

**The Impact of Tree Species, Elevated Nitrogen Deposition, Stand Age, and  
Environmental Factors on Herbaceous Plant Communities in a Central Appalachian  
Hardwood Forest**

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## Abstract

### The Impact of Tree Species, Elevated Nitrogen Deposition, Stand Age, and Environmental Factors on Herbaceous Plant Communities in a Central Appalachian Hardwood Forest

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Although the herb layer represents less than 1% of the biomass of temperate forests, this layer may contain up to 90% of the plant species in the forest and can contribute up to 20% of the foliar litter, thus playing an essential role in forest biodiversity and nutrient cycling. The objectives of this study were to investigate the differences in cover, species richness, Shannon-Wiener diversity, and evenness of herb layer plants a) under tree species associated with contrasting soil nitrogen levels and b) in watersheds that vary in nitrogen deposition, stand age, and watershed aspect at the Fernow Experimental Forest in West Virginia. In the watersheds evaluated, overstory tree species, N deposition level, stand age, and other environmental factors influenced herb layer characteristics. This study demonstrated 1) a sugar maple effect, i.e. sugar maple having a positive effect on understory cover, at intermediate levels of soil fertility in reference watersheds and in an N-fertilized watershed by improving the nutrient microenvironment for herb layer plants, 2) that long-term N enrichment can reduce ecosystem biodiversity by favoring nitrophilic species, and 3) that herb layer characteristics can be influenced by stand age, with more recent disturbance being reflected in higher herb layer abundance and diversity. This study justifies further examination of tree-herb layer interactions for a wider range of tree species, N-deposition levels, and stand ages in future studies in order to inform adaptive forest management in the light of climate change and other continuing anthropogenic influences.

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## TABLE OF CONTENTS

<b>ABSTRACT .....</b>	<b>II</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>III</b>
<b>LIST OF TABLES.....</b>	<b>V</b>
<b>LIST OF FIGURES .....</b>	<b>VI</b>
<b>CHAPTER 1 .....</b>	<b>1</b>
INTRODUCTION.....	1
METHODS .....	10
RESULTS .....	22
WS3 vs WS7.....	29
WS7 vs WS13.....	30
WS10 vs WS13.....	31
DISCUSSION.....	32
RED MAPLE VS SUGAR MAPLE.....	32
FERTILIZATION.....	35
STAND AGE.....	36
ASPECT.....	38
CONCLUSION.....	40
LITERATURE CITED .....	42
<b>CHAPTER 2 .....</b>	<b>49</b>
INTRODUCTION.....	49
METHODS .....	51
RESULTS .....	54
DISCUSSION.....	58
LITERATURE CITED .....	60
<b>APPENDICES.....</b>	<b>62</b>
TABLE A1: LOCATION, ELEVATION, AND DBH OF TREE SPECIES IN THIS STUDY .....	62
TABLE A2: THE FIVE CLOSEST NEIGHBORING ECTOMYCORRHIZAL AND ARBUSCULAR TREES TO THE PLOT-CENTER MAPLES. ....	64
TABLE A3: TOTAL COVER OF ALL UNDERSTORY TAXA IN THIS STUDY IN EACH WATERSHED FOR EACH OVERSTORY MAPLE SPECIES.....	66
TABLE A4. ANOVA RESULTS TESTING THE EFFECT OF WATERSHED, OVERSTORY MAPLE SPECIES AND THEIR INTERACTION FOR 22 INDIVIDUAL HERB-LAYER SPECIES ....	69
TABLE A5. DISTANCE BETWEEN PLOT CENTER MAPLES WITHIN A PLOT PAIR. ....	74
TABLE A6. ANOVA TESTS OF EFFECT SLICES .....	75
FIGURE A1. LOCATION OF SITES 1-9 WITHIN EACH WATERSHED .....	76

### **List of Tables: Chapter 1**

<b>Table 1.</b> Characteristics of the watersheds in the study.....	13
<b>Table 2.</b> Results of the statistical analyses (ANOVA).....	28
<b>Table 3.</b> Streamwater nutrient concentrations at the Fernow Experimental Forest .....	37

### **List of Tables: Chapter 2**

<b>Table 1.</b> Characteristics of the watersheds in the study.....	51
<b>Table 2.</b> Regression components for estimating aboveground biomass of 42 plant species and four functional groups using the linear equation.....	57

## List of Figures: Chapter 1

<b>Figure 1.</b> Location of Fernow Experimental Forest and watersheds used in this study .....	<b>11</b>
<b>Figure 2.</b> Slopes of the study watersheds (WS3, WS7, WS10, WS13) at the Fernow Experimental Forest.....	<b>12</b>
<b>Figure 3.</b> Experimental design of plot pairs at each site for collecting herb composition and cover.....	<b>15</b>
<b>Figure 4.</b> Visual illustration of the hand-area method.....	<b>17</b>
<b>Figure 5.</b> Visual illustration of how the five closest neighboring trees were separated by ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) association.....	<b>19</b>
<b>Figure 6.</b> Relative cover of 8 understory taxa and other .....	<b>24</b>
<b>Figure 7.</b> Mean cover per of the most common understory plant species in WS3, WS7, WS10, and WS13 .....	<b>25</b>
<b>Figure 8.</b> Herb characteristics in red and sugar maple plots in each of the studied watersheds .....	<b>26</b>
<b>Figure 9.</b> Biotic and abiotic factors influencing study plots under red and sugar maples in each of the watersheds.....	<b>27</b>
<b>Figure 10.</b> Conceptual diagram of how sugar and red maple may affect herb layer biomass.....	<b>35</b>

## List of Figures: Chapter 2

<b>Figure 1.</b> Visual illustration of the hand-area method.....	<b>52</b>
<b>Figure 2.</b> Relationships of log-transformed cover and biomass for the dominant herb species per functional group .....	<b>54</b>
<b>Figure 3.</b> Linear regressions between aboveground biomass and cover .....	<b>55</b>

## Chapter 1

### Effects of Tree Species, Land Use History, and Environmental Factors on Herbaceous Plant Community Characteristics

#### Introduction

##### Importance of the herb layer in forest ecosystems

The importance of the herbaceous layer in forest ecosystems has received increasing attention in recent decades (Gilliam 2014). While it represents less than 1% of the biomass of the forest, it contains between 75 and 91% of the plant species of the forest and can contribute up to 20% of the foliar litter mass to the forest floor (Gilliam 2007).

Biodiversity, defined as the variety of living organisms, is linked to ecosystem stability and productivity (Hooper et al. 2005). Biodiversity increases ecosystem productivity, nutrient use and retention, and ecosystem resilience (Tilman et al. 1997, Liang et al. 2015). According to the niche-efficiency model, a plant's individual rate of productivity is highest in a diverse system, and, conversely, the loss of biodiversity will decrease a plant's individual rate of productivity (Liang et al. 2015). Better utilization of nutrients in diverse communities can reduce the leaching of nutrients, including nitrogen and calcium, which will increase the sustainability of nutrient cycling and soil fertility at least in grassland ecosystems (Tilman et al. 1997). Greater plant biodiversity can also produce a greater mixture of root exudates, which can further create a more diverse soil microbial community (Lavelle et al. 1995, Broekling 2008, Fu et al. 2015), leading to a more resilient soil environment that is better protected against stress and disturbances (Brussaard et al. 2007).

Litter decomposition is one of the major drivers of nutrient cycling and nutrient availability in an ecosystem (Freschet et al. 2013). Live leaves of typical herb layer species have high concentrations of N, P, K, and Mg and low concentrations of lignin and cellulose. After senescence, the quality of leaf litter, measured as litter C:N ratio and carbon quality, correlates strongly with corresponding parameters in living leaves (Chapin 2003). The plant will reabsorb approximately half of their nitrogen and phosphorus pools and very little of the initial carbon pool, regardless of the environment in which they grow (Chapin and Kedrowski 1983, Aerts and Chapin 2000). Litter decomposability varies across plant species (Freschet et al. 2013) and, more broadly, between spring and summer herbs. Spring and summer herbs differ in their metabolic rates, which may be caused by differences in light and nutrient availability at their time of growth. During the summer months, nutrients are less available due to microbes and woody plants immobilizing large quantities, causing the summer herbs, living in the shade of the forest trees, to maintain low metabolic rates (Gilliam 2014, Taylor and Pearcy 1976). In contrast, spring ephemeral herbs, with their short period of growth and reproduction completed before the canopy closes (Lapointe 2001, Vezina and Grandtner 1965), have high rates of photosynthesis and respiration, requiring a high mineral nutrient supply. This led to the vernal dam hypothesis, stating that spring ephemerals can serve as a short-term nutrient sink, preventing nutrients from being leached into streams and, therefore, from being removed from the ecosystem (Muller and Bormann 1976). In addition, following the decomposition of senesced aboveground biomass, areas with more spring ephemerals may have higher soil nutrient concentrations than areas with fewer ephemerals (Muller 1978). In sum, spring and summer herb litter, with its low C:N ratio and high C quality, on average decomposes more than twice as rapidly as tree litter (Mueller 2003; Melillo et al. 1989), thus providing a rapid pathway for the recycling of nutrients and underscoring the importance of the herb layer despite its tiny biomass footprint in the forest (Elliott et al. 2015).

## Interactions between Trees and the Herb Layer

The forest overstory has a direct effect on the availability of resources for herb layer plants (Gilliam 2014). Most obviously, trees decrease the amount and quality of light reaching the forest floor. The overstory can also decrease nutrient and moisture availability via uptake by fine roots (Gilliam 2014) located in the O-horizon (i.e., below the litter, in the soil layer with the highest organic matter content) and via interception of precipitation and atmospheric deposition by foliage.

Stemflow, the portion of precipitation that runs down the tree trunk and branches, could be important in establishing soil moisture and mineral gradients around the tree base (Carlisle et al. 1967). Thus, stemflow could be a determining factor of herb distribution by affecting and establishing microhabitats underneath the overstory (Carl and Ralph 1984).

However, the herbaceous layer can also positively affect overstory plants. Elliott et al. (2015) reported greater rates of nitrogen mineralization, nitrogen availability, greater tree litterfall mass, and total tree litterfall N in experimental plots with added herbaceous biomass compared to plots where the herbaceous layer had been removed.

## Microbial Interactions

The understory also has an effect on soil microbial abundance. Wu et al. (2011) observed that removing the understory significantly reduced the amount of phospholipid fatty acids (PLFAs) (an estimate of microbial biomass) found in the soil and reduced the fungi to bacteria ratio, concomitant with an increase in soil temperature and reduced N availability. Similarly, Xiaoli et al. (2015) found that increased understory abundance and tree diversity led to an increase of the fungi to bacteria ratio in the soils of plantation forests. If the greater fungi to bacteria ratio was due to an increase in mycorrhizal fungi, increased understory abundance would lead to better plant growth (positive feedback) as increased colonization of plant roots by

mycorrhizal fungi has been found to be associated with higher plant biomass and phosphorus content (Treseder 2013).

In temperate deciduous forests, the most common types of mycorrhiza are arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM). Examples of AM associated trees are maples (*Acer* spp.), tulip poplar (*Liriodendron tulipifera*), and black cherry (*Prunus serotina*); examples of ECM associated trees are oaks (*Quercus* spp.) and American beech (*Fagus grandifolia*) (Allen et al. 1995). Mycorrhizal type correlates with tree growth and nutrient dynamics (Cornelissen et al. 2001). For example, trees with AM associations tend to have a faster relative growth rate, higher levels of N and P in their leaves, faster litter decomposition, and have a lower rate of carbon sequestration than trees associated with ECM. ECM trees invest more energy in structural (lignin) and chemical defenses, necessitating higher carbon sequestration rates yet potentially slowing their growth rate (Coley 1988; Cornelissen et al. 1998; Cornelissen et al. 2001). High-lignin litter from ECM trees breaks down more slowly than litter of AM trees (Cornelissen et al. 2001); the different nutrient turnover rates around ECM than AM trees may affect the herbaceous layer.

#### Effects of Nitrogen on the Herb Layer

Nitrogen is the fourth most abundant element in cellular biomass, a macronutrient essential for growth and reproduction (Stein and Klotz 2016). Nitrogen cycling in ecosystems is originally derived from three main sources: biological nitrogen fixation, mineralization, and atmospheric deposition (Bobbink et al. 2010). During biological nitrogen fixation, plants (mostly legumes) convert atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ) with the help of symbiotic bacteria in their roots. Mineralization comprises the processes of converting organic nitrogen into inorganic forms of nitrogen (via microbes) and can vary with soil temperature, moisture, and aeration. Atmospheric deposition is the process of nitrogen being transferred from the

atmosphere to Earth's surface in the form of particles or gases (dry deposition) or dissolved in precipitation (wet deposition) (Paerl et al. 2002).

Excess nitrogen from anthropogenic activities has led to an increase in nitrogen deposition in the eastern U.S. forests (Driscoll et al. 2003). An increase in atmospheric nitrogen deposition is known to reduce plant diversity in forests, especially in the herb layer (Gilliam 2006). As the herb layer is sensitive to nutrient availability, nitrogen additions can create a competitive environment (Muller 2014) supporting the survival and growth of nitrophilic species while decreasing plant biodiversity and species richness (Rajaniemi 2002, Bobbink et al. 2010). Nitrophilic species are commonly measured by the Ellenberg N score, which is based on the natural occurrence of plant species along a soil nitrogen gradient (Moreau et al. 2013). The index ranges from 1 to 9 based on their attraction toward N, with 9 being the highest level of nitrophily (Walter et al. 2017). Species loss due to nitrophilic species was demonstrated by Walter et al. (2016) at the Fernow Experimental Forest (FEF) in the Appalachian Mountains of West Virginia. After 25 years of adding nitrogen fertilizer to a watershed, the cover of nitrophilic *Rubus* spp. had increased from 1 to 19 % of total herb cover. Walter et al. (2016) concluded that the increase in *Rubus* cover was consistent with the N homogenization hypothesis (Gilliam 2006), stating that, as an ecosystem shifts from N limitation to N saturation due to the homogenous supply of soil nutrients, species richness decreases due to the exclusion of N-efficient species by nitrophilic species (Gilliam 2016).

#### Sugar Maple and Red Maple as Predictors of Soil Nitrogen

Sugar maple (*Acer saccharum* Marsh.) and red maple (*Acer rubrum* L.) are dominant and widely distributed tree species of the hardwood forests of the northeastern US. Studies have shown that these species are unique and critical with regard to nitrogen cycling in forest ecosystems (Lovett and Mitchell 2004, Peterjohn et al. 2015). Peterjohn et al. (2015) investigated associations of tree species and nitrate availability across spatial scales at the FEF.

The researchers observed that, at the scale of individual trees, small plots, and entire watersheds, sugar maples—in contrast with red maples—strongly correlated with higher soil nitrate availability. At the scale of individual trees, the study found lower soil C:N ratios and higher soil pH values around sugar maples relative to red maples.

The distribution and abundance of sugar and red maple may be changing. In some locations, sugar maple populations may be increasing due to reduction of one of their main competitors, American beech (*Fagus grandifolia* Ehrh.), which is affected by beech bark disease (Lovett et al. 2010). However, several factors, including acid deposition, climate change, and the introduction of a new insect pest (i.e., the Asian long-horned beetle), threaten populations of sugar maple in the future. Acid deposition can increase sulfate and nitrate leaching from soils. These fluxes can accelerate leaching of other nutrients, e.g. Ca, and thus negatively affect the growth of sugar maple trees. This occurs predominately in trees growing on base-cation-poor sites (Horsley et al. 2000, Lovett and Mitchell 2004). Climate change can limit or shift the range of sugar maple by affecting the amount of moisture and nutrients in the soil (Fei et al. 2017). The Asian long-horned beetle prefers to feed on maples and bores large holes into the trunk of the tree, which will eventually cause mortality by disrupting the tree's vascular tissues and weakening the wood structure (Lovett and Mitchell 2004; Haack et al. 2010).

Red maple does reasonably well in a wide variety of habitats and ecological conditions. It possesses the physiological, morphologically, and growth characteristics of both early and late successional tree species, which might explain the difference between sugar and red maple in terms of soil nitrate availability (Abrams 1998). These traits, which are rare among plant species, have allowed red maple to increase in numbers despite a wide range of environmental changes. As an early successional species, red maple can be opportunistic, following forest disturbances and agricultural abandonment. Subsequently, red maple can persist and spread in many forest types due to requiring relatively lower amounts of water, nutrients, and light for

survival. This species has also increased in many forests that were affected by deer overabundance or gypsy moths. Red maple is less preferred by herbivores than most other trees, because of the influence of alkaloids in their foliage (Barbosa and Krischik 1987, Abrams 1998).

Changes in the abundance of sugar maple could lead to major changes in nitrogen retention in watersheds in the eastern US. As mentioned above, sugar maples are associated with soils having high rates of nitrification, i.e. nitrate production. Nitrate, the most mobile form of inorganic nitrogen, is readily lost from an ecosystem by leaching below the rooting zone (Lovett and Mitchell 2004). While the study of Peterjohn et al. (2015) could not determine whether tree species cause or reflect patterns of soil nitrate availability, their results supported the hypothesis that the nature of leaf litter alters soil C:N ratios in ways that influence rates of nitrification (Lovett et al. 2002, Lovett and Mitchell 2004). With the herb layer potentially contributing a significant amount of litter, and with its high foliar concentrations of N, P, K, and Mg and low concentrations of lignin and cellulose (Chapin 2003), the herb layer has the potential to lower soil C:N ratios and increase nitrification rates thus either amplifying the stimulating effect of sugar maple or buffering against the dampening effect of red maple on nutrient cycling (Figure 10).

#### Long-term Effects of Forest Harvest on the Herb Layer

Anthropogenic disturbances have largely replaced natural disturbances in forest ecosystems (Oheimb and Härdtle 2009). The intensity of the disturbance can affect the amount of time for the forest to return to its natural state (Gilliam 2007). These disturbance events, mostly tree harvesting, can create gaps in the canopy that allow more light to reach the forest floor increasing species richness with disturbance severity. At the Fernow Experimental Forest, Walter et al. (2016), observed that *Rubus* spp. cover increased with increasing canopy openness under adequate N supply. While these light-mediated short-term effects are well

known (Gotmark et al. 2005), less is known of how (or if) longer time periods since the last harvest affect understory in a closed-canopy forest.

### Effects of Abiotic Factors on the Herb Layer

Slope and aspect can affect the amount of solar radiation received by vegetative and soil surfaces. Solar radiation creates microclimates influencing decomposition and nutrient availability by affecting soil temperature and soil moisture content (Bennie et al. 2008; Cantlon 1953; Maren et al. 2015). Aspect can influence soil moisture by effecting the rate of evapotranspiration (Hawley et al. 1983), with south-facing slopes generally having drier soils than north-facing slopes. Mudrick et al. (1994) reported that litter decomposition was faster on northerly than on southerly aspects in hardwood forests. With northerly aspects having more soil moisture and higher microbial activity (Kang et al. 2009), N availability can be expected to be higher relative to watersheds with southerly aspects (Peterjohn et al. 2015). Slope steepness can also affect soil moisture with steeper slopes having drier soils than gentle slopes (Bennie et al. 2008). As soil moisture stress is one of the most important abiotic stresses that affects yield and reproduction in plants, slope and aspect certainly play an important role in understory cover and diversity.

### Research Justification and Objectives

Compared to the tree component, relatively little is known about the contributions of the biodiversity and abundance of the herbaceous layer to forest functioning in general and to the functioning of forests with varying nitrogen deposition levels, land use history, and abiotic factors in particular. It is also unknown whether herb layer characteristics vary beneath trees of different species. Given that different tree species have been associated with different levels of soil nitrate availability and that biodiversity may be positively correlated with nutrient retention, the herbaceous layer under different tree species warrants investigation. With climate change,

atmospheric N deposition, introduced pests, management practices, and other factors influencing forest ecosystems, a better understanding of the contributions of *all* components of the ecosystem to its functions will help practitioners adaptively manage forest resources for sustained delivery of ecosystem goods and services. This research project primarily aimed to fill a current knowledge gap and, secondarily, to provide the basis for studying the role of understory nitrogen cycling in future studies. The specific objectives were to:

1. Quantify herb species composition, cover, and biomass under two different *Acer* species

$H_0$ : There is no difference in herb layer characteristics under sugar maple and red maple within a given watershed.

