p = 0.005). The decline observed at PAIN was not statistically significant (Figure 14). STAN, NFDR, and PINE were below the leakage threshold of 20, as defined by Cole et al. (2002) and Johnson and Lindberg (1992). In 2000 and 2017, only PINE was below the threshold. Results from the ANOVA and *t* test showed that, in 2000, the mean C:N ratios in STAN, NFDR, and PINE were significantly different from PAIN and WOR. Results from the 2017 ANOVA showed that PAIN, PINE, and STAN were also significantly different from each other.



*Figure 14.* Results of C:N ratio sampling from 2000 and 2017 sampling events. Bars represent the mean C:N ratio at each watershed; error bars show the standard deviation from each group of samples. Blue bars and orange bars distinguish the 2000 and 2017 sampling events, respectively. The black, dashed line shows the threshold at which any watershed lower than 20 exhibits increased nitrogen output. No sampling was conducted at NFDR and WOR in 2017.

## Discussion

Nitrogen cycling in temperate forest systems, such as SHEN, is a highly complicated process. Tools, such as nitrogen budgets, can help better synthesize multivariable systems and reveal areas of study that require further investigation. In this present study, the nitrogen budget revealed that the greatest drivers of nitrogen export are most likely those that we have the least amount of information about. This primarily pertains to the soil storage element of the budget.

In this study, a fixed value was applied to the soil storage term, however, results from the C:N ratio analysis and the previous literature have shown that this is not the case. Soil storage can vary significantly based on location (Yang et al., 2011). Forest nitrogen studies have proven that soils and litter are the largest reservoir of nitrogen in a forested watershed (Heilman & Gessel, 1963; Johnson et al., 2003; Mead & Pritchett, 1975; Melin et al., 1983; Raison et al., 1990). Additionally, forest soils have been found to be the most significant regulator of nitrate export in streams (Kawakami & Honoki, 2008).

Forest floor C:N ratios have been proposed by several studies as indicators of nitrate leaching from forest watersheds (Aber, 1992; Dise et al., 1998; Emmett et al., 1998; Gundersen et al., 1998, Lewis et al., 2014). In 1981, Gosz hypothesized that vegetation on nitrogen-rich sites produces litter with high nitrogen concentrations, leading to faster rates of decomposition and nitrogen mineralization. In nitrogen-poor ecosystems, plants grow slower, use nitrogen more efficiently, and produce lower quality litter and greater C:N ratios. At low C:N ratios, decomposer organisms are not nitrogen limited, and a net release of inorganic nitrogen to the soil solution occurs. Low soil

nitrogen availability leads to high nitrogen use efficiency and the production of litter with a higher C:N ratio (Bonito et al., 2003; Vitousek, 1982). It is well known that there is a strong relationship between the C:N ratio and soil decomposition rates (Janssen, 1996; Kelly et al., 2007; Quan et al., 2014; Springob & Kirchmann, 2003; White & Haines, 1988). In this study, watersheds with the greatest stream nitrate concentrations and the highest variability (PINE and NFDR) were also the watersheds with the lowest C:N ratios. This supports the findings by several studies (Cole et al., 2002; Johnson & Lindberg, 1992), suggesting soils with a C:N ratio greater than 20 are more effective at retaining nitrogen.

In addition to soil retention, it is possible that nitrogen deposition is driving nitrogen fluxes. NFDR and PINE, both watersheds with high nitrogen export, also had received large amounts of total nitrogen deposition before the 2000s. However, there is large uncertainty associated with the nitrogen deposition calculations before TDEP maps were available. Despite this, many studies have, in fact, found that the intensity of nitrogen deposition and the degree of watershed nitrogen saturation are closely related (Johnson & Lindberg, 1992; Stoddard, 1994; Williams et al., 1996). In a study of northeastern watersheds, it was determined that atmospheric nitrogen deposition explained 30% of summer surface nitrate concentrations (Aber et al., 2003). Although there was an overall decline in total nitrogen deposition in SHEN, dry deposition of NH<sub>3</sub> has increased in SHEN watersheds. This is most likely a result of increased agricultural activities in the United States. The Clean Air Act targeted nitrogen emissions from fossil fuels but never affected nitrogen usage on farms. Additionally, large variability in total nitrogen deposition was observed between SHEN watersheds. Variability in nitrogen

deposition can be controlled by factors including prevailing wind direction, elevation, climate, and proximity to the source of deposition (Seinfeld & Pandis, 2016)

