TREE-MEDIATED WATER-NUTRIENT FLUXES FROM THE MICROBIAL TO REGIONAL SCALE: INSIGHTS FROM MIXED-DECIDUOUS FORESTS IN THE NORTHEASTERN UNITED STATES

by

Janice E. Hudson

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Geography

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Most people consider trees in passing. Trees provide necessary ecosystem services, chief among them are the production of oxygen and sequestration of carbon dioxide through photosynthesis, the creation of stable and fertile soils, and reduction of storm run-off. Additionally, humans utilize tree biomass as fuel, consumable products, building material, and foodstuffs. However, trees are rarely thought of as agents of biogeochemical influence and change. In the Northeast region of the United States, knowledge of the ways in which trees influence and change watersheds are key, as the surface waters held within these watersheds are essential for human activities and often the primary source of drinking water. Not only do trees experience changes in their environment at different temporal scales (e.g., individual storm events, periodic disturbances, seasons), they also create a changing environment for chemical and microbial processes within those conditions. Further, trees not only exist in but are themselves, a habitat. This dissertation explores some ways in which trees can influence a watershed.

The decomposition of broadleaved tree leaves can contribute a substantial amount of energy to forested watersheds via dissolved organic matter, nutrients, and biological activity. Less is known about how these inputs may vary within a single tree species that is known to have two genetically distinct and geographically separate populations, or how these inputs may change throughout autumn senescence and abscission. We analyzed the morphological and chemical leaf traits, and leachates
from *Fagus grandifolia* (American beech) leaves during three phenophases: fresh green leaves, senescing leaves, and fallen leaves collected from four sites along a geographic transect stretching from Vermont to North Carolina (over 1400 km). Leachates were analyzed for routine solutes and nutrients, as well as fluorescent and UV-visible absorbance indices. Results suggest significant differences in leached nutrients among sites and optical properties and nutrients among phenophases. These results also suggest that geographically (or genetically) separate populations of the same species do not experience senescence in the same way and that implicit assumptions of intraspecific uniformity of leaf-litter leachate chemistry for a given tree species may be invalid.

Precipitation incident on a forest canopy is partitioned into throughfall, stemflow, and interception loss. Throughfall is the dominant subcanopy water flux and is responsible for numerous nutrient cycles between the atmosphere, canopy, and soil. Events impacting the eastern United States (Maryland, Rhode Island, and Vermont) were sampled, and throughfall chemistry was explored for each site in the lens of large-scale storm events. Season was found to be the strongest driver of base cation flux differences among the study sites. Additionally, strong regional deposition played a key role in the discrimination of throughfall chemistry in the region, overriding the influence of the synoptic scale storm events.

The second subcanopy water flux, stemflow, differs from throughfall in nutrient enrichment and residence time. Stemflow coupled with bark texture and hypothesized bark microclimates could provide a refuge for bark surface bacteria. Additionally, it is expected that microbial communities will differ along an urban to rural gradient. This study found that there were indeed multiple phyla of microbial life
on the bark surface, and while their biological functions are not understood at this
time, there is a difference in composition and location along the tree bole between the
urban and rural sites. Stemflow also acts to translocate microbial biomass during
precipitation events creating sources and sinks of microbial diversity on the bark.
1.1 Relevance of Proposed Work

Wooded ecosystems occupy over 30% of the earth’s ice-free land mass (Hooke, Martin-Duque, & Pedraza, 2012) and provide invaluable services to humans and other wildlife. Over 23% of the Earth’s forests are classified as temperate forest (Ohte & Tokuchi, 2011). Temperate forests are widespread, and vegetation within them is variable in both species and density, although they typically consist of some mix of broadleaved deciduous and evergreen canopy species. These forests also host large numbers of native plant species, many of which have exclusively dependent relationships with each other, as well as with native fauna and microorganisms (Bonan, 2008). In addition to immeasurable biodiversity, forests are also responsible for the improvement of water and soil quality, and the sequestration of 1-3 gigatons of carbon each year (Dixon et al., 1994; FAO, 2012; Houghton, 2003). Vegetation influences chemical and microbial processes as they relate to water, especially how vegetation structure and function affect chemistry and hydrology. These interactions are complicated. Not only do trees experience changes in their environment at different temporal scales (e.g., individual storm events, periodic disturbances, seasons), they also create a changing environment for chemical and microbial processes with those conditions. Trees specifically exist in and are themselves, a habitat. The tree, and specifically the bark of a tree (also known as the cortisphere), provides a microenvironment in which the turbulence of air, moisture, chemistry, and...
pH will all be altered from the larger environment due to bark texture (which is itself a product of tree species and age) and aspect. Additionally, similar forests at differing latitudes or elevations are also expected to be different in their microclimates.

Figure 1.1: Urban areas create an array of differences in the ecological climatology of forest fragments located within them.

Similarly, urban areas create an array of additional differences in the ecological climatology of the forest fragments located within them (Figure 1.1). These differences include but are not limited to: altered air conditions, increased temperatures, altered precipitation patterns, increased non-point pollution and particulate deposition, alteration of nutrient inputs to soil and water, and altered biodiversity, including the addition of non-native species, whether intentional or not (Bonan, 2008). Although spatially less extensive than temperate forests (less than 3%
of land area (Earth Institute Columbia University, 2018)), urban regions are home to more than half of the world’s population (World Health Organization, 2018). In the United States, nearly 80% of people live in urban areas, and there are more than 70 million acres of urban forest ecosystems cared for by the U.S. Forest Service (United States Department of Agriculture & U.S. Forest Service, 2018). Consequently, urban regions and their forests, which have over the last few decades become the subject of much in-depth study, will continue to experience significant alterations to their hydrological and biogeochemical cycles worldwide (Bonan, 2011).

1.2 Rainfall and Nutrient Partitioning

During a rain event, approximately 70-90% of incident precipitation that is intercepted by a forest canopy reaches the ground, depending on forest type, season and meteorological factors (Figure 1.2; Levia & Frost, 2003). Of that, approximately 90% often falls as throughfall, precipitation either passing directly through the canopy or coalescing and releasing from leaves and woody surfaces (Levia & Frost, 2006; Levia et al., 2011). Throughfall often has a short to medium residence time in the canopy and is highly variable in time, space, and chemistry (Levia & Frost, 2006). Throughfall has been shown to be enriched in foliar leachable nutrients relative to incident precipitation and gross dry deposition (Staelens et al., 2007, Lovett and Lindberg 1984), and has an influence on fine root development (Ford and Deans 1978). The other approximately <10% of intercepted precipitation, per unit canopy area, is funneled over the bark surface, down stems and branches, as stemflow. Although it is typically less than 10% of intercepted precipitation, and sometimes overlooked, stemflow represents a highly enriched, point-source input of water and nutrients at the base of trees in a forested ecosystem due to its long residence time on
foliar and woody surfaces (Carlyle-Moses et al., 2018, Johnson & Lehmann, 2006; Levia & Herwitz, 2005; Levia et al., 2012; Levia & Herwitz, 2002; Levia & Herwitz, 2000; Llorens & Domingo, 2007). Stemflow is often high in particulate organic matter and dissolved organic carbon (DOC) has been shown to be enriched in stemflow up to 2372% relative to incident precipitation (Johnson and Lehmann, 2006). The remaining intercepted water never reaches the ground and may be stored on foliar and woody surfaces, sometimes up to 1.3 mm (Zinke, 1967). This water will evaporate as interception loss, and timing will depend on the meteorological conditions and tree species (Carlyle-Moses & Gash, 2011). Sub-canopy water fluxes are important energy and nutrient pathways in forests and urban forests. Throughfall, which is largely a diffuse input but can be concentrated along drip points from leaves and branches, can affect biogeochemical processes in the litter layer and soil on a broader spatial scale than stemflow. Unfortunately, our understanding of elemental fluxes in stemflow in urban forests is still limited, and comparison of urban to rural forests is often complicated by geographic factors like differing soils (Michopoulos, 2011). Nonetheless, the long residence time of water on bark, both as stemflow and water storage, not only allows for the chemical enrichment of stemflow but also provides the medium for the existence of bacteria.
Figure 1.2: Subcanopy water fluxes are important energy and nutrient pathways in forests and urban forests. The foci of this research are a few of the multiple ways in which these water fluxes may be influenced by a tree including: 1. greenfall and senescence (Chapter 3); 2. throughfall (Chapter 4); and 3. stemflow (Chapter 5).
Broadleaved trees are a major source of nutrient supply to streams (Bernhardt and Likens, 2002; Cory and Kaplan, 2012). Both throughfall (precipitation falling through the canopy) and stemflow (precipitation funneled down branches and stems to the bole) transfer nutrients and water to the base of the plant creating a “fertile island” in soils surrounding the trunk (Kuzyakov & Blagodatskaya, 2015; Levia & Frost, 2003; Levia, Keim, Carlyle-Moses, & Frost, 2011; Whitford, Anderson, & Rice, 1997). During autumn senescence, nutrients from decomposing leaves are mobilized via leaching and various other biological and physical mechanisms. Fisher and Likens (1973) found that leaf decomposition accounted for 29% of the annual energy budget of a second-order New England stream. Within days of submersion in a stream, up to 40% of the dissolved organic matter (DOM) of a leaf can be leached (McDowell and Fisher, 1976; Cuss and Gueguen, 2013).

The DOM of leaf-litter leachate changes dramatically during autumn as tree leaves experience senescence, nutrient resorption (the translocation of nutrients from leaves to other tissues during senescence), and abscission. At northern latitudes, photoperiod is the ultimate determinant for the onset of senescence, while seasonal changes in air temperature become more important at lower latitudes (Estiarte and Penuelas, 2015). The main nutrients resorbed are nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), because they are mobile in the phloem, unlike calcium (Ca), which is accreted (Killingbeck, 2004). Even after abscission, DOM of leaf-litter leachate may be affected by temperature changes (Bärlocher, 2005). Nutrient resorption efficiency and proficiency differ among tree species, resulting in interspecific variation in leaf-litter leachate DOM (Killingbeck, 1996; 2004).
Recent studies have also shown that leaf-litter leachates from differing species can have a complex interaction with microbial populations in soil and stream water, especially with regards to nutrient cycling and bioavailability (Wymore et al., 2015; Joly et al., 2016). Depending on forest composition, these dissimilarities in leachate quality can have a substantial impact on soil and stream ecosystems through alterations of available DOM, nitrogen and other nutrients as well as activity and respiration of microbial communities (Bernhardt and Likens, 2002; Cory and Kaplan, 2012; Wymore et al., 2015). Even though it plays an important role in energy and biogeochemical cycling in forests, especially in autumn, less is known about how the quality of leaf-litter leachate may differ throughout autumn as leaves senesce and abscise or how this differs intraspecifically between trees at different geographical and latitudinal locations. Furthermore, autumnal phenology is a critical stage in the success of plant function, propagation, and morphology, especially for determinant growth species such as *Fagus grandifolia* Ehrh. (American beech). And, while long-term climate data begin to indicate changes in the autumnal season timing and length, there remains little agreement on the effect of climate change on plant phenology.

One average deciduous tree whose trunk is 30 centimeters in diameter may have nearly 97 m² of stem and branch bark surface area (Levia & Herwitz, 2005; Whittaker & Woodwell, 1967) and it would take the bark surface of only 55 trees of this size to cover the area of an entire American football field. The basal area of a tree (in this same case, only .61 square feet) is important in the calculation of two ecohydrological contributions of stemflow to a watershed: the *amount* of water delivered to the base of the tree (funneling ratio) and *enrichment* of water delivered to the base of the tree (enrichment ratio).
Leaf area index, a measure of projected canopy surface area per unit ground area, ranges between 2-5 at the Fair Hill Natural Resource Management Area (NRMA). This translates to 2-5 times more surface area is available to intercept and enrich incident precipitation as it moves through the canopy. Additionally, this surface area can scavenge particulate matter and transmit it to the subcanopy via throughfall and leaf fall. Further, this foliar surface area, upon abscission or detachment as green fall due to periodic disturbance can end up in the stream as fodder for primary producers and provide yet another way in which trees may influence downstream water quality, chemistry, and more.

While rainfall and nutrient partitioning studies have gained significant momentum in temperate regions over the past decade, there are still substantial gaps in our understanding of these processes (Lambais et al., 2014; Levia & Germer, 2015; Ohte & Tokuchi, 2011). One of these gaps pertains to the communities of microorganisms living on and within the bark surfaces of plants. In a recent review article, Levia & Germer (2015) highlighted questions that have yet to be addressed in the field of ecohydrology regarding microbial communities. These questions, with relevance to the bark surface, are how species composition of microorganisms varies by ecoregion and phenoseason, how these communities effect stemflow chemistry, and how bark characteristics that are unique to individual woody plant species might impact the composition of microorganisms present on the bark. In our attempt to address these questions, we will examine how communities of cortisphere microorganisms affect the chemistry of water routed through canopies of native Quercus spp., and how these microbial communities vary with land use. The same review of stemflow literature also highlights the paucity of knowledge available on
these microorganism’s interactions with stemflow and nutrient cycling, especially at the plant-soil interface (Levia & Germer, 2015).

Imagine this same tree-surface area from above multiplied by the number of hectares of forest landcover in the United States, or the world. This surface is the same amount of surface area on which corticular (bark surface) microbial communities may colonize and contribute to the chemical alteration of water as it passes along bark surfaces. Even the most detailed of urban forest research initiatives overlook microbial biodiversity. Scientists estimate that we know less than 10% of microbial species across all environments (Haegeman, Moriarty, Neal, Dushoff, & Weitz, 2013; Pace, 1997; Selama, James, Nateche, Wellington, & Hacène, 2013)! Moreover, the functions, effects, and interrelationships of microbes with the macroscopic world are still not well understood (Andrews & Harris, 2000; Caporaso et al., 2011; Graham et al., 2016; Lambais, Lucheta, & Crowley, 2014; Levia & Germer, 2015; Pace, 1997). We do know that both fungi and bacteria inhabit tree canopies and root systems, but we have very little knowledge of life in the cortisphere (Bai et al., 2015; Bruez et al., 2015; Grube et al., 2015; Khan et al., 2016; Laforest-Lapointe, Messier, & Kembel, 2016; Lindow & Leveau, 2002; Mason, Pfammatter, Holeski, & Raffa, 2015; Ptatscheck et al., 2018; Sridhar & Karamchand, 2009). Each of the biotic and abiotic factors subject to change by urban regions may have the ability to alter microclimatic conditions at the bark surface, thus influencing populations of microbes living there and their consequent impact on biogeochemical cycling.

1.3 Rationale

Exploring microbial diversity in the cortisphere through the lens of ecohydrology allows us to understand better the impacts of microbes on the air we
breathe and water we drink. Microbes regulate processes involving the fixation or release of various nitrogen species, carbon dioxide, and methane, and their regulation of these processes is intimately coupled with the presence or absence of water. Known microbes are more diverse and numerous than their macroscopic counterparts, but they and their functions within the environment are still poorly understood. Accurate estimates of microbial diversity remain unavailable. Each new microbial study, no matter how small, contributes a whole new level of insight into the environmental roles microbes play, and how they affect humans via climate, food, pollutants and biogeochemical processes (Schloss and Handelsman, 2004; Schloss et al., 2016).

Regarding biodiversity, we cannot know a species’ significance to an ecosystem if it is yet undiscovered, which makes studies such as this of the utmost importance to expanding our limited knowledge of microbial populations on an inadequately explored surface. Hot spots and hot moments of nutrient inputs, to which bacterial communities are likely to contribute, are often unaccounted for in decision making and long-term planning. Growing cities and shrinking budgets require urban foresters to be strategic in maintenance and preventative planning for the lands they maintain. Improved knowledge of plant-microbe interactions and the applicability of beneficial bacterial communities in these instances may be vital in dealing with the effects of climate change on trees, particularly microbial or fungal pathogens, which may be more damaging without the presence native, benign microbial communities.

These systems are highly complex. Our understanding of these processes at each scale must be imposed upon those of the other scales, as many of these interactions are highly dependent. For example, abiotic factors at the patch, regional, and global scales determine vegetation type and moisture availability, which in turn
determines plant morphology and thus precipitation partitioning, specifically influencing the *quantity and quality of the sub-canopy flux and habitat available to microorganisms* at the plant scale.

1.4 Research Objectives

This study aims to address existing knowledge gaps by supplying key data and insights into regional and species vegetation-driven variations in chemical enrichment, the species of bacteria inhabiting the cortisphere, and their importance to biogeochemical cycling (Graham et al., 2016; Kirchman, 2012). The cross-scale knowledge gained from this study (the micro, plant, and patch, and regional scales, and interactions thereof) will, in the future, be transferable to the disciplines of ecohydrology and biogeochemistry, microbial ecology, forestry, and urban forestry.

To begin to address the aforementioned knowledge gaps, this dissertation asks the following questions:

- Chapter 3: Do nutrient inputs to watersheds from leaf leachates vary with geography, genetics, and phenophase? Do trees of the same species experience senescence and contribute to watershed biogeochemistry in the same way over regional transects?
- Chapter 4: Do throughfall chemistry, DOM character, or nutrient inputs to watersheds from throughfall vary based on geography and season? Of these, which is the strongest driver of differences in nutrient inputs from throughfall?
- Chapter 5: Do communities of microorganisms in the cortisphere change between urban and rural land use with storm events? And more importantly, how might these communities influence nutrient fluxes at the plant-soil interface?
Chapter 2

STUDY AREA

The first two studies were conducted as part of the NEWRnet project, a $6 million NSF-funded, three-state grant with the overarching goal of high-frequency water quality monitoring along a large transect of the northeastern United States (Figure 2.1; Grant No. IIA-1330238, IIA-1330446, and IIA-1330406). This grant was awarded to the University of Vermont, University of Rhode Island, and the University of Delaware. Leaf samples were collected in association with those three states (MD, RI, and VT) and an additional beech forest in North Carolina near the home of a co-author. Throughfall samples were collected in three states associated with the NEWRnet grant (MD, RI, and VT).
Specimens collected in Maryland (MD) were gathered at Fair Hill Natural Resource Management Area, with whom the university maintains a long-term use agreement, in Cecil County (39.71°N, 75.85°W). The NRMA is the site of a 10-year joint funded research site between the NSF and University of Delaware (Figure 2.2). The NRMA is historically a mix of both farms and woodland, a patchwork of open fields and mixed-deciduous forest. This site receives 1205 mm mean annual precipitation. The 1981–2010 30 year mean maximum air temperature is 19.1°C, and the 30-year mean minimum temperature is 6.8°C [National Climatic Data Center.
Soils in the area are primarily loam and silt loams of igneous, metamorphic, phyllite, and schist parent material, specifically Baile silt loam with 0–3% slopes, Delanco-Codorus-Hatboro complex with 0–8% slopes, Glenelg loam with 8–15% slopes, and Manor loam with 15–25% slopes [U.S. Department of Agriculture Natural Resources Conservation Service (USDA NRCS), 2012] at 70 m elevation. Soils were found to be 0.129% total N. The forest type is mixed deciduous, containing primarily yellow poplar, beech, and sweet birch (Betula lenta).

Figure 2.2: Images of Maryland collection sites.

Rhode Island (RI) leaves were collected in an old-growth beech forest found within the Aquidneck Land Trust’s Oakland Forest in Newport County (41.56°N, 71.26°W). Rhode Island throughfall samples were collected along a 2.25km transect of Bailey Brook in Middletown (Figure 2.3). Dominant species at the collection sites
consisted of *Salix* spp., *Acer platanoides*, and *Nyssa sylvatica*. The 1981–2010 30-year mean maximum temperature is 15.1°C, and the 30-year mean minimum temperature is 6.4°C [NCDC, 2016]. The average annual precipitation is 1176 mm. Soils in the site consist of Newport, Pittstown, and Stissing silt loams, with slopes of less than 8% [USDA NRCS, 2012] at an elevation of about 47 m and consist of 1.065% total N.