$H_a$ : Herb layer characteristics differ under sugar maple and red maple.

2. Quantify herb species composition and cover under two different *Acer* species in watersheds with different levels of N deposition

$H_0$ : There is no difference in herb layer characteristics between a fertilized watershed (WS3) and an unfertilized reference watershed (WS7).

$H_a$ : Herb layer characteristics differ between a fertilized watershed (WS3) and an unfertilized reference watershed (WS7).

3. Quantify herb species composition and cover under two different *Acer* species in watersheds with different stand ages

$H_0$ : There is no difference in herb layer characteristics between a watershed with 60-year-old trees (WS7) and a watershed with 110-year-old trees (WS13).

$H_a$ : Herb layer characteristics differ between a watershed with 50-year-old trees (WS7) and a watershed with 110-year-old-trees (WS13).

4. Quantify herb species composition and cover under two different *Acer* species in watersheds with different aspects

$H_0$ : There is no difference in herb layer characteristics between a watershed with a northerly aspect (WS13) and a watershed with a southerly aspect (WS10).

$H_a$ : Herb layer characteristics differ between a watershed with a northerly aspect (WS13) and a watershed with a southerly aspect (WS10).

## Materials and Methods

### Study Site

This study took place at the Fernow Experimental Forest (FEF), located in Tucker County in north-central West Virginia (Figure 1). The FEF lies within an area classified as the Allegheny Mountain Section of the Central Appalachian Broadleaf Forest (Adams et al. 1993). The growing season extends from May through October, with tree leaves emerging in late April and being fully developed by mid-June. Leaves begin to fall in October. The mean annual precipitation is approximately 1460 mm yr<sup>-1</sup> (122 mm per month on average) with somewhat higher precipitation occurring during the growing season (March through August) (Adams et al. 1994, Gilliam et al. 1994). The mean monthly air temperatures range from -2.8 °C in January to 20.4 °C in July (Adams et al. 2012). The most common soil at FEF is Calvin channery silt loam (loamy-skeletal, mixed, active, mesic Typic Dystrudept) (Adams 2012). All study watersheds support vegetation originating from natural regeneration. The dominant overstory species include sugar maple (*Acer saccharum* Marsh.), sweet birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrh.), yellow tulip poplar (*Liriodendron tulipifera* L.), black cherry (*Prunus serotina* Ehrh.), and northern red oak (*Quercus rubra* L.) (Adams et al. 2006), but their relative importance varies both within and between the watersheds being studied.

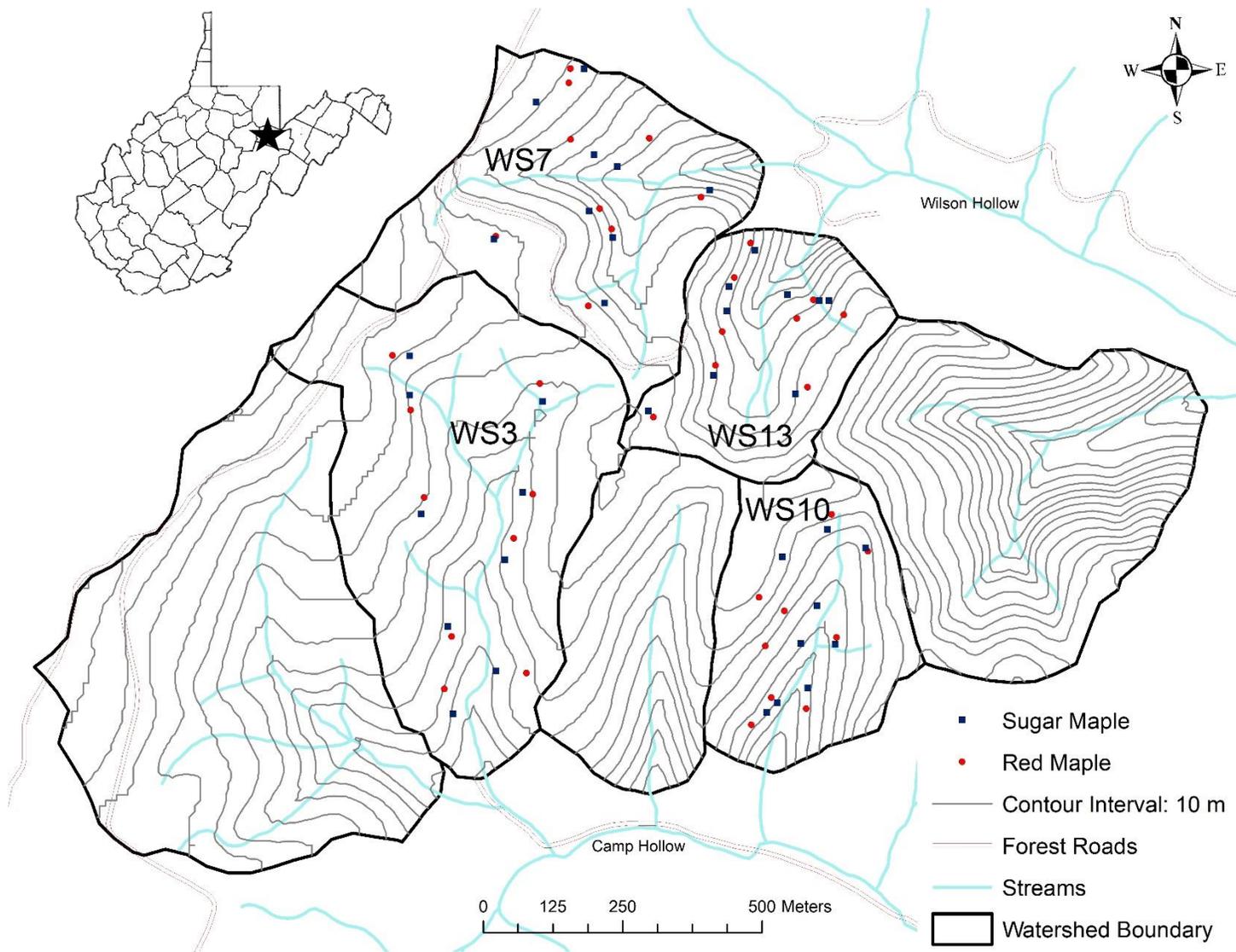


Figure 1. Location of Fernow Experimental Forest (insert) and watersheds used in this study (WS3, WS7, WS10, WS13). Location of study plots are marked by the tree at the plot center (square - sugar maple, circle - red maple).

Watersheds are in close proximity to each other (Figure 1) and are similar with respect to soil series, geology, climate, and natural disturbance history; watersheds differ in regard to slope (Figure 2, Table 1) and human-related disturbance history (detailed below).

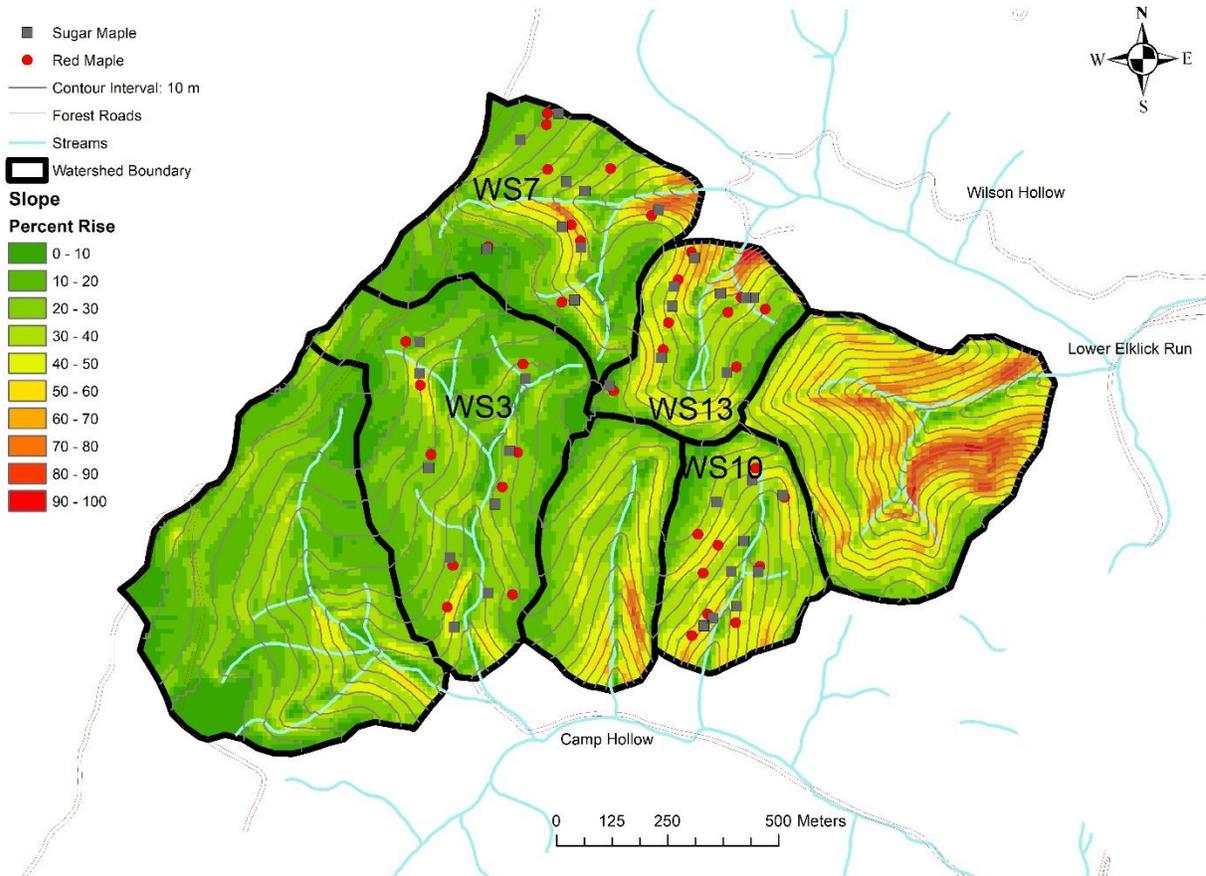


Figure 2. Slopes (in %) of the study watersheds (WS3, WS7, WS10, WS13) at the Fernow Experimental Forest. Location of study plots are marked by the tree at the plot center (square - sugar maple, circle - red maple).

Table 1. Characteristics of the watersheds in the study.

Watershed ID	Location*	Area (ha)	Elevation (m)	Aspect	Average Slope (Range) %	Dominant Tree Species**	Stand Age (yr)	Treatment
3	39.05413N 79.68625W	34.3	730-860	S	20.6 (0-60)	black cherry, red maple sugar maple	~50	Fertilization
7	39.06388N 79.68029W	24.2	730-860	E	25.8 (0-90)	tulip poplar, sweet birch black cherry	~50	Reference
10	39.05411N 79.68029W	15.2	695-805	S	33.4 (0-70)	red maple American beech, chestnut oak	~110	Reference
13	39.06280N 79.67917W	14.2	610-830	NE	35.2 (0-100)	sugar maple American beech red oak	~110	Reference

\* Lowest point in the watershed, i.e. the location of the stream weir

\*\* Tree species are listed in order of descending cover/importance (see text below)

### *Watershed 3*

Watershed 3 (WS3) has received fertilization/acidification treatments to study the effects of atmospheric N deposition since 1989. Since then, granular ammonium sulfate has been applied aerially three times a year at a rate of 7.1 kg N ha<sup>-1</sup> in March and November, and 21.2 kg N ha<sup>-1</sup> in July (Peterjohn 2017). WS3 was logged around 1910 and only undesirable trees were left behind. In the 1940's, chestnut trees affected by chestnut blight were also removed from this watershed and the other watersheds in this study. Partial cuts were made in 1958, 1963, and 1968. In 1958, 14% of trees with a DBH ≥ 12.7 cm (5.0 in) were cut and removed. In 1963, 9% of the trees with the same DBH were removed, and finally in 1968, 6% of trees of the same DBH were removed. The watershed was subsequently clearcut in 1969-1970; all saplings with a DBH between 2.5 cm and 12.5 cm (1 in to 4.9 in) were sprayed with herbicide and trees with a DBH > 12.7 cm (5.0 in) were cut (Trimble 1986); a 3-ha riparian/protection buffer strip (approximately 10-20 m wide and 730 m long) was initially left along each side of the perennial stream to help protect water quality until it was removed in 1972 (Adams et al. 1994; Aubertin and Patric 1974; Peterjohn 2017). In 2004, the dominant tree species (% basal area) in this

watershed were black cherry (51), red maple (11.5), sugar maple (11.3), sweet birch (5.1), and American beech (2.5) (Adams et al. 2007).

#### *Watershed 7*

To assess fertilization/acidification effects in WS3, watershed 7 (WS7) is used as a reference watershed since these watersheds have a similar stand age. The upper half of the watershed was clearcut from 1963 – 1964; the lower half was clearcut from 1966 – 1967. Following the clearcuts, the watershed was then herbicided annually till 1969, allowing vegetation to naturally regenerate thereafter (Peterjohn 2017, Adams 2012). In 2004, the dominant vegetation (% basal area) for this watershed are yellow tulip poplar (26.2), sweet birch (20.5), black cherry (20.5), red maple (8.2), and sugar maple (4.9) (Adams et al. 2007).

#### *Watershed 10*

Watershed 10 (WS10) serves as an “unmanaged” reference watershed (Peterjohn 2017). The last known clearcut in this watershed took place between 1905 and 1910. In 2000, the dominant tree species (as relative importance values) in this watershed were red maple (22%), American beech (15%), chestnut oak (12%), red oak (12%), and sugar maple (7%). Relative importance values are calculated by averaging the relative dominance (based on basal area), relative density, and relative frequency of each of the tree species (Peterjohn et al. 2015).

#### *Watershed 13*

Watershed 13 (WS13) also serves as an “unmanaged” reference watershed (Peterjohn 2017). Around 1910, it was heavily cut, along with WS10 (Trimble 1986). In the early 1950s, the last harvesting (a partial cut) took place in this watershed. In 2000, the dominant tree species (relative importance values) in WS13 were sugar maple (23%), American beech (15%), red oak (12%), and red maple (11%) (Peterjohn et al. 2015). While both WS13 and WS10 serve as reference watersheds, they differ in watershed aspect (Table 1) and have contrasting maple

importance values (WS13: sugar maples (23%), red maples (11%) versus WS10: sugar maples (7%), red maples (22%).

### Experimental Design

Nine plot pairs per watershed were established in the summer of 2017. Two adjacent plots, with one centering around a stem of sugar maple and the other around a stem of red maple, represent a site (Figure 3, Figure A1). The distance between plot centers of a red and sugar maple pair averaged 33 m (range: 6 – 83 m) while the average distance between neighboring plot pairs (closest plot centers) was 113 m (range: 16 – 258 m) (Figure 1). Selected plot-center trees were vigorous and had a diameter at breast height (DBH) of at least 10 cm (Table A1, Appendix). Tree locations were marked using a Garmin eTrex20 (Table A1, Appendix). Within each plot (summing up to a total of 72 plots across four watersheds), data on herb layer species composition, cover, and factors that may influence the herb layer were collected from June through August 2018.

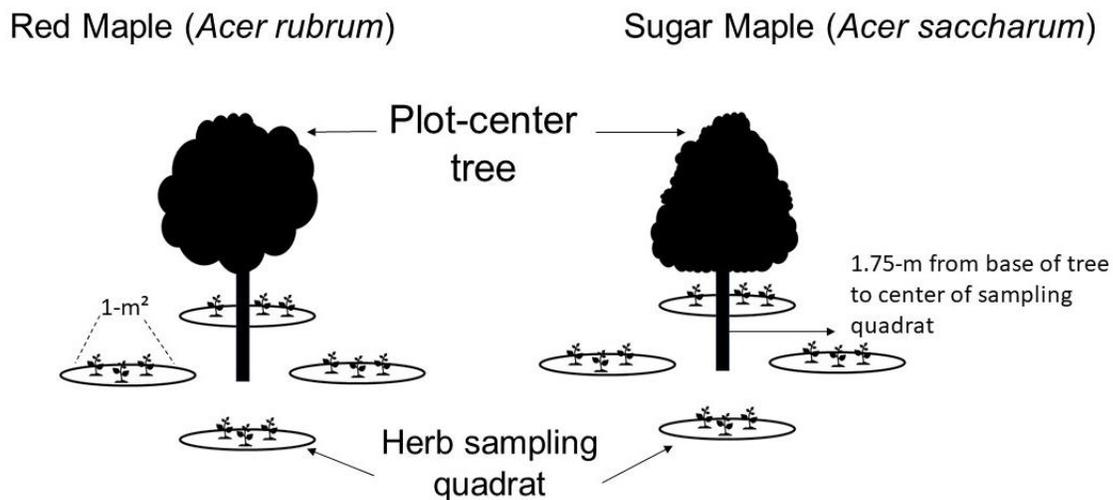


Figure 3. Experimental design of plot pairs at each site (replicated nine times in each watershed) for collecting herb composition and cover.

## Data Collection

Characteristics of the herbaceous layer < 1 m tall were assessed in four circular 1-m<sup>2</sup> herb sampling quadrats established at the four cardinal directions (N, S, E, W) from the plot center tree (Figure 3). The sampling quadrat's center was 1.75 m away from the base of the plot center tree, roughly halfway between the stem and the edge of the crown to avoid stem flow and canopy drip.

Within each sampling quadrat, herb layer composition was determined by identifying most plants to the species level, with exceptions for grasses and sedges (identified as graminoids), and *Rubus* spp., *Viola* spp., and *Anemone* spp. (identified to genus level). These taxonomically difficult groups were not identified to species level to limit misidentification. For example, in the Fernow Experimental Forest, the vast majority of *Rubus* individuals are *Rubus allegheniensis* (blackberry), but there is also *Rubus idaeus* (raspberry) at this location. *Rubus* species can hybridize and are difficult to identify without fruit or flowers (Walter et al. 2016). These exceptions likely resulted in an underestimation of species richness. Diversity indices would have been affected to a lesser extent. As graminoids and *Anemone* species had low abundance and *Rubus* was likely *R. allegheniensis*, these taxa would have been appropriately represented in diversity and evenness calculations.

For each taxon, cover was measured as leaf area using the hand-area (HA) method (Walter et al. 2015). In brief, the HA method compares the area of a hand with the area of the individual leaves of a plant or species. The observer places a hand, palm side down, and fingers closed, directly above the leaves or leaflets of the species they are measuring within the sampling quadrat. The observer then determines the size of the leaf in relation to their hand, either as individual or group, until all leaf or leaflet surfaces are observed within the quadrant. A full hand is equivalent to 1% of 1 m<sup>2</sup> and half a hand is 0.5% (Figure 4). To improve the

accuracy and precision, a) observer hands were adjusted to 1 dm<sup>2</sup> by folding under the thumb and or fingertips depending on the actual size of the observer's hand and b) two observers independently recorded cover, and the average of the two estimates was recorded (Walter et al. 2015). Cover is expressed as total cover (dm<sup>2</sup> of plant surface) per individual species or all species combined, or as relative cover (total cover of 1 taxon / sum of total cover of all species) per watershed.

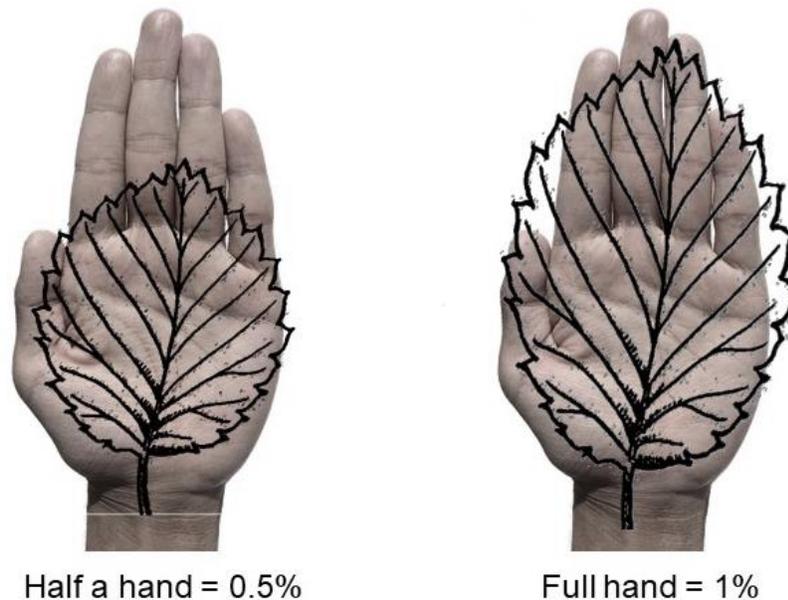


Figure 4. Visual illustration of the hand-area method. Half of a hand is equivalent to 0.5% and a full hand is equivalent to 1% of a 1-m<sup>2</sup> sampling quadrat (assuming the hand area is 1 dm<sup>2</sup>).