But nitrogen deposition, alone, does not control variability of stream nitrate export and nitrogen saturation in watersheds. In addition to nitrogen deposition rates and soil retention, nitrogen saturation and stream nitrogen export can be driven by stand age and successional status (Vitousek & Reiners, 1975), climate (Cole, 1992), and land use (Johnson & Turner, 2014; Magill et al., 1996).

SHEN has a long agricultural history. Prior to its establishment in 1935, hundreds of families worked the land, planted crops and orchards, and used the mountains for logging and mining (National Park Service, 2018). Agriculture, logging, and fire history could describe why some watersheds are better at retaining nitrogen than others. Silsbee and Larson (1982) found that in the Great Smokey National Park watersheds, which had been logged before its establishment as a national park, had significantly lower C:N ratios than that of unlogged watersheds.

Fires can also affect a watershed's nitrogen-retaining ability (Hornbeck et al., 1997), and low- to moderate-intensity fires can enhance nitrogen retention (Dow & DeWalle, 1997). High-intensity fires can have significant effects on long-term nitrogen budgets (Johnson & Turner, 2014) because up to 13% of forest nitrogen can be lost through fire (Adams & Attiwil, 2011; Grier, 1975). Land use history maps (Fievet et al., 2003) document that 36% of PINE was unforested before 1941, owing to logging and agricultural activities (Table 6). Additionally, significant fires in 1930 and 1981 occurred in the PINE watershed. Each fire covered more than one-fifth of the PINE watershed.

PINE's watershed extensive disturbances could have also contributed to its low C:N ratio and susceptibility to nitrogen saturation.

The mature successional stage results in a slowed uptake rates of nitrogen (Lovett & Rueth, 1999; Magill et al., 2000). Aggrading forests have lower nitrate losses because of their higher demand for nitrogen (Vitousek & Reiners, 1975). Based on the results from the biomass nitrogen accumulation analyses, SHEN watersheds are mature successional forests that no longer accumulate large amounts of nitrogen. Additionally, there was no observed relationship between SHEN annual stream nitrate and biomass accumulation of nitrogen.

Table 6

WOR

Watershed	% Open Area
NFDR	0.2
PAIN	.15
PINE	36.0
STAN	12.6

Percent Open Area in SHEN Study Watersheds Before 1941

Note. Information based off data collected in Fievet et al. (2003).

Through the use of Lovett and Goodall's mass balance equation the residual term shown in the nitrogen budget should represent denitrification rates. Based on this analysis denitrification composes a significant portion of nitrogen sinks. Yanai et al. (2013) also determined that denitrification was a large portion of the budget. However, there is a large degree of uncertainty associated with this term as it relies on estimates from all the

0.6

other terms. The residual term also has large error due to the fact that biological nitrogen fixation and soil storage are represented as a range of values.

Based on data collected in this study, it is possible that some watersheds in SHEN are well on their way to becoming nitrogen saturated. Aber (1992) defined nitrogen saturation as the (a) absence of vegetation growth despite additional nitrogen, (b) the initiation of nitrate leaching in streams and groundwater, and (c) the lack of a net nitrogen accumulation in ecosystems as seen in sources and sinks. PINE has recently exhibited an increase in stream nitrate, which could be the result of a reduction in its soil nitrogenretaining abilities. Increases in stream nitrate could also be the result of insect invasions; however, results from the previous chapter found no significant link between stream nitrate concentrations and ash coverage in the synoptic data. The PINE watershed soils do have a low C:N ratio, proving soils are already reaching their nitrogen retention capacity. Although the NFDR watershed has no current stream nitrate data, it has previously released high amounts of nitrate relative to the PAIN, STAN, and WOR watersheds. NFDR also has weak nitrogen-retaining abilities, as indicated by its low C:N ratio. Based on low C:N ratios, historically high stream nitrate concentrations, mature stand age, disturbance history, and Aber's (1992) nitrogen saturation definition, PINE and NFDR are exhibiting symptoms of nitrogen saturation. The nitrogen saturation status of these watersheds is driving the high nitrogen export that is seen in the stream nitrate records.