![Figure 2.3: Images of Rhode Island collection sites.](image)

In Vermont (VT), leaves and throughfall were collected in the Wade Brook watershed near Montgomery Center (44.86°N, 72.55°W) near an elevation of 390 m. Primary species in this forest are sugar maple, red maple, yellow birch, American beech, and white ash. The 1981–2010 30 year mean maximum temperature is 13.3°C, and the 30-year mean minimum temperature is 1.1°C [NCDC, 2016]. The average annual precipitation is 1060 mm. Soils in the site are primarily Peru fine sandy loam,
with 3 to 15% slopes, at times very stony [USDA NRCS, 2012], and are 0.355% total N.

Leaves from North Carolina (NC) were collected in a beech-dominated forest on Rich Mountain, Boone, NC, above 1400 m elevation (36.24°N, 81.71°W). This site receives 1337 mm annual mean precipitation. The 1981–2010 30 year mean maximum temperature is 16.3°C, and the 30-year mean minimum temperature is 4.0°C [NCDC, 2014]. Soils in the site consist of Burton-Craggey-Rock Outcrop with 15 to 95% slopes (gravelly to cobbly sandy loam profile) and Burton-Wayah Complex with 15 to 50% slopes (gravelly loam to fine sand-loam to gravelly loamy sand profile) [USDA NRCS, 2012]. Soils consist of 0.382% total N.

Soil samples were collected in August 2016 and analyzed by the University of Delaware’s Soil Testing Lab. One-half kg samples were analyzed for pH, P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, Al, estimated cation exchange capacity, base saturation, TNb, ammonium-N (NH\textsubscript{4} -N), and nitrate-N (NO\textsubscript{3} -N). This lab participates in the North American Proficiency Testing Program for Agricultural Testing Laboratories operated by the Soil Science Society of America, the USDA Northeastern Coordinating Committee for Soil Testing NEC1312, and the Mid Atlantic Soil Testing and Plant Analysis Workgroup.
Figure 2.4: Native range of *Quercus rubra* and location of field sites for Chapter 5.

The microbial sample collection for the microbial study took place in the mid-Atlantic region, at one rural forested area and one urban forest fragment (Figure 2.4). For ease of access and timely collection and processing of samples before and after rain events, we selected sites near the University of Delaware, where current research is already being conducted. Rural samples were collected at Fair Hill NRMA. Urban samples were collected in Banning Park, located less than 20 miles away, in metropolitan Wilmington, DE, and a site with which the research group maintains a permit and an established research relationship. Primary tree species in Banning Park
are similar to those in Fair Hill NRMA, but the Banning Park forest fragment is bordered by residential neighborhoods, the SEPTA and AMTRAK regional lines, a major roadway, and is located within a mile of a campus occupied by the second largest chemical manufacturing company in North America.

*Quercus rubra* L. (northern red oak) was selected for the microbial study due to its presence and abundance at both sites (Figure 2.4). Morphological advantages of using red oak for this study include the deeply furrowed bark texture (ideal for bark-surface-scale microclimatology studies) and a round canopy with branch architecture that will encourage stemflow production (Gilman & Watson, 1994). Finally, the frequent use of red oak in suburban and urban areas as “street trees” across most of the country (Gilman & Watson, 1994) will expand the applicability of the results of this study.
Table 2.1:  Common sample acronyms found within this dissertation

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH</td>
<td>Fair Hill NRMA</td>
</tr>
<tr>
<td>BP</td>
<td>Banning Park</td>
</tr>
<tr>
<td>Environmental Fluid</td>
<td>Either gross precipitation, stemflow, or throughfall</td>
</tr>
<tr>
<td>PG</td>
<td>gross precipitation</td>
</tr>
<tr>
<td>SF</td>
<td>stemflow</td>
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<tr>
<td>TF</td>
<td>throughfall</td>
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<tr>
<td>MD</td>
<td>Maryland</td>
</tr>
<tr>
<td>VT</td>
<td>Vermont</td>
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<tr>
<td>RI</td>
<td>Rhode Island</td>
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<tr>
<td>DJF</td>
<td>December-January-February (winter)</td>
</tr>
<tr>
<td>MAM</td>
<td>March-April-May (spring)</td>
</tr>
<tr>
<td>JJA</td>
<td>June-July-August (summer)</td>
</tr>
<tr>
<td>SON</td>
<td>September-October-November (autumn)</td>
</tr>
<tr>
<td>Open</td>
<td>gross precipitation - collected at FH</td>
</tr>
</tbody>
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Chapter 3

AMERICAN BEECH LEAF-LITTER LEACHATE CHEMISTRY: EFFECTS OF GEOGRAPHY AND PHENOPHASE


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Key words: organic matter, fluorescence, Fagus grandifolia, biogeochemical cycling, deciduous forest.

20
3.1 Abstract

The decomposition of broadleaved tree leaves can contribute a substantial amount of energy to forested watersheds via dissolved organic matter, nutrients, and biological activity. Less is known about how these inputs may vary within a single tree species that is known to have two genetically distinct and geographically separate populations, or how these inputs may change throughout autumn senescence and abscission. It is often implicitly assumed that intraspecific differences in leaf-litter leachate chemistry do not significantly differ geographically. We analyzed the morphological and chemical leaf traits, and leachates from *Fagus grandifolia* (American beech) leaves (*n*=360) during three phenophases: fresh green leaves, senescing leaves, and fallen leaves. During each phenophase, leaves were collected from four sites along a geographic transect stretching from Vermont to North Carolina (over 1400 km), with two sites representing each genetic population and differing climatic conditions. Leachates were analyzed for routine solutes and nutrients, as well as fluorescent and UV-visible absorbance indices. Amounts of macro- and micronutrients were highly variable among sites and phenophases but tended to be lowest during the fallen-leaf phase, while measured fluorescence and absorbance indices tended to increase during the senescing-leaf phase and plateau. Results suggest significant differences in leached nutrients among sites and optical properties and nutrients among phenophases. Aromaticity and molecular weight of DOM in leachates was generally low, and aromaticity and humification of leachates both increased over time with leaf-age. These results also suggest that geographically (or genetically) separate populations of the same species do not experience senescence in the same way and that implicit assumptions of intraspecific uniformity of leaf-litter leachate chemistry for a given tree species may be invalid.
3.2 Introduction

Broadleaved trees are a major source of nutrient supply to streams (Bernhardt and Likens, 2002; Cory and Kaplan, 2012), especially during autumn senescence, when nutrients from decomposing leaves are mobilized via leaching and various other biological and physical mechanisms. Fisher and Likens (1973) found that leaf decomposition accounted for 29% of the annual energy budget of a second-order New England stream. Within days of submersion in a stream, up to 40% of the dissolved organic matter (DOM) of a leaf can be leached (Cuss and Guéguen, 2013; McDowell and Fisher, 1976).

The DOM of leaf-litter leachate changes dramatically during autumn as trees experience resorption, senescence, and abscission. At northern latitudes, photoperiod is the ultimate determinant for the onset of senescence, while seasonal changes in air temperature become more important at lower latitudes (Estiarte and Peñuelas, 2014). The main nutrients resorbed are nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) because they are mobile in the phloem, unlike calcium (Ca) and magnesium (Mg), which are accreted (Killingbeck, 2004). Even after abscission, DOM of leaf-litter leachate may be affected by temperature changes (Bärlocher, 2005). Nutrient resorption efficiency and proficiency differ between tree species, resulting in interspecific variation in leaf-litter leachate DOM (Killingbeck, 1996, 2004). Other factors that influence leaf-litter leachate DOM include soil acidification, which can result in a decrease in leaf cation concentration (Duquesnay et al., 2000); inter-tree variability and year-to-year fluctuations (Fluckiger and Braun, 1998; Duquesnay et al., 2000); leaf-litter age and solar radiation, which can decrease the bioavailability of leachate dissolved organic matter (Fellman et al., 2013); xeric conditions; tropospheric ozone conditions (Bussotti et al. 2005); and differences in realized and potential
resorption rates. Factors that influence realized, versus potential, resorption include the plant’s physiological status, which depends on available energy, nutrient concentration, and enzymatic activity, the existence of a sink demand for nutrients, disturbances, water stress, and timing of leaf senescence (Killingbeck, 2004).

The inherent variability of interspecific leachates is known, but recent research using hybrids and genotypes of related species have also revealed differences of leachate composition even within species genotypes (Wymore et al. 2015). Recent studies have also shown that leaf-litter leachates from differing species can have a complex interaction with microbial populations in soil and stream water, especially with regards to nutrient cycling and bioavailability (Joly et al., 2016; Wymore et al. 2015). Depending on forest composition, these dissimilarities in leachate quality can have a substantial impact on soil and stream ecosystems through alterations of available dissolved organic carbon, nitrogen and other nutrients as well as activity and respiration of microbial communities (Bernhardt and Likens, 2002; Cory and Kaplan, 2012, Wymore et al. 2015). Even though it plays an important role in energy and biogeochemical cycling in forests, especially in autumn, less is known about how the quality of leaf-litter leachate may differ throughout autumn as leaves senesce and abscise, or how this differs intraspecifically between trees at different geographical and latitudinal locations. Furthermore, autumnal phenology is a critical stage in the success of plant function, propagation, and morphology, especially for determinant growth species such as Fagus grandifolia Ehrh. (American beech). And, while long-term climate data begins to indicate changes in the autumnal season timing and length, there remains little agreement on the effect of climate change on plant phenology (Richardson et al., 2013).
*Fagus grandifolia* is a common species in eastern North America that is characterized by high adaptability, enabling it to grow in a wide variety of conditions (*Bussotti* et al., 2005). A smooth-barked, shade-tolerant climax species, the native range of *F. grandifolia* spans from eastern Texas and northern Florida, northward to Nova Scotia and Maine (*Tubbs* and *Houston*, 1990). *Fagus grandifolia* possesses large intraspecific variation in its chemical, phenotypic, and reproductive characteristics: *Boerner* (1984) found that among sampled broadleaved deciduous trees, the foliar P content was most variable in *F. grandifolia*; *Bresson* et al. (2011) found genetic variation to account for 0-28% of the total phenotypic variation between populations. Furthermore, through comparisons of allelic components, *Kitamura* and *Kawano* (2001) found differences between northern and southern populations at several loci, indicating an extended period of isolation between the two populations due to historical glaciations. The southern population ranges from the Gulf and eastern coastal plains to the Piedmont and Ozark Plateaus; the northern population is found in northern glaciated territories and parts of the Appalachian Mountains (*Kitamura* and *Kawano*, 2001). Even though two genetically distinct populations have been found in *F. grandifolia* and latitudinal-specific differences between northern and southern *F. grandifolia* populations could result in intraspecific differences in leaf-litter leachate DOM, regional modeling of DOM rarely accounts for genetically-linked geographical differences between *F. grandifolia* stands.

Our research seeks to answer the following question: are there differences in the leachates as senescence progresses, and if so, what factors control these differences? Accordingly, we present our findings as to how nutrient concentration and leaf-litter leachate chemistry and composition from three phenological leaf stages
(fresh green, senescing, and leaf-litter) differ intraspecifically among *F. grandifolia* trees in Vermont (VT), Rhode Island (RI), Maryland (MD), and North Carolina (NC). Because leaf-litter leachate plays a substantial role in the biogeochemical cycles of forested watersheds, we: (1) quantify the temporal and intraspecific variation in *F. grandifolia* leaf-litter leachate; and then (2) employ discriminant function analysis to divulge and clarify the most influential discriminatory variables that account for the intraspecific differences in *F. grandifolia* leaf-litter leachate chemistry as a function of both geography and phenophase. We specifically delve into the intricacies of intraspecific variation in leaf-litter leachate chemistry to investigate whether it is reasonable to assume uniformity in the biogeochemistry of leaf-litter leachates for a particular tree species (*F. grandifolia* in this case) across a large portion of its biogeographic range. Rejection of this assumption would indicate that DOM models at regional, national, or international scales should consider the intrinsic intraspecific differences in leaf-litter leachate chemistry that are partly the result of site-specific factors (e.g., soils, geology). We hope that this work will move us closer to understanding the fundamental linkage of *F. grandifolia* leaf-litter leachate contribution (and by extension, any tree population covering large ranges) to watershed biogeochemistry.

3.3 Materials and Methods

3.3.1 Site Descriptions

For this study, beech leaves were collected in 2015 from four field sites in the eastern United States, spread across a distance of approximately 1,450 km and 8° of
latitude, with elevations ranging from 47 to 1,400 m. More detailed sites descriptions can be found in Wheeler et al. (2017).

Leaves from North Carolina were collected in a beech-dominated forest, 1,400 m elevation above mean sea level in the Blue Ridge Mountains near Boone (36°13'58" N, 81°41'48" W). This site receives 1,338 mm annual mean precipitation, and the 30-year mean temperature is 9°C (NCEI, 2014). Soils in the site consist of steep rocky outcrops with 15 to 95% slopes and a gravelly-loam to fine-sand-loam to gravelly-loamy-sand profile with 15 to 50% slopes (USDA NRCS, 2012). Fresh leaves were collected on 24 August, senescing leaves were collected on 12 October, and fallen leaves were collected on 25 October.

In Maryland, leaves were gathered at Fair Hill Natural Resource Management Area (39°43'10" N, 75°49'56" W). This site receives approximately 1,200 mm annual mean precipitation. The 30-year mean temperature is 13°C (NCEI, 2016). Soils in the area are primarily loam, and silt loams with 0-25% slopes (USDA NRCS, 2012) at an average 70 m elevation above mean sea level. The forest type is mixed deciduous. Fresh leaves were collected on 29 June, senescing leaves were collected on 5 November, and freshly fallen leaves were collected on 11 November.

Rhode Island leaves were collected in an old-growth beech forest found within the Aquidneck Land Trust’s Oakland Forest in Portsmouth (41°33'23" N, 71°15'38" W). The 30-year mean temperature is 11°C, and the average annual precipitation is 1,176 mm (NCEI, 2016). Soils in the site consist of silt loams, with slopes of less than 8% (USDA NRCS, 2012) at an elevation of about 47 m above mean sea level. Fresh leaves were collected on 6 July, senescing leaves were collected on 6 November, and fallen leaves were collected on 15 December.
In Vermont, leaves were collected in the Wade Brook watershed near Montgomery Center around 390 m above mean sea level (44°52’31” N, 72°34’34” W). Primary species in this forest are sugar maple, red maple, yellow birch, American beech, and white ash. The 30-year mean temperature is 7°C, and the average annual precipitation is 1,060 mm (NCEI, 2016). Soils in the site are primarily fine sandy loam, with 3 to 15% slopes (USDA NRCS, 2012). Fresh leaves were collected on 13 July, senescing leaves were collected 19 October, and freshly fallen leaves were collected 30 October.

3.3.2 Sampling

Thirty F. grandifolia leaves in good condition with the petiole intact were collected for each of the three phenological conditions (fresh, senescing, and freshly fallen; hereafter “phenophase” or “phase”) from forested watersheds in each of the four states (n=360; hereafter “states”). Because leaf traits can vary significantly even on the same tree, fresh and senescent leaves were collected from multiple individual beech trees of varying size and age in each of these watersheds and were collected on varying sides and from varying heights of those trees, in both shade and sun, to capture the broadest range of chemical and functional traits possible (Bussotti and Pollastrini, 2015). It is assumed, for the purposes of this study, that leachates from fresh leaves accurately represent the DOM and chemical contribution of vegetative greenfall or stormfall to a watershed system. Given that the climatological normals vary for each of the four locations, each state's collection took place during what would be considered its 'peak' for each phenophase using empirical observations of long-term researchers at the respective field sites. To ensure leaves were collected in their most natural environment, freshly fallen leaves were collected from the top-most
portion of the litter layer on the forest floor, under the trees from which fresh and senescent leaves were taken. Freshly fallen leaves are not assumed to have come from the individual tree under which fallen leaves were found, but it is assumed that surrounding beech in the same area experienced similar conditions.

Immediately after collection, leaves from North Carolina, Rhode Island, and Vermont were shipped standard overnight to the University of Delaware where they were put into storage at 4°C. Leaves collected in Maryland were taken directly to the laboratory and kept at 4°C for 48 hours in order to standardize the storage conditions to those being shipped. After receipt, or at the 48-hour mark, leaves were placed through a LI-COR LI-3100 leaf area meter (Lincoln, NE USA), photographed with scale, and measured for leaf thickness and fresh weight (Bussotti and Pollastrini, 2015). Leaves were briefly rinsed (< 5 seconds) with deionized water to minimize contributions of geographically-dependent dry deposition to the leachate study. Dry weight was also obtained for all leaf sets after oven drying at 70°C for 72 hours (Bussotti and Pollastrini, 2015).

For each set of 30 leaves, individual dried leaves with petioles removed were cut in half to ensure a complete fit into separate pre-combusted 500 mL borosilicate glass beakers filled with 200 mL NANOpure™ deionized water. Individual leaf masses were left intact to mimic leachate concentrations in field conditions, especially with regard to DOC (Cuss and Guéguen, 2013). A pre-combusted glass stirrer was used to keep the leaf fully submerged in the beaker of water, which was covered to keep out contaminants and placed in the refrigerator at 4°C for 72 hours (Cuss and Guéguen, 2013). At the end of the 72-hour period, leaves were removed from the beakers, and the thirty individual leaf-litter leachates were pooled to yield five
composite samples (six individual leachates yielding one composite leachate sample). Composite samples were filtered immediately using a 0.7 µm glass fiber filter (Millipore). Filtered samples were placed into a 250 mL HDPE sample container for chemical analysis and a 40 mL amber glass vial container for fluorescence and UV-visible absorbance analysis. Samples were refrigerated at 4°C until instrumental analysis could be completed.

The University of Delaware Soil Testing Laboratory performed the chemical analyses. Solution pH and EC were measured using an Accumet pH meter model AB15 with a SymPHony pH electrode and a VWR Model 1052 conductivity meter with a platinum dip cell, respectively. P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, and Al were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Elemental Intrepid II XSP Duo View, Madison, WI, USA). DIC/DOC and total bound N (TNb) were measured with an Elementar Vario-Cube TOC Analyzer (Mount Holly, NJ, USA), while nitrate-N and ammonium-N were measured with a Bran & Luebbe AutoAnalyzer 3 (Model AA3, Buffalo Grove, IL, USA). A table of soil chemistry results can be found in the supplementary tables of Wheeler et al. (2017). Fluorescence and UV-visible absorbance analysis were conducted by the Watershed Sciences Research Group at the University of Delaware on a Horiba Aqualog® Benchtop Fluorometer for DOM (Kyoto, Japan). For more detailed information on instrumental analysis, see Wheeler et al. (2017). The following indices were of particular interest for this study: percent humic-like fluorescence, percent fulvic-like fluorescence, percent protein-like fluorescence, humification index (HIX), biological index (BIX), fluorescence index (FI), spectral slope ratio (SR), and specific
ultraviolet absorbance at 254 nm (SUVA\textsubscript{254}). For a comprehensive summary table of these DOM quality indices, see Inamdar et al. (2012).

Finally, soil samples were collected from each site in late summer 2016, following the protocol provided by the University of Delaware Soil Testing Laboratory. One-half kg samples were analyzed for pH, P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, Al, estimated cation exchange capacity, base saturation, TNb, ammonium-N (NH\textsubscript{4}\textsuperscript{+}-N), and nitrate-N (NO\textsubscript{3}\textsuperscript{-}-N).