Species Richness (S), Shannon-Wiener Diversity (H), and Pielou's Evenness (J) were calculated from the collected data. Species Richness is the number of species per unit of area. Shannon-Wiener Diversity, an index commonly used to characterize species diversity, accounts for both abundance and evenness of species present (Begon 1996). H will increase with increasing species richness and with increasingly equitable contributions of the species to the community. Pielou's Evenness is another measure of diversity. Values for J are the ratio of

actual  $H$  to maximally possible  $H$  (if all species were present in equal proportion). Thus, values for  $J$  are constrained between 0 and 1; with  $J = 1$  indicating that all species in each area are present in equal proportions; if  $J$  is close to zero, it indicates the presence of a strongly dominant species (Pielou 1975; Begon 1996).

The Shannon-Wiener Diversity Index is calculated as

$$H = - \sum_{i=1}^s P_i \ln P_i$$

where  $P_i$  is the relative abundance of each herbaceous species in the total quadrat and where  $s$  is the number of species (Shannon and Weaver 1949).

Pielou's Evenness Index is calculated as

$$J = \frac{H}{H_{max}} = H / \ln S$$

where  $H$  is the Shannon-Wiener Diversity Index and  $S$  is the total number of species (Pielou 1975).

To characterize the abiotic environment of the herb layer, several additional measurements were conducted either on the same day as herb characteristics were measured (i.e. canopy cover) or in August. In order to quantify the light environment for the understory, percent canopy cover was measured with a densiometer over each sampling quadrat while facing the plot-center tree. The four measurements were then averaged in each plot. Slope was measured using a clinometer and aspect using a compass; both measurements were taken at the plot center facing downhill. The distance from the plot center to perennial stream was quantified using ArcMap, as water availability can affect the abundance and diversity of herbaceous plants. To align aspect with productivity and to be able to analyze it as a continuous variable, aspect was transformed into a linear scale that ranges from 0-2, with the value zero

being southwest (lowest productivity) and the value two being northeast (highest productivity) using the formula:  $A' = \sin(A + 45) + 1$ , where  $A'$  is the transformed aspect code and  $A$  is the aspect defined as the direction of the prevailing slope (Beers et al. 1966).

To assess potential influence of neighboring trees on herb characteristics below the plot center tree, DBH of the five closest neighboring trees was measured. Neighbor trees were separated by ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) association and basal area (BA,  $\text{m}^2 \text{ha}^{-1}$ ) was calculated for each association (Figure 5). The fifth-nearest tree from the plot center was used as the radius of the plot area (ha), by which the sum of the stem cross-sectional area ( $\text{m}^2$ ) of ECM or AM trees was divided (Table A2, Appendix).

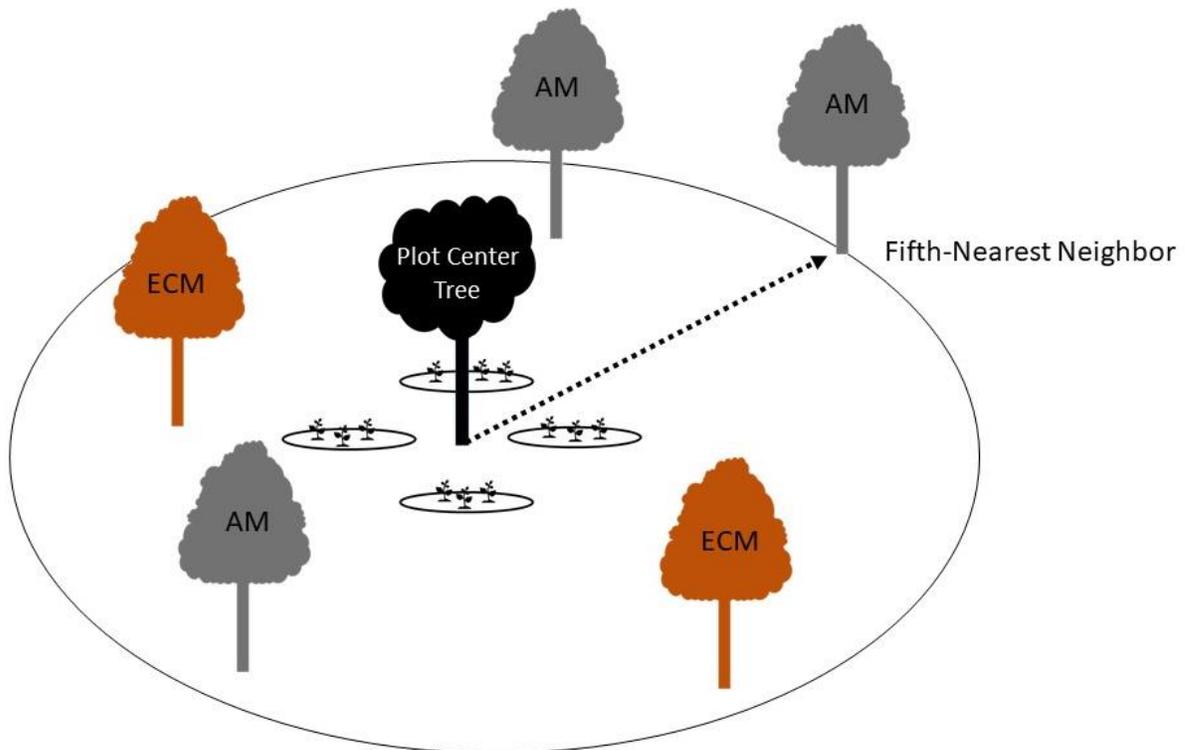


Figure 5. Visual illustration of how the five closest neighboring trees were separated by ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) association. Basal area (BA,  $\text{m}^2 \text{ha}^{-1}$ ) was calculated for each association by using the fifth-nearest tree as the radius of the plot area (ha) by which the sum of basal area ( $\text{m}^2$ ) of ECM or AM trees were divided.

## Statistical analysis

Analysis of variance (ANOVA) was conducted separately for the following watershed pairs: WS3-WS7 (differ in fertilization level; similar, “younger” stand age); WS7-WS13 (differ in stand age; similar aspect); WS10-WS13 (differ in watershed aspect; similar, “older” stand age). Watersheds were analyzed in these pairs to prevent statistical confounding, as the watersheds differed in various known independent characteristics (stand age, recent fertilization, aspect). Residuals were checked for normality and variables were transformed when necessary. Graphs and tables presented use untransformed data. Repeated measures ANOVA were undertaken to account for the spatial correlation between sugar maple/red maple plot pairs within each site (Figure 3) (SAS code: Repeated Tree / Subject = Site\*WS). All models evaluated the main effects of watershed (WS) and overstory maple species (sugar and red maple) (M) and watershed × maple species interactions (WS × M) on herb layer characteristics (cover, S, H, J). Watershed is used in the sense of “treatment”, i.e. fertilized vs unfertilized in WS3 and WS7, respectively, younger vs older (referring to the stand age) in WS7 and WS13, respectively, and northerly aspect vs southerly watershed aspect in WS13 and WS10, respectively. Additional predictor variables included in the initial full (saturated) model were slope, DBH of plot-center tree, percent tree canopy cover, aspect, distance to stream, and basal area (BA) of neighboring ectomycorrhizal trees (BA ECM) and arbuscular mycorrhizal trees (BA AM). In watershed pair WS10-WS13, plot-level aspect is used as a predictor variable in addition to watershed (WS). Inclusion of plot-level aspect is justified by individual plots not having strict north versus south aspects as the whole watersheds (WS13 and WS10, respectively); and including watershed, while hypothesized to represent watershed aspect, is justified as it retains the capability to detect unmeasured differences between the watersheds. A reduced model was created from the full model, by removing predictor variables (other than watershed, overstory maple species, and their interaction) if they had high p-values. Final model selection (full versus reduced model)

was determined by the lowest AIC value. Four pairwise comparisons were conducted within each watershed pair (using the slicing option in SAS Proc Mixed), comparing herb layer responses a) between the two overstory maple species per watershed and b) between the two watersheds under a given overstory tree species. There was no adjustment made for multiple comparisons, but the number of comparisons were minimized to four in order to reduce false positives (Type I error). In all statistical analyses, significance criterion alpha for all tests was 0.05 and a statistical trend was declared when  $P \leq 0.1$ .

In order to determine associations between individual understory plant species with watershed and overstory maple species, we first selected individual herb species that occurred in multiple sites in at least two watersheds. A total of 22 herb species (or genus for those identified to only genus level) were selected to test whether variations in cover were a response to watershed, overstory maples species, and  $WS \times M$  interactions in each of the three defined watershed pairs. Since the probability of a Type I error increases with the number of tests conducted (familywise error rate), the Benjamini-Hochberg method was applied to control the false discover rate (i.e. a false positive or a Type I error). The concept is similar to the Bonferroni adjustment for multiple comparisons, yet less conservative in order to account for the large number of tests conducted (McDonald, 2014). To perform the Benjamini-Hochberg method, P-values from the 22 individual ANOVAs are ranked from smallest to largest. The smallest P-value receives a rank (i) of 1, the next larger P-value receives the rank of 2 and so on. Next, each P-value is compared to the Benjamini-Hochberg critical value  $(i/m)Q$ , where i is the rank, m is the total number of tests (total number of individual P-values ranked), and Q is the false discovery rate selected by the researcher (McDonald 2009). In this study, the false discovery rate was set at 0.10. For the cover of a plant species to vary significantly by WS, M, or  $WS \times M$ , the P-value must be smaller than the Benjamini-Hochberg critical value. For example, with 22 species and 2 main effects (WS, M) and an interaction effect, there is a family of 66

analyses (i.e.  $n = 66$ ). For the smallest P-value obtained by ANOVA to be considered significant, it would have to be smaller than  $1/66 * 0.1 = 0.0015$ ; the P-value at rank 2 would have to be smaller than  $2/66 * 0.1 = 0.003$ , etc. All P-values that are greater than the Benjamini-Hochberg critical value are considered not significant.

While the analyses of the overstory maple species effect on herb characteristics are truly replicated, the analyses of differences between watersheds is pseudo-replicated, i.e. no inference can be made beyond the watershed pairs used in this case study. Statistical analyses were carried out using JMP and SAS software (JMP®, Version Pro 12.2, SAS Institute Inc., Cary, NC, Copyright ©2015; SAS®, Version 9.3, SAS Institute Inc., Cary, NC, Copyright ©2002-2010).

## Results

Among all four watersheds, there were a total of 64 taxa recorded: 58 species, three genera, two families, and one group of unknown species (mostly young seedlings). Twenty-nine taxa were found in plots in WS3, 47 in WS7, 35 in WS13, and 42 in WS10. Dominant herb layer species (expressed in relative cover) in plots of WS3 were *Rubus* spp. (40%) and *Dryopteris intermedia* (14.4%), in WS7 *Dryopteris intermedia* (19.6%) and *Rubus* spp. (17.4%), in WS13 *Rubus* spp. (27.3%) and *Quercus rubra* (11.6%) and in WS10 are *Smilax rotundifolia* (17.7%) and *Rubus* spp. (16%) (Figure 6). The taxa that were identified only to genus - *Rubus* spp., *Viola* spp., and *Anemone* spp. - comprised 33%, 6% and 0.2% of total cover, respectively. Interpretation of the results should take this into account as the diversity indices may be lower as a result of not identifying to species level for these taxa. Graminoids (found in 13 sampling quadrats across 9 plots) and the unknown species group (found in four sampling quadrats across 3 plots) contributed 0.23 and 0.04 % to total cover and, thus, had a negligible effect on diversity indices.

Effects of overstory maple species (M) and watershed (WS) on total cover of dominant herb layer species are shown in Figure 7. Results of the statistical analyses are shown in Table 2. In the analyses (ANOVA) of herb characteristics in watershed pairs, overstory maple species (M) was not statistically significant as main effect in any of the models but there was a statistically significant effect or a trend of the WS × M interaction in seven of the 12 models (Figure 8, Table 2). In these models, herb cover and/or diversity indices were lower under red than sugar WS3 and WS13 but not in WS7 and WS10. Watershed, in the sense of “treatment”, affected the herbaceous layer in watershed pairs WS3 vs WS7 and WS7 vs WS13. In the fertilized watershed (WS3), species richness, diversity, and evenness were significantly lower than in the unfertilized watershed (WS7). In the comparison of the watershed with the younger stand (“younger watershed”, WS7) with the watershed with the older stand (“older watershed”, WS13), the younger watershed had higher cover, diversity, and evenness in comparison to the older watershed. In the comparison of the reference watershed with a northerly aspect (WS13) with the reference watershed with southerly aspect (WS10), cover and diversity indices did not differ (Figure 8, Table 2). Other biotic and abiotic factors differed between watersheds (Figure 9), and, as expected, also affected herb characteristics (Table 2).

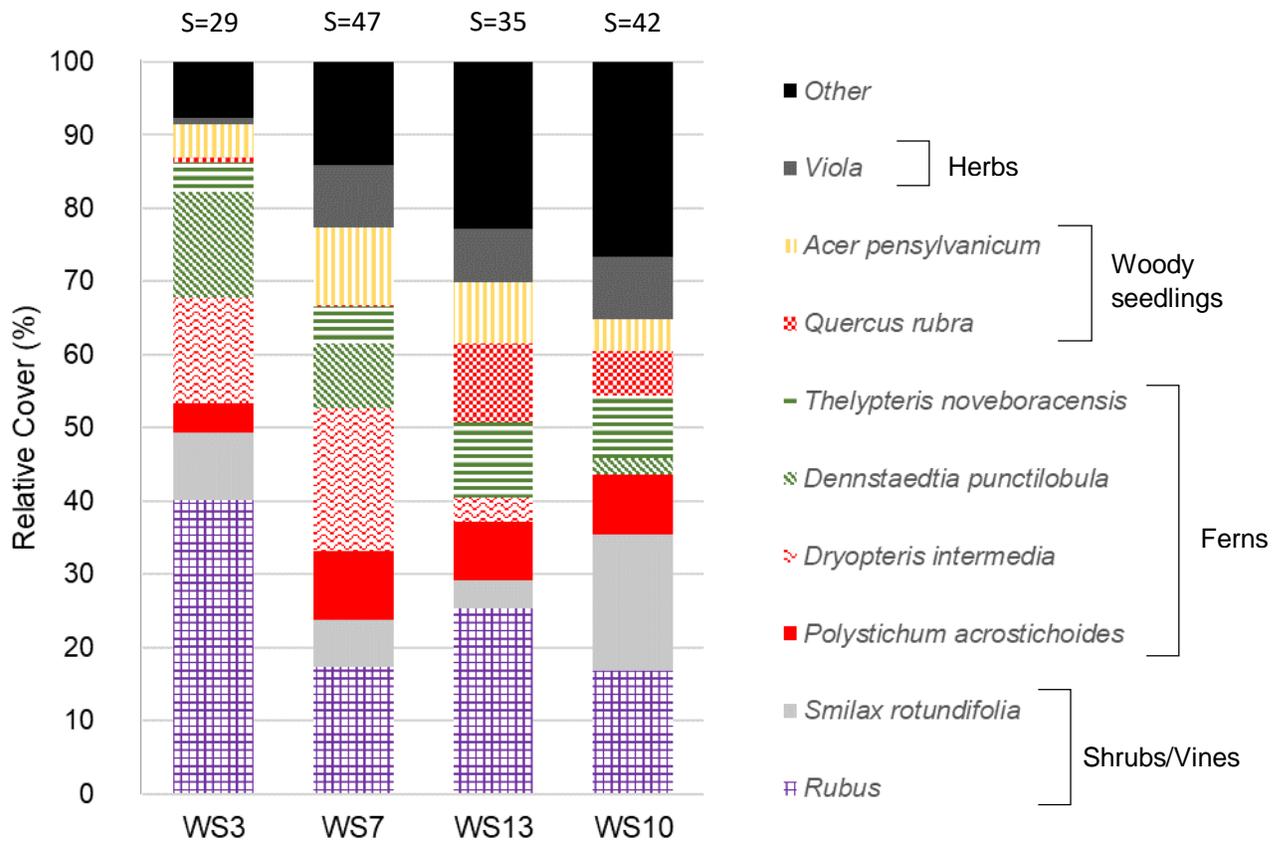


Figure 6. Relative cover of nine understory taxa and all other taxa combined in each watershed. S indicates species richness in each watershed.

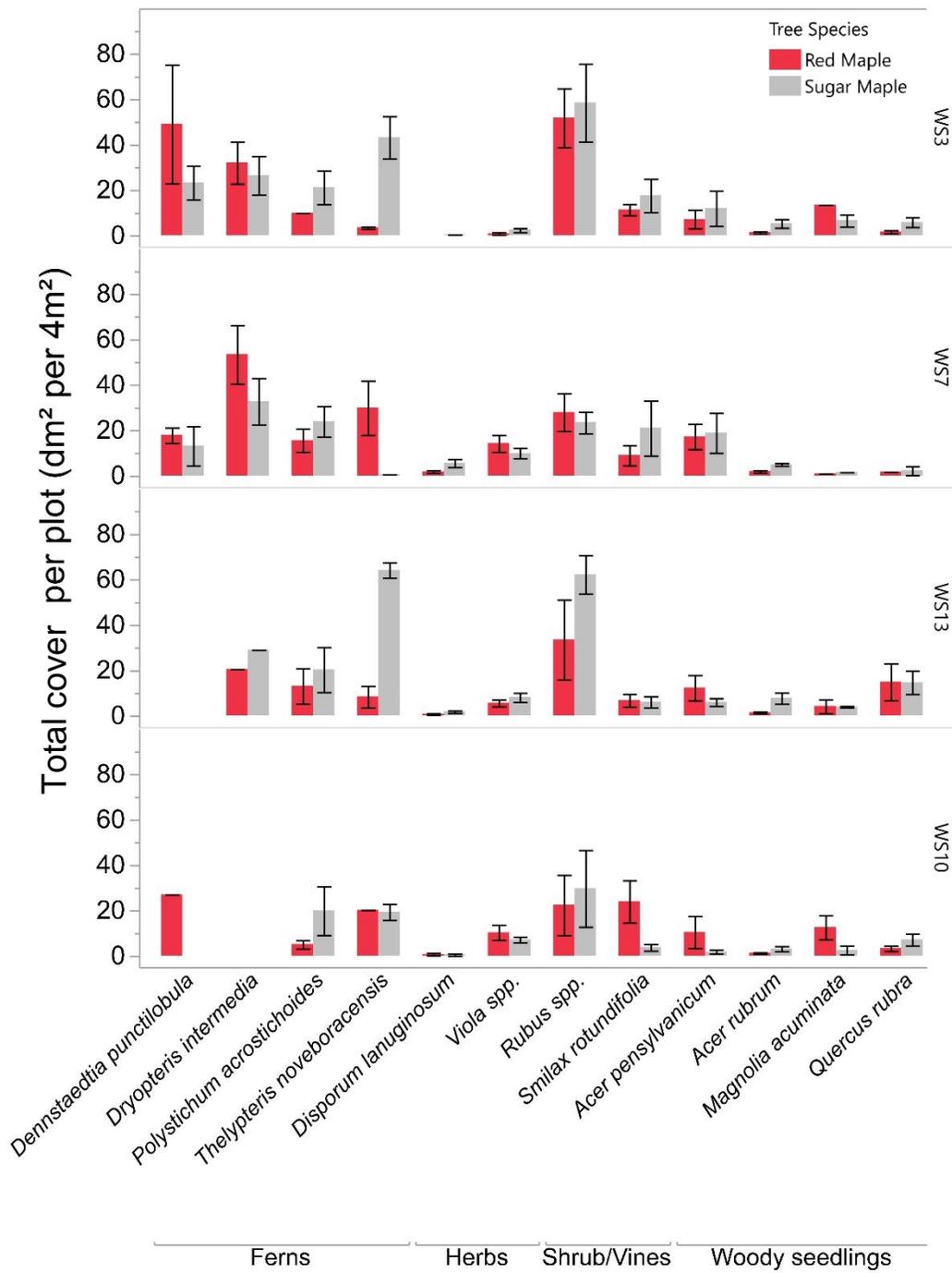


Figure 7. Total cover per plot (dm<sup>2</sup> per 4m<sup>2</sup>) of the most common understory plant species in WS3, WS7, WS10, and WS13. Error bars represent 1 SE.