**Uncertainty.** It is challenging to estimate the degree of uncertainty associated with nutrient budgets. Nutrient budgets rarely include error analysis, making it difficult to establish the statistical significance of the results (Yanai et al., 2013). Creating a closed nutrient budget could help provide insight about ecosystem processes and future research

ideas. Sources of uncertainty can be classified into those arising from imperfect knowledge and those arising from the inherent variability in the system studied (Harmon et al., 2008; Yanai et al., 2013). The results of this study should be seen as providing the best estimates with the best, most-available data. In this analysis, a much higher degree of uncertainty is associated with all of the values-based estimates from the literature than those based on the data collected in SHEN.

# Chapter 4: Summary and Prospects for Future Research Summary and Implications

Effect of invasive insects on stream nitrate export. Chapter 2 analyzed the relationship between ash tree abundance and stream nitrate concentrations in 12 watersheds in SHEN, Virginia. Two separate stream sampling datasets, quarterly and synoptic, were compared to remote-sensing-based vegetation maps in each watershed. The synoptic stream sampling dataset revealed no significant link between stream nitrate and presume ash coverage in any watershed. However, a significant relationship was observed when quarterly data was compared to stream nitrate in the studied watersheds. The significant relationship revealed that sites with a large potential for ash coverage experienced a higher export of nitrate in streams from 2013 to 2017. An early infestation of the EAB is still extremely difficult to detect. An early signal seen in stream nitrate concentrations can aid forest managers in EAB control and eradication.

Effect of internal cycling of nitrogen on stream nitrate export. In Chapter 3, a comprehensive nitrogen budget was prepared for five watersheds in SHEN, which have been monitored since the 1980s. The nitrogen budget was used to determine which sources and sinks are driving the variability seen in stream nitrate export in the SHEN watersheds. Elements of the nitrogen budget studied included atmospheric deposition, biological nitrogen fixation, denitrification, biomass accumulation, soil retention, and export of nitrate via streams. Individual methods were enacted for each element of the budget. Given the methodology and data available, some elements of the budget were

known with greater certainty than others. It was determined from the budget that the soil had a weaker ability to retain nitrogen in the PINE and NFDR watershed, which caused the high export of nitrogen throughout their periods of record. Additionally, nitrogen export from these watersheds was exacerbated by the high amounts of atmospheric deposition they received. Because of this, both of these watersheds are more susceptible to nitrogen saturation. This is the first time a nitrogen budget has been compiled in the SHEN watersheds. The nitrogen budget was a valuable tool for expanding our comprehension of forest nitrogen cycling in SHEN and an excellent indicator of how future research should proceed.

#### **Future Research Opportunities**

The EAB is a relatively new pest, and it is anticipated to worsen in SHEN. As the EAB persists, the stream nitrate signal will likely be more robust because the EAB will become more established in the park and ash mortality will increase. It is critical to maintain observation and control of this pervasive pest. The concentration of nitrate varies seasonally, thus collection of nitrate samples in different seasons would show differing results. Improved timing of sampling, when nitrate is at its highest in streams, would provide more comprehensive data and results. Additionally, utilization of higher quality remote-sensing products would provide better estimates of actual ash coverage in SHEN.

The biogeochemical cycle of nitrogen in forested watersheds is an extremely complex system due to the many transformations and biotic pools. Nitrogen cycling is further complicated by external forces such as atmospheric deposition and insect invasions. The results from Chapter 3 reveal that soil is likely the main driver of nitrogen

retention and release in watersheds. Unfortunately, soil is one of the least understood and studied elements of the nitrogen budget in SHEN. A study that gains better estimates of the soil and its carbon and nitrogen pools would provide insight into the degree of nitrogen storage in watersheds and reduce uncertainty in the current nitrogen budget results. Additionally, uncertainty would be reduced in this study if estimates of biological nitrogen fixation and denitrification were based on watershed experiments rather than estimates from the literature.

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