3.3.3 Statistical Analysis

We employed statistical methods to determine differences in the leachates. These methods include comparing descriptive statistics among the central tendency and dispersion of the variables. A simple non-parametric Kruskal-Wallis test was used to determine if leachates of different phenophases were different within the same state. Analysis of Variance (ANOVA) was used to evaluate leachate variation by both state and phenophase, and a forward-stepwise discriminant function analysis (DFA) was used to classify leachates both by state and phenophase. Variables relating directly to leaf-litter leachate composition were standardized by the variable mean and standard deviation and then run through the DFA using JMP Pro 12. Variables that showed little-to-no variance or significance in either the Kruskal-Wallis or ANOVA were still included in the DFA on the chance that they may have some discriminating power. While nutrient concentrations in leachate samples were not corrected by mass due to the complicated weighting that would have been necessary for our pooled samples, physical leaf characteristics for each state were included in the DFA on the belief that the size of the leaf might impact nutrient concentration within the leachate sample. Variables that could be used to over-parameterize the model by state or phenoseason,
such as climatological data, latitude, longitude, and soil data, were withheld from the DFA and rather used to assist in the interpretation of the results of the analysis.

3.4 Results and Discussion

Patterns of macro- and micronutrients, non-essential constituents (i.e., Al), and leaf areas and weights showed very little consistency among states and phenophases (Tables 3.1 and 3.2). Generally, nutrient content of leachate either decreased over time or peaked during the senescing-leaf phase. We propose that this senescent-peak pattern of nutrient content in leachate samples over time may be a function of cellular deterioration. During the fresh-leaf phase, cell walls are mostly intact, allowing little leaching of essential nutrients over time. Alternatively, during the senescing-leaf phase, cell walls are in the process of destabilization, so one may see the highest amounts of some nutrients being leached at this time – especially those nutrients that are most closely related to biological membranes and enzymatic function, such as Ca, Mg, P, and S (Taiz and Zeiger, 2002). Leaves from the fallen phenophase, which were collected post-senescence and after any resorption had taken place, generally retained little-to-no nutrient content in their leachates.
Table 3.1: Mean (± 1 SD) nutrient content and fluorescence and UV-visible absorbance indices of *F. grandifolia* leaf-litter leachates for all states and phenophases, as well as each phenophase individually.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Fresh</th>
<th>Senescing</th>
<th>Freshly Fallen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample pH</td>
<td>5.40 ± 0.43</td>
<td>5.43 ± 0.632</td>
<td>5.415 ± 0.342</td>
<td>5.37 ± 0.234</td>
</tr>
<tr>
<td>EC (mmhos/cm)</td>
<td>0.02 ± 0.008</td>
<td>0.023 ± 0.008</td>
<td>0.022 ± 0.003</td>
<td>0.014 ± 0.01</td>
</tr>
<tr>
<td>Al (mg L⁻¹)</td>
<td>0.005 ± 0.007</td>
<td>0.014 ± 0.007</td>
<td>0.001 ± 0.003</td>
<td>0.001 ± 0.002</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>0.02 ± 0.012</td>
<td>0.031 ± 0.016</td>
<td>0.015 ± 0.003</td>
<td>0.014 ± 0.003</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>0.46 ± 0.34</td>
<td>0.285 ± 0.183</td>
<td>0.595 ± 0.248</td>
<td>0.496 ± 0.476</td>
</tr>
<tr>
<td>Cu (mg L⁻¹)</td>
<td>0.002 ± 0.002</td>
<td>0.002 ± 0.002</td>
<td>0.002 ± 0.001</td>
<td>0.001 ± 0.002</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>0.034 ± 0.023</td>
<td>0.052 ± 0.011</td>
<td>0.029 ± 0.014</td>
<td>0.022 ± 0.03</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>3.27 ± 1.601</td>
<td>4.62 ± 1.292</td>
<td>3.405 ± 0.698</td>
<td>1.787 ± 1.247</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>0.31 ± 0.18</td>
<td>0.349 ± 0.177</td>
<td>0.312 ± 0.17</td>
<td>0.285 ± 0.201</td>
</tr>
<tr>
<td>Mn (mg L⁻¹)</td>
<td>0.07 ± 0.074</td>
<td>0.042 ± 0.027</td>
<td>0.095 ± 0.104</td>
<td>0.074 ± 0.064</td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>0.54 ± 0.33</td>
<td>0.637 ± 0.562</td>
<td>0.508 ± 0.032</td>
<td>0.47 ± 0.054</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>0.24 ± 0.171</td>
<td>0.3 ± 0.138</td>
<td>0.269 ± 0.225</td>
<td>0.155 ± 0.097</td>
</tr>
<tr>
<td>S (mg L⁻¹)</td>
<td>0.79 ± 0.562</td>
<td>0.47 ± 0.501</td>
<td>1.237 ± 0.018</td>
<td>0.667 ± 0.63</td>
</tr>
<tr>
<td>Zn (mg L⁻¹)</td>
<td>0.037 ± 0.007</td>
<td>0.039 ± 0.006</td>
<td>0.039 ± 0.006</td>
<td>0.032 ± 0.006</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>7.66 ± 4.146</td>
<td>9.721 ± 4.732</td>
<td>7.646 ± 1.862</td>
<td>5.613 ± 4.316</td>
</tr>
<tr>
<td>TNb (mg L⁻¹)</td>
<td>0.59 ± 0.74</td>
<td>1.345 ± 0.807</td>
<td>0.352 ± 0.344</td>
<td>0.071 ± 0.14</td>
</tr>
<tr>
<td>% Humic Like</td>
<td>11.57 ± 4.471</td>
<td>8.04 ± 1.147</td>
<td>10.28 ± 3.003</td>
<td>16.38 ± 3.547</td>
</tr>
<tr>
<td>% Fulvic Like</td>
<td>13.47 ± 3.594</td>
<td>10.98 ± 1.898</td>
<td>13.26 ± 3.847</td>
<td>16.18 ± 2.719</td>
</tr>
<tr>
<td>% Protein Like</td>
<td>74.96 ± 7.498</td>
<td>80.99 ± 2.744</td>
<td>76.46 ± 5.789</td>
<td>67.45 ± 5.792</td>
</tr>
<tr>
<td>HIX</td>
<td>0.32 ± 0.097</td>
<td>0.206 ± 0.024</td>
<td>0.353 ± 0.076</td>
<td>0.393 ± 0.047</td>
</tr>
<tr>
<td>BIX</td>
<td>0.46 ± 0.153</td>
<td>0.278 ± 0.038</td>
<td>0.564 ± 0.113</td>
<td>0.537 ± 0.074</td>
</tr>
<tr>
<td>FI</td>
<td>1.68 ± 0.161</td>
<td>1.556 ± 0.128</td>
<td>1.801 ± 0.167</td>
<td>1.697 ± 0.066</td>
</tr>
<tr>
<td>SUVA₂₅₅ (L mg C⁻¹ cm⁻¹)</td>
<td>0.02 ± 0.009</td>
<td>0.009 ± 0.005</td>
<td>0.021 ± 0.005</td>
<td>0.025 ± 0.005</td>
</tr>
<tr>
<td>Sr</td>
<td>1.44 ± 0.614</td>
<td>1.091 ± 0.433</td>
<td>1.781 ± 0.538</td>
<td>1.459 ± 0.664</td>
</tr>
</tbody>
</table>
Table 3.2: Physical leaf data and average leachate sample DOC

<table>
<thead>
<tr>
<th></th>
<th>n=</th>
<th>Leaf Area (cm²)</th>
<th>Leaf Fresh Weight (g)</th>
<th>Leaf Dry Weight (g)</th>
<th>Water Content</th>
<th>Foliar Biomass (g/cm²)</th>
<th>Average Sample DOC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh MD</td>
<td>30</td>
<td>1171.80</td>
<td>4.22</td>
<td>Missing Data</td>
<td></td>
<td></td>
<td>9.46</td>
</tr>
<tr>
<td>Fresh RI</td>
<td>30</td>
<td>1928.83</td>
<td>12.31</td>
<td>6.66</td>
<td>5.65</td>
<td>0.003</td>
<td>13.34</td>
</tr>
<tr>
<td>Fresh VT</td>
<td>30</td>
<td>1531.34</td>
<td>8.35</td>
<td>4.18</td>
<td>4.17</td>
<td>0.003</td>
<td>7.97</td>
</tr>
<tr>
<td>Fresh NC</td>
<td>30</td>
<td>1304.75</td>
<td>9.80</td>
<td>4.25</td>
<td>5.55</td>
<td>0.004</td>
<td>8.10</td>
</tr>
<tr>
<td>Senescing MD</td>
<td>30</td>
<td>1261.53</td>
<td>6.95</td>
<td>2.83</td>
<td>4.12</td>
<td>0.003</td>
<td>6.23</td>
</tr>
<tr>
<td>Senescing RI</td>
<td>30</td>
<td>1534.85</td>
<td>5.37</td>
<td>4.08</td>
<td>1.29</td>
<td>0.001</td>
<td>9.04</td>
</tr>
<tr>
<td>Senescing VT</td>
<td>30</td>
<td>1727.51</td>
<td>11.14</td>
<td>4.10</td>
<td>7.04</td>
<td>0.002</td>
<td>7.61</td>
</tr>
<tr>
<td>Senescing NC</td>
<td>30</td>
<td>1531.63</td>
<td>12.28</td>
<td>5.29</td>
<td>6.99</td>
<td>0.005</td>
<td>7.70</td>
</tr>
<tr>
<td>Fallen MD</td>
<td>30</td>
<td>1502.00</td>
<td>8.14</td>
<td>4.36</td>
<td>3.78</td>
<td>0.003</td>
<td>2.89</td>
</tr>
<tr>
<td>Fallen RI</td>
<td>30</td>
<td>1506.40</td>
<td>4.96</td>
<td>4.68</td>
<td>0.28</td>
<td>0.000</td>
<td>2.58</td>
</tr>
<tr>
<td>Fallen VT</td>
<td>30</td>
<td>1602.82</td>
<td>10.76</td>
<td>5.44</td>
<td>5.32</td>
<td>0.003</td>
<td>4.66</td>
</tr>
<tr>
<td>Fallen NC</td>
<td>30</td>
<td>1501.13</td>
<td>12.09</td>
<td>7.70</td>
<td>4.39</td>
<td>0.005</td>
<td>12.33</td>
</tr>
</tbody>
</table>

Measured fluorescence indices also tended to increase during the senescing-leaf phase and plateau at those values (Table 3.1). The HIX showed a shift to longer wavelengths over time due to lower H:C ratios as leaves deteriorated, becoming more aromatic and less bioavailable during the fallen phase (Senesi, 1990; Ohno, 2002). With few exceptions, the BIX remained below the threshold of 1, indicating that there was little to no biological activity within the samples (Huguet et al., 2009). The FI threshold between extra-cellular release and plant-terrestrial DOM is above ~1.4, and typically, the senescing leaf samples fell above this threshold, supporting our hypothesis that cell wall deterioration is highest at this time, when DOM is also more bioavailable (McKnight et al., 2001). Percent fulvic-like and percent humic-like organic acids increased while percent protein-like decreased, suggesting that over time, there is simply less bioavailable material to be leached (Fellman et al., 2009).
The $S_R$ followed patterns similar to those of protein and organic acids because it is a measure of the inverse molecular weight, and thus bioavailability. SUVA$_{254}$ values were generally quite low ($0.02 \pm 0.01 \text{ L mg C}^{-1} \text{ cm}^{-1}$) but were within typical values for leachates, between $0.0004$ and $0.025 \text{ L mg C}^{-1} \text{ cm}^{-1}$ (Strauss and Lamberti, 2002), indicating low aromaticity and unstable organic compounds, especially during senescence. Many of our measured values are within the range of those found for mixed forest leachates at the same field site in Maryland by Inamdar et al. (2012).

For each state, many of the nutrient variables were significantly different over the three phenophases. For North Carolina leachates, 44% of nutrients varied significantly among phenophases while 83% of nutrients were significantly different for Maryland, and 89% for Rhode Island and Vermont (Table 3.3). Additionally, the optical properties of the samples from each state were significantly different over the three phenophases for over 87% of the measured variables (Table 3.3). It should therefore be unsurprising that an ANOVA would reveal significant differences among leaf characteristics between sites due to nutritional differences in the soils and genetic populations at each site, and among fluorescence indices and, even more significantly, the nutritional composition between phenophases, due to changes in the breakdown of OM and release of nutrients over time (Table 3.3).
Table 3.3: Results of Kruskal-Wallis Test of variance of phenophases by state (DF = 2) and results of the analysis of variance of F. grandifolia leaf-litter leachates by state (DF = 2) and phenophase (DF = 3). Significant results indicated by bold-italic.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>NC</th>
<th>MD</th>
<th>RI</th>
<th>VT</th>
<th>State</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi²</td>
<td>Prob Chi²</td>
<td>Chi²</td>
<td>Prob Chi²</td>
<td>Chi²</td>
<td>Prob Chi²</td>
</tr>
<tr>
<td>Sample pH</td>
<td>10.194</td>
<td>0.006</td>
<td>4.044</td>
<td>0.132</td>
<td>4.528</td>
<td>0.104</td>
</tr>
<tr>
<td>EC (mmhos/cm)</td>
<td>1.916</td>
<td>0.384</td>
<td>7.272</td>
<td>0.026</td>
<td>11.305</td>
<td>0.004</td>
</tr>
<tr>
<td>Al (mg L⁻¹)</td>
<td>6.007</td>
<td>0.050</td>
<td>12.522</td>
<td>0.002</td>
<td>11.180</td>
<td>0.004</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>8.640</td>
<td>0.013</td>
<td>8.029</td>
<td>0.018</td>
<td>9.780</td>
<td>0.008</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>8.340</td>
<td>0.016</td>
<td>3.780</td>
<td>0.151</td>
<td>10.238</td>
<td>0.006</td>
</tr>
<tr>
<td>Cu (mg L⁻¹)</td>
<td>2.204</td>
<td>0.332</td>
<td>6.346</td>
<td>0.042</td>
<td>0.960</td>
<td>0.619</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>1.520</td>
<td>0.468</td>
<td>12.500</td>
<td>0.002</td>
<td>12.500</td>
<td>0.002</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>4.340</td>
<td>0.114</td>
<td>7.849</td>
<td>0.020</td>
<td>12.500</td>
<td>0.002</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>8.640</td>
<td>0.013</td>
<td>6.016</td>
<td>0.049</td>
<td>10.820</td>
<td>0.005</td>
</tr>
<tr>
<td>Mn (mg L⁻¹)</td>
<td>7.220</td>
<td>0.027</td>
<td>7.440</td>
<td>0.024</td>
<td>7.619</td>
<td>0.022</td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>3.025</td>
<td>0.220</td>
<td>11.580</td>
<td>0.003</td>
<td>6.320</td>
<td>0.042</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>7.740</td>
<td>0.021</td>
<td>10.260</td>
<td>0.006</td>
<td>10.820</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>mg L⁻¹</td>
<td></td>
<td>mg L⁻¹</td>
<td></td>
<td></td>
<td>mg L⁻¹</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td><strong>S</strong></td>
<td>7.273</td>
<td><strong>0.026</strong></td>
<td>12.020</td>
<td><strong>0.003</strong></td>
<td>12.500</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Total Dissolved C</td>
<td>5.040</td>
<td>0.081</td>
<td>10.820</td>
<td><strong>0.005</strong></td>
<td>10.820</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td><strong>DIC</strong></td>
<td>0.720</td>
<td>0.698</td>
<td>5.460</td>
<td>0.065</td>
<td>12.500</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td><strong>DOC</strong></td>
<td>5.660</td>
<td>0.059</td>
<td>10.820</td>
<td><strong>0.005</strong></td>
<td>10.220</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td><strong>TNb</strong></td>
<td>3.571</td>
<td>0.168</td>
<td>11.195</td>
<td><strong>0.004</strong></td>
<td>12.727</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td>1.340</td>
<td>0.512</td>
<td>6.260</td>
<td><strong>0.044</strong></td>
<td>10.260</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td><strong>Optical Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Fulvic</td>
<td>9.060</td>
<td><strong>0.011</strong></td>
<td>8.720</td>
<td><strong>0.013</strong></td>
<td>11.580</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>% Humic</td>
<td>9.620</td>
<td><strong>0.008</strong></td>
<td>8.340</td>
<td><strong>0.016</strong></td>
<td>12.500</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>% Protein</td>
<td>10.820</td>
<td><strong>0.005</strong></td>
<td>9.920</td>
<td><strong>0.007</strong></td>
<td>12.500</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td><strong>BIX</strong></td>
<td>9.420</td>
<td><strong>0.009</strong></td>
<td>12.500</td>
<td><strong>0.002</strong></td>
<td>9.620</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td><strong>FI</strong></td>
<td>9.920</td>
<td><strong>0.007</strong></td>
<td>9.360</td>
<td><strong>0.009</strong></td>
<td>9.780</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td><strong>HIX</strong></td>
<td>12.500</td>
<td><strong>0.002</strong></td>
<td>9.500</td>
<td><strong>0.009</strong></td>
<td>9.420</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td><strong>Sr</strong></td>
<td>10.820</td>
<td><strong>0.005</strong></td>
<td>3.860</td>
<td>0.145</td>
<td>12.500</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td><strong>SUVA₂₅₄</strong></td>
<td>11.520</td>
<td><strong>0.003</strong></td>
<td>7.460</td>
<td><strong>0.024</strong></td>
<td>11.180</td>
<td><strong>0.004</strong></td>
</tr>
</tbody>
</table>

**Leaf Characteristics**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Leaf Thickness (cm)</td>
<td>4.528</td>
<td><strong>0.007</strong></td>
<td>3.248</td>
<td><strong>0.046</strong></td>
</tr>
<tr>
<td>Leaf Thickness Standard Deviation</td>
<td>8.008</td>
<td><strong>0.000</strong></td>
<td>1.001</td>
<td>0.374</td>
</tr>
<tr>
<td>Dry Weight (g)</td>
<td>4.353</td>
<td><strong>0.008</strong></td>
<td>0.680</td>
<td>0.511</td>
</tr>
<tr>
<td>Fresh Weight (g)</td>
<td>9.237</td>
<td>&lt;.0001</td>
<td>1.643</td>
<td>0.202</td>
</tr>
<tr>
<td>Leaf Area (cm²)</td>
<td>1.901</td>
<td>0.140</td>
<td>1.955</td>
<td>0.151</td>
</tr>
</tbody>
</table>
3.4.1 State-based Discriminant Function Analysis

Forward-stepwise discriminant function analysis of the standardized data identified seven variables with which it was possible to validate a model: sample pH, electrical conductivity (EC), B, P, leaf thickness standard deviation, leaf area, and dry weight. The model was cross-validated, by training using 36 of the 60 cases and 24 cases withheld as a validation sample. All model tests were significant at the < 0.0001 level, and only 4% of the excluded cases were misclassified (one RI fallen leaf was classified as a VT leaf). Results of the DFA by state imply that the phosphorous content of the leachate samples is the most influential variable for differentiating between states (Table 3.4).
Table 3.4: Discriminant weights of each variable to the state's function; absolute value reflects relative contribution to the function. Highest contributions are shown in bold. In gray, discriminant loadings for the states, showing the variance that the variables have with the discriminant function, to be interpreted in factor-loading fashion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MD</th>
<th>NC</th>
<th>RI</th>
<th>VT</th>
<th>Canon1</th>
<th>Canon2</th>
<th>Canon3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-4.467</td>
<td>-7.568</td>
<td>7.829</td>
<td><strong>12.900</strong></td>
<td>-0.695</td>
<td>-0.159</td>
<td><strong>0.626</strong></td>
</tr>
<tr>
<td>EC (mmhos/cm)</td>
<td>-1.488</td>
<td>-6.282</td>
<td>8.083</td>
<td>0.301</td>
<td>-0.281</td>
<td>-0.269</td>
<td>0.122</td>
</tr>
<tr>
<td>B (mg L(^{-1}))</td>
<td>-3.868</td>
<td>2.268</td>
<td>1.042</td>
<td>3.151</td>
<td>-0.122</td>
<td>0.347</td>
<td>0.059</td>
</tr>
<tr>
<td>P (mg L(^{-1}))</td>
<td><strong>9.896</strong></td>
<td>6.974</td>
<td>-14.982</td>
<td>-4.485</td>
<td><strong>0.792</strong></td>
<td>0.098</td>
<td>0.183</td>
</tr>
<tr>
<td>Leaf Thickness Std Dev</td>
<td>8.244</td>
<td>1.459</td>
<td>-5.315</td>
<td>-8.404</td>
<td>0.201</td>
<td>-0.261</td>
<td>0.443</td>
</tr>
<tr>
<td>Leaf Area (cm(^2))</td>
<td>7.088</td>
<td>0.195</td>
<td>-4.877</td>
<td>-2.923</td>
<td>0.525</td>
<td>0.255</td>
<td>0.296</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>-6.919</td>
<td>1.764</td>
<td>0.282</td>
<td>6.320</td>
<td>-0.105</td>
<td><strong>0.601</strong></td>
<td>0.305</td>
</tr>
<tr>
<td>Constant</td>
<td>11.573</td>
<td>6.731</td>
<td>16.197</td>
<td>11.221</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This is especially true for the first canonical function, which discriminates between Maryland-North Carolina in the positive direction, and Vermont-Rhode Island in the negative direction. Here, P also has a negative relationship with sample pH, which is unsurprising given phosphorus’ availability is quite sensitive to pH (Marschner, 1986). The second canonical function, which appears to discriminate between North Carolina-Vermont and Rhode Island-Maryland, is weighted almost exclusively by dry leaf weight, which has relatively large contributions to Vermont and Maryland functions, albeit in the opposite direction (Table 3.4). The third canonical function is almost exclusively sample pH, for which North Carolina and Vermont have the highest function contribution, and it appears to discriminate between the North Carolina-Rhode Island groups and the Maryland-Vermont groups (Table 4). In a previous study by Possen et al. (2014), specific leaf area (a ratio of leaf area to dry weight) was one of two best parameters for predicting growth in field conditions. Here again, leaf area and dry weight are suggested indicators for predicting leaf-leachate origin. For the four states studied, while the variables P, pH, and dry weight carry much of the discriminatory power (Table 3.4), removal of the additional, and seemingly unnecessary variables (such as B which showed no significant difference between states) weakens the discriminatory power of the functions greatly, and confirms that this reduced set of variables is likely the best of those measured for discriminating between these states.