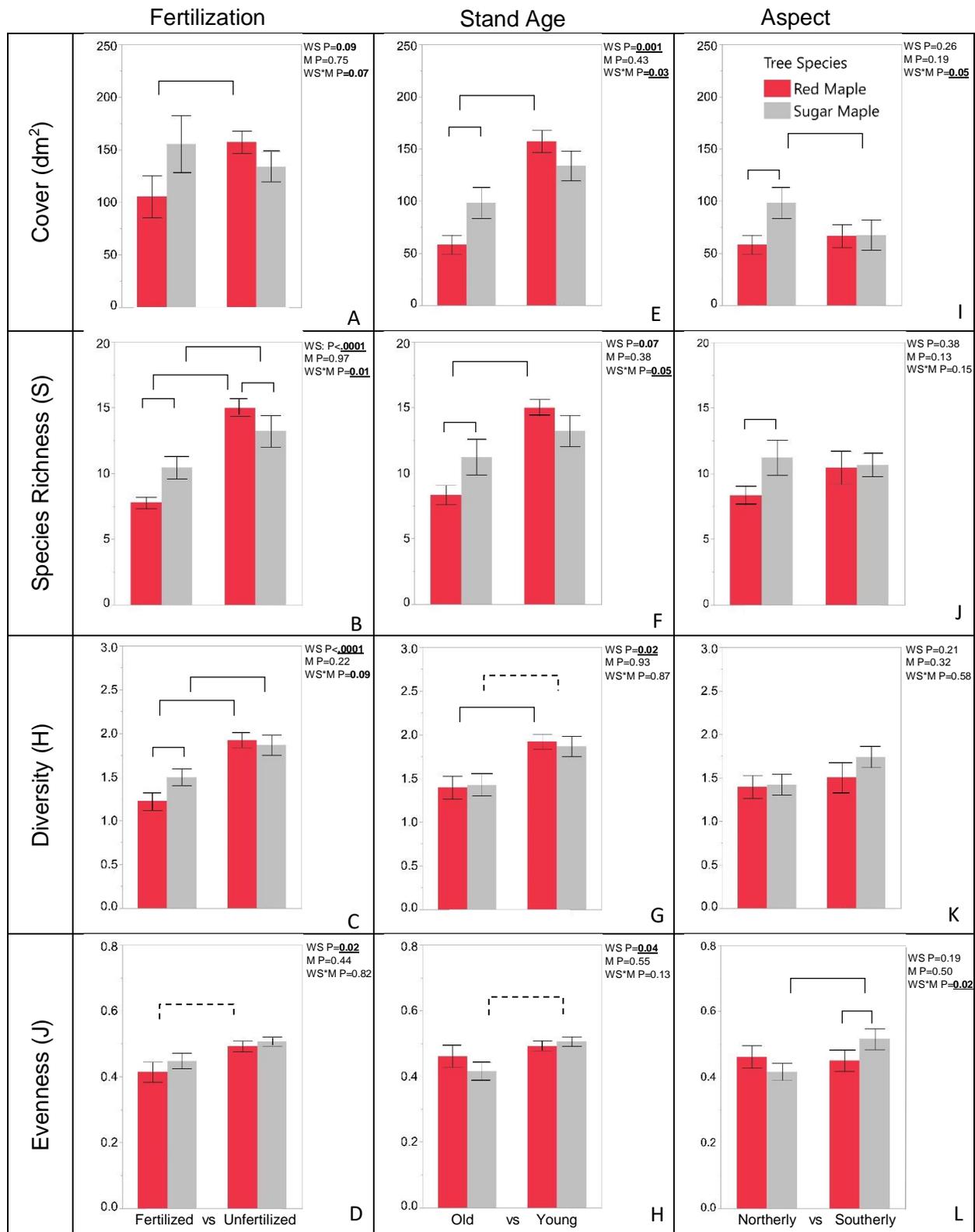


Figure 8. Herb characteristics in red and sugar maple (M) plots (4m<sup>2</sup> sampling area) in each of the studied watersheds (WS). Watershed comparisons are: fertilized (WS3) vs. unfertilized (WS7) younger stands (A-D), younger stand (WS7) vs. older (WS13) reference stand (E-H), and older stands with northerly aspect (WS13) vs. southerly aspect (WS10) (I-L). Error bars represent 1 SE. Statistical results of main and interaction effects are excerpts from the final ANOVA model (Table 2 and Table A6). Horizontal brackets represent pairwise comparisons at P ≤ 0.05 (solid line) and P ≤ 0.1 (dashed line).

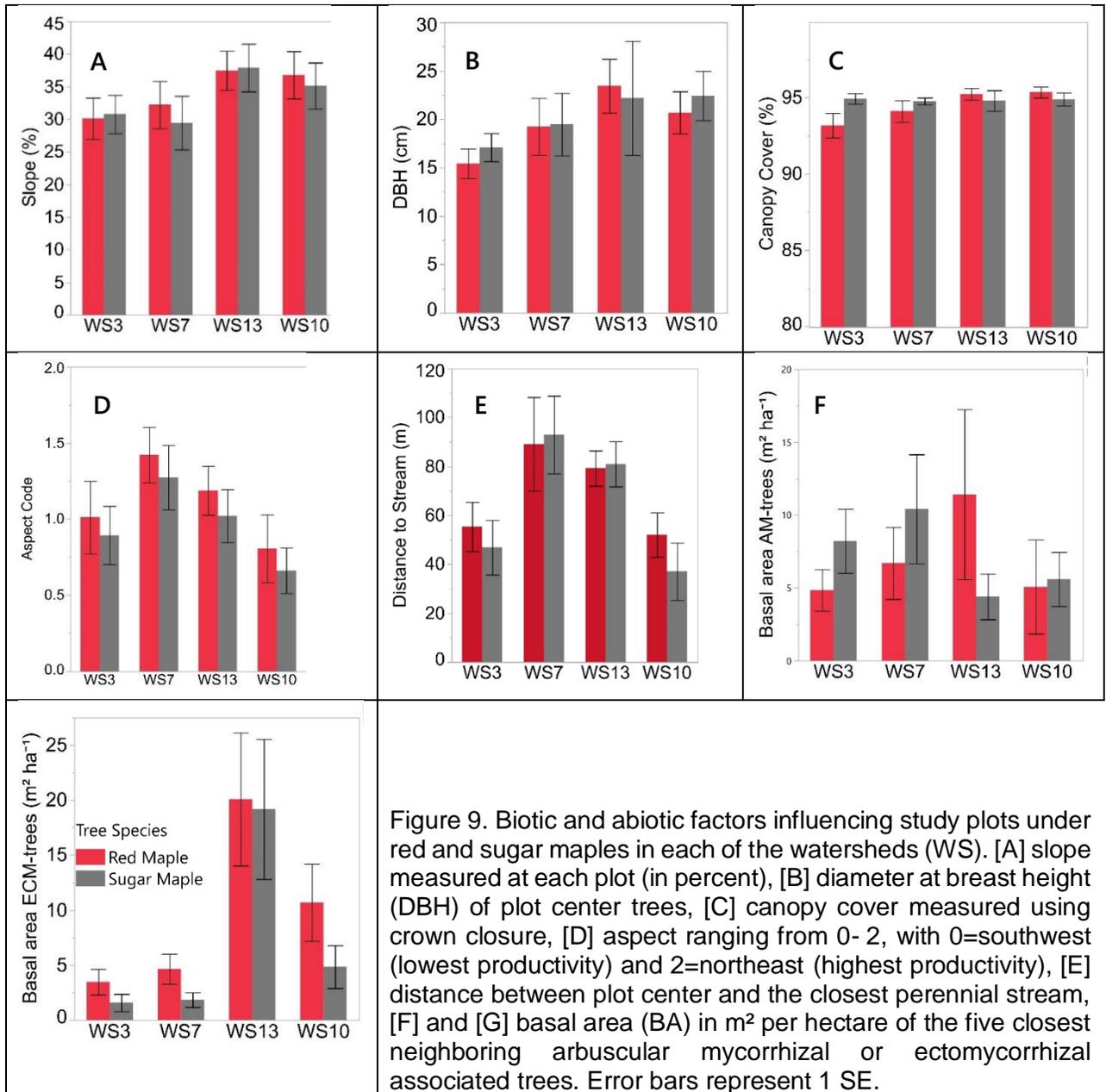


Figure 9. Biotic and abiotic factors influencing study plots under red and sugar maples in each of the watersheds (WS). [A] slope measured at each plot (in percent), [B] diameter at breast height (DBH) of plot center trees, [C] canopy cover measured using crown closure, [D] aspect ranging from 0- 2, with 0=southwest (lowest productivity) and 2=northeast (highest productivity), [E] distance between plot center and the closest perennial stream, [F] and [G] basal area (BA) in  $m^2$  per hectare of the five closest neighboring arbuscular mycorrhizal or ectomycorrhizal associated trees. Error bars represent 1 SE.

Table 2. Results of the statistical analyses (ANOVA). P-values are shown below for full and reduced models. The final models selected for this study have the lowest AIC value (shown in bold) in the comparison between full and reduced model. Continuous predictor variables are explained in Figure 9.

	Total Cover		Species Richness, S		Diversity, H		Evenness, J	
<b>WS3 vs WS7</b>								
	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model
<b>AIC</b>	<b>313.9</b>	321.4	152.7	<b>148.2</b>	53.7	<b>34.8</b>	-22.3	<b>-62.0</b>
Watershed (WS)	<b>0.09</b>	<b>0.08</b>	<u>&lt;.0001</u>	<u>&lt;.0001</u>	<u>0.0002</u>	<u>&lt;.0001</u>	<b>0.06</b>	<u>0.02</u>
Overstory Maple (M)	0.75	0.66	0.90	0.97	0.62	0.22	0.58	0.44
WS × M	<b>0.07</b>	<b>0.05</b>	<b>0.004</b>	<b>0.003</b>	0.17	<b>0.09</b>	0.96	0.82
Slope	0.93		<u>0.01</u>	<u>0.01</u>	<u>0.03</u>	<u>0.02</u>	0.13	
DBH	0.37	0.28	0.77		0.43		0.26	
Tree Cover	0.91		0.68		0.36		<b>0.08</b>	0.19
Aspect	0.36	0.31	<u>0.02</u>	<u>0.01</u>	<u>0.03</u>	<u>0.02</u>	<b>0.09</b>	<b>0.09</b>
Distance to stream	0.11	<b>0.08</b>	<u>0.02</u>	<u>0.02</u>	<b>0.07</b>	<b>0.08</b>	0.33	
AM-trees	0.47	0.41	0.53	0.46	0.89		0.81	
ECM-trees	<b>0.08</b>	<b>0.06</b>	<u>0.02</u>	<u>0.01</u>	0.63		0.33	
<b>WS7 vs WS13</b>								
	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model
<b>AIC</b>	<b>301.7</b>	319	<b>171.3</b>	171.6	62	<b>54.3</b>	-16	<b>-34.4</b>
Watershed (WS)	<u>0.001</u>	<u>&lt;.0001</u>	<b>0.07</b>	<u>0.05</u>	<u>0.02</u>	<u>0.01</u>	<b>0.10</b>	<u>0.04</u>
Overstory Maple (M)	0.43	0.42	0.38	0.38	0.91	0.93	0.6	0.55
WS × M	<b>0.03</b>	<b>0.01</b>	<b>0.05</b>	<b>0.03</b>	0.82	0.87	0.25	0.13
Slope	0.83		<b>0.04</b>	<u>0.03</u>	<b>0.10</b>	<b>0.08</b>	0.44	0.18
DBH	0.76		0.42	0.30	0.14	<b>0.06</b>	0.13	<u>0.04</u>
Tree Cover	0.84		0.12	<b>0.06</b>	0.20	0.16	0.25	<u>0.33</u>
Aspect	0.64	0.35	<b>0.09</b>	<u>0.04</u>	0.35	0.33	0.91	
Distance to stream	0.67		0.96		0.88		0.54	
AM-trees	0.59	0.43	0.93		0.78		0.42	
ECM-trees	0.75		0.66		0.29	0.28	0.15	0.15
<b>WS10 vs WS13</b>								
	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model	Full Model	Reduced Model
<b>AIC</b>	<b>295</b>	309.6	179.5	<b>174.8</b>	70.5	<b>51.3</b>	-11.3	<b>-29.6</b>
Watershed (WS)	0.26	0.21	0.68	0.38	0.20	0.21	0.16	0.19
Overstory Maple (M)	0.19	0.14	0.22	0.13	0.23	0.32	0.45	0.50
WS × M	<b>0.05</b>	<b>0.04</b>	0.19	0.15	0.42	0.58	<u>0.02</u>	<u>0.02</u>
Slope	0.67		0.93		0.59		0.44	
DBH	0.40		0.83		0.11	0.14	<u>0.01</u>	<u>0.009</u>
Tree Cover	0.17	0.24	0.73	0.24	0.31		<b>0.09</b>	<b>0.10</b>
Aspect	<b>0.04</b>	<b>0.02</b>	0.65	<u>0.02</u>	0.19	0.34	<u>0.01</u>	<u>0.007</u>
Distance to stream	<u>0.02</u>	<u>0.03</u>	0.56	<u>0.03</u>	0.43		<b>0.08</b>	<b>0.09</b>
AM-trees	0.42		0.45	0.23	0.14	<b>0.10</b>	<b>0.09</b>	<b>0.10</b>
ECM-trees	0.37		0.78		0.70		0.73	

**Bold font** =  $P \leq 0.1$ , **Underlined bold font** =  $P \leq 0.05$

### *WS3 vs WS7: Effects of fertilization and overstory maple species*

In the comparison of the two watersheds with younger stands, watershed (WS) and the watershed  $\times$  overstory maple interactions (WS  $\times$  M) were statistically significant explanatory variables for most herb characteristics but overstory maple species (M) as a main effect was not. Herb cover overall tended to be lower in the fertilized watershed (WS3) than the unfertilized watershed (WS7) ( $P=0.09$ ). Cover tended to be greater under sugar maples than red maples in the fertilized watershed with an opposite pattern in the unfertilized watershed (WS  $\times$  M  $P=0.07$ ) (Figure 8A). This pattern was also observed for species richness (WS  $P<0.0001$  and WS  $\times$  M  $P=0.01$ ) (Figure 8B)). Twenty-three herb layer species found in the unfertilized watershed were absent from the fertilized watershed (with four species absent in WS7 but present in WS3). Most of these 23 species in WS7 had low relative cover (on average 0.08%) (Table A3). Shannon-Wiener diversity was lower in the fertilized watershed than the unfertilized watershed when averaged across maple species (WS  $P<0.0001$ ) and tended to be lower underneath red maples compared to sugar maples in WS3 but not WS7 (WS  $\times$  M  $P=0.09$ ) (Figure 8C). Evenness overall was lower in the fertilized watershed than in the unfertilized watershed (WS  $P=0.02$ ) and there was no WS  $\times$  M interaction ( $P>0.05$ ) (Figure 8D). Analyses of individual herb species showed that cover of three species varied with watershed or overstory maple species. Cover of *Viola* spp. and jack-in-the-pulpit (*Arisaema triphyllum*) was significantly lower in WS3 than in WS7 (WS  $P=0.001$  and WS  $P=0.003$ , respectively). The cover of New York fern (*Thelypteris noveboracensis*) was greater beneath red maples than sugar maples in WS3 with an opposite pattern in WS7 (WS $\times$ M  $P=0.002$ ) (Figure 7).

### *WS3 vs WS7: Effect of abiotic and biotic variables*

Various environmental factors explained significant variation in herb characteristics, but with independent factors being less consistent between diversity indices than WS, M, and WS × M (Table 2). Richness and diversity decreased with increasing distance from the stream ( $P=0.02$  and  $P=0.07$ ). Richness and diversity decreased with increasing slope steepness ( $P=0.01$  and  $P=0.02$ ). Richness, diversity and evenness tended to increase with increasing aspect code (i.e. aspect changing from southwest toward northeast) ( $P=0.01$ ,  $P=0.02$ ,  $P=0.09$ , respectively). Species richness decreased as the basal area of ECM-associated neighbor trees increased ( $P=0.01$ ) (Table 2).

### *WS7 vs WS13: Effects of stand age and overstory maple species*

In comparison of the two watersheds with different stand age, watershed (WS) and the watershed × overstory maple interactions (WS × M) were statistically significant explanatory variables for most of the herb characteristics, but overstory maple (M) as a main effect was not. Herb cover was greater in the younger watershed (WS7) than in the older watershed (WS13) (WS  $P=0.001$ ). Cover was higher under sugar maples than under red maples in the older watershed, with an opposite pattern in the younger watershed (WS × M  $P=0.03$ ) (Figure 8E). Species richness tended to be greater in the younger watershed than in the older watershed (WS  $P=0.07$ ). Richness was greater under sugar maples than under red maples in the older watershed, with an opposite pattern in the younger watershed (WS × M  $P=0.05$ ) (Figure 8F). Diversity and evenness were higher in the younger watershed (WS  $P=0.02$  and  $P=0.04$ ) than the older watershed and did not vary by overstory maple species (WS × M  $P>0.05$ ) (Figure 8G,8H) (Table 2). Considering individual understory species, the cover of red maple seedlings was greater beneath sugar maples than red maples in both watersheds (M  $P=0.0003$ ) (Figure 7).

*WS7 vs. WS13: Effects of abiotic and biotic variables*

Environmental factors, such as slope, aspect, and DBH, explained significant variation in herb characteristics, but with less consistency between the four herb characteristics (Table 2). Herb cover was not affected by any of the abiotic or biotic factors tested. Richness decreased as slope increased and richness increased with increasing aspect code (i.e. toward the NE aspect) ( $P=0.04$  and  $P=0.09$ , respectively). Diversity tended to decrease as slope increased and it decreased with increasing DBH of the plot center tree ( $P=0.08$  and  $P=0.06$ ). Evenness decreased as the DBH of the plot center trees increased ( $P=0.04$ ) (Table 2).

*WS10 vs WS13: Effect of watershed aspect and overstory maple species*

In comparison of the two watersheds with the same (older) stand age but varying watershed aspect, watershed (WS) and overstory maple species (M) were not statistically significant explanatory variables for any of the herb characteristics. Watershed  $\times$  overstory maple interaction (WS $\times$ M) was a statistically significant explanatory variable for cover and evenness. Herb cover under sugar maples was greater than under red maples in the north-facing watershed (WS13), while there was no difference between tree species in the south-facing watershed (WS10) (WS  $\times$  M  $P=0.05$ ) (Figure 8I). Evenness was greater under sugar than red maples in WS10, with an opposite pattern in WS13 (WS  $\times$  M  $P=0.02$ ) (Figure 8L). Species richness and diversity did not differ between watersheds (Figure 8J, 8K) (Table 2). Considering individual understory species, the cover of red maple seedlings was greater beneath sugar maples than red maples (M  $P=0.0003$ ) (Figure 7).

*WS10 vs WS13: Effect of abiotic and biotic variables*

A few environmental factors explained significant variation in herb characteristics (Table 2). Herb cover and species richness increased with increasing aspect code ( $P=0.04$  and  $P=0.02$ , respectively) and decreased with increasing distance to stream ( $P=0.02$  and  $P=0.03$ ,

respectively). Shannon-Wiener Diversity tended to increase with increasing basal area of AM associated trees ( $P=0.1$ ). Evenness decreased with increasing DBH and aspect ( $P=0.009$  and  $P=0.007$ , respectively). As canopy cover increased, evenness increased as well ( $P=0.1$ ).