3.4.2 Phenophase-based Discriminant Function Analysis

Forward-stepwise discriminant function analysis of the standardized data identified eight variables with which it was possible to validate a model: K, Mg, Al, B, percent humic-like, BIX, FI, and SUVA$_{254}$. The model was cross-validated using 36 of
the 60 cases for training and 24 cases withheld as a validation sample. All model tests were significant at the < 0.0001 level. One hundred percent of the training cases were correctly classified, and only 16.6% of the excluded cases were misclassified; one fresh leaf was classified as senescing, one senescing leaf was classified as fallen, and two fallen leaves were classified as senescing. While the ANOVA suggested that there were no significant differences in the variances for B between phenophases (Table 3.3), it stands out as the highest relative contribution for all three leaf phenophase functions (Table 3.5). The first canonical function of the DFA suggests that phenophases are being discriminated primarily by the amount of extra-cellular release; all variates of the first canonical function (K, Mg, Al, B) are directly related to cell-wall structure, biosynthesis, and secondary metabolic functions suggesting that as growth ends, leaf functionality slows, and breakdown of the leaf tissues progresses over time, these nutrients are most representative of the change in activity and growth (Table 3.5). As the biological and fluorescence indices increase on this canonical function, there is a negative relationship with the mobile micronutrients essential for enzymatic and biosynthetic activity (Marschner, 1986; Taiz and Zeiger, 2002). The second canonical function is loaded almost entirely by “percent humic-like,” suggesting the decomposition stage of the OM may be the final necessary variate needed to discriminate between the phenophases (Table 3.5). These results are similar to those of Fellman et al. (2013), who found significant correlations between increasing leaf-litter age and increasing bioavailability of DOM in leaf-litter leachates. The result of this DFA should, therefore, be unsurprising given that decomposition stage is essentially a proxy for leaf age, and thus phenophase.
Table 3.5: Discriminant weights of each variable to the phase's function; absolute value reflects relative contribution to the function. Highest contributions are shown in bold. In gray, discriminant loadings for the phases, showing the variance that the variables have with the discriminant function, to be interpreted in factor-loading fashion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fresh</th>
<th>Senescing</th>
<th>Fallen</th>
<th>Canon1</th>
<th>Canon2</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (mg L(^{-1}))</td>
<td>-3.733</td>
<td>-1.356</td>
<td>4.021</td>
<td><strong>-0.638</strong></td>
<td>-0.461</td>
</tr>
<tr>
<td>Mg (mg L(^{-1}))</td>
<td>5.985</td>
<td>-1.902</td>
<td>-5.516</td>
<td>-0.146</td>
<td>0.042</td>
</tr>
<tr>
<td>Al (mg L(^{-1}))</td>
<td>-9.421</td>
<td>5.903</td>
<td>3.571</td>
<td><strong>-0.838</strong></td>
<td>0.136</td>
</tr>
<tr>
<td>B (mg L(^{-1}))</td>
<td><strong>-12.722</strong></td>
<td>11.605</td>
<td><strong>8.018</strong></td>
<td><strong>-0.847</strong></td>
<td>0.120</td>
</tr>
<tr>
<td>% Humic</td>
<td>2.450</td>
<td>3.336</td>
<td>-7.066</td>
<td>0.581</td>
<td><strong>0.721</strong></td>
</tr>
<tr>
<td>BIX</td>
<td>7.313</td>
<td>-5.748</td>
<td>-3.658</td>
<td><strong>0.883</strong></td>
<td>-0.217</td>
</tr>
<tr>
<td>FI</td>
<td>6.172</td>
<td>-3.005</td>
<td>0.509</td>
<td><strong>0.659</strong></td>
<td>-0.143</td>
</tr>
<tr>
<td>SUVA(_{254}) (L mg C(^{-1}) cm(^{-1}))</td>
<td>12.183</td>
<td>-3.935</td>
<td>-5.808</td>
<td><strong>0.897</strong></td>
<td>0.030</td>
</tr>
<tr>
<td>Constant</td>
<td>26.435</td>
<td>9.488</td>
<td>11.351</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Surprisingly, the addition of a dummy variable for genetic population did not provide enough discriminatory power to be entered into either the state or phenophase analysis, and we found no significant latitudinal or genetic differences in intraspecific DOM quality for *F. grandifolia*.

### 3.5 Conclusions

DOM quality indices were useful for discriminating between leaf phenophases while sample chemistry, rather than quality indices, was more useful for discriminating between the geographic states. The results of this study indicate that it is highly likely that geographically or genetically separate populations of the same species do not experience senescence in the same way. This has implications for (a) phenoseasonal timing of nutrient and DOM input to watersheds and subsequent impacts to soil and stream ecosystems, and (b) regional modeling of the contribution of plant nutritional status and DOM to biogeochemical cycling in forested watersheds,
especially when the model is based on remotely sensed optical leaf property data. For example, while the return value of remotely sensed spectroscopy data may provide a similar spectral distribution at the same time or even at the same point during a phenophase, we cannot assume that a ‘same’ signal means the same thing for the nutritional or phenological status of trees that are geographically separate. Similarly, we have shown that the chemical contributions from leaf-litter leachate to local watersheds may be considerably variable, even for the same species, regardless of genetic population. Thus, one should take into account the intrinsic intraspecific differences in leaf-litter leachate chemistry that are partly the result of site-specific factors (e.g., soils, geology). It is no longer sufficient to consider forests a black box of regional biogeochemical cycling. As Earth’s climate continues to change, the composition and biodiversity of forested watersheds will also change. Understanding these variations at the watershed and regional scales and how they change over time is a critical piece of the energy and nutrient cycling puzzle for watersheds at a global scale.

3.6 Acknowledgments

This work was made possible with financial support from the U.S. National Science Foundation (Grant No. IIA-1330238, IIA-1330446, and IIA-1330406), as part of the larger North East Water Resources Network Project, and the authors would like to acknowledge the hard work of the many other members of this project who have worked alongside us for the duration of the grant. The authors would like to thank the Inamdar Watershed Hydrochemistry Group and the University of Delaware Soil Testing Lab for their laboratory analyses. All field sites are thanked for their
permission to collect leaves. Marilou Wheeler, Jennifer Kane, and Kristin McDermott are recognized for their assistance with leaf collection at their respective sites.

3.7 Addendum to Published Work

During the defense, the committee asked questions and made suggestions for future investigation, which has led to the addendum for this published chapter of the dissertation.

Removal of boron from the DFA, which showed no significant difference between states in tests of significance, substantially weakened the discriminatory power of the functions of the state-based discriminant function analysis. We were unsure why boron was having such an essential role in the DFA, but it is well known that B is critical during growth and cell wall development. Upon further investigation of our data, leaves from RI have some of the highest average leaf thicknesses, highest boron concentrations in fresh leaf leachates, and highest concentrations of boron in soil samples. Of the top 20% of values of boron concentrations per mass, RI leaves make up 40% (NC makes up the other 60%). Given this, and the fact that many of the other physical leaf traits showed strong discriminatory power in the functions of the state-based DFA, it seems likely that B is showing up in the DFA as an analog for a physical characteristic of the leaves. That is, in addition to dry weight, leaf area, and leaf thickness standard deviation, B may be discriminating as an analog for leaf thickness and 'mass.'

Growth rates for the two genotypes were under question as a possible tracer for the plant use of boron. Unfortunately, literature seems to list all *Fagus grandifolia* as slow growing, with a lifespan of approximately 300 years, but according to Kitamura
and Kawano (2001), there has not yet been a determination of what differences may exist in terms of genetic function for those genes that are different between the two genotypes, including differences in growth rates.

Finally, there was a query as to whether these leachate results might be related to deposition. Aside from Zn, there seems to be a minimal relationship between nutrient concentrations in leachate as a function of measured soil concentration (see Appendix C for these biplots). For this study, we followed methods as best we could to eliminate the possibility of atmospheric deposition as a source. Unfortunately, given the data that we have, without knowing the exact transpiration and uptake rates for these trees at all times, and without knowing soil conditions for each period which would inherently impact moisture, pH, and nutrient uptake, there is very little way to prove or disprove a definitive relationship between the leaf leachate and soil nutrient concentration or atmospheric deposition. We believe that our methods sufficiently eliminated atmospheric contributions as a source and believe that the leaching process directly from leaves is distinct enough from soil conditions to say that our results are in line with what is being removed from the leaf itself.
REFERENCES


Chapter 4

A REGIONAL PERSPECTIVE OF THROUGHFALL BASE CATION CHEMISTRY IN MIXED DECIDUOUS FORESTS OF THE NORTHEASTERN UNITED STATES

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4.1 Introduction

Forest ecosystems are hotspots of hydrological and biogeochemical cycling and carbon storage (Hanson and Wullschleger, 2003). Studies have shown that long-term change in regional climates is likely to and already is inducing changes to precipitation regimes (Hanson and Wullschleger, 2003; Trenberth, 2011). Studies of both deciduous and coniferous forests show that altered precipitation amounts, event
timing, and source regions will result in physiological, morphological, phenological, and production related modifications to these forests at all scales (Hanson and Wullschleger, 2003; McKenzie et al., 2003).

Forests and forest canopies are spatially heterogeneous. This variability is a function of species, species distribution, topography, soils, as well as a host of biotic characteristics such as canopy gap size and branching structure, leaf shape and distribution, and stand age (Pypker et al., 2011). Even small changes to climate will modify forest composition and characteristics, including the succession of dominant species, vulnerability to insect driven herbivory (Michalzik et al., 2016), alteration of the timing of phenoseason and coinciding biogeochemical inputs (Van Stan et al., 2012) and the aforementioned biotic characteristics of the canopy will be redistributed (Pypker et al., 2011).

Because the same characteristics which make a forest physically variable similarly control the movement of water and nutrients through a forest canopy, it follows that the way trees redistribute water and nutrients will also change. During rain events, trees partition incident precipitation into throughfall and stemflow, with the remaining volume being stored in the canopy and on bark surfaces and subsequently lost to evaporation (Carlyle-Moses and Schooling 2015). For deciduous trees during the leaf-on season(s), throughfall (rain falling through, dripping from, or splashing off of the canopy) is approximately 74% of incident precipitation (Staelens et al., 2011). Approximately 4% of incident precipitation is funneled down stems, and the trunk as stemflow and the other approximately 22% is lost to canopy interception. Throughfall has short to medium residence times (depending on atmospheric and species-specific factors), is highly variable in time, space and chemistry (Keim et al.,
2005; Allen et al., 2014)), and is often highly enriched in foliar leachable nutrients relative to incident precipitation and gross dry deposition (Levia and Herwitz, 2005; Johnson and Lehmann, 2006a; Levia and Germer, 2015). Because throughfall is a diffuse input under the canopy, it has an influence on fine-root development (Ford and Deans 1978).

A significant amount of nutrients are deposited onto a forest canopy during either wet, dry, or occult deposition (Weathers and Ponette-Gonzalez, 2011). For each of these depositional processes, additional interactions occur as precipitation moves through the canopy either simultaneously or during a later event. The amount of time precipitated water is in contact with foliar or woody surfaces enhances leaching from those surfaces (Johnson and Lehmann, 2006b; Levia and Frost, 2006; Levia et al., 2011; Pypker et al., 2011).

Variability of nutrients in throughfall may be introduced to forests via industrial and natural sources of nutrients and pollutants, prevailing wind direction, storm sources and characteristics, season, location and structure of individual trees, canopy structure, insects, and edge effects produced by distance from tree to forest edge (Durocher, 1990; Price et al., 1997; Crockford and Richardson, 2000; Carlyle-Moses et al., 2004; Devlaeminck et al., 2005; Levia and Frost, 2006; Pypker et al., 2011; Bischoff et al., 2015; Michalzik et al., 2016). Trees near the edge of a forest have been shown to have increases in base cations and decreases in potassium and calcium, while sulfate inputs remained the same (Hojjati et al., 2008; Pypker et al., 2011). Canopy structure can also drive the creation of hot spots and hot moments of nutrient and water input beneath the canopy (Pypker et al., 2011).
This study seeks to better our understanding of base cation inputs in relation to changing precipitation regimes, and how throughfall chemistry may change as a function of synoptic storm conditions or increased frequency of events along a 640 km transect of the northeastern USA. With the exception of the national throughfall monitoring program in Sweden which covers an area somewhat larger than our transect here (Knulst, 2004), studies for which ‘regional’ represents a sampling along <200 km watershed, which is larger than the typical study stand (Mason et al., 1997; Martin-Stpaul et al., 2013), a majority of regional throughfall studies are based on annual fluxes using databases of published data (Armbruster et al., 2002; Langusch et al., 2003). So many of these throughfall and precipitation partitioning datasets can be easily compared to each other to create regional assessments, and this work is of the utmost importance, but to our knowledge, no other study has coordinated simultaneous event collection along a transect of this size. Additionally, we present that while averaging annual regional fluxes is necessary to further our understanding of forest rainfall partitioning and nutrient transport, the results of this study highlight that 1) comparisons of same meteorological events (especially those at the regional or synoptic scale) may not accurately describe the nutrient fluxes based on inputs alone, as large air masses with generally uniform qualities and provenance may not result in the same input to the watersheds over which they precipitate 2) although annual fluxes are important, the timing and distribution of events, and antecedent conditions (including atmospheric deposition) can greatly impact watershed inputs at the event scale, which is very much important to watershed stakeholders.
This work seeks to answer, do base cations inputs to watersheds from throughfall vary based on geography and/or season? Of these, which is the strongest driver of differences in nutrient inputs from throughfall?

4.2 Materials and Methods

4.2.1 Sampling

This study was conducted as part of the North East Water Resource Network (NEWRnet) project, a $6 million NSF-funded, three-state grant with the overarching goal of high-frequency water quality monitoring along a large transect of the northeastern United States. For this study, volume-weighted throughfall samples were collected at randomly selected, non-roving points under the selected canopies from September 2014 to July 2017, from three field sites in the eastern United States, spread across a distance of approximately 640 km, with elevations ranging from 47 to 400 m. The broader interest of the NEWRnet project was to understand the sources and movement of watershed chemistry better, and thus, our goal was to look at throughfall as the input of this chemistry. Throughfall is rarely homogenous, and therefore our intention was to consider the canopy weighted mix of throughfall that could be making its way into the stream at each site. To achieve this, we sampled throughfall under the dominant tree species, knowing that the small n does not reflect spatial variability, but rather creates an analog which reflects the stream-destined subcanopy water as a mix of canopy sources (Scudlark et al., 2005). Open precipitation samples were also collected at each site for the same events.

Throughfall collectors were deployed under the canopy at each forested site (MD n=5; RI n=3; VT n=5). The throughfall collectors and the single open
precipitation collector consisted of an 8” HDPE funnel connected to a 1000 ml amber glass bottle, secured atop a post at a height of 1.5m. Pyrex glass wool was inserted into the funnel to prevent large debris from falling into the sample collector. The open precipitation sample was collected as a single sample. For each site, a volume-weighted composite sample from the n throughfall collectors was created. Each of these samples (precipitation and throughfall composite) was then divided into (1) 40 ml amber glass vial for fluorescence analysis and (1) 250 ml HDPE sampling bottle for chemical analysis. Fluorescence analyses results were not included in this manuscript.

Precipitation events forecasted to affect the entire study region were sampled, whereupon throughfall and open precipitation collectors were deployed at each site, minimizing the influence of dry deposition. Rain and throughfall samples were collected within 24 hours of the event and cold-shipped to the Ecohydrology Lab at the University of Delaware. Samples requiring storage were kept in a refrigerator at 4°C until shipped (for example events collected when shipping providers were closed for the weekend or holidays). Upon receipt at the University of Delaware, samples were filtered using a 0.7 µm glass fiber filter (Millipore). Filtered samples were placed back into DI-rinsed 250 mL HDPE sample container for chemical analysis and a 40 mL amber glass vial container for fluorescence and UV-visible absorbance analysis. Samples were refrigerated at 4°C until analysis could be completed.

The University of Delaware Soil Testing Laboratory performed the chemical analyses. Solution pH and EC were measured using an Accumet pH meter model AB15 with a SymPHony pH electrode and a VWR Model 1052 conductivity meter with a platinum dip cell, respectively. Here, we focus on base cations due to their
similar behaviors and importance to forest vegetation in both dissolved and particulate form, but P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, and Al were all measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Elemental Intrepid II XSP Duo View, Madison, WI, USA). DIC/DOC and total bound N (TNb) were measured with an Elementar Vario-Cube TOC Analyzer (Mount Holly, NJ, USA), while nitrate-N and ammonium-N were measured with a Bran & Luebbe AutoAnalyzer 3 (Model AA3, Buffalo Grove, IL, USA). A table of soil chemistry results can be found in Appendix E.

4.2.2 Site Descriptions

In Maryland, throughfall samples were collected in a mixed deciduous forest at Fair Hill Natural Resource Management Area (39°43’10" N, 75°49’56" W). Throughfall collectors were placed under Betula lenta L., Fagus grandifolia Ehrh., and Liriodendron tulipifera L. trees. This site receives approximately 1,200 mm annual mean precipitation. The 30-year mean temperature is 13°C (NCEI, 2016). Soils in the area are primarily loam, and silt loams with 0-25% slopes (USDA NRCS, 2012) at an average 70 m elevation above mean sea level.

Rhode Island throughfall samples were collected along a 2.25 km transect of Bailey Brook in Middletown. The 30-year mean temperature is 11°C, and the average annual precipitation is 1,176 mm (NCEI, 2016). Soils in the site consist of silt loams, with slopes of less than 8% (USDA NRCS, 2012) at an elevation of about 47 m above mean sea level. Dominant species at the collection sites consisted of Salix spp., Acer platanoides L., and Nyssa sylvatica Marsh.

In Vermont, throughfall samples were collected in the Wade Brook watershed near Montgomery Center around 390 m above mean sea level (44°52’31" N, 72°34’34"
W). Primary species in this forest are *Acer saccharum* Marsh., *Acer rubrum* L., *Betula alleghaniensis* Britt., *F. grandifolia* Ehrh., and *Fraxinus americana* L. The 30-year mean temperature is 7°C and the average annual precipitation is 1,060 mm (NCEI, 2016). Soils in the site are primarily fine sandy loam, with 3 to 15% slopes (USDA NRCS, 2012).

### 4.2.3 Hierarchical Cluster Analysis

To determine which samples were most similar, and on which characteristics those similarities were based, hierarchical cluster analysis was employed. Samples that are most similar will cluster together, and the result is a distinct cluster (or multiple clusters. Hierarchical cluster analysis with Ward's method minimizes the total within-cluster variance to create clusters of similar objects. In practice, each step calculates the pair of clusters that leads to the least increase in total within-cluster variance after merging the two pairs (JMP 2018). This method was chosen because it is similar to but more forgiving than k-means clustering and will cluster samples into their natural groups with relative ease, assuming that the samples are representative, and the variables are not correlated. For the purpose of this dissertation, we assume these conditions are met.

### 4.3 Results

Between September 2014 and July 2017, 54 precipitation events were sampled at the three sites, of these, 8 events were coordinated sampling events where the precipitation event affected the entire study region which is the focus of this manuscript (Table 4.1). The most common storm type sampled were cold fronts, including two post-tropical cyclone associated fronts. Seasonally, for JJA, the
Maryland site had the highest average precipitation (27.94 +/- 11.87 mm), followed by Vermont (24.34 +/- 7.09 mm) then Rhode Island (11.88 +/- 8.8 mm). Forecast maps for these dates can be found in Appendix F.

Table 4.1: Descriptions of coordinated storm events and air mass provenance for each event and state. For air mass origin, C = Continental, M = Marine origin, derived from the HYSPLIT model. Total event precipitation recorded at the Fair Hill DEOS station in mm. Depth equivalents in mm calculated from collector volume and area. For storm type, CF = cold front. Associated with Hurricane Bill. Associated with Hurricane Patricia. Collectors considered to be full, therefore actual depth equivalent may be greater than listed.

<table>
<thead>
<tr>
<th>Event</th>
<th>Event Date</th>
<th>Season</th>
<th>Pg MD</th>
<th>Pg RI</th>
<th>Pg VT</th>
<th>Storm Type</th>
<th>Air mass origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20-Jun-15</td>
<td>JJA</td>
<td>23.8</td>
<td>12.8</td>
<td>29.3</td>
<td>CF^3</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>20-Aug-15</td>
<td>JJA</td>
<td>21</td>
<td>7.1</td>
<td>18</td>
<td>CF</td>
<td>M/M/M</td>
</tr>
<tr>
<td>3</td>
<td>29-Sep-15</td>
<td>SON</td>
<td>32</td>
<td>30.8*</td>
<td>30.8*</td>
<td>M</td>
<td>M/M/M</td>
</tr>
<tr>
<td>4</td>
<td>9-Oct-15</td>
<td>SON</td>
<td>9.7</td>
<td>10.8</td>
<td>18.7</td>
<td>CF</td>
<td>C/C/C</td>
</tr>
<tr>
<td>5</td>
<td>28-Oct-15</td>
<td>SON</td>
<td>46</td>
<td>30.6</td>
<td>missing data</td>
<td>CF^4</td>
<td>M/M/C</td>
</tr>
<tr>
<td>6</td>
<td>5-Jun-16</td>
<td>JJA</td>
<td>18</td>
<td>5.3</td>
<td>28.2</td>
<td>OF</td>
<td>C/C/C</td>
</tr>
<tr>
<td>7</td>
<td>21-Aug-16</td>
<td>JJA</td>
<td>29</td>
<td>7.4</td>
<td>15.4</td>
<td>CF</td>
<td>M/M/M</td>
</tr>
<tr>
<td>8</td>
<td>6-Jul-17</td>
<td>JJA</td>
<td>47.9</td>
<td>26.8</td>
<td>30.8*</td>
<td>CF</td>
<td>M/M/C</td>
</tr>
</tbody>
</table>

In general, pH was higher for open precipitation than throughfall in all three states, while cation concentration was greater for throughfall at all sites. Of the three sites, precipitation in Rhode Island had the highest concentration of Ca, K, Mg, and Na (Table 4.2, Figure 4.1) Concentrations of sodium, magnesium, and calcium were...
substantially higher in Rhode Island throughfall than in any of the other sample (Table 4.2).

Table 4.2: Base cation concentrations of precipitation and throughfall for selected events by state.

<table>
<thead>
<tr>
<th>State</th>
<th>Event</th>
<th>pH</th>
<th>EC- (mmhos/cm)</th>
<th>Ca-(mg/l)</th>
<th>K-(mg/l)</th>
<th>Mg-(mg/l)</th>
<th>Na-(mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>Pg</td>
<td>6.58</td>
<td>0.02</td>
<td>0.33</td>
<td>0.20</td>
<td>0.13</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>6.27</td>
<td>0.04</td>
<td>1.19</td>
<td>4.35</td>
<td>0.57</td>
<td>2.48</td>
</tr>
<tr>
<td>RI</td>
<td>Pg</td>
<td>6.70</td>
<td>0.12</td>
<td>3.04</td>
<td>0.41</td>
<td>0.82</td>
<td>17.57</td>
</tr>
<tr>
<td></td>
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Figure 4.1: Base cation concentration of open precipitation for all three states. Values of Ca and Na are notably higher in Rhode Island.
While the highest base cation concentration observed in precipitation was Na in Rhode Island, the highest observed throughfall flux is K at the Maryland site, although fluxes of K are higher than other nutrient fluxes at all three states (Figure 4.2). K fluxes are highest at all three sites in the September-October-November season (Figure 4.5) Rhode Island has the highest flux of Na in throughfall, followed by Maryland, then Vermont. The flux timing of Na matches that of K, with the maximum flux and concentration in precipitation occurring the SON season. Median values of Ca flux are similar; however, the Maryland data has greater non-outlier spread than the other two sites.
Figure 4.3: Hierarchical cluster map of throughfall and open precipitation samples for all events. Maryland throughfall samples cluster together (+). Rhode Island samples of open precipitation and throughfall tend to cluster together as well ( Vinci, Δ, ▼). Precipitation samples from Maryland and Vermont also tend to cluster together, joined by only a handful of throughfall samples from Rhode Island (X). Green circles indicate JJA clusters, while blue circles indicate SON clusters.

Hierarchical clustering, using all measured solutes (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Zn) and shown in Figure 4.3, created three primary clusters for all samples collected, and these groups are largely separated by 1) Maryland throughfall samples, 2) all Rhode Island samples, and 3) Maryland precipitation samples. Vermont and Maryland samples are very similar in summer, and therefore those samples appear together in the clusters, while Rhode Island has a distinct pattern due
to very high levels of calcium in the samples. 63% of Rhode Island samples are separated by these distinct calcium concentrations. In autumn, Maryland concentrations can be similar to both Rhode Island and Vermont, but Rhode Island and Vermont are almost always distinct from each other (Figure 4.5). Cluster analysis using only the base cation fluxes shows some clustering by season, but seemingly not enough variables to create true clusters based on these fluxes alone (Figure 4.4).
Figure 4.4: Hierarchical cluster map of growing season base cation fluxes for all events. JJA samples cluster together (red), and SON samples cluster together (blue) but states are mixed within these clusters (MD, ○; VT, ◇; RI, +).

These events demonstrate little pattern in solute concentration (Figure 4.3), but linear least squares regression, and following ANOVA show that solutes are
significantly different within the samples, and more often as a function of the site, rather than the event itself (Table 4.3).

Figure 4.5: Total base cation fluxes for all three states by the coordinated event. Throughfall base cation fluxes for events show fluxes are much higher during autumn than summer, particularly in K.
Table 4.3: Results of analysis of variance following linear least squares regression. Base cations concentrations show significant differences among the samples as a result of the state.

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4.4 Discussion and Conclusions

Base cations are highly variable among and between sites, events, and along the transect in its entirety. Results suggest that control of differences in base cation fluxes between sites is dominated by season, likely because season plays a major role in aerosol deposition to forested regions and the types of large-scale precipitation events occurring at a location. Nearly all nutrients show maximum concentrations during summer. The knowledge of variations in leachable foliar nutrients of throughfall, especially with the season, is not new (Gallardo et al., 1998; Scudlark et al., 2005; Van Stan et al., 2012). Nor is the deposition of Na in coastal areas surprising. What is novel and of interest here is that, at the event and seasonal scales at the regional level, results suggest that the seasonal control can be easily overridden by local geographic factors (which shows up as “state” in the ANOVA). In the case of our study locations, these factors include nearby agriculture at Fair Hill NRMA and high deposition of Na and other solutes from ocean breezes at the Rhode Island sites. This local-to-regional geography explains why the effect tests suggest that, after season, the state is the primary driver of differences in solute concentrations of throughfall between these sites.
For many of our analyses, Maryland and Vermont (or Maryland and Rhode Island) shared characteristics for both throughfall and open precipitation, but rarely did Vermont and Rhode Island share characteristics within samples. This result is surprising, given that our leachate study (Hudson et al., 2018) suggested very strong geographical dissimilarities of leachate impacts in watersheds, especially by latitude, and we expected to see a similar trend of distinct groups for the base cations by the state in this study as well but did not.

The dominance of the effect of the season in our analysis highlights the need for large spatial-scale comparisons such as this to monitor the potential impacts of anthropogenic climate change on the shifting of precipitation regimes, as these regimes have the potential alter the timing and type of solute input to forested watersheds. Additionally, the influence of local geographic factors may be altered in the future by short term land use changes, altering the aerosol deposition on forested watersheds.

4.5 Acknowledgments

This work was made possible with financial support from the U.S. National Science Foundation (Grant No. IIA-1330238, IIA-1330446, and IIA-1330406), as part of the larger North East Water Resources Network Project, and the authors would like to acknowledge the hard work of the many other members of this project who have worked alongside us for the duration of the grant. The authors would like to thank the Inamdar Watershed Hydrochemistry Group and the University of Delaware Soil Testing Lab for their laboratory analyses. All field sites are thanked for their permission to collect precipitation and throughfall samples.
Chapter 5

MAPPING BARK BACTERIA: INITIAL INSIGHTS FROM STEMFLOW COLLECTED FROM URBAN AND RURAL TREES

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5.1 Summary

Urban areas create differences in the ecological climatology of forest fragments located within them. Unfortunately, even the most detailed of urban forest research initiatives often overlook microbial biodiversity and bark surfaces are underrepresented in the literature. Given our knowledge of microbial diversity in the phyllosphere and rhizosphere, we hypothesize that bark may create a microenvironment that selects for specific microbes. To better understand the bacterial biogeography and the resultant impact on hydrological processes for individual trees as a function of land use, bacterial samples were collected from bark and stemflow from trees in rural and urban forest fragments in the mid-Atlantic before and after a
rain event. Results obtained using well-established DNA extraction methods, modern sequencing, and the latest bioinformatics software suggest significant differences in amplicon sequence variants between the rural and urban sites and between the northerly and southerly aspects of the sampled trees. The primary driver may be increased nutrient loads at the rural site. Major groups of bacteria present on bark at both sites include *Acidobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes*, while cultured stemflow samples had higher counts of *Bacteroidetes*. Using these new data, we hope to investigate the reciprocal effects of microbial communities on stemflow in forested ecosystems.

5.2 Introduction

5.2.1 Plant-microbe Interactions in Forested Ecosystems

The last decade has given way to a great amount of learning in microbial ecology. Advances in sequencing technology have allowed us to sequence plant genomes more than six hundred times the size of the human genome (Frazier et al., 2001; Zimin et al., 2014). We’ve come much closer understanding the important relationships between symbiotic (and pathogenic) microbial communities on and within hosts and surfaces such as the human gut, city concrete, and insects who burrow in the sapwood of trees (Maresca et al., 2017; Ceppa et al., 2018; McAllister et al., 2018; Vangay et al., 2018; Pasolli et al., 2019). We now know that microbial communities are responsible for the greatest amount of nitrogen fixation and biogeochemical cycling at the global scale (Kirchman, 2012). A recent study even shows that there are strong seasonal patterns to airborne microbial communities, which may have great impacts on the seasonal ecology of forests and other land uses.
(Cáliz et al., 2018). And at a basic level, we’ve even come to understand better how microorganisms should be classified, and just how little we really do know about the number and function of these organisms.

Great strides have also been made in our understanding of microbial communities in forested ecosystems. Baldrian (2017) highlighted many advances in our understanding of forest microbes, including those in tree habitats such as the phyllosphere, wood, bark surface lichens, and soil (Baldrian, 2017). Still, even in this recent review, bark surface bacteria outside of free-living cyanobacteria are unknown. The functions, effects, and interrelationships of microbes with the macroscopic world are still not thoroughly understood (Pace, 1997; Andrews and Harris, 2000; Caporaso et al., 2011; Lambais et al., 2014; Levia and Germer, 2015; Graham et al., 2016). We do know that both fungi and bacteria inhabit tree canopies and root systems, but with the exception of Leff et al. 2015 which explicitly quantified bacterial communities on the bark of three Gingko biloba in an arboretum in Boston, Massachusetts we have very little knowledge of bacterial life on the bark surface, also known as the cortisphere (Lindow and Leveau, 2002; Sridhar and Karamchand, 2009; Bai et al., 2015; Bruez et al., 2015; Grube et al., 2015; Mason et al., 2015; Khan et al., 2016; Laforest-Lapointe et al., 2016).

Similarly, rainfall and nutrient partitioning studies have gained significant momentum in temperate regions over the past decade, but there are still substantial gaps in our understanding of these processes including those processes pertaining to the communities of microorganisms living on and within the bark surfaces of plants (Ohte and Tokuchi, 2011; Lambais et al., 2014; Levia and Germer, 2015). In a recent review article, Levia & Germer (2015) highlighted some questions which have yet to
be addressed in the field of ecohydrology regarding microbial communities. With relevance to the bark surface, these questions include how species composition of microorganisms varies by ecoregion and phenoseason, how these communities affect stemflow chemistry, and how the bark characteristics that are unique to individual woody plant species might impact the composition of microorganisms present on the bark. The same review of stemflow literature also highlights the paucity of knowledge available on these microorganism’s interactions with stemflow and nutrient cycling, especially at the plant-soil interface (Levia and Germer, 2015). In fact, a keyword search (cortisphere, bark, stemflow, bacteria, microbe) on Web of Science produces only a handful of studies in which authors from a broad range of specialties attempting to address related hypotheses merely suggest the need for further research in this realm (Whitford et al., 1997; Bischoff et al., 2015; Köhler et al., 2015; Van Stan and Pypker, 2015). Additionally, only a limited number of studies have attempted to investigate the inverse of these questions, in which the impacts of stemflow on the soil microbial and epiphytic communities are considered (Ceccherini et al., 2008; Rosier et al., 2016).

Both throughfall (precipitation falling through the canopy) and stemflow (precipitation funneled down branches and stems to the bole) transfer nutrients and water to the base of the plant creating a “fertile island” in soils surrounding the trunk, and another recent study has found stemflow to be a way in which rotifers and nematodes can be transferred to the soil (Whitford et al., 1997; Levia and Frost, 2003; Levia et al., 2011; Kuzyakov and Blagodatskaya, 2015; Ptatscheck et al., 2018). Surprisingly, we could find no study which demonstrates the direct interactions of cortisphere communities on stemflow chemistry.
5.2.2 Trees: Habitat and Rainfall Partitioning

A number of studies have shown that microbial communities on plants are very species-specific, and trees are no exception (Lambais et al., 2006, 2014; de Bello et al., 2010; Bruez et al., 2015; Paine et al., 2015). Each tree, and specifically the bark of a tree, likely provides a unique microenvironment in which the turbulence of the air, moisture, and pH will be altered from the larger environment due to bark texture, aspect, and position within the stand (which are all additionally a product of tree species and age). Trees can also serve as a source of enrichment in forested watersheds through rainfall partitioning. During a rain event, approximately 70-90% of incident precipitation that is intercepted by a forest canopy reaches the ground, depending on forest type and meteorological factors (Levia and Frost, 2003). Of that, 90% of precipitation per projected canopy area often falls as throughfall, precipitation passing directly through the canopy or coalescing and dropping from leaves and woody surfaces (Levia and Frost, 2006; Levia et al., 2011). Throughfall often has a short to medium residence time in the canopy (Levia and Frost, 2006). The other <10% percent of intercepted precipitation per projected canopy area is funneled on the bark surface, down stems and branches, as stemflow. Although it is typically less than 10% of intercepted precipitation, and sometimes overlooked, stemflow represents a highly enriched, point-source input of nutrients at the base of trees in a forested ecosystem due to its long residence times on tree surfaces (Levia Jr. and Herwitz, 2000; Levia and Herwitz, 2002, 2005; Johnson and Lehmann, 2006b; Llorens and Domingo, 2007; Levia et al., 2012; Carlyle-Moses et al., 2018). The remaining intercepted water never reaches the ground and may be stored on foliar and woody surfaces, sometimes ranging from 1.3 mm up to 4 mm (Zinke, 1967; Llorens and Gallart, 2000; Link et al.,
This water will evaporate as interception loss, and timing will depend on the meteorological conditions and tree species (Carlyle-Moses and Gash, 2011).

In the United States, nearly 80% of people live in urban areas, and there are more than 70 million acres of urban forest ecosystems cared for by the U.S. Forest Service. Urban areas create an array of differences in the ecological climatology of forest fragments located within them. In urban areas, partitioning of precipitation by trees can help to slow the movement of rainwater to stormwater infrastructure, reducing peaky and flashy hydrographs and moderating runoff within an urban catchment (Michopoulos, 2011). Unfortunately, our understanding of elemental fluxes in stemflow in urban forests is still limited, and comparison of urban to rural forests is often complicated by geographic factors like differing soils (Michopoulos, 2011).

Regardless of location, the long residence time of water on bark, both as stemflow and water storage, not only allows for the chemical enrichment of stemflow but also provides the medium for the existence of bacteria. Previous studies have shown contrasting results when observing the effects of the distance between trees of the same species on bacterial communities associated with the phylloosphere. We can extend this to the cortisphere and hypothesize that the bark characteristics of the individual trees will induce changes in bacterial community composition present on tree bark due to the unique microclimate generated by bark surface morphology. Due to differences in temperature, nonpoint pollution, precipitation, nutrient inputs at the base of the tree, biodiversity, and air conditions between a rural forested site and urban forest fragment, it is hypothesized that species composition will also be different between sites.
Because baseline information of corticular bacteria is deficient, we conducted this pilot study as a foundation for further research, and here provide the community results thereof. Using this data as the starting point, we hope to attempt to understand the differences between corticular microbial communities based on land use and the subsequent enrichment of stemflow.