## Discussion

The forest understory plays important roles in deciduous forests through its influence on nutrient cycling, provision of habitat, and overstory regeneration. This study evaluated understory composition beneath red and sugar maple, tree species that differ in their influences on nutrient cycling (St Clair and Lynch 2005, Abrams 1998, Lovett et al. 2004). The overstory tree species effect was studied in tandem with watershed effects and other factors that are well-known to influence the understory. The watersheds evaluated at the Fernow Experimental Forest varied in N deposition level, stand age, and aspect, factors which comprise or influence the abiotic and biotic environment for understory growth (Gilliam 2006, Gilliam et al. 2016, Olivero and Hix 1998, Hawley et al. 1983). This study showed that understory communities can be affected by numerous factors, including overstory tree species, environmental factors including aspect and slope, and anthropogenic activities, such as N fertilization and time since last harvest.

### *Herb Layer Responses to Overstory Red Maple vs. Sugar Maple [H1]*

This study revealed that there was no consistent response of the herb layer to the overstory tree species (i.e. no statistically significant M main effect in any model), but that the herb response differed by maple species depending on the watershed. In seven of the 12 models (four herb characteristics and three watershed pairs) there was a significant effect (or trend) of the WS x M interaction, indicating that herb layer cover and diversity indices were lower under red maple relative to sugar maple in WS7 and WS13, but not in the other watersheds (Figure 8).

Red maples and sugar maples also behave differently in terms of nitrogen cycling. Chapman et al. (2006) separated plants into nitrogen-conservative and nitrogen-extravagant groups depending on the plant species' and mycorrhizal symbionts' strategies and the environment they inhabit. Nitrogen-conservative plant traits include (a) high levels of mycorrhizal colonization, (b) reliance on mycorrhizal fungi for nutrient uptake, (c) ability to access organic nitrogen, and (d) production of low quality litter. Soils beneath nitrogen-extravagant plants are more prone to nitrogen leaching and these plants (a) do not support high levels of mycorrhizal colonization, (b) are unable to access organic N, and (c) produce high quality litter (Chapman et al. 2006). On native soil, red maple compared to sugar maple has been shown to have higher levels of mycorrhizal colonization and photosynthesis, with higher photosynthesis likely being a consequence of the higher mycorrhizal colonization as this symbiosis can increase nutrient uptake and water use efficiency in plants (Caravaca et al. 2003). The quality and quantity of tree litter also differs between red maples and sugar maples. Soils beneath sugar maple have significantly less forest floor biomass, a lower C:N ratio in the mineral soil (Finzi et al. 1998, Vitousek et al. 1982), and significantly more soil calcium than under the red maples (Vitousek et al. 1982). Thus, red maple demonstrates nitrogen-conservative traits and sugar maples nitrogen-extravagant traits.

Sugar maples are associated with soils with higher rates of nitrification and nitrate production than red maples (Peterjohn et al. 2015). Being highly mobile in soil, nitrate may be easily accessible to understory plants, but also more susceptible to leaching (Boudsocq et al. 2012). The process of nitrate production (nitrification) slightly decreases the pH of the soil, resulting in increased concentrations of cations in soil solution. Once in soil solution, increased mobility of cations, such as calcium is increased (Peterjohn et al. 1996). Dijkstra (2002) showed that calcium availability varied beneath tree species, with mineral soil calcium being highest under sugar maples and lowest under red maples among all species studied. Vitousek et al.

(1982) also found that sugar maples had more than double the amount of calcium availability in the soil than red maples.

In sum, the two maple species can affect the spatial pattern of nutrient availability, and thus influence the abundance and possibly diversity of the understory. The higher availability of nitrogen and calcium beneath sugar maples creates an environment that better supports a higher abundance of understory plants (“sugar maple effect”) as seen in WS3 and WS13 (Figure 10). This study indicates that a “sugar maple effect” may not manifest under some conditions as seen in the younger reference watershed (WS7) and the older reference watershed with a southerly watershed aspect (WS10). While this study did not investigate causation, it may be possible that, in the absence of fertilization, a sugar maple effect is detectable under the intermediate levels of soil fertility of WS13, but not under the more fertile conditions of WS7 (as evidenced by relatively high streamwater nitrate concentrations, Table 3) or under conditions of low soil fertility/moisture of WS10 (as evidenced by relatively low streamwater nitrate concentrations, Table 3). Under conditions of high external N inputs in WS3, sugar maple may be able to buffer against nutrient imbalances following excessive nitrate leaching (Table 3) by relatively high rates of calcium mineralization.

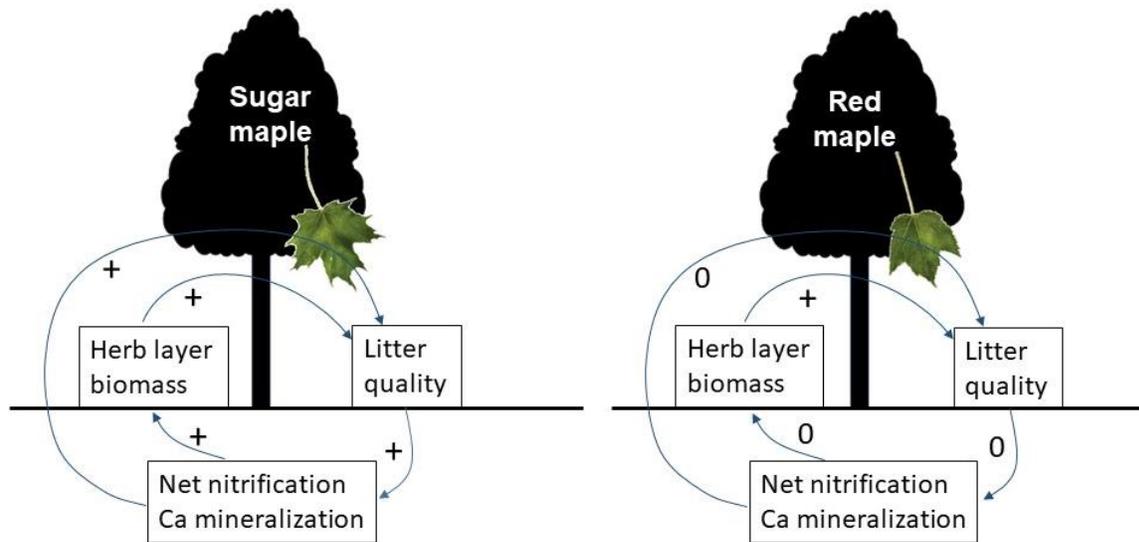


Figure 10. Conceptual diagram of how sugar and red maple may affect herb layer biomass through neutrally (0) or positively (+) influencing soil nitrate and calcium availability.

*Herb Layer Responses to Fertilization: WS3 vs WS7 [H2]*

In this study, the herbaceous layer cover and diversity indices were consistently negatively affected by N amendments to watershed 3 compared to its unfertilized reference watershed (WS7). As the applied fertilizer is ammonium sulfate, changes in the understory may reflect direct responses to  $\text{NH}_4^+$  or concomitant changes in soil properties (i.e. soil acidification, calcium loss), and indirect responses via competitive exclusion from nitrophilic species. These effects may be modified by the dominant tree species, which differentially influence N and Ca cycling (Dijkstra 2002, Abrams 1998).

Diversity indices (S, H, J) were lower in the fertilized than unfertilized watershed. This pattern is in agreement with the N homogenization hypothesis (Gilliam 2006), stating that, as an ecosystem shifts from N limitation to N saturation, species richness decreases from the displacement of N-efficient species by nitrophilic species. In this study, as in the study of Gilliam

et al. (2016), N-efficient species were displaced by species like *Rubus* spp., altering community composition and decreasing biodiversity. The N homogenization hypothesis also states that the response time of the herbaceous layer to fertilization will depend on ambient N deposition. For example, an environment with low N would react more quickly to additional N than an environment with high N. As sugar maples are known to be associated with higher N availability than red maples (Peterjohn et al. 2015), the vicinity of sugar maples could potentially delay the negative effects of fertilization on understory richness, diversity and evenness. However, since fertilization began almost 30 years ago, the current “sugar maple effect” is likely due to sugar maple buffering against nutrient imbalances (Figure 10).

This study provided additional data to the research by Gilliam et al. (2016) comparing herb layer characteristics in WS4 (similar to WS10/WS13) and fertilized WS3 during the first 25 years of N fertilization. In their study, there was an increase in cover in the fertilized young watershed (WS3) in comparison to the older unfertilized watershed (WS4). In our study, the younger but unfertilized watershed (WS7) also had a higher cover than the older watershed (WS13), indicating that the findings of Gilliam et al. (2016) may also represent an age effect rather than fertilization effect and, thus, highlighting the need for careful selection of reference watersheds in case studies.

#### *Herb Layer Responses to Land Use History (Stand Age): WS7 vs WS13 [H3]*

Herb cover and diversity indices (S, H, J) were consistently greater in the younger watershed (WS7) compared to the older watershed (WS13). The results of this study are in agreement with Bormann and Likens (1979), who noted greater diversity in recently disturbed stands (25-75 years since disturbance) relative to mature stands in the Hubbard Brook Experimental Forest in New Hampshire. Small and McCarthy (2005) found that the herb layer had greater richness and abundance in a 7-year old stand than in a 125-year old stand in Athens County, Ohio. One reason for these pattern is that younger stands tend to have

increased spatial heterogeneity and increased resource availability compared to mature stands (Getzin et al. 2008). Although this study did not detect any differences in light availability by measuring crown openness with a densiometer, WS7 and WS13 may have varied in light quality because of differing crown structure (Peterjohn 2017). However, the two watersheds did vary in soil nutrient resources. Streamwater nitrate and calcium export, reflecting high availability of these ions to plants, were higher in the younger watershed (WS7) than in the older watershed (WS13) (Table 3). Assuming that streamwater chemistry of reference watersheds reflects watershed fertility (as opposed to tree composition, see Peterjohn et al. 2015), and since both watersheds are similar in other characteristics, it is likely that WS7 supported higher cover and herb layer diversity than WS13 due to higher soil nutrient levels as a result of more recent disturbance. In contrast, in fertilized WS3, where excess N (Table 3) could theoretically support the highest herb layer cover (out of all watersheds in the study), this potential for highest cover may have been counterbalanced by detrimental Ca losses due to increased nitrate leaching.

Table 3. Average streamwater nutrient concentrations in the Fernow Experimental Forest over a 30-year period (1983-2015) (from Peterjohn 2017). The linear regression between streamwater nitrate and calcium yielded an  $R^2 = 0.99$  ( $n=4$ ).

<b>Watershed ID</b>	<b>Calcium Export (mg/L)</b>	<b>SD</b>	<b>Nitrate Export (mg/L)</b>	<b>SD</b>	<b>Treatment</b>	<b>Stand Age (yr)</b>
3	2.8	0.21	8.5	1.14	Fertilization	~50
7	2.07	0.14	4.6	0.67	Reference	~50
13	1.76	0.18	1.96	0.63	Reference, North Aspect	~110
10	1.60	0.20	0.83	0.32	Reference, South Aspect	~110

\*SD= standard deviation

#### *Herb Layer Responses to Watershed Aspect: WS13 vs WS10 [H4]*

For the two reference watersheds, there was no main effect of WS (i.e. watershed aspect) explaining herb characteristics. Herb cover was higher under sugar maples than red maples in WS13 and cover under sugar maple in WS13 was higher than cover under either maple species in WS10 (Figure 8I). This difference in cover was in part due to the higher abundance of nitrophilic *Rubus* spp. under sugar maple than under red maple in WS13 or either maple in WS10 (Figure 7). While species richness and diversity of the understory did not differ between watersheds and overstory maple species, evenness was higher beneath sugar maples in WS10 in comparison to red maples with an opposite pattern in WS13. The lack of a watershed effect between the two reference watersheds supports the assumption that FEF watersheds in close proximity are similar enough that, while pseudoreplicated, differences found in comparisons between watersheds (WS3 vs WS7, WS7 vs WS13) are likely due to the treatment (i.e. fertilization and stand age, respectively).

While not a focus of this study, the differences in overall watershed aspect between WS13 and WS10 could possibly explain the differences in relative importance values for sugar maples and red maples found in WS13 and WS10 by Peterjohn et al. (2015). WS13 has a relative importance value for sugar maple of 23% and red maple of 11%. In contrast, WS10 has a relative importance value for sugar maple of 7% and red maple of 22%. The contrasting importance of maple species in these watersheds may be a response to soil moisture availability. Red maples grew better in hotter and drier soil environments and took up more ammonium than sugar maples in a soil warming experiment at Harvard Forest (Butler et al. 2017). South-facing WS10 could be expected to have higher soil temperature and lower soil moisture, than the north-facing WS13, with WS10 thus potentially providing an advantage for red maple.

### *Influence of other environmental factors*

At the plot-level, slope steepness and aspect (WS3 vs WS7 and WS7 vs WS13) or plot aspect alone (WS13 vs WS10) explained significant variability of two or three herb characteristics ( $P \leq 0.1$ , Table 2) in each watershed pair with lower values on steeper slopes and in plots with lower aspect codes. The influence of these abiotic factors (via soil moisture) on vegetation has long been established and are not surprising in the mountainous terrain of the Fernow Experimental Forest.

Distance to stream, tree cover, and DBH of plot center trees also explained variation in some herb characteristics in this study. Increasing distance between plot center and stream decreased species richness and diversity in two of the watershed pairs, potentially due to reduced light availability or lower soil moisture, reducing diversity (Guo et al. 2019). Canopy cover and DBH of the plot-center maple mostly affected evenness. Increasing canopy cover generally increased evenness in watershed pairs WS7 vs WS13 and WS10 vs WS13. Increasing DBH decreased evenness in WS7 and WS13 and WS13 vs WS10. Since these effects were mostly statistical trends, they are not further discussed.

### *Mycorrhizal Associations*

In this study, different mycorrhizal association of neighboring trees affected the younger watershed pair (WS3 and 7) than the older reference watershed pair (WS10 and 13) but with a similar outcome. In the younger watershed pair, an increase of basal area of ECM trees near herb sampling quadrats decreased understory species richness (and cover as a trend); in the older reference watershed pair, the increase of basal area of AM trees increased diversity and evenness. In this study, neighboring trees, growing on average 4 m away from the plot-center maple (range 0.5-11.6 m) could influence the litter quality in the study plots. Trees associated with ectomycorrhizal fungi (ECM) grow more slowly and their litter breaks down more slowly

than that from arbuscular mycorrhizal (AM) trees (Cornelissen et al. 2001). This differing rate in nutrient turnover may affect the herbaceous layer by impacting nutrient access to the understory (Chapman et al. 2005).

## **Summary & Conclusions**

In the watersheds evaluated at the Fernow Experimental Forest, overstory tree species, N deposition level, stand age, and other environmental factors, but not watershed aspect, influenced herb layer characteristics. This study demonstrated 1) a sugar maple effect, i.e. sugar maple having a positive effect on understory cover, at intermediate levels of soil fertility (in reference watersheds) and in an N-fertilized watershed by improving the nutrient microenvironment for herb layer plants, 2) that long-term N enrichment can reduce ecosystem biodiversity by favoring nitrophilic plant species, and 3) that herb layer characteristics can be influenced by stand age, with more recent disturbance being reflected in higher herb layer abundance and diversity. The lack of a “watershed” effect in the comparison of the two older reference watersheds is a strong indicator that differences found between the fertilized and unfertilized watersheds and between the watersheds with younger and older stands are due to the fertilization and stand age rather than site effects.

While lower herb layer cover and diversity indices in older relative to younger stands may be part of the natural successional trajectory in forests recovering from disturbance, lower herb layer cover and diversity in the N-fertilized watershed indicates that anthropogenic activity may have fundamentally altered the overall structure and function of the eastern deciduous forest over the past decades of high atmospheric N deposition. Looking into the future, as maple species are shifting in abundance in the eastern United States (Fei and Steiner 2007), concomitant change in the understory can be expected along with changes in ecosystem function due to feedbacks between diversity and productivity. Given the urgent need to adapt forest management to meet the challenges of climate change and other anthropogenic

influences, this study justifies further examination of tree-herb layer interactions for a wider range of tree species, N-deposition levels and stand ages in future studies.

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## Chapter 2

### Estimating Herb Layer Biomass from Cover Derived by the Hand-Area Method

#### Introduction

Quantifying biomass is fundamental for understanding carbon stocks and biogeochemical cycling in terrestrial ecosystems (Luo et al. 2002). Biomass can reflect the level of productivity in ecosystems and, when assessed at species level, can easily indicate dominant plant species at a site, and thus, which species control water, nutrient, and solar resources (Fassnacht et al. 2014). The amount and distribution of plant biomass defines structure, has implication for processes, and helps maintain stability in northeastern hardwood forests. In forests, efforts have focused on estimating tree biomass and the contribution of various tissues based on measurements of DBH and basal area (Jenkins et al. 2003; Jenkins et al. 2004; Campbell et al. 1985; Martin et al. 1998). This approach has also been employed for estimating forest shrub biomass from basal diameter (Brown 1976). Less attention has been paid to the herbaceous understory due to its small biomass footprint (Gilliam 2014). However, quantifying forest herbs is important because live leaves (i.e., biomass) of typical herb layer species have high concentrations of N, P, K, and Mg and are a major driver of nutrient cycling and nutrient availability in an ecosystem (Freshet et al. 2013).

Estimating herb biomass is common in rangeland settings. There are several “classic” techniques of estimating biomass, all requiring at least some destructive sampling and none practical when the biomass of many individual species is of interest. The harvest method determines the amount of ground vegetation at the time of harvest. This method can separate weight by species and can reflect seasonal and annual fluctuations in climate. Removal of vegetation to measure biomass prevents repeated sampling, limits long-term growth sampling, and with repeated sampling, can negatively impact the community (Ohsowski et al. 2016).

Contemporary techniques for non-destructive biomass estimation include image analyses and frequency methods (Coulloudon et al. 1999; Bollandas et al. 2013). Repeatability is the main advantage in these techniques, but the majority of these methods do not quantify cover or biomass, only presence or absence. Photographs are a simple technique that requires no destructive sampling to adequately portray dominant vegetation. Rottgermann et al. (2000), established linear relationship between cover and biomass for an inland sand dune community via image analysis, which is a computerized extension of the quadrat charting method, but this can only measure the topmost vegetative layer (Dietz and Steinlein 1996).

Quadrat frequency is a very common technique that uses a quadrat sampler, generally a square or circle, that allows the user to quantify absence or presence, cover, density, and frequency of vegetation that is rooted inside the quadrat and. The dry weight rank method is type of quadrat frequency method that determines species composition by observing and ranking the three dominant species. Rank 1 corresponds to 70% composition, rank 2 to 20%, and rank 3 to 10% of species composition of the quadrat. The value recorded for each species is then divided by the total of the weighted column to get a percent composition for each of the species, totaling to 100 percent (Coulloudon et al. 1999).

In contrast, simple, low-tech, yet accurate methods in estimating biomass involve relating non-destructively sampled plant cover to previously established relationships with biomass. However, few such relationships are available in the literature for forest herbs. As an exception, Gilliam and Turrill (1993) developed a universal relationship between cover and biomass for the understory at the Fernow Experimental Forest in the Appalachian Mountains of West Virginia. Expanding on the work of Giliam and Turrill (1993), this study developed linear regressions relating aboveground biomass to cover for a) 42 individual species and b) four functional groups (woody seedlings, herbs, ferns, and shrubs/vines).

## Methods and Materials

This study took place at the Fernow Experimental Forest, located near Parsons, West Virginia. In the summer of 2018, 18 circular 1-m<sup>2</sup> sampling quadrats were placed throughout each of four study watersheds; watershed 3 (WS3) watershed 7 (WS7), watershed 13 (WS13) and watershed 10 (WS10). Watersheds harbor closed-canopy eastern deciduous forests that have arisen from natural regeneration since the last major disturbance (i.e. harvest) 50 to 110 years ago; watersheds differ in fertilization level and some abiotic conditions (Table 1). Species within each sampling quadrat were identified and cover was recorded; subsequently all aboveground biomass was destructively harvested.

Table 1. Characteristics of the watersheds in the study.