5.3 Results

5.3.1 Storm Event and Stemflow

The stratiform rain event took place 18-19 November 2017, and samples were collected on November 17 and November 19. In the two weeks leading up to the storm event, the average air temperature was 7°C. The study trees, and most surrounding canopy trees, still had their foliage. The storm produced 8 mm of precipitation and had winds averaging 1.4 m s\(^{-1}\) out of the west-northwest (average 276°). This event produced 150 mL of stemflow at Fair Hill and 23,911 mL of stemflow at Banning Park.

5.3.2 Sequencing Results

For this study, we collected a total of 68 bark samples (32 samples from the bark of each tree), three stemflow fluid samples (two from Banning Park, one from Fair Hill NRMA), and one sample from the open precipitation. Each of these samples was analyzed using 16S rRNA gene sequencing (see Experimental Procedures). The experimental procedures used in this pilot study produced over a million sequences from bark and stemflow.
Figure 5.1: Stacked bar chart of relative percentages of amplicon sequencing variants. In the Banning stemflow sample (BSF), greatest percentages are Bacteroidetes and Firmicutes. In the Fair Hill stemflow (FH SF) and Open (rainfall), the greatest percent of the sample are Gammaproteobacteria. Largest percentages are of bark samples are Acidobacteria, Alphaproteobacteria, and unassigned bacteria.
Figure 5.2: Heatmap of abundance for both bark tissue and environmental fluids. A. Abundance in environmental fluids, where those fluids are two samples of stemflow from Banning Park, one sample of stemflow from Fair Hill, and the open precipitation sample from Fair Hill. Primary abundance in FH SF and Open Pg is *P. agglomerans*, followed by *Gammaproteobacteria* genus and *Bacteroidetes*. B. Unassigned bacteria are most abundant on bark tissue samples, as well as *Acidobacteria* and *Proteobacteria*. C. Bark samples group primarily by site more than by height, direction, or time, although these factors are critical for individual phyla.
Banning Park bark community samples were more tightly related to each other than the Fair Hill bark community samples, which had more ecological variation among samples and several samples that were very different from the others (ex. 10 and 26; pre- and post-event, respectively, both east-facing, at 50cm height), although the Shannon diversity indices suggest the two sites have similar amounts of bark community diversity (1.96 for Fair Hill and 1.93 for Banning, when $H_{\text{max}}$ is 3.43). The largest percentage of reads assigned to bacteria, but not a defined phylum (Figures 5.1 & 5.2). The major phyla of the bark samples include *Acidobacteria*, *Actinobacteria*, *Proteobacteria* (especially *Alphaproteobacteria*), and *Bacteroidetes*, with smaller relative abundances of *Verrucomicrobia* (Figure 5.1 & 5.2). Although community differences were driven largely by site, some of the major taxa also varied significantly with the aspect from which the sample was taken (*Bacteroidetes*, $p = 0.0242$; *Proteobacteria*, $p = 0.0372$; *Verrucomicrobia*, $p = 0.0257$) and with height (*Actinobacteria*, $p = 0.0475$; *Chloroflexi*, $p = 0.0005$; unassigned, $p = 0.0043$). Of course, it is simple enough to throw all the samples into the software “pot” if you will and say that height, aspect, and time are not significant, but the real story is a bit subtler than that. To determine the role of stemflow in microbial translocation, i.e., phyla being washed down the woody surface, the phyla abundance data before the event was subtracted from the post-event values ($\Delta$ abundance). Locations with positive $\Delta$ abundance values have higher abundance data for a given phylum *after* the precipitation event relative to the pre-event conditions, values around zero (+/- 20) show no difference in abundance after the precipitation event, and negative values indicate higher abundance numbers before the precipitation event. Only at Banning Park, at the 4m sampling height, for the north and east sampling points were all $\Delta$
abundance positive for all selected phyla. Results for selected phyla are shown in Table 5.1 and Figures 5.3 to 5.10 and are presented below. For some bacteria (e.g., Chloroflexi, Deferribacteres, Fibrobacteres, Fusobacteria, Gracilibacteria, Hydrogenedentes, Microgenomates, Parcubacteria, Spirochaetae, unassigned bacteria, Tenericutes, Chlamydiae, Elusimicrobia, FBP, Gemmatimonadetes, Saccharibacteria), initial abundance is little to none, and there is little to no change with event (median abundance of less than ~15), and so for this analysis, they are not included.
Table 5.1: Δ abundance values for select phyla at all four heights for both sites.

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<td>227</td>
<td>1592</td>
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<td>1057</td>
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<td>1453</td>
<td>1051</td>
<td>757</td>
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<tr>
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<td>459</td>
<td>-66</td>
<td>1004</td>
<td>-673</td>
<td>818</td>
<td>2715</td>
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<td>-1935</td>
<td>-1407</td>
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<td>-867</td>
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<tr>
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<td>481</td>
<td>-223</td>
<td>882</td>
<td>678</td>
<td>848</td>
<td>-110</td>
<td>6</td>
<td>-3780</td>
</tr>
<tr>
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<td>-476</td>
<td>-746</td>
<td>2421</td>
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<td>-698</td>
<td>19</td>
<td>-369</td>
</tr>
<tr>
<td>0.5</td>
<td>270</td>
<td>493</td>
<td>-1946</td>
<td>1473</td>
<td>-6</td>
<td>467</td>
<td>-694</td>
<td>-4</td>
<td>-265</td>
</tr>
</tbody>
</table>
Strongly positive Δ abundance for Acidobacteria at Banning Park at the 4m level occurred in all aspects except south, where a highly negative Δ abundance value was observed (Figure 5.3). At 2.4m, positive Δ abundance values are found on the south and west faces of the tree, with negative Δ abundance values at all other aspects. Positive Δ abundance values occurred for all aspects at 1.5 meters. High positive Δ abundance values were noted at all aspects except a highly negative Δ abundance value at the west sample point at 0.5 meters. For Fair Hill, positive Δ abundance values were found for all aspects except south, which was highly negative, at 4 meters. At 2.4m, positive Δ abundance was found at all aspects except for south, with north and east values being strongly positive, and the southern value being highly negative. At 1.5m, positive Δ abundance values occur at the east and west aspect sampling points, and negative Δ abundance values present at the north and south sampling points. Below the stemflow collar, positive Δ abundance values are found on the east, and south faces of the tree bole and a strongly negative Δ abundance value occurred at the north sample location.
Figure 5.3: Contour plot of *Acidobacteria* Δ abundance. Phylum Δ abundance was visualized as a contour plot with the y-axis representing the height of the sampling location (m), and the x-axis representing aspect. North direction is shown at both ends to represent the closing of the cylinder, which is the tree bole. Data were smoothed using a 3rd order polynomial spline to aid in the interpretation of bark surface patterns of abundance. Blue vertical line represents the predominant wind direction for the storm event sampled, while the horizontal dashed line represents the height of the stemflow collar. Green areas show where a given phylum is more abundant after the storm event, white show areas of no difference and the brown end of the color map represents areas where phylum is less abundant after the storm event.
High positive Actinobacteria Δ abundance values at Banning Park at the 4m height were found at the north and east sample points (Figure 5.4). At 2.4m positive Δ abundance were found for all aspects except for north. For 1.5m, positive Δ abundance values occur only on the north and east aspect sample points. Below the stemflow collar (.5m) positive Δ abundance is only found on the east facing side of the tree, with strongly negative Δ abundance values occurring on the south and west faces. At the 4m sample height at Fair Hill, positive Δ abundance values occur on the north, east, and west sides of the tree. At 2.4M, positive Δ abundance occurs in all aspects except for the south, which is highly negative. At 1.5m, all aspects produced high, positive Δ abundance values except for the west facing sample location. For the 0.5m sample height, high positive Δ abundance values occurred at the east and west sample points, with a highly negative Δ abundance value occurring on the north side of the tree.
Figure 5.4: Contour plot of *Actinobacteria* Δ abundance. Refer to Figure 5.3 for a full figure caption.

Bacteroidetes Δ abundance was positive at the 4m sampling height for all aspects except the western aspect location at Banning Park (Figure 5.5). At 2.4m, all aspects had positive to strongly positive Δ abundance values. For the 1.5m sampling height, positive Δ abundance values were found at the east and south sampling points, with negative values at north and west. At 0.5 meters, positive Δ abundance values were found for all aspects except a strongly negative Δ abundance value for the west facing sample point. At the Fair Hill site, positive Δ abundance values were found for all aspects at the 4m sample height. At 2.4m, all aspects had positive Δ abundance
except for a highly negative value on the south sampling point. For the 1.5m samples, Δ abundance values range positive to highly positive for all aspects except for a highly negative value observed on the west face of the tree. All Δ abundance values were negative below the stemflow collar at 0.5m.

Figure 5.5: Contour plot of Bacteroidetes Δ abundance. Refer to Figure 5.3 for a full figure caption.

Positive Δ abundance for Cyanobacteria at the 4m height at Banning Park is found at all aspects except west (Figure 5.6). At 2.4m, positive Δ abundance is only found at the west aspect sampling point, with all other aspects having negative Δ
abundance values. At 1.5m, positive Δ abundance values are observed at the south and west sampling points with all other aspects having negative Δ abundance values. Below the stemflow collar, all aspects except for north had positive Δ abundance values. At Fair Hill, positive Δ abundance values at 4m were observed at the north and west sampling points, with all other aspect having negative Δ abundance values. At 2.4m, strongly positive Δ abundance values were observed at the north and east sampling points, with a highly negative Δ abundance value occurring at the southern aspect point. At 1.5, positive Δ abundance values were only observed at the north aspect point, with strong negative Δ abundance values found at the east and west points. At .5m, positive Δ abundance was found at the north aspect sample point, with strong negative Δ abundance values occurring at the east and west sampling locations.
Firmicutes Δ abundance values were positive for all aspects at the 4m height at Banning Park except the western aspect (Figure 5.7). At 2.4m, all Δ abundance values were negative. At 1.5m, Δ abundance values were positive for the north aspect point and negative for the south and west sample locations. Below the stemflow collar, Δ abundance values were positive at the east and south sample points, and negative at the west facing sampling point. For Fair Hill, Δ abundance values at the 4m height were negative, for all aspects. At 2.4m, Δ abundance values were positive for all aspects except the south. At 1.5m, Δ abundance values were positive at all aspects.
except north. Below the stemflow collar, all Δ abundance were positive except at the west aspect site, which was negligibly negative.

Figure 5.7: Contour plot of Firmicutes Δ abundance. Refer to Figure 5.3 for a full figure caption.

Higher Δ abundance values for Planctomycetes at Banning Park at the 4m sampling height are found for all aspects except the western aspect (Figure 5.8). At the 2.4m level, only the south facing point has positive Δ abundance values, while all other aspects have negative Δ abundance values. Positive Δ abundance values at the 1.5m height are only found at the east facing location, with negative values occurring
at the south and west aspect points and negligible difference at the north facing point. Below the stemflow collar (.5m) positive Δ abundance values are found at the north and east sampling points, while negative Δ abundance values occur at the south and west sample points. At Fair Hill, higher Δ abundance values at the 4m elevation are only found at the west aspect sampling point; with negative Δ abundance occurring at all other aspects. At 2.4 meters, positive Δ abundance values are found at all aspects except the south aspect sampling point. At 1.5m, positive Δ abundance values are found at the east, and south aspect points with negative values found at the north and west points. Below the stemflow collar (.5m), positive Δ abundance values only occur at the east and west sampling points.
Proteobacteria had positive Δ abundance values at the 4m height at Banning Park for all aspects (Figure 5.9). At 2.4 m, the only positive Δ abundance value occurred at the southern aspect, with a strongly negative value recorded at the north sampling point. At 1.5m, Δ abundance values were positive at the east and south sampling points, negative at the west point, and strongly negative at the north location. Below the stemflow collar, Δ abundance values were positive at the north and east sample points, negative at the south sample point, and strongly negative at the west sampling location. At Fair Hill, Δ abundance values were positive at the north and
west sample point, and negative to strongly negative at the south and east sample points respectively. At 2.4m, Δ abundance was either strongly positive at the north and west aspect points or strongly negative at the east and south locations. For the 1.5m height, Δ abundance was positive at the north and south locations, negative at the west sample point, and strongly negative at the eastern sample point. Below the stemflow collar, all Δ abundance values were negative to strongly negative.

Figure 5.9: Contour plot of *Proteobacteria* Δ abundance. Refer to Figure 5.3 for a full figure caption.
Verrucomicrobia Δ abundance values were positive at 4m sampling height at Banning Park for all aspects except west (Figure 5.10). At 2.4m, positive Δ abundance values are observed at the south and west sampling locations, with negative Δ abundance values present at the north and east sample points. At 1.5m, positive Δ abundance values are observed for the east and south sampling locations, with negative values for the north and west positions. At 0.5m, the 1.5m pattern is replicated, with positive values for the east and south locations, and negative values at the north and west locations. At Fair Hill, for the 4m sampling location, positive Δ

Figure 5.10: Contour plot of Verrucomicrobia Δ abundance. Refer to Figure 5.3 for a full figure caption.
abundance values are present at the north and west sampling locations, with negative Δ abundance values for all other aspects. At 2.4m, all Δ abundance are positive except the south facing sample position. At 1.5m, highly positive Δ abundance values are observed for the east and south sample locations, with negative values at the north and west positions. The 0.5m sample height had all positive Δ abundance values except for the southern aspect sampling points.

On the Fair Hill NRMA tree bark above the stemflow collar, *Cyanobacteria*, and *Planctomycetes* populations decreased after the rain event, while *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* all showed increased populations after the rain event (Figure 5.11B). Below the stemflow collar on the tree at Fair Hill, *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* corticular populations decreased after the rain event, while *Verrucomicrobia*, *Acidobacteria*, *Planctomycetes*, and *Actinobacteria* populations all increased after the rain event (Figure 5.11A).
Figure 5.11: Difference plots show that there are differences in the populations which are above the stemflow collar before and after rain events: Abundances near zero (less than removed, as they indicate little to no change in abundances with rain event. A) Change in abundances on bark surface below the stemflow collar with rain event. Values below the zero line indicate depletion of the population on bark after a rain event; values above the zero line indicate an increase in population on bark after a rain event. B) Change in abundances on bark surface above the stemflow collar with a rain event. Values below the zero line indicate depletion of the population on bark after a rain event; values above the zero line indicate an increase in population on bark after a rain event. C) Most abundant phyla are remaining in stemflow after the subtraction of bulk precipitation. Values above the zero line indicate greater abundances of respective phyla in stemflow than rainfall.
On the Banning Park tree *above* the stemflow collar, *Firmicutes* and *Planctomycetes* corticular populations decreased after the rain event, while *Cyanobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Proteobacteria* populations on bark increased after the rain event (FIG 5.11B). Below the stemflow collar, *Actinobacteria* and *Proteobacteria* populations were both less after the rain event, and *Acidobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia*, and *Planctomycetes* all showed larger corticular populations after the rain event (FIG 5.11A).

Banning Park stemflow samples were more diverse than the stemflow collected at Fair Hill NRMA (Shannon Diversity indices of $H=1.52$ and $H=0.88$, respectively). These indices are reasonable, given that other natural aquatic and soil diversity indices can range from 1.5 to 6. Fair Hill NRMA stemflow and precipitation samples were mostly dominated by *Proteobacteria* (particularly *Gammaproteobacteria*), *Bacteroidetes*, and *Firmicutes* (Figure 5.1 & 5.2). On average, the abundance of *Cyanobacteria* and *Planctomycetes* was greater in stemflow at Fair Hill than in the open precipitation, while *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* show greater populations in precipitation than in stemflow (Figure 5.11C). Banning park stemflow was dominated by *Bacteroidetes* and *Firmicutes*, and on average, *Firmicutes* and *Planctomycetes* show greater populations in stemflow than in precipitation, while *Cyanobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Proteobacteria* populations are greater in the open precipitation (Figure 5.11C). These delta values can also be seen in Table 5.1 and 5.2.
Table 5.2: For clarity, the following tables provide the mean, median, and upper and lower percentile values of the delta abundances for dominant phyla at the two sites, both above and below the stemflow collar.

<table>
<thead>
<tr>
<th>Fair Hill changes in abundances (Δabundance) above stemflow collar with rain event for dominant phyla</th>
<th>Banning Park changes in abundances (Δabundance) above stemflow collar with rain event for dominant phyla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>358.00</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>788.00</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>415.50</td>
</tr>
<tr>
<td>Cyanobacteria</td>
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</tr>
<tr>
<td>FBP</td>
<td>7.00</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>17.50</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>16.50</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>-12.50</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>47.00</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>225.50</td>
</tr>
<tr>
<td>Phyla</td>
<td>Fair Hill Median</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Acidobacteria</td>
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</tr>
<tr>
<td>Actinobacteria</td>
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<tr>
<td>Bacteroidetes</td>
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</tr>
<tr>
<td>Chloroflexi</td>
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</tr>
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<tr>
<td>FBP</td>
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</tr>
<tr>
<td>Firmicutes</td>
<td>58.25</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>167.75</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>1975.50</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>538.25</td>
</tr>
</tbody>
</table>
5.3.3 Odds Ratios

Results of the odds ratios calculations show similar patterns to the contour plots. Comparing Figures 5.3 to 5.10 with the following table, Table 5.3, you can see for example that there are slightly greater odds for decreasing phyla Δ abundance with rain event on the north aspect of the tree at Fair Hill, except for *Cyanobacteria* and *Verrucomicrobia*.

Table 5.3: Odds ratios for the major phyla at the two sites for both 1) north, east, south, and west aspects, and 2) 0.5 m, 1.52 m, 2.44 m, and 3.96 m heights.

<table>
<thead>
<tr>
<th>1. Odds Ratios - Aspect</th>
<th>Fair Hill</th>
<th>Banning Park</th>
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<tbody>
<tr>
<td></td>
<td>N E S W</td>
<td>N E S W</td>
</tr>
<tr>
<td><em>Planctomycetes</em></td>
<td>1.14 0.74 0.99 0.74</td>
<td>0.98 0.97 1.26 1.22</td>
</tr>
<tr>
<td><em>Cyanobacteria</em></td>
<td>0.74 1.32 1.77 2.98</td>
<td>1.35 1.47 0.91 0.59</td>
</tr>
<tr>
<td><em>Actinobacteria</em></td>
<td>1.08 0.55 1.06 0.70</td>
<td>0.73 0.79 1.48 1.36</td>
</tr>
<tr>
<td><em>Acidobacteria</em></td>
<td>1.17 0.63 0.98 0.86</td>
<td>0.66 0.76 0.87 1.19</td>
</tr>
<tr>
<td><em>Verrucomicrobia</em></td>
<td>0.72 0.58 0.87 0.90</td>
<td>1.10 1.12 0.90 0.99</td>
</tr>
<tr>
<td><em>Bacteroidetes</em></td>
<td>1.05 0.78 0.77 1.34</td>
<td>0.84 0.78 0.89 0.96</td>
</tr>
<tr>
<td><em>Firmicutes</em></td>
<td>1.00 0.61 1.37 0.69</td>
<td>0.82 0.91 1.07 1.43</td>
</tr>
<tr>
<td><em>Proteobacteria</em></td>
<td>1.14 2.11 0.99 0.92</td>
<td>1.32 1.21 0.99 0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Odds Ratios - Height</th>
<th>Fair Hill</th>
<th>Banning Park</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 1.52 2.44 3.96</td>
<td>0.5 1.52 2.44 3.96</td>
</tr>
<tr>
<td><em>Planctomycetes</em></td>
<td>0.71 0.96 0.80 1.14</td>
<td>0.94 0.98 1.44 0.94</td>
</tr>
<tr>
<td><em>Cyanobacteria</em></td>
<td>1.18 1.59 0.77 1.12</td>
<td>0.87 1.35 1.37 0.79</td>
</tr>
<tr>
<td><em>Actinobacteria</em></td>
<td>0.92 0.68 0.71 0.89</td>
<td>1.40 0.81 0.83 1.21</td>
</tr>
<tr>
<td><em>Acidobacteria</em></td>
<td>0.73 0.99 0.93 0.97</td>
<td>0.91 0.71 0.99 0.79</td>
</tr>
<tr>
<td><em>Verrucomicrobia</em></td>
<td>0.60 0.76 0.72 0.94</td>
<td>0.84 1.19 0.93 1.06</td>
</tr>
<tr>
<td><em>Bacteroidetes</em></td>
<td>1.14 0.76 1.07 0.74</td>
<td>0.89 1.05 0.42 1.25</td>
</tr>
<tr>
<td><em>Firmicutes</em></td>
<td>0.41 0.67 0.78 1.63</td>
<td>0.90 1.20 1.95 0.51</td>
</tr>
<tr>
<td><em>Proteobacteria</em></td>
<td>1.34 1.26 1.52 1.10</td>
<td>1.10 1.13 1.23 1.11</td>
</tr>
</tbody>
</table>
5.4 Discussion

Our results are similar to a study by Leff et al., (2015) using *G. biloba*, which found large numbers of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* on trunk and bark tissue, some differences with height, and greatest phylotype richness on trunk and bark tissues (Leff et al., 2015). The Shannon diversity indices reported above seem to be on par with other aqueous and environmental samples.

We believe that these results demonstrate that there are dominant stable communities of bacteria present on the bark surface and that some bacteria are deposited in the canopy and washed down the trunk via precipitation partitioning. In this study, the stemflow collar, placed at 1.3 m above the ground, created differences in abundances above and below the collar. Differences between the populations above and below the stemflow collar suggest that stemflow may, in fact, be a mechanism of “repopulation” for these phyla. The stemflow collar effectively captures and diverts a majority of stemflow that would otherwise flow all the way to the soil; if the stemflow is blocked, there is no increase in some phyla below the collar for those events, which suggests that stemflow is a source for the deposition of bacteria to the trunk bark surface. The Δ abundance values below the stemflow collar represent the change in abundance with stemflow excluded, meaning, there would be no downward flux of ASVs, and any changes in values are due to direct precipitation interception by the trunk, soil splash, or biotic response to lessened atmospheric evaporative demand. But the Fair Hill stemflow collar was placed just before the event, while the Banning Park stemflow collar had been in place for two years. It is possible that the Banning park
tree could have developed a “stemflow-less” microbial ecosystem, while the Fair Hill tree below the stemflow collar may still represent a more stemflow dependent ecosystem. Regardless, the phyla with the highest levels of depletion post-event, are the same phyla that show up most commonly in stemflow: *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*.

Fair Hill NRMA does seem to have a local external source of *Proteobacteria* (*P. agglomerans*, possibly from hay baling in this semi-agricultural area) and this external source could have ended up in the precipitation sample as well, creating artifacts in the results for the Banning Park *Actinobacteria* and *Proteobacteria* values. In future work, this could potentially be confirmed by checking our samples for chloroplasts that may have been screened out from the Fair Hill samples during processing.

We are unsure what may have been the cause of the depletion patterns of different phyla with the rain event. We expected that the most important controlling factor of differences in these populations would be the rain itself and that we would see greater separations of the populations before and after the event due to moisture availability. This turned out to be the least important factor after site, height, and aspect. The third most important factor, aspect (or direction in Figure 5.2) has a lot to do with insolation. South facing bark will experience more solar radiation throughout the day and therefore be warmer and drier than the north facing bark, which will be cooler and wetter. The higher moisture availability and likely existence of shade and damp loving mosses and fungi with which bacteria are commonly associated lead us to believe that this factor would also be more important. Height turned out to be an important factor in differentiating of phyla, which we expected would be due to soil
bacteria nearer the roots and leaf bacteria at the higher sampling locations, but again, even with this factor’s importance, there seems to be very little discernable pattern as to why. Using the odds ratios (Table 5.3), we can directly compare the odds of a phylum increasing with aspect or height. It is possible to see that, beneath the stemflow collar, odds ratios favor increases in most phyla after the rain event, while *Proteobacteria* are most likely to decrease or stay the nearly the same at all sampling locations. Regardless, few patterns exist within these results. Among other shortcomings of the study, a lack of good meteorological data beneath the canopies means that we can only assume the impacts of predominant wind direction and rain inclination on the microbial populations beneath the canopy on trees that are removed from the edges of the stand (Table 5.4). No aspect shows consistent Δ abundance at all heights, as one might expect. But, we propose that, in addition to the disruption of stemflow (and therefore the transport of microbiota) by the collar, changing furrow networks of the bark may be responsible for the clear lack of sequence or order to samples by either aspect or height on the trunk. Additionally, our sampling methodology of removing the very bark surface may have had more impact than we expected. In future work, it would be of great benefit to 1) conduct the study on both smooth and rough-barked trees, to see if there is a possibility of eliminating the furrow variable, and perhaps finding a pattern of depletion or deposition, and 2) develop a horizontal, non-destructive sampling methodology, one that also included sample cultivation, to eliminate those additional opportunities for artifacts in the data (Table 5.4).

Most importantly, results of this pilot study indicate that an ecologically diverse community of bacteria are present on the bark surface *Quercus rubra* at these
two sites, which to our knowledge has not been shown before. Our understanding of the current literature leads us to believe that it is highly likely that these communities may be responsible for the chemical enrichment of or at least interaction with stemflow at this, and likely in all forested areas. The function(s) of these communities, albeit outside the scope of this pilot study, may be connected to forest health and plant resilience, especially if one considers 1) the plant benefits of known bacteria in the phyllosphere and rhizosphere, particularly those benefits involving biogeochemistry, and the existence of many ubiquitous soil bacteria colonizing these trees (e.g., *Pseudomonas, Sphingomonas, Curtobacterium, Hymenobacter, Macilaginibacter*; 2) the many examples of niches elsewhere in ecology in which certain mutualistic or commensurate symbiotic relationships exist and disallow the existence of parasitic or pathogenic bacteria, for example, *S. rhizophila*, or the complete opposite case in which a typically mutualistic plant-associated bacteria cooperate with a pathogen to increase disease severity, as in the case of *P. agglomerans*. It is our hope that the significant scientific implications of the presence of species- and bark-selected bacteria on the bark surface of trees alone will be motivation for future studies and expanding the literature on corticular communities. Secondly, we were able to show that site (Fair Hill NRMA (rural) or Banning Park (urban fragment), Figure 5.2) is the most influential factor driving the differences in ecology between samples. Higher stemflow enrichment ratios at the Banning Park urban site could be a key factor in the differing relative abundances at that site, as the wealth of nutrients lowers the need for competition, specialization. Each solute tested has a unique relationship with pH and is therefore either influencing or influenced by the pH at the site. Influencing solutes suggest more alkaline environments at the Fair Hill site, and the acidity of the Banning
Park site could also be a controlling factor in the dominant phyla. Future work will require replicated sampling for multiple events and on multiple trees, as well as simultaneous testing of solutes in stemflow for those events. Additional future testing will also include measurement of pH for both bark and stemflow and in-situ measurements of the optical properties of the bark surface.
Table 5.4: Future work in corticular microbial ecology

<table>
<thead>
<tr>
<th>Include</th>
<th>Why</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check for chloroplasts</td>
<td>to determine the impact of agricultural flora at Fair Hill (i.e., P. agglomerans)</td>
</tr>
<tr>
<td>meteorological data collection below the canopy</td>
<td>to better understand microclimate conditions at a scale more appropriate for this type of study</td>
</tr>
<tr>
<td>smooth bark trees and trees without stemflow collars</td>
<td>to account for disruption of stemflow by furrows and collar</td>
</tr>
<tr>
<td>a horizontal, non-destructive, cultivation inclusive sampling methodology</td>
<td>to eliminate additional opportunities for artifacts in the data caused by removing bark and the downstream ramifications of that small change, which may be larger than anticipated</td>
</tr>
<tr>
<td>simultaneous testing of solutes in stemflow</td>
<td>for comparison with the community and functional data</td>
</tr>
<tr>
<td>the pH of both bark and stemflow</td>
<td>pH is extremely important in the distribution of bacteria and has a unique relationship with each solute that may be moving or changed during these processes</td>
</tr>
<tr>
<td>optical properties of the bark</td>
<td>to determine what impacts the bark qualities may have on radiative transfer near the bark surface (as part of microclimate)</td>
</tr>
<tr>
<td>replicates for multiple events and trees</td>
<td></td>
</tr>
<tr>
<td>expanded genetic sequencing to include marker genes for nutrient cycling</td>
<td>to better understand functional information about the bacteria, what roles they are playing in situ in terms of biogeochemistry, and when</td>
</tr>
<tr>
<td>radio cesium + microbes</td>
<td>to understand if Cs, an analog for K, may have long-term impacts on bacterial populations in wooded ecosystems, especially potassium solubilizing bacteria (KSB)</td>
</tr>
</tbody>
</table>

5.5 Experimental Procedures

5.5.1 Study Site Details

Sample collection for this study took place in the mid-Atlantic region of the United States, in one rural forested area (Fair Hill Natural Resource Management Area (NRMA)), and one urban forest fragment (Banning Regional Park). The University of Delaware maintains a long-term use agreement with Fair Hill NRMA, which is the location of a 10-year NSF and University of Delaware joint-funded research site. Fair Hill NRMA is in Cecil County, MD (elevation 73 m), in a humid subtropical region of the Köppen climate classification. The study tree is located on a south-west facing...
slope in a 12-hectare watershed within the NRMA near the Maryland-Pennsylvania border (39°43'15"N 75°49'51"W). The NRMA is historically a mix of both farms and woodland, a patchwork of open fields and mixed-deciduous forest.

The Banning Park urban forest fragment is in New Castle County, DE. The forest fragment is approximately 28.5 hectares (elevation 10 m, gentle south-east slope) located less than 20 miles east of the NRMA in a small, unincorporated area on the outskirts of metropolitan Wilmington, DE (39°43'03"N 75°35'45"W). Banning Park is a site with which the research group maintains a permit and an established research relationship. Primary tree species in Banning Park are similar to those in Fair Hill NRMA, but the Banning Park forest fragment is bordered by lower-density urban neighborhoods, the SEPTA and AMTRAK regional lines, major roadways (I-95 and I-495 interchange, route US 202, DE-141, and DE-4), and is located within a mile of the GM Boxwood Industrial Brownfield site (operating 1947-2009) and its accompanying railyard, and an active campus occupied by BASF, the second largest chemical manufacturing company in North America.

*Quercus rubra* L. (northern red oak) was selected for this study due to its presence and abundance at both sites. Morphological advantages of using red oak for this study include the deeply furrowed bark texture (ideal for bark-surface-scale microclimatolgy studies) and a round canopy with branch architecture that will encourage stemflow production (Gilman and Watson, 1994). Finally, the frequent use of red oak in suburban and urban areas as “street trees” across most of the country (Gilman and Watson, 1994) will expand the applicability of the results of this study.
5.5.2 Collection: Stemflow and Stemflow Chemistry

Uniform bark tissue cores were collected at four heights (0.5 m, 1.5 m, 2.4 m, 4 m) and in four cardinal directions (N, S, E, W) from a single red oak tree at each site before and after one rain event using a hollow leather punch. The wood punch was sterilized in bleach and rinsed in deionized water after each sample was collected, and the sample placed into a labeled Nasco Whirl-pak bag. Pre-storm samples were collected before one forecasted frontal storm system, and post-storm samples were collected within 24 hours after the end of the event. Between lab procedures, samples were stored in a freezer to prevent changes to bacterial populations and the degradation of bacterial DNA. Bacterial DNA was extracted from the bark tissue using a Qiagen DNeasy PowerSoil Kit (Hilden, Germany).

Rainfall depth and intensity in the open were measured using two meteorological stations closest to the study sites, equipped with tipping bucket gauges (Fair Hill, MD, and Wilmington, DE – Talleyville) which are part of the Delaware Environmental Observing System. Stemflow collectors were attached to the two trees being sampled for microbial diversity. Stemflow collectors consisted of a polyethylene stemflow collar stapled and then sealed around the bole of the tree which directed generated stemflow into a polyethylene storage container (Levia et al., 2010). Stemflow volume was measured, and a stemflow sample was collected into a 200 ml HDPE container for extraction of DNA using Qiagen DNeasy PowerSoil Kit (Hilden, Germany). Stemflow samples were collected after the same events, at the same time as post-storm bark samples, from the same trees for which microbial samples from bark were collected.

Sequencing data were compared with average hydrochemical data, which was collected between April 2016 and August 2017. Stemflow was collected
for ten events from four red oaks at Banning Park and five red oaks at Fair Hill NRMA (Dowtin, in preparation). These stemflow samples were sent for hydrochemical analysis at the UD Soil Testing lab (ICP-OES for S, Cu, Fe, Mg, Mn, P, Zn conducted on Thermoscientific iCAP TM for samples processed before 15 July 2016, and Thermoscientific Iris Intrepid II XSP Duo View ICP OES Analyzer for samples processed after 15 July 2016; NH 4 -N and NO 3 -N analyzed on Brand & Luebbe Autoanalyzer 3 Flow Injection Analyzer; Dowtin, in press). Hydrochemical data was processed using Tibco Statistica, version 13.3 (Dowtin, in press).

5.5.3 DNA Extraction

Aqueous stemflow samples were vacuum filtered onto a 0.22 μm mixed cellulose GSWP filter (Millipore, Burlington MA) to collect microbial cells and immediately frozen at -20°C before DNA extraction with milliQ water as a negative control. 100 mL of material was filtered for each sample, except for Fair Hill NRMA, which had 60 mL filtered. The outer most layer of the tree bark samples was separated using a razor blade and saved for subsequent DNA extraction. DNA was extracted using the Qiagen DNeasy PowerSoil Kit (Hilden, Germany). Each filter or tree bark scraping was placed directly into a provided bead beating tube, and manufacturer’s protocol was followed. DNA yield was determined using a Qubit fluorometer (Qubit 1.0, Life Technologies, Carlsbad, CA) and DNA was stored at -20°C.

16S rRNA Gene Sequencing and Analysis

Paired-end Illumina MiSeq (Illumina, San Diego, CA) sequencing of the 16S rRNA gene was performed at the University of Chicago using the universal V4 region primer set (515f/806r; (Caporaso et al., 2010). QIIME2 (version 2018.4.0; https://qiime2.org; (Caporaso et al., 2010; Bolyen et al., 2018) was used to analyze the
sequences, which included denoising using DADA2, calculating alpha and beta diversity metrics, and taxonomic assignments using the SILVA132 database. In the denoising process, both forward and reverse reads were trimmed to 146 bp to eliminate regions with low overall quality scores as determined by visual inspection in QIIME2. Before diversity measurements and taxonomic assignments, unassigned sequences, chloroplasts, and mitochondria were removed from the dataset, which was subsequently rarefied to 3,813 sequences per sample for downstream analysis.

5.5.4 Additional Visualization and Analysis

Phylum Δ abundance was visualized as a contour plot with the Y-axis representing the height of the sampling location (m), and the X-axis representing aspect. North direction is shown at both ends to represent the closing of the cylinder, which is the tree bole. Data were smoothed using a 3rd order polynomial spline to aid in the interpretation of bark surface patterns of abundance. Blue vertical line represents the predominant wind direction for the storm event sampled, while the horizontal dashed line represents the height of the stemflow collar. Green areas show where a given phylum is more abundant after the storm event, white show areas of no difference and the brown end of the color map represents areas where phylum is less abundant after the storm event.

Odds ratios, a measurement of association between exposure and outcome, were used to understand the presence of phyla at the site further. In this case, "exposure" is the rain event, and the outcome is either an increase or decrease in the given phylum. The closer the value is to 1, the more likely it is that the selected factor has little effect on the odds of that outcome (e.g., rain events have hardly any effect on the odds of finding an increase or decrease in Proteobacteria on south facing bark in

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Banning Park and Fair Hill). A result of >1 means the exposure to the rain event is associated with higher odds of decreasing numbers of that phyla, while results <1 means exposure to the rain event is associated with lower odds decreasing numbers of that phylum (or an increase).
Chapter 6
SYNTHESIS AND CONCLUSIONS

The aim of this dissertation research was to supply key data and insights into 1) regional and species vegetation-driven variations in chemical enrichment, and 2) the species of bacteria inhabiting the cortisphere, and their importance to biogeochemical cycling (Graham et al., 2016; Kirchman, 2012). The cross-scale knowledge gained from this study (the micro, plant, and patch, and regional scales, and interactions thereof) are transferable across the disciplines of ecohydrology and biogeochemistry, microbial ecology, forestry, urban forestry, and even watershed management if one considers the downstream implications of these nutrient inputs.

In chapter 3 we sought to learn if nutrient inputs to watersheds from leaf leachates vary with geography, genetics, or phenophase, and whether trees of the same species experience senescence and contribute to watershed biogeochemistry in the same way over regional transects. We found that there were differences in chemistry and DOM quality for both phenophase and geographic state, and these differences could be measured through DOM quality indices and sample chemistry. The most remarkable implications of this study indicate that it is highly likely that geographically separate populations of the same species do not experience senescence in the same way, and that it is therefore no longer sufficient to consider forests a black box of regional biogeochemical cycling. These differences in chemistry and DOM quality have implications for both phenoseasonal timing of nutrient and DOM input to watersheds and subsequent impacts to soil and stream ecosystems, and regional
modeling of the contribution of plant nutritional status and DOM to biogeochemical cycling in forested watersheds. Results also demonstrate that the chemical contributions from leaf-litter leachate to local watersheds may be considerably variable, even for the same species, regardless of genetic population. Thus, one should take into account the intrinsic intraspecific differences in leaf-litter leachate chemistry that are partly the result of site-specific factors (e.g., soils, geology). As Earth’s climate continues to change, the composition and biodiversity of forested watersheds will also change. Understanding these variations at the watershed and regional scales and how they change over time is a critical piece of the energy and nutrient cycling puzzle for watersheds at a global scale.

In chapter 4, we sought to learn if throughfall chemistry varied based on storm event, geography, and season, and of these variables, which was the strongest driver of differences of inputs from throughfall. Here, we found that more than anything, differences in throughfall vary largely on season, as season has much influence on the movement of aerosols being deposited on a forest, but that these differences can be overridden by strong local signals of atmospheric deposition, e.g., the ocean, and other local geographic factors, such as small differences in climate and rainfall characteristics.

In chapter 5, we attempted to address a literature gap by determining whether communities of corticular bacteria existed at all in our research area. Remarkably, possibly the most remarkable result of the dissertation, was that we were able to show that there are communities of microorganisms in the cortisphere and that they are different between urban and rural land uses. A still important question remains as to why the communities are being translocated and how these communities might
influence nutrient fluxes at the plant-soil interface. Continuing to identify these microbes and their functions could be transformative to the field of ecohydrology. Given the current lack of data on these microbial communities, we believe that the size, exploratory nature, and novel (and possibly profound) results of this study, although not exhaustive, are rather like opening the door to a lifetime’s worth of research. Different atmospheric deposition composition, tree species, different forests, different climates, and rain events may all have different impacts on the species and populations within a microbial community, which may all affect the nutrient cycling taking place in the stemflow on the bark surface. The continuation of this research is of the utmost importance.