<b>Watershed ID</b>	<b>Area (ha)</b>	<b>Elevation (m)</b>	<b>Aspect</b>	<b>Average Slope (Range) %</b>	<b>Stand Age (yr)</b>	<b>Treatment</b>
3	34.3	730-860	S	20.6 (0-60)	~50	Fertilization
7	24.2	730-860	E	25.8 (0-90)	~50	Reference
10	15.2	695-805	S	33.4 (0-70)	~110	Reference
13	14.2	610-830	NE	35.2 (0-100)	~110	Reference

Among all sampling quadrats, there were a total of 39 individual species plus three taxa that could only be identified to the genus level (*Rubus* spp., *Viola* spp., and *Anemone* spp.). Each sampling quadrat contained between one and eleven understory species. All understory species encountered in this study were less than 1 meter in height.

For each species (genus for three taxa), cover was measured as leaf area using the hand-area (HA) method (Walter et al. 2015). In brief, the HA method compares the area of a

hand with the area of the individual leaves of a plant or species. The observer places a hand, palm side down, and fingers closed, directly above the leaves or leaflets of the species they are measuring within the sampling quadrat. The observer then determines the size of the leaf in relation to their hand, either as individual or group, until all leaf or leaflet surfaces are observed within the quadrat. A full hand is equivalent to approximately 1% of 1-m<sup>2</sup> and half a hand is 0.5% assuming the palm area is 1 dm<sup>2</sup> (Figure 1). To improve the accuracy and precision, a) observer hands were adjusted to 1 dm<sup>2</sup> by folding under the thumb and/ or fingertips depending on the actual size of the observer's hand and b) two observers independently recorded cover, and the average of the two estimates was recorded (Walter et al. 2015). After measuring the plant cover in a quadrat, all the herbs inside the quadrat were clipped at ground level, sorted by species (or genus for three taxa, see above), dried at 70°C for 48 hours, and weighed.

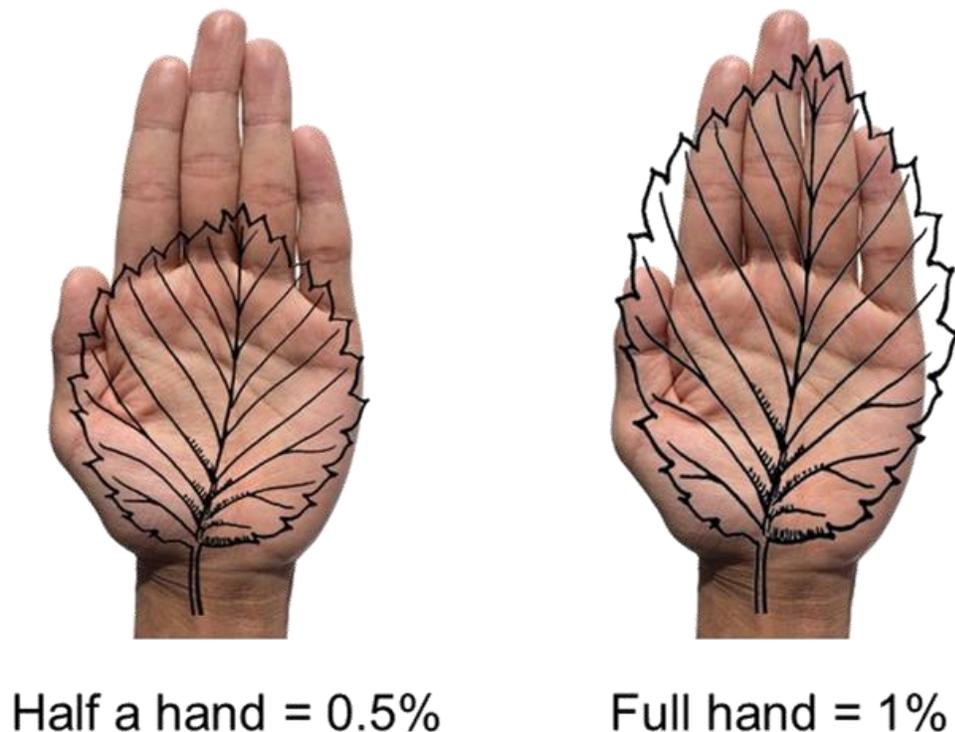


Figure 1. Visual illustration of the hand-area method. Half of a hand is equivalent to 0.5% and a full hand is equivalent to 1% of a 1-m<sup>2</sup> sampling quadrat (assuming the hand area is 1 dm<sup>2</sup>).

## Statistical methods

A relationship of biomass to cover was developed via linear regression for each of the 42 taxa and for species combined into four groups (woody seedling, herb, fern, and shrubs/ vines) (Table 2). Residuals of each species were checked for normality and transformed using natural logarithm on both biomass and cover. Untransformed biomass and cover contained values  $<1$ , therefore unity was added prior to transformation. Statistical analyses were carried out using JMP and SAS software (JMP®, Version Pro 12.2, SAS Institute Inc., Cary, NC, Copyright ©2015; SAS®, Version 9.3, SAS Institute Inc., Cary, NC, Copyright ©2002-2010).

## Results

This study found linear relationships between the natural logarithms of cover and the dependent variable of aboveground biomass (Table 2). Of the 42 taxa collected, 22 were found in more than two sampling quadrats. The coefficient of determination ( $R^2$ ) for these species ranged from 0.49 – 0.90 (Table 2); the  $R^2$ -value for the four most common species ranged from 0.69 – 0.90 (Figure 2). When combining species into the four functional groups, the coefficient of correlation ranged from 0.69-0.78 (Table 2, Figure 3). Comparing regression parameters, the slope of the Fern group was different from the slope of the Woody Seedlings group ( $P = <0.0001$ ), the slope of the Shrubs/vines group was different from Woody Seedlings group ( $P=0.006$ ), and the slope of Herb group was not different from the Woody seedlings group ( $P=0.69$ ). There was no apparent difference in cover-biomass relationships of functional groups between the four watersheds (Figure 3).

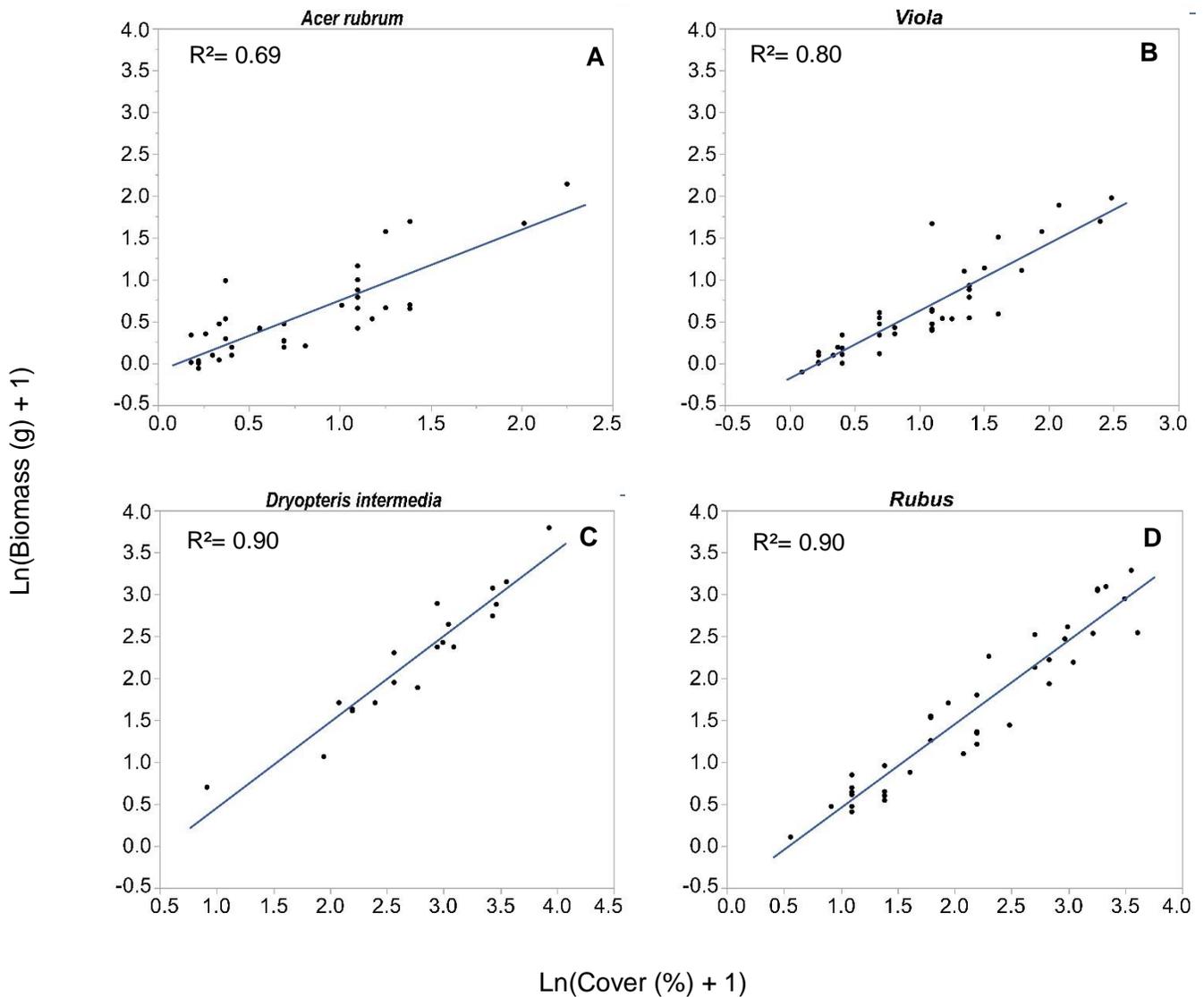


Figure 2: Relationships of log-transformed cover and biomass for the dominant herb species per functional group. A) woody seedlings: *Acer rubrum* (red maple), B) herbs: *Viola* sp. (violets), C) ferns: *Dryopteris intermedia* (intermediate wood fern), D) shrubs/vines: *Rubus* (blackberry).

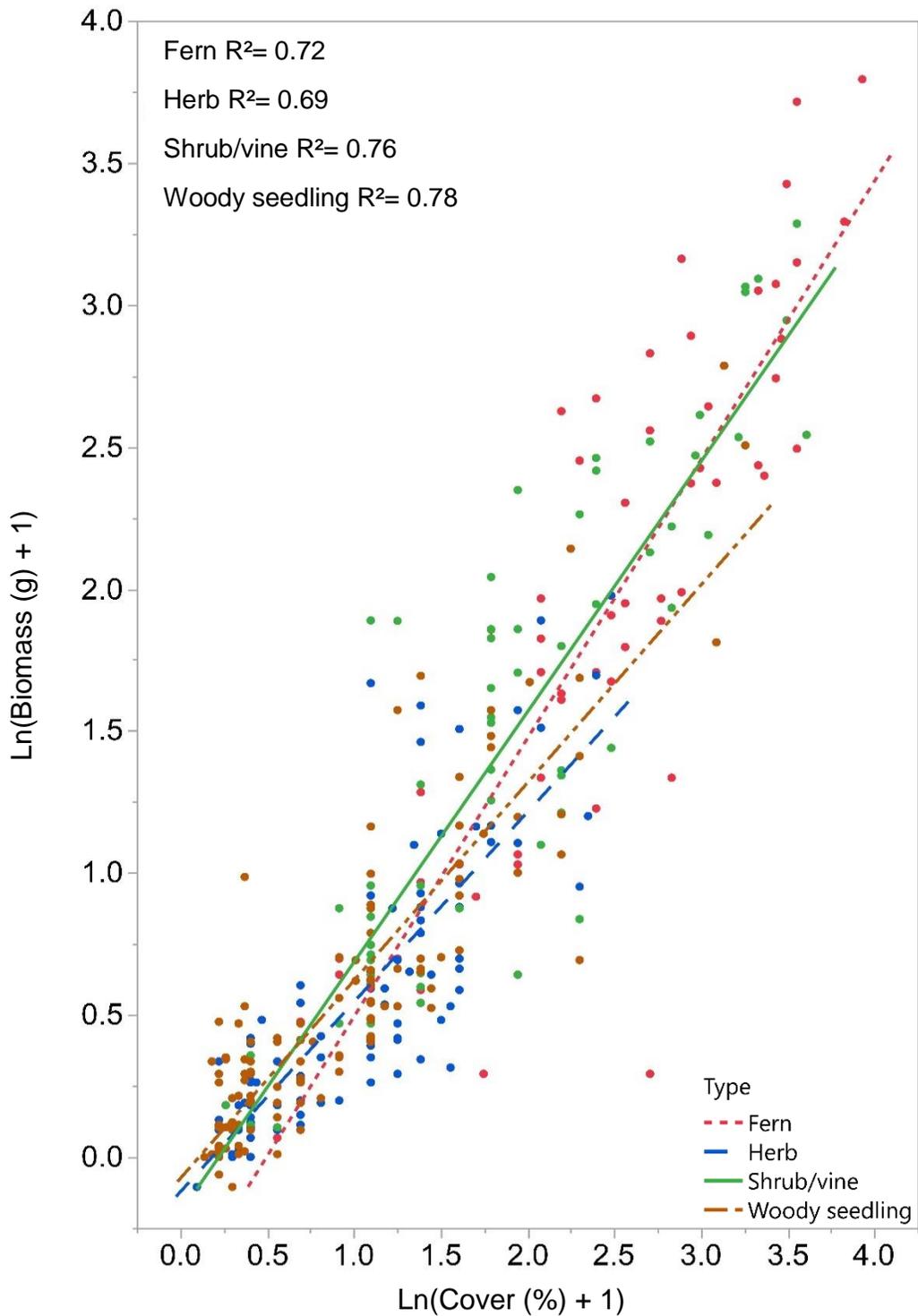


Figure 3. Linear regressions between aboveground biomass and cover (both natural-log transformed) for four functional groups of forest understory plants; woody seedlings, herbs, ferns, and shrubs/vines.

Table 2. Regression components for estimating aboveground biomass of 42 plant species and four functional groups using the linear equation:  $\ln[\text{biomass (g)} + 1] = a + b \ln[\text{cover (\%)} + 1]$ .

Species	<i>n</i>	<i>a</i>	<i>b</i>	<i>R</i> <sup>2</sup>	SD
<b>Woody Seedlings</b>					
ash ( <i>Fraxinus americana</i> )	10	0.11	0.38	0.78	0.17
big leaf holly ( <i>Ilex montana</i> )	2	0.71	0.00	0.00	0.24
birch ( <i>Betula lenta</i> )	2	0.26	0.30	1.00	0.18
bitternut hickory ( <i>Carya cordiformis</i> )	2	-0.10	0.43	1.00	0.27
black cherry ( <i>Prunus serotina</i> )	7	-0.07	0.69	0.78	0.20
cucumber tree ( <i>Magnolia acuminata</i> )	1	0.09	0.00		
Ironwood ( <i>Ostrya virginiana</i> )	1	0.99	0.00		
red maple ( <i>Acer rubrum</i> )	37	-0.09	0.84	0.69	0.53
red oak ( <i>Quercus rubra</i> )	18	-0.17	0.79	0.89	0.73
sassafras ( <i>Sassafras albidum</i> )	2	-0.04	0.20	1	0.06
striped maple ( <i>Acer pensylvanicum</i> )	25	-0.02	0.59	0.77	0.54
sugar maple ( <i>Acer saccharum</i> )	11	-0.14	0.74	0.93	0.45
tulip poplar ( <i>Liriodendron tulipifera</i> )	4	-0.26	0.66	0.97	0.61
white oak ( <i>Quercus alba</i> )	1	0.02	0		
witch hazel ( <i>Hamamelis virginiana</i> )	1	0.11	0		
<b>Combined species</b>	<b>126</b>	<b>-0.07</b>	<b>0.69</b>	<b>0.78</b>	<b>0.25</b>
<b>Herbs</b>					
wintergreen ( <i>Gaultheria procumbens</i> )	1	0.33	0.00		
bedstraw ( <i>Galium lanceolatum</i> )	1	0.48	0.00		0.62
bellwort ( <i>Uvularia sessilifolia</i> )	3	-0.31	0.81	0.98	0.48
chickweed ( <i>Stellaria pubera</i> )	23	-0.19	0.83	0.75	
sweet cicily ( <i>Osmorhiza claytonia</i> )	1	1.10	0.00		0.14
fairybells ( <i>Disporum lanuginosum</i> )	6	0.01	0.24	0.67	0.27
false nettle ( <i>Bohemeria cylindrica</i> )	2	-0.47	0.97	1	0.31
heartleaf aster ( <i>Symphyotrichum cordifolium</i> )	8	0.02	0.50	0.64	0.31
Indian cucumber root ( <i>Medeola virginiana</i> )	7	-0.01	0.38	0.69	0.021
Japanese knotweed ( <i>Reynoutria japonica</i> )	2	0.26	0.03	1	
nettle ( <i>Urtica dioica</i> )	3	-0.18	0.54	0.80	0.25
smooth carrionflower ( <i>Smilax herbacea</i> )	5	-0.19	0.55	0.78	0.19
trillium ( <i>Trillium erectum</i> )	1	0.10	0.00		0.56
violets ( <i>Viola</i> spp.)	41	-0.18	0.80	0.80	
wood anemone ( <i>Anemone</i> spp.)	1	0.92	0.00		0.08
yam ( <i>Dioscorea quaternata</i> )	2	0.31	0.13	1	
<b>Combined species</b>	<b>107</b>	<b>-0.12</b>	<b>0.66</b>	<b>0.69</b>	<b>0.26</b>
<b>Ferns</b>					
Christmas fern ( <i>Polystichum acrostichoides</i> )	14	-0.46	1.16	0.81	1.01
cinnamon fern ( <i>Osmunda cinnamomea</i> )	1	0.69	0.00		
hay-scented fern ( <i>Dennstaedtia punctilobula</i> )	13	-0.53	0.85	0.56	0.81
intermediate fern ( <i>Dryopteris intermedia</i> )	20	-0.57	1.02	0.90	0.74
lady fern ( <i>Athyrium filix-femina</i> )	2	-0.0	0.74	1	1.38
New York fern ( <i>Thelypteris noveboracensis</i> )	7	-0.43	0.78	0.92	0.79
<b>Combined species</b>	<b>57</b>	<b>-0.48</b>	<b>0.97</b>	<b>0.72</b>	<b>0.49</b>
<b>Shrubs and Vines</b>					
greenbrier ( <i>Smilax rotundifolia</i> )	23	0.07	0.79	0.49	0.78
blackberry/raspberry ( <i>Rubus</i> spp.)	38	-0.54	0.99	0.90	0.91
blueberry ( <i>Vaccinium pallidum</i> )	2	-0.96	1.55	1	0.76
<b>Combined species</b>	<b>63</b>	<b>-0.19</b>	<b>0.88</b>	<b>0.76</b>	<b>0.42</b>

SD=standard deviation

## Discussion

The results of this study show that cover can be used to estimate biomass with reasonable accuracy. Results are similar to those of the study by Gilliam and Turrill (1993) showing that biomass could be predicted from understory with the relationship:  $[\text{biomass,g}] = 0.18 [\text{cover,\%}]^{1.29}$  for all species combined with an  $R^2$  of 0.71. For comparison, in this presented study, the power relationship was  $[\text{biomass,g}] = 0.37 [\text{cover,\%}]^{1.12}$  ( $R^2=0.78$ ). The  $R^2$  values for all species combined are similar to the  $R^2$  values the for functional groups (0.69-0.78, Table 2) of this study. Furthermore, Gilliam and Turrill's (1993) equation appeared to be robust across watersheds with different stand ages (WS4 with stand age of 80 years vs WS3 with stand age of 20 years at the time of their study). Visual analysis of the functional groups in the four watersheds in this study (Figure 3) confirmed their results.

Rottgermann et al. (2000) observed that a linear relationship existed between biomass and cover in inland sand dune communities, but with limitations. Herbaceous plants that grew in productive, nutrient rich sites grew larger and deviated from the linear relationship by increasing supportive plant biomass (e.g., stem) without increasing cover. Their overall coefficient of correlation for all nine species combined was 0.84, with  $R^2$  values of individual species ranging from 0.61 to 0.95. The species with a lower  $R^2$  value tended to have more supportive structure and deviated from the linear relationship (Rottgermann et al. 2000).

In this study, greenbrier (*Smilax rotundifolia*) and hay-scented fern (*Dennstaedtia punctilobula*) had relatively low  $R^2$  values (0.5 and 0.56, respectively). Greenbrier samples collected varied widely in cover and weight, depending on the amount of woody stem/vines present. As greenbrier leaves are often absent due to deer browsing, the cover of horizontal stems was also included in cover measurements. While cover estimates for linear structures (stems) are more difficult than for leaves, including stem cover for vines should theoretically improve biomass prediction but needs to be verified. For both hay-scented fern and greenbrier,

no outliers were removed so as to not reduce an already small sample size. This also contributed to the low  $R^2$  values for these two species.

Consistent cover-biomass relationships within functional groups and across environmental conditions indicate that regression equations of functional groups provide a reasonable approach to estimating the biomass of species for which no regression is available. The results of this study also showed that the relationships between cover and biomass differ between functional groups. Therefore, functional groups represent an advantage over just a single relationship for all understory plants lumped together (Brown 1976).

In sum, cover measurements for forest understory, in combination with the linear relationships provided by this study, whether for individual species or functional groups, can be used to estimate biomass in a simple and reasonably accurate manner without the need for time-consuming destructive sampling, expensive equipment, or image analysis. An expanded use of quantifying forest understory would contribute to a better understanding of the role of herb layer plants in important ecosystem processes, such as nutrient cycling, ecosystem productivity, and carbon storage.

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## Appendix

Table A1. Diameter at breast height (DBH) and location of plot-center maple species and plot elevation for each of the 72 plots in this study.

WS	Site	Maple Species	DBH (cm)	Latitude	Longitude	Elevation (m)	Sampling Dates
3	1	red	11.9	39.05563	-79.686827	774.72	6/25/2018
3	2	red	20.3	39.05643	-79.686568	791.00	6/26/2018
3	3	red	20.3	39.05873	-79.687191	821.09	6/27/2018
3	4	red	21.4	39.06014	-79.687446	844.86	6/26/2018
3	5	red	11.1	39.06103	-79.687805	842.89	6/26/2018
3	6	red	12.2	39.06054	-79.68476	829.60	6/26/2018
3	7	red	10	39.05875	-79.68494	801.37	6/25/2018
3	8	red	13.4	39.05805	-79.685348	791.76	6/25/2018
3	9	red	18.2	39.05587	-79.685122	775.77	6/26/2018
3	1	sugar	10	39.05522	-79.68666	777.52	6/26/2018
3	2	sugar	14.2	39.05663	-79.686742	804.70	6/26/2018
3	3	sugar	13.8	39.05845	-79.687258	821.76	6/27/2018
3	4	sugar	19.5	39.06038	-79.687458	829.64	6/26/2018
3	5	sugar	13.4	39.06101	-79.687448	841.69	6/26/2018
3	6	sugar	19.9	39.06024	-79.684704	827.64	6/25/2018
3	7	sugar	20.6	39.05878	-79.685146	801.87	6/25/2018
3	8	sugar	23.4	39.0577	-79.685536	785.58	6/25/2018
3	9	sugar	19	39.0559	-79.685757	795.72	6/26/2018
7	1	red	40.7	39.06351	-79.681365	775.42	6/21/2018
7	2	red	16.5	39.06334	-79.683466	798.83	6/19/2018
7	3	red	10.6	39.06301	-79.683226	794.48	6/21/2018
7	4	red	15	39.06178	-79.683734	805.12	6/21/2018
7	5	red	19	39.06292	-79.685628	823.73	6/21/2018
7	6	red	19.9	39.06538	-79.684067	832.12	6/20/2018
7	7	red	11.8	39.06561	-79.684033	824.98	6/20/2018
7	8	red	20.4	39.06447	-79.68405	814.28	6/20/2018
7	9	red	19.3	39.06447	-79.682418	796.32	6/20/2018
7	1	sugar	17	39.06362	-79.681182	773.40	6/21/2018
7	2	sugar	14.5	39.0633	-79.683691	807.91	6/19/2018
7	3	sugar	33.5	39.06288	-79.683209	791.80	6/21/2018
7	4	sugar	17.5	39.06182	-79.683399	802.26	6/21/2018
7	5	sugar	14.2	39.06288	-79.685665	827.76	6/21/2018
7	6	sugar	13.5	39.06508	-79.684753	823.15	6/20/2018
7	7	sugar	38.8	39.06561	-79.683621	823.70	6/20/2018
7	8	sugar	12.2	39.06422	-79.683568	797.58	6/20/2018
7	9	sugar	14	39.06402	-79.683089	805.40	6/20/2018

Table A1 continued.

WS	Site	Maple Species	DBH (cm)	Latitude	Longitude	Elevation (m)	Sampling Dates
10	1	red	17.6	39.05498	-79.680481	747.99	6/28/2018
10	2	red	17.3	39.05541	-79.680061	725.63	6/28/2018
10	3	red	19	39.05625	-79.680165	773.50	7/2/2018
10	4	red	26.5	39.05681	-79.679763	783.52	7/2/2018
10	5	red	18.6	39.05704	-79.680283	778.22	7/4/2018
10	6	red	35.4	39.05836	-79.678755	791.06	7/4/2018
10	7	red	16.5	39.05775	-79.678014	785.68	7/4/2018
10	8	red	21.2	39.05637	-79.678687	745.19	7/4/2018
10	9	red	14	39.0554	-79.679092	758.87	6/28/2018
10	1	sugar	30.9	39.05518	-79.680156	736.39	6/28/2018
10	2	sugar	14.6	39.05533	-79.679933	741.57	6/28/2018
10	3	sugar	12.7	39.05627	-79.679427	760.42	7/2/2018
10	4	sugar	35.5	39.05688	-79.679087	757.54	7/2/2018
10	5	sugar	20.1	39.05768	-79.679786	787.91	7/4/2018
10	6	sugar	28.1	39.05811	-79.67885	793.11	7/4/2018
10	7	sugar	21	39.0578	-79.678062	790.15	7/4/2018
10	8	sugar	17.3	39.05625	-79.678721	745.43	7/4/2018
10	9	sugar	21.5	39.05556	-79.679299	746.87	6/26/2018
13	1	red	40.6	39.06158	-79.678441	774.05	7/3/2018
13	2	red	21.5	39.06182	-79.679064	767.79	7/3/2018
13	3	red	31.5	39.06153	-79.679415	758.90	7/3/2018
13	4	red	17.5	39.06041	-79.679214	784.02	7/3/2018
13	5	red	21.3	39.05997	-79.682419	818.14	6/28/2018
13	6	red	14.8	39.06079	-79.681116	787.32	6/28/2018
13	7	red	15.4	39.06133	-79.680963	774.13	7/3/2018
13	8	red	27	39.0622	-79.680698	759.31	7/4/2018
13	9	red	21.3	39.06275	-79.680348	774.39	7/4/2018
13	1	sugar	68	39.06181	-79.678745	757.39	7/3/2018
13	2	sugar	14.9	39.06181	-79.678949	759.22	7/3/2018
13	3	sugar	21	39.06191	-79.679603	762.69	7/3/2018
13	4	sugar	17.5	39.06033	-79.679519	769.52	7/3/2018
13	5	sugar	19.9	39.06007	-79.682522	828.61	6/28/2018
13	6	sugar	21	39.06062	-79.681162	796.00	7/3/2018
13	7	sugar	16.9	39.06166	-79.680866	789.30	7/3/2018
13	8	sugar	12.5	39.06206	-79.680811	784.58	7/4/2018
13	9	sugar	8	39.06264	-79.680252	776.96	7/4/2018

Table A2. Basal area (BA) of the five nearest neighbor trees to plot-center maples, separated by arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) association. The distance of the fifth-nearest tree was used to calculate the ground area (ha) by which the tree cross sectional area (m<sup>2</sup>) was divided.

WS	Plot	Plot-center Maple Species	Farthest Tree (meters)	Plot Area (m <sup>2</sup> )	BA (m <sup>2</sup> /ha) of AM Trees	BA (m <sup>2</sup> /ha) of ECM Trees
3	1	red	4.3	58.06	0.61	6.11
3	1	sugar	5.5	94.99	0.45	6.39
3	2	red	3.8	45.34	12.68	5.24
3	2	sugar	4	50.24	17.06	4.78
3	3	red	5.5	94.99	4.33	10.55
3	3	sugar	4.7	69.36	11.96	0.00
3	4	red	6.8	145.19	4.67	0.00
3	4	sugar	8	200.96	2.70	0.86
3	5	red	4.7	69.36	3.25	2.80
3	5	sugar	4.1	52.78	18.80	0.47
3	6	red	11.6	422.52	0.42	0.12
3	6	sugar	5.9	109.30	4.48	0.00
3	7	red	5.2	84.91	10.68	2.13
3	7	sugar	3.6	40.69	7.10	0.89
3	8	red	5.7	102.02	1.50	0.00
3	8	sugar	5.9	109.30	9.05	0.00
3	9	red	5.9	109.30	5.42	4.31
3	9	sugar	4.8	72.35	2.30	0.83
7	1	red	5.4	91.56	0.00	6.19
7	1	sugar	5.4	91.56	15.06	6.02
7	2	red	7.4	171.95	6.30	8.98
7	2	sugar	4.3	58.06	2.11	2.08
7	3	red	4.1	52.78	11.10	11.05
7	3	sugar	5.1	81.67	3.18	1.75
7	4	red	5.2	84.91	7.70	6.51
7	4	sugar	4	50.24	0.98	2.37
7	5	red	5.7	102.02	0.63	1.26
7	5	sugar	6.6	136.78	4.32	0.76
7	6	red	5.4	91.56	0.58	1.54
7	6	sugar	4.8	72.35	7.07	0.00
7	7	red	5.5	94.99	1.78	0.00
7	7	sugar	6.6	136.78	12.55	3.57
7	8	red	5.6	98.47	8.99	6.30
7	8	sugar	2.9	26.41	37.32	0.00
7	9	red	3.2	32.15	23.07	0.00
7	9	sugar	3.5	38.47	11.00	0.00

Table A2 continued.

WS	Plot	Plot-center Maple Species	Farthest Tree (meters)	Plot Area (m <sup>2</sup> )	BA (m <sup>2</sup> /ha) of AM Trees	BA (m <sup>2</sup> /ha) of ECM Trees
10	1	red	6.2	120.70	2.30	0.00
10	1	sugar	6.8	145.19	1.43	0.42
10	2	red	5.2	84.91	9.70	19.10
10	2	sugar	6.7	140.95	0.70	6.44
10	3	red	7.3	167.33	0.92	15.51
10	3	sugar	7.5	176.63	1.97	0.00
10	4	red	7.3	167.33	0.21	0.38
10	4	sugar	5.6	98.47	10.14	0.00
10	5	red	9.9	307.75	0.90	12.62
10	5	sugar	4.8	72.35	2.20	12.70
10	6	red	5.5	94.99	0.63	0.52
10	6	sugar	3.9	47.76	15.56	15.86
10	7	red	11.4	408.07	0.61	0.33
10	7	sugar	4	50.24	7.56	0.55
10	8	red	6.8	145.19	0.75	23.28
10	8	sugar	5.6	98.47	10.77	5.05
10	9	red	5.4	91.56	29.57	24.50
10	9	sugar	6.4	128.61	0.00	2.59
13	1	red	6.8	145.19	1.79	14.66
13	1	sugar	6	113.04	0.00	1.36
13	2	red	5.1	81.67	0.38	25.32
13	2	sugar	5.9	109.30	2.14	0.00
13	3	red	4.5	63.59	18.24	0.45
13	3	sugar	4	50.24	2.44	45.32
13	4	red	5.1	81.67	3.34	0.26
13	4	sugar	7.2	162.78	8.44	35.60
13	5	red	5.2	84.91	54.02	39.79
13	5	sugar	5.8	105.63	3.60	10.40
13	6	red	6.2	120.70	2.05	0.00
13	6	sugar	5.8	105.63	0.80	39.96
13	7	red	4.6	66.44	3.58	46.00
13	7	sugar	6.1	116.84	14.73	0.00
13	8	red	6.8	145.19	1.05	37.90
13	8	sugar	8.3	216.31	5.88	34.16
13	9	red	5.8	105.63	18.17	16.41
13	9	sugar	7.4	171.95	1.52	5.78

Table A3. Total cover (in dm<sup>2</sup> per 36 m<sup>2</sup>) of all understory taxa in this study in each watershed for each overstory maple species (R= red maple, S= sugar maple).

Watershed and plot-center maple									
Understory species		WS3 R	WS3 S	WS7 R	WS7 S	WS10 R	WS10 S	WS13 R	WS13 S
<i>Acer pensylvanicum</i>	Striped maple	36	72	120.5	131.7	41.75	7.35	86.15	42
<i>Acer rubrum</i>	Red maple	11.09	45.55	13.14	32	17.6	19.3	26.8	47.92
<i>Acer saccharinum</i>	Silver maple	4	0	0	0	0	0	0	0
<i>Acer saccharum</i>	Sugar maple	0	0	16.95	1.5	0.5	3.8	1.55	2.87
<i>Actaea racemosa</i>	Black cohosh	0	0	2	0	0	0	0	0
<i>Anemone</i> spp.	Wood thimble	0	0	0	5.15	0	5.5	0	0.5
<i>Arisaema triphyllum</i>	Jack-in-the-pulpit	0.1	0.05	2.5	3.25	1	1	0	0.5
<i>Aruncus dioicus</i>	Goat's beard	0	0	0.75	0	0	0	0	0
<i>Aster divaricatus</i>	Heartleaf aster	0	0	8.25	2.5	8.6	5.6	3.1	2.5
<i>Athyrium filix-femina</i>	Lady fern	4.75	0	2.75	1.05	0	0	0	5
<i>Betula lenta</i>	Sweet birch	12	4.75	0.5	0	3	9.75	0	0
<i>Carya cordiformis</i>	Bitternut hickory	0	0	3	0	0	0	0	0
<i>Castanea dentata</i>	American chestnut	0	0	0	0	0	6.5	0	0.25
<i>Conopholis americana</i>	Squawroot	0	0	0	0	0.5	1	0	0
<i>Cornus alternifolia</i>	Alternate leaf dogwood	0	0	8	4.25	0	0	0	0
<i>Cypripedium acaule</i>	Lady slipper	0	0	2	0	2.25	0	0.45	0
<i>Dennstaedtia punctilobula</i>	Hay-scented fern	155.5	180.5	127	86.1	0	27	0	0
<i>Dioscorea quaternata</i>	Wild yam	1	0	12	7.5	4.85	4.7	2	7.85
<i>Disporum lanuginosum</i>	Fairy bells	0	0.5	20.5	28.75	1.3	1.25	5.1	10.85
<i>Dryopteris intermedia</i>	Intermediate wood fern	170.9	169	228.25	235.3	0	0	49.5	0
<i>Fagus grandifolia</i>	American beech	0	0	0	0	1.3	0	7	0.75

Table A3 continued.

Watershed and plot-center maple									
Understory species		WS3 R	WS3 S	WS7 R	WS7 S	WS10 R	WS10 S	WS13 R	WS13 S
<i>Fraxinus americana</i>	White ash	0	14	1.95	2.25	2.5	41.5	0.9	12.75
<i>Galium asprellum</i>	Bedstraw	0	0	0	0.2	0	0	0	0
<i>Galium lanceolatum</i>	Wild licorice	0	0	0.2	0	0	0.5	0	0
<i>Galium triflorum</i>	Three leaf bedstraw	0	0	0.25	0	0	0	0	0
<i>Goodyera pubescens</i>	Downy rattlesnake plantain	0	0	0	1.75	0	0	0	0.5
Graminoids	Grasses, sedges	0	0	5.7	9.1	0.5	0.2	0.2	1.5
<i>Hamamelis virginiana</i>	Witch hazel	0	2.25	0	0	2.25	4.75	0	0
<i>Ilex Montana</i>	Big leaf holly	1.15	1.25	0	0	0	0	0	0
<i>Kalmia latifolia</i>	Mountain laurel	0	0	0	0	1.15	0	0	0
<i>Laportea canadensis</i>	Nettle	0	3	34.5	60.25	0	3	0	0
<i>Lindera benzoin</i>	Spicebush	1.3	0	0	0	2.5	0	0	0
<i>Liriodendron tulipifera</i>	Tulip poplar	0	0	0	1.5	14.2	14.15	1.3	6.95
<i>Magnolia acuminata</i>	Cucumber tree	13.5	13.25	0	2.5	27.4	53	5.15	15.25
<i>Magnolia fraseri</i>	Umbrella tree	0	0	0	0	0	4	0	1.25
<i>Maianthemum racemosum</i>	False solomon's seal	0	0	0	0	0	0	3	0
<i>Medeola virginiana</i>	Indian cucumber root	8.45	2.5	15.75	4.6	2.85	0.1	1	1.9
<i>Menziesia pilosa</i>	Minniebush	0	0	0	0	1	2.75	0	0
<i>Mitchella repens</i>	Partridge berry	0.15	0	0	0	1	0.75	0	0
<i>Monotropa uniflora</i>	Indian pipe	0	0	0	0	0	0.2	0	0
<i>Nyssa sylvatica</i>	Blackgum	0	0	0	1.5	0	0	0	0
<i>Osmorhiza claytonii</i>	Sweet cicely	0	0	3	1.7	0	0	0	0
<i>Ostrya virginiana</i>	Ironwood	1.37	0	0.5	7	7	4	4.5	16.75

Table A3 continued.

Watershed and plot-center maple									
Understory species		WS3 R	WS3 S	WS7 R	WS7 S	WS10 R	WS10 S	WS13 R	WS13 S
<i>Parthenocissus quinquefolia</i>	Virginia creeper	0	0	1.5	0	0	0	0	0
<i>Polygonatum biflorum</i>	Solomon's seal	0	0	10.25	4.75	0	0	0	2.6
<i>Polygonum cuspidatum</i>	Japanese knotweed	5.5	1	12	2.5	0	0	0	1
<i>Polystichum acrostichoides</i>	Christmas fern	24.25	70.5	99.65	121.5	15.75	79.25	46	74.75
<i>Prunus serotina</i>	Black cherry	3.8	6.5	3.4	7.1	1.05	4.15	4.15	6.9
<i>Quercus alba</i>	White oak	0	0	0	1	3.25	0	0.25	0
<i>Quercus prinus</i>	Chestnut oak	0	0	0	0	2.4	1.5	0	2.75
<i>Quercus rubra</i>	Red oak	7.5	9.25	1.75	4.5	33.95	36.75	75.15	87.9
<i>Rhododendron calendulaceum</i>	Azalea	0	0	0	0	18.25	3.15	0	0
<i>Rosa multiflora</i>	Multiflora rose	0	0	0	29.75	0	0	0	0
<i>Rubus</i> Spp.	Blackberry	436.6	504.25	198.4	212.5	29.9	163.75	106.75	276.25
<i>Sambucus nigra</i>	Elderberry	0	0	5	0	0	0	0	0
<i>Sassafras albidum</i>	Sassafras	0	0	12.5	6.25	0	0.75	0	0
<i>Smilax herbacea</i>	Smooth carrionflower	1.75	0	1.5	0.5	0	0	0	0
<i>Smilax rotundifolia</i>	Greenbrier	103.7	111.25	33.65	122.5	150.8	63.5	31	27.55
<i>Stellaria pubera</i>	Star chickweed	0.75	16.9	83.25	53.1	4.4	3.25	6.25	11.85
<i>Thelypteris noveboracensis</i>	New York fern	52.5	41	80.5	39.5	35.75	62	85.5	68
<i>Trillium</i>	Trillium	0	0	5.35	0	0	0	0	0
<i>Vaccinium pallidum</i>	Blueberry	0	0	0	0	8.75	12.5	0	1.25
<i>Viburnum acerifolium</i>	Maple leaf viburnum	0	0.25	0.25	0	5.85	1.8	0	2.75
<i>Viola</i> Spp.	Violets	11.7	9.05	122.25	80.75	44.55	54.35	48.55	61.5
<i>Unknown</i>	Unknown species	0	0	0	2.25	0	0.67	0.5	0

Table A4. ANOVA results testing the effect of watershed (WS), overstory maple species (M) and their interaction (WS×M) for 22 individual herb-layer species in the three watershed pairs. The Benjamini Hochberg method was applied to account for the familywise error rate. ANOVA P-values smaller than the Benjamini-Hochberg critical value (BH FDR.1) (in bold font) indicate that the discoveries are not a false-positives (based on an assumed false discovery rate (FDR) of 0.1).

WS3 vs WS7							
Species	Effect	NumDF	DenDF	FValue	ProbF	Rank	BH FDR.1
Violets	WS	1	24	32.48	<b>0.0001*</b>	1	0.001818
New York fern	WS×M	1	5	33.74	<b>0.0021*</b>	2	0.003636
Jack-in-the-pulpit	WS	1	5	25.85	<b>0.0038*</b>	3	0.005455
Sweet/Black Birch	WS	1	4	16.62	0.0151	4	0.007273
Fairy Bells	WS	1	11	7.62	0.0185	5	0.009091
Fairy Bells	M	1	11	7.26	0.0209	6	0.010909
Red Maple	M	1	25	5.63	0.0257	7	0.012727
Star chickweed	WS	1	18	5.55	0.03	8	0.014545
Sweet/Black Birch	M	1	4	6.45	0.064	9	0.016364
New York fern	WS	1	5	5.61	0.0641	10	0.018182
Blackberry	WS	1	29	3.6	0.0676	11	0.02
Violets	WS×M	1	24	3.42	0.0769	12	0.021818
White Ash	WS	1	3	5.76	0.0959	13	0.023636
Jack-in-the-pulpit	M	1	5	3.05	0.1411	14	0.025455
Hay-scented fern	M	1	19	2.28	0.1477	15	0.027273
Cucumber tree	WS	1	1	13.03	0.1721	16	0.029091
Indian Cucumber	WS×M	1	8	1.79	0.2172	17	0.030909
Japanese knotweed	M	1	2	3.09	0.2211	18	0.032727
Japanese knotweed	WS	1	2	2.69	0.2424	19	0.034545
Intermediate wood fern	M	1	19	1.38	0.2554	20	0.036364
hay-scented fern	WS	1	19	1.37	0.2559	21	0.038182
Roundleaf greenbrier	WS×M	1	23	1.24	0.2762	22	0.04
New York fern	M	1	5	1.44	0.2843	23	0.041818
Indian Cucumber	M	1	8	1.29	0.2882	24	0.043636
Red Oak	WS×M	1	4	1.15	0.3434	25	0.045455
Intermediate wood fern	WS	1	19	0.93	0.3476	26	0.047273

Table A4 continued.

WS3 vs WS7							
Species	Effect	NumDF	DenDF	FValue	ProbF	Rank	BH FDR.1
Striped Maple	WS	1	21	0.92	0.3486	27	0.049091
Violets	M	1	24	0.8	0.3802	28	0.050909
Black Cherry	M	1	23	0.76	0.3916	29	0.052727
Black Cherry	WS	1	23	0.7	0.4118	30	0.054545
Red Oak	WS	1	4	0.69	0.4537	31	0.056364
Red Maple	WS	1	25	0.55	0.4635	32	0.058182
Cucumber tree	WSxM	1	1	1.18	0.4742	33	0.06
Christmas fern	M	1	12	0.53	0.4819	34	0.061818
Red Maple	WSxM	1	25	0.46	0.5044	35	0.063636
Wild Yam	WS	1	6	0.48	0.5162	36	0.065455
Sugar Maple	M	1	5	0.4	0.5526	37	0.067273
Indian Cucumber	WS	1	8	0.38	0.5546	38	0.069091
roundleaf greenbrier	M	1	23	0.26	0.6123	39	0.070909
Red Oak	M	1	4	0.22	0.6623	40	0.072727
Star chickweed	M	1	18	0.15	0.706	41	0.074545
Black Cherry	WSxM	1	23	0.13	0.7218	42	0.076364
Roundleaf greenbrier	WS	1	23	0.1	0.7535	43	0.078182
Cucumber tree	M	1	1	0.12	0.7838	44	0.08
Striped Maple	M	1	21	0.07	0.7945	45	0.081818
Intermediate wood fern	WSxM	1	19	0.06	0.8025	46	0.083636
Japanese knotweed	WSxM	1	2	0.05	0.8472	47	0.085455
Christmas fern	WSxM	1	12	0.04	0.8498	48	0.087273
White Ash	M	1	3	0.03	0.8703	49	0.089091
Blackberry	M	1	29	0.02	0.8834	50	0.090909
Wild Yam	M	1	6	0.01	0.9153	51	0.092727
Blackberry	WSxM	1	29	0.01	0.9359	52	0.094545
Hay-scented fern	WSxM	1	19	0	0.9506	53	0.096364
Christmas fern	WS	1	12	0	0.9676	54	0.098182
Striped Maple	WSxM	1	21	0	0.9935	55	0.1

Table A4 continued.

WS7 vs WS13							
Species	Effect	NumDF	DenDF	FValue	ProbF	rank	BH FDR.1
Red Maple	M	1	27	16.73	<b>0.0003*</b>	1	0.001724
Fairy Bells	WS	1	20	8.7	0.0079	2	0.003448
Star chickweed	WS	1	19	8.52	0.0088	3	0.005172
New York fern	WSxM	1	6	13.29	0.0108	4	0.006897
Fairy Bells	M	1	20	6.94	0.0159	5	0.008621
Jack-in-the-pulpit	WS	1	5	8.27	0.0347	6	0.010345
Red Oak	WS	1	10	5.77	0.0372	7	0.012069
Violets	WS	1	29	2.85	0.1023	8	0.013793
Blackberry	WSxM	1	20	2.53	0.1271	9	0.015517
New York fern	WS	1	6	3.04	0.1319	10	0.017241
Blackberry	M	1	20	2.36	0.1405	11	0.018966
Hay-scented fern	M	1	11	2.44	0.1469	12	0.02069
Jack-in-the-pulpit	M	1	5	2.87	0.1512	13	0.022414
Blackberry	WS	1	20	1.73	0.2029	14	0.024138
Cucumber tree	WS	1	3	2.54	0.2091	15	0.025862
Tulip/Yellow Poplar	M	1	2	3.25	0.213	16	0.027586
Indian Cucumber	WSxM	1	5	1.92	0.2242	17	0.02931
Indian Cucumber	WS	1	5	1.78	0.2392	18	0.031034
Violets	WSxM	1	29	1.26	0.2707	19	0.032759
Wild Yam	WS	1	10	1.26	0.2885	20	0.034483
Roundleaf greenbrier	WS	1	17	0.97	0.3375	21	0.036207
Sugar Maple	WSxM	1	9	0.93	0.3598	22	0.037931
Striped Maple	WS	1	24	0.83	0.3717	23	0.039655
Sugar Maple	WS	1	9	0.84	0.3827	24	0.041379
Tulip/Yellow Poplar	WS	1	2	1.04	0.4157	25	0.043103
White Ash	M	1	7	0.55	0.4818	26	0.044828
Roundleaf greenbrier	WSxM	1	17	0.51	0.4842	27	0.046552
Japanese knotweed	WS	1	1	0.86	0.5247	28	0.048276
Intermediate wood fern	WSxM	1	9	0.43	0.5304	29	0.05
Christmas fern	WS	1	14	0.4	0.5372	30	0.051724
Japanese knotweed	M	1	1	0.78	0.5394	31	0.053448
Roundleaf greenbrier	M	1	17	0.39	0.5429	32	0.055172
Christmas fern	M	1	14	0.39	0.5444	33	0.056897
Indian Cucumber	M	1	5	0.37	0.5718	34	0.058621
Striped Maple	WSxM	1	24	0.31	0.5822	35	0.060345
New York fern	M	1	6	0.33	0.5851	36	0.062069
Cucumber tree	M	1	3	0.32	0.6094	37	0.063793

Table A4 continued.

WS7 vs WS13							
Species	Effect	NumDF	DenDF	FValue	ProbF	rank	BH FDR.1
White Ash	WSxM	1	7	0.28	0.6137	38	0.065517
Red Oak	WSxM	1	10	0.27	0.6177	39	0.067241
Black Cherry	WSxM	1	16	0.21	0.651	40	0.068966
Fairy Bells	WSxM	1	20	0.2	0.657	41	0.07069
Star chickweed	WSxM	1	19	0.17	0.6843	42	0.072414
Intermediate wood fern	WS	1	9	0.1	0.7543	43	0.074138
Striped Maple	M	1	24	0.07	0.8008	44	0.075862
Wild Yam	M	1	10	0.06	0.817	45	0.077586
Red Maple	WSxM	1	27	0.05	0.8278	46	0.07931
Intermediate wood fern	M	1	9	0.04	0.8504	47	0.081034
Black Cherry	WS	1	16	0.04	0.8516	48	0.082759
White Ash	WS	1	7	0.03	0.8768	49	0.084483
Red Oak	M	1	10	0.02	0.8943	50	0.086207
Wild Yam	WSxM	1	10	0.01	0.9136	51	0.087931
Black Cherry	M	1	16	0.01	0.9179	52	0.089655
Violets	M	1	29	0.01	0.927	53	0.091379
Christmas fern	WSxM	1	14	0	0.9452	54	0.093103
Cucumber tree	WSxM	1	3	0	0.9512	55	0.094828
Sugar Maple	M	1	9	0	0.981	56	0.096552
Red Maple	WS	1	27	0	0.9841	57	0.098276
Star chickweed	M	1	19	0	0.9979	58	0.1
WS10 vs WS13							
Red Maple	M	1	30	16.28	<b>0.0003*</b>	1	0.001852
Red Oak	WS	1	20	5.57	0.0285	2	0.003704
Tulip/Yellow Poplar	WSxM	1	8	4.45	0.0678	3	0.005556
New York fern	WSxM	1	6	3.26	0.1211	4	0.007407
Red Maple	WS	1	30	2.44	0.1284	5	0.009259
New York fern	M	1	6	2.87	0.1412	6	0.011111
Striped Maple	M	1	18	2.33	0.1444	7	0.012963
Roundleaf greenbrier	M	1	19	2.23	0.1518	8	0.014815
White Ash	M	1	8	2.5	0.1522	9	0.016667
Sugar Maple	WSxM	1	8	2.44	0.1569	10	0.018519
Striped Maple	WS	1	18	1.97	0.1779	11	0.02037
Roundleaf greenbrier	WSxM	1	19	1.93	0.1812	12	0.022222
Indian Cucumber	WSxM	1	3	2.43	0.2166	13	0.024074
Jack-in-the-pulpit	WS	1	2	3.01	0.225	14	0.025926
Fairy Bells	WS	1	11	1.54	0.2401	15	0.027778

Table A4 continued.

WS10 vs WS13							
Species	Effect	NumDF	DenDF	FValue	ProbF	rank	BH FDR.1
Cucumber tree	WSxM	1	9	1.41	0.2647	16	0.02963
Sugar Maple	WS	1	8	1.41	0.2695	17	0.031481
Blackberry	WSxM	1	11	1.16	0.3042	18	0.033333
Blackberry	WS	1	11	1.1	0.317	19	0.035185
Red Maple	WSxM	1	30	1.01	0.3229	20	0.037037
Christmas fern	M	1	10	0.97	0.3477	21	0.038889
Fairy Bells	WSxM	1	11	0.94	0.3535	22	0.040741
Blackberry	M	1	11	0.9	0.362	23	0.042593
Indian Cucumber	M	1	3	0.85	0.4255	24	0.044444
Jack-in-the-pulpit	M	1	2	0.97	0.4284	25	0.046296
Sweet/Black Birch	M	1	1	1.47	0.4392	26	0.048148
Red Oak	M	1	20	0.61	0.4434	27	0.05
Violets	WS	1	24	0.58	0.4532	28	0.051852
Striped Maple	WSxM	1	18	0.56	0.4646	29	0.053704
Roundleaf greenbrier	WS	1	19	0.55	0.4685	30	0.055556
White Ash	WS	1	8	0.58	0.4687	31	0.057407
Cucumber tree	M	1	9	0.55	0.4776	32	0.059259
Tulip/Yellow Poplar	M	1	8	0.52	0.492	33	0.061111
Black Cherry	WSxM	1	10	0.42	0.5307	34	0.062963
Violets	WSxM	1	24	0.39	0.5401	35	0.064815
White Ash	WSxM	1	8	0.3	0.5965	36	0.066667
Star chickweed	M	1	9	0.29	0.6058	37	0.068519
Black Cherry	WS	1	10	0.15	0.7081	38	0.07037
Christmas fern	WSxM	1	10	0.15	0.709	39	0.072222
Violets	M	1	24	0.1	0.7515	40	0.074074
Sugar Maple	M	1	8	0.11	0.7528	41	0.075926
Christmas fern	WS	1	10	0.1	0.7574	42	0.077778
Black Cherry	M	1	10	0.07	0.7967	43	0.07963
Fairy Bells	M	1	11	0.06	0.8099	44	0.081481
New York fern	WS	1	6	0.06	0.8211	45	0.083333
Tulip/Yellow Poplar	WS	1	8	0.05	0.8371	46	0.085185
Wild Yam	WS	1	8	0.04	0.8472	47	0.087037
Wild Yam	WSxM	1	8	0.04	0.8488	48	0.088889
Star chickweed	WSxM	1	9	0.03	0.8579	49	0.090741
Red Oak	WSxM	1	20	0.02	0.8782	50	0.092593
Wild Yam	M	1	8	0.01	0.9164	51	0.094444
Star chickweed	WS	1	9	0.01	0.9352	52	0.096296
Indian Cucumber	WS	1	3	0	0.98	53	0.098148
Cucumber tree	WS	1	9	0	0.9936	54	0.1

Table A5. Distances between plot center maples (red and sugar maple) within a plot pair (site) and distance between a member of a plot pair to the closest member of a neighboring plot pair. Site 1 is closest to the weir to the left of the stream when facing upstream; numbering proceeds clockwise with Site 9 being closest to the weir on the right side of the stream when facing upstream (See Figure A1).

Watershed	Site	Within Site Distance (m)	Between Site distance (m)
3	1	47.5	94
3	2	18.7	94
3	3	30.7	154.4
3	4	22.9	69
3	5	30.9	69
3	6	34	169.8
3	7	17.8	89
3	8	40.4	89
3	9	56	98.9
<b>Average</b>			<b>103.01</b>
7	1	19.3	141.8
7	2	20.8	56.7
7	3	15.7	56.7
7	4	29.3	109.3
7	5	6.2	174.8
7	6	68.1	37.3
7	7	24.9	37.3
7	8	50.8	46.7
7	9	76.3	46.7
<b>Average</b>		<b>34.6</b>	<b>78.6</b>
13	1	36.85	17.9
13	2	10.9	17.9
13	3	45.7	43
13	4	16	119.1
13	5	14	131
13	6	19.4	61.2
13	7	37.4	61.2
13	8	19.7	60.6
13	9	15.6	61
<b>Average</b>		<b>23.95</b>	<b>63.7</b>
10	1	39.2	28.6
10	2	14.4	28.6
10	3	63.8	63
10	4	60.2	58.5
10	5	83.1	58.5
10	6	26.2	74.1
10	7	6.6	74.1
10	8	13.2	65.8
10	9	37.5	53.7
<b>Average</b>		<b>38.2</b>	<b>56.1</b>

Table A6. P-values for individual pairwise comparisons of herb characteristics beneath a given overstory maple species between two watersheds and the comparison of herb characteristics beneath the two overstory maple species within a watershed (SAS AVOVA Effect Slices of the watershed (WS) and overstory maple species (M) interaction).

<b>WS3 vs WS7</b>						
<b>Effect</b>	<b>Overstory Maple Species</b>	<b>Watershed (WS)</b>	<b>Total Cover (%)</b>	<b>Species Richness (S)</b>	<b>Diversity (H)</b>	<b>Evenness (J)</b>
<b>WS x M</b>	Red maple		0.01	<.0001	<.0001	0.08
<b>WS x M</b>	Sugar maple		0.91	0.0073	0.01	0.13
<b>WS x M</b>		3	0.32	0.03	0.04	0.49
<b>WS x M</b>		7	0.11	0.04	0.72	0.67
<b>WS7 vs WS13</b>						
<b>WS x M</b>	Red maple		0.0001	0.008	0.03	0.53
<b>WS x M</b>	Sugar maple		0.17	0.83	0.06	0.01
<b>WS x M</b>		13	0.03	0.05	0.86	0.13
<b>WS x M</b>		7	0.27	0.39	0.95	0.51
<b>WS13 vs WS10</b>						
<b>WS x M</b>	Red maple		0.77	0.11	0.59	0.69
<b>WS x M</b>	Sugar maple		0.05	0.68	0.18	0.02
<b>WS x M</b>		10	0.64	0.96	0.27	0.04
<b>WS x M</b>		13	0.02	0.04	0.76	0.23

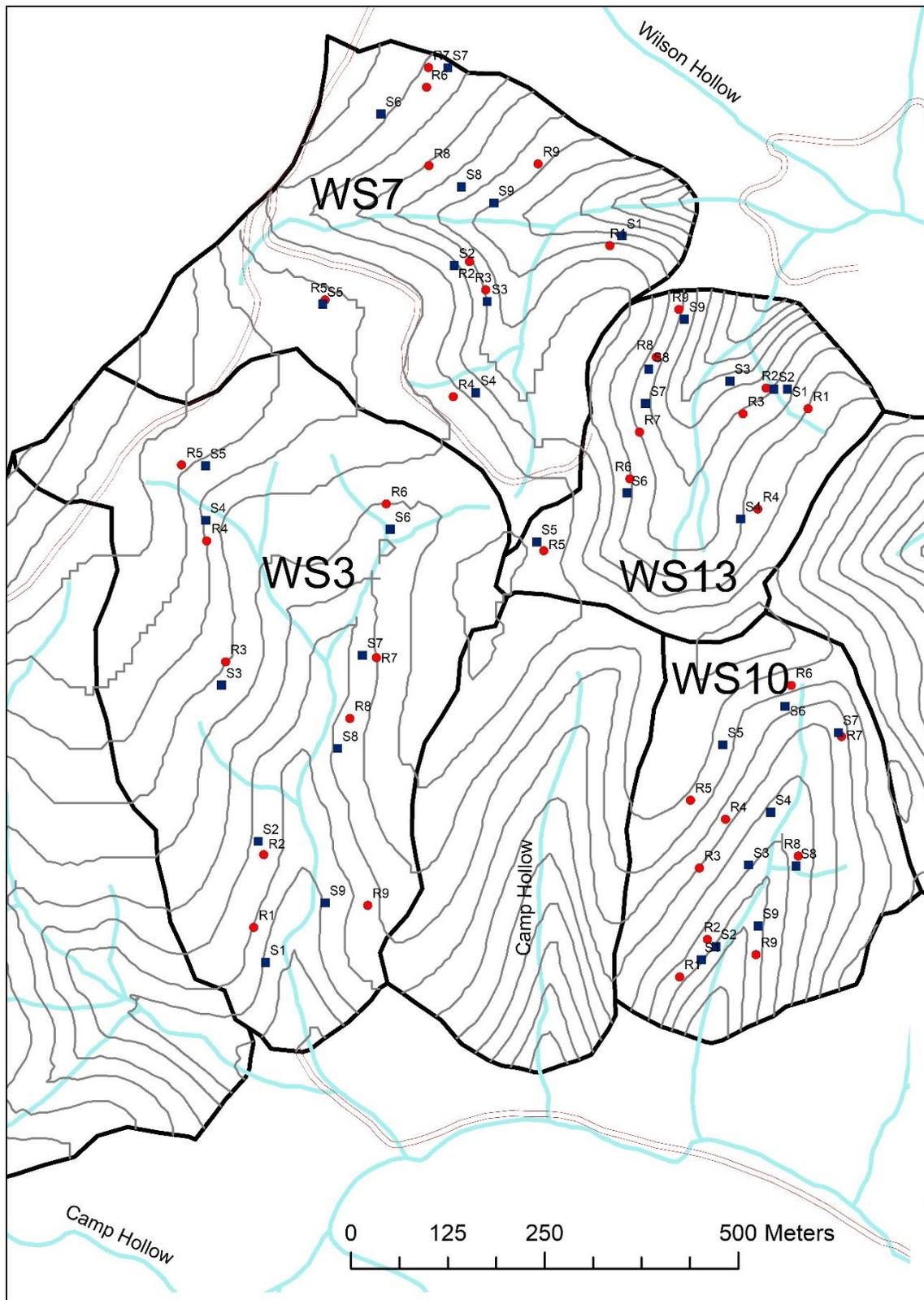


Figure A1. Location of sites 1-9 within each watershed. Plot center trees are marked with a square for sugar maple and a circle for red maple.