This work has confirmed that temperate forests and temperate urban forest fragments can cause highly variable inputs of nutrients and bacteria to forested watersheds through leaf leachates, throughfall, and stemflow. The combined impact of corticular and leaf surface areas of a single tree can be massive in these ecosystems. Along large regional transects, season and phenoseason are the primary drivers of differences to nutrient inputs, and these transects are what make the research novel. In both the throughfall and leachate studies, we were surprised to find that the controls we expected would be governing these processes over long distances played a very small role in the reality of things.

One would have expected similar outcomes in throughfall to those of the leachate paper, in terms of nutrient concentrations. We expect that this was not seen in the throughfall paper given the short residence time of precipitation on leaf surfaces during rain events. The direct impact of leaves on throughfall is likely well below the signal of atmospheric deposition.
Future work with the leaf-leachate data will include attempting to tie our data set back to the data from the in-stream sensors, especially at the phenophase scale.

For throughfall, future work would benefit from increased frequency of coordinated events, as well as increased points of sampling along the transect, to be able to compare other maritime sites with that of Rhode Island. This study can also be tied back to the in-stream sensor data and synoptic climatology, and an additional manuscript for this topic is now in the planning stages.

Future work for the bacterial geography experiment will include replicated sampling for multiple events and on multiple trees, as well as simultaneous testing of solutes (especially C) in stemflow for those events. Additional future testing will also include measurement of pH for both bark and stemflow and in-situ measurements of the optical properties of the bark surface. Finally, expanded genetic sequencing to include marker genes for nutrient cycling would be helpful to understand functional information about the bacteria better, what roles they are playing in situ in terms of biogeochemistry, and when.
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Revealed by Over 150,000 Genomes from Metagenomes Spanning Ag. *Cell* **176**: 1–14.


Appendix A

American beech leaf-litter leachate chemistry: Effects of geography and phenophase

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Abstract

The decomposition of broadleaved tree leaves can contribute a substantial amount of energy to forested watersheds via dissolved organic matter (DOM), nutrients, and biological activity. Less is known about how these inputs may vary within a single tree species that is known to have two genetically distinct and geographically separate populations, or how these inputs may change throughout the growing season. Senescent leaves are often implicitly assumed that intraspecific differences in leaf-litter chemistry do not significantly differ geographically. We analyzed the morphological and chemical leaf traits and leachates from Fagus grandifolia (American beech) leaves (n = 300) during three phenophases: fresh green, senescent leaves, and leaf litter. During each phenophase, leaves were collected from four sites along a geographic transect stretching from Vermont to North Carolina (over 1200 km) with two sites representing each genetic population and differing climatic conditions. Leachates were analyzed for routine solutes and nutrients, as well as fluorescent and UV-visible absorbance indices. Amounts of macro- and micronutrients were highly variable among sites and phenophases but tended to be lowest during the senescent stage, while measured fluorescence and absorbance indices tended to increase during the senescent stage. Results suggest significant differences in leached nutrients among sites and, optical properties and nutrients among phenophases. Aromaticity and molecular weight of DOM in leachates was generally low, and aromaticity and humification of leachates both increased over time with leaf age. These results also suggest that geographically (or genetically) separate populations of the same species do not experience senescence in the same way and that implicit assumptions of intraspecific uniformity of leaf-litter leachate chemistry for a given tree species may be unwarranted.

Key words: biogeochemical cycling / deciduous forest / Fagus grandifolia / fluorescence / organic matter

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1 Introduction

Broadleaved trees are a major source of nutrient supply to streams (Bennett and Scherler, 2002; Cory and Kaplan, 2012), especially during autumn senescence, when nutrients from decomposing leaves are mobilized via leaching and various other biological and physical mechanisms. Fisher and Ures (1973) found that leaf decomposition accounted for 29% of the annual energy budget of a second-order New England stream. Within days of submergence in a stream, up to 40% of the dissolved organic matter (DOM) of a leaf can be leached (McClellan and Fisher, 1976; Duarte and Gherardi, 1993).

The DOM of leaf-litter leachate changes dramatically during autumn as trees experience senescence, nutrient resorption (the translocation of nutrients from leaves to other tissues during senescence), and assimilation. At northern latitudes, photosynthesis is the ultimate determinant for the onset of senescence, while seasonal changes in air temperature become more important at lower latitudes (Esteban and Peniné, 2015). The main nutrients resorbed are nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), because they are mobile in the phloem, unlike calcium (Ca), which is accumulated (Killingbeck, 2004). Even after assimilation, DOM of leaf-litter leachate may be affected by temperature changes (Jaeger and Gherardi, 1993). Nutrient resorption efficiency and partitioning differ among tree species, resulting in inter-specific variation in leaf-litter leachate DOM (Killingbeck, 1996, 2004). Other factors that influence leaf-litter leachate DOM include soil acidification, which can result in a decrease in leaf cation concentration (Duquesney et al., 2000), inter-specific variation and year-to-year fluctuations (DU et al., 1998; Duquesney et al., 2000); leaf-litter age and solar radiation, which can decrease the bioavailability of leachate dissolved organic matter (Feiman et al., 2013); xeric conditions; tropospheric ozone conditions (Buseck et al., 2005); and differences in realized and potential resorption rates. Factors that in-
fluence realized, versus potential, resorption induce the plant's physiological status, which depends on available energy, nutrient concentration, and enzymatic activity, existence of a sink demand for nutrients, disturbances, water stress, and timing of leaf senescence (Killingbeck, 2004).

The inherent variability of interspecific leaf leachate is known, but recent research using hybrids and genotypes of related species have also revealed differences of leachate composition even within species genotypes (Wymore et al., 2015). Recent studies have also shown that leaf-litter leachates from differing species can have a complex interaction with microbial populations in soil and stream water, especially with regard to nutrient cycling and bioavailability (Wymore et al., 2015; Joly et al., 2016). Depending on forest composition, these dissimilarities in leachate quality can have a substantial impact on soil and stream ecosystems through additions of available DOM, nitrogen and other nutrients as well as activity and respiration of microbial communities (Bernhardt and Likens, 2002; Clow and Kaplan, 2012; Wymore et al., 2015). Even though it plays an important role in energy and biogeochemical cycling in forests, especially in summer, less is known about how the quality of leaf-litter leachate may differ throughout autumn as leaves senesce and abscise or how this differs intraspecifically between trees at different geographical and altitudinal locations. Furthermore, autumnal phenology is a critical stage in the success of plant function, propagation, and morphology, especially for determinate growth species such as Fagus grandifolia Eth (American beech). And, while long-term climate data begin to indicate changes in the autumnal season timing and length, there remains little agreement on the effect of climate change on plant phenology (Richardson et al., 2013).

Fagus grandifolia is a common species in eastern North America that is characterized by high adaptability enabling it to grow in a wide variety of conditions (Bussiotti et al., 2005). A smooth-barked, shade-intolerant climax species, the native range of F. grandifolia spans from eastern Texas and northern Florida, northward to Nova Scotia and Maine (Tobe and Houston, 1990). Fagus grandifolia possesses large intraspecific variation in its chemical, phenotypic, and reproductive characteristics. Boerner (1984) found that among sampled broadleaved deciduous trees, the foliar P concentration was mostly in F. grandifolia. Bresco et al. (2011) found genetic variation to account for up to 29% of the total phenotypic variation between populations. Furthermore, through comparisons of allelic components, Kitanura and Kawai (2001) found differences between northern and southern populations at several loci, indicating an extended period of isolation between the two populations due to historical glaciations. The southern population ranges from the Gulf and eastern coastal plains to the Piedmont and Ozark Plateaus; the northern population is found in northern glaciated terrains and parts of the Appalachian Mountains (Kitanura and Kawai, 2001). Even though two genetically distinct populations have been found in F. grandifolia and latitudinal differences between northern and southern F. grandifolia populations could result in intraspecific differences in leaf-litter leachate DOM, regional mapping of DOM rarely accounts for genetically linked geographical differences between F. grandifolia stands.

Our research seeks to answer the following question: are there differences in the leachates as senescence progresses, and if so, what factors control these differences? Accordingly, we present our findings as to how nutrient concentration and leaf-litter leachate chemistry and composition from three phenological leaf stages (fresh green, senescing, and leaf-litter) differ intraspecifically among F. grandifolia trees in Vermont (VT), Rhode Island (RI), Maryland (MD), and North Carolina (NC). Because leaf-litter leachate plays a substantial role in the biogeochemical cycles of forested watersheds, we (1) quantify the temporal and intraspecific variation in F. grandifolia leaf-litter leachate and then (2) employ discriminant function analysis to divide and clarify the most influential discriminatory variables that account for the intraspecific differences in F. grandifolia leaf-litter leachate chemistry as a function of both geography and phenophase. We specifically delve into the intricacies of intraspecific variation in leaf-litter leachate chemistry to investigate whether it is reasonable to assume uniformity in the biogeochemistry of leaf-litter leachates for a particular tree species (F. grandifolia in this case) across a large portion of its biogeographic range. Rejection of this assumption would indicate that DOM models at regional, national, or international scales should consider the intrinsic intraspecific differences in leaf-litter leachate chemistry that are partly the result of site-specific factors (e.g., soils, geology). We hope that this work will move us closer to understanding the fundamental linkage of F. grandifolia leaf-litter leachate composition and, by extension, any tree population covering large ranges to watershed biogeochemistry.

2 Material and methods

2.1 Site Descriptions

In 2015, beech leaves were collected from four field sites in the eastern United States, spread across a distance of approximately 1,450 km and 8° of latitude, with elevations ranging from 47 to 1,400 m. More detailed sites descriptions can be found in Wheeler et al. (2017). Leaves from North Carolina were collected in a beech-dominated forest, 1,400 m elevation above mean sea level in the Blue Ridge Mountains near Boone (36°13′38″ N, 81°41′48″ W). This site receives 1,338 mm annual mean precipitation and the 30-year mean temperature is 9°C (NCEI, 2016). Soils in the site consist of steep rocky outcrops with 15 to 95% slopes and a gravelly loam to fine-sand-loam to gravelly-loamy-sand profile with 15 to 60% slopes (USDA, 2012). Fresh leaves were collected on August 24, senescing leaves were collected on October 12, and fallen leaves were collected on October 25. In Maryland, leaves were gathered at Fair Hill Natural Resource Management Area (39°42′10″ N, 75°45′56″ W). This site receives approximately 1,200 mm annual mean precipitation. The 30-year mean temperature is 13°C (NCEI, 2016). Soils in the area are primarily loam and silt loams with soil types as 0.25 to 60% slopes (USDA, 2012) at an average 70 m elevation above mean sea level. The forest type is mixed deciduous. Fresh leaves were collected on June 29, senescing leaves were collected on November 5, and freshly fallen leaves were collected on November 11. Rhode Island leaves were collected in an old-growth beech forest found within the Aquidneck Land Trust's...
Oakland Forest in Portsmouth (41°33'23" N, 71°15'36" W). The 30-year mean temperature is 11°C and the average annual precipitation is 1,170 mm (NCASI, 2010). Soils in the site consist of silt loams, with slopes of less than 2% (USDA, 2012) at an elevation of about 47 m above mean sea level. Fresh leaves were collected on July 6, senescing leaves were collected on November 6, and fallen leaves were collected on December 15. In Vermont, leaves were collected in the Wade Brook watershed near Montgomery Center around 350 m above mean sea level (44°52'30" N, 72°36'30" W). Primary species in this forest are sugar maple, red maple, yellow birch, American beech, and white ash. The 30-year mean temperature is 7°C and the average annual precipitation is 1,058 mm (NCASI, 2011). Soils in the site are a sandy loam, with 3 to 15% slopes (USDA, 2012). Fresh leaves were collected on July 13, senescing leaves were collected October 19, and freshly fallen leaves were collected October 30.

2.2 Sampling

Thirty Folliage leaves in good condition with the petiole intact (n = 360) were collected for each of the three phenological conditions (fresh, senescing, and freshly fallen; hereafter “phenophase” or “phase”) from forested watersheds in each of the four states (hereafter “states”). Because leaf traits can vary significantly even on the same tree, fresh and senescent leaves were collected from multiple individual beech trees of variable size and age in each of these watersheds, and were collected on varying slides and from varying heights of those trees, in both shade and sun, to capture the broadest range of chemical and functional traits possible (Buscot and Polle, 2015). It is assumed, for the purposes of this study, that leaves from fresh leaves accurately represent the DOM and chemical contribution of vegetative greenfall or stormfall to a watershed system. Given that the climatological normative values vary for each of the four locations, each state's collection took place during what would be considered its peak for each phenophase using empirical observations of long-term research at each field site. To ensure leaves were collected in their most natural environment, freshly fallen leaves were collected from the top-most portion of the litter layer on the forest floor, under the trees from which fresh and senescent leaves were taken. Freshly fallen leaves are not assumed to have come from the individual tree under which fallen leaves were found, but it is assumed that surrounding beech in the same area experienced similar conditions.

Immediately after collection, leaves from North Carolina, Rhode Island, and Vermont were shipped standard overnight to the University of Delaware where they were put into storage at 4°C. Leaves collected in Maryland were taken directly to the laboratory and kept at 4°C for 48 h in order to standardize the storage conditions to those being shipped. After receipt, or at the 48 h mark, leaves were placed through a LiCOR Li-3100 leaf area meter (Lincoln, NE USA), photographed with scale, and measured for leaf thickness and fresh weight (Buscot and Polle, 2015). Leaves were briefly rinsed (< 5 s) with deionized water to minimize contributions of geographically dependent dry deposition to the leachate study. A dry weight was also obtained for all leaf sets after oven-drying at 70°C for 72 h (Buscot and Polle, 2015). For each set of 30 leaves, individual dried leaves with petioles removed were cut in half to ensure a complete fit into separate pre-combusted 500-ml borosilicate glass beakers filled with 200 ml NANOpure™ deionized water. Individual leaf masses were left intact to mimic leachate concentrations in field conditions, especially with regard to DOC (Cuss and Due Guerrero, 2013). A pre-combusted glass stirrer was used to keep the leaf fully submerged in the beaker of water which was covered to keep out contaminants and placed in the refrigerator at 4°C for 72 h (Cuss and Due Guerrero, 2013). At the end of the 72 h period, leaves were removed from the beakers, and the thirty individual leaflet leachates were pooled to yield five composite samples (six individual leachates yielding one composite leachate sample). Composite samples were filtered immediately using a 0.7-μm glass fiber filter (Millipore). It should be noted that this filter size does allow a small amount of low-end particulate organic carbon (POC) to pass into the aqueous sample, but the inclusion of such POC, if present, is not problematic since the results would remain unaltered. Filtered samples were placed into a 250-ml HDPE sample container for chemical analysis and a 40-ml amber glass vial container for fluorescence and UV-visible absorbance analysis. Samples were refrigerated at 4°C until instrumental analysis could be completed.

The University of Delaware Soil Testing Laboratory performed the chemical analyses. Solution pH and EC were measured using an Accumet pH meter model A-815 with a SYMPhony pH electrode and a WTW Model 1952 conductivity meter with a platinum dip cell, respectively. P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, and Al were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Elemental Intrepid II XP Duo View, Madison, WI, USA). DIC/DOC and total bound N (TNH) were measured using an Elementar VarioCUIC TOC Analyzer (Mount Holy, NJ, USA). While nitrate-N and ammonium-N were measured with a Bran & Luebbe AutoAnalyzer 3 (Model AA3, Buffalo Grove, IL, USA). A table of soil chemistry results can be found in the supplementary tables of Wheeler et al. (2017). Fluorescence and UV-visible absorbance analysis was conducted by the Watershed Sciences Research Group at the University of Delaware on a Horiba Fluoromax-3 Benchtop Fluorometer for DOM (Kyoto, Japan). For more detailed information on instrumental analysis, see Wheeler et al. (2017). The following indices were of particular interest for this study: percent humic-like fluorescence, percent fulvic-like fluorescence, percent protein-like fluorescence, humification index (HI), biological index (BIx), fluorescence index (FI), spectral slope ratio (3ε), and specific ultraviolet absorbance at 254 nm (SUVA254). For a comprehensive summary of these DOM quality indices see Inamdar et al. (2012).

Finally, soil samples were collected from each site in late summer 2016, following the protocol provided by the University of Delaware Soil Testing Laboratory. One-half kg samples were analyzed for pH, P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, Al, and estimated cation exchange capacity, base saturation, TNH, ammonium-N (NH₄-N), and nitrate-N (NO₃-N).
2.3 Statistical Analysis

We employed statistical methods to determine differences in the leachates. These methods include comparing descriptive statistics among the central tendency and dispersion of the variables. A simple non-parametric Kruskal–Wallis test was used to determine if leachates of different phenophases were different within the same state. Analysis of variance (ANOVA) was used to evaluate leachate variation by both state and phenophase, and a forward-stepwise discriminant function analysis (DFA) was used to classify leachates both by state and phenophase. Variables relating directly to leaf-litter leadside composition were standardized by the variable mean and standard deviation and then run through the DFA using JMP Pro 12. Variables that showed little-to-no variance or significance in either the Kruskal–Wallis or ANOVA were still included in the DFA on the chance that they may have some discriminating power. While nutrient concentrations in leachate samples were not corrected by mass due to the complicated weighting that would have been necessary, for our pooled samples, physical leaf characteristics for each state were included in the DFA on the belief that the size of the leaf might affect nutrient concentration within the leachate sample. Variables could be used to over-parametrize the model by state or phenoseason, such as climatological data, latitude, longitude, and soil data, were withheld from the DFA and rather used to assist in the interpretation of the results of the analysis.

3 Results and discussion

Patterns of macro- and micronutrients, non-essential constituents (i.e., Al), and leaf areas and weights showed very little consistency among states and phenophases (Tables 1 and 2). Generally, nutrient concentrations of leachate either decreased over time or peaked during the senescing-leaf phase. We propose that this senescent-leaf peak pattern of nutrient concentrations in leachate samples over time may be a function of cellular deterioration during the fresh leaf phase.

Table 1. Mean (± SD) nutrient concentrations and fluorescence and UV-visible absorbance indices of F. grandifolia leaf-litter leachates for all states and phenophases, as well as each phenophase individually.

<table>
<thead>
<tr>
<th>Sample pH</th>
<th>Fresh</th>
<th>Senescing</th>
<th>Freshly fallen</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.40 – 0.43</td>
<td>5.43 – 0.63</td>
<td>5.41 – 0.34</td>
<td>5.37 – 0.23</td>
</tr>
<tr>
<td>EC (mmhos cm⁻¹)</td>
<td>0.020 – 0.008</td>
<td>0.023 – 0.008</td>
<td>0.022 – 0.003</td>
</tr>
<tr>
<td>Al (mg L⁻¹)</td>
<td>0.000 – 0.007</td>
<td>0.014 – 0.007</td>
<td>0.001 – 0.003</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>0.020 – 0.012</td>
<td>0.031 – 0.016</td>
<td>0.015 – 0.003</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>0.460 – 0.340</td>
<td>0.285 – 0.183</td>
<td>0.595 – 0.248</td>
</tr>
<tr>
<td>Cu (mg L⁻¹)</td>
<td>0.002 – 0.002</td>
<td>0.002 – 0.002</td>
<td>0.002 – 0.001</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>0.034 – 0.023</td>
<td>0.052 – 0.011</td>
<td>0.029 – 0.014</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>3.270 – 1.601</td>
<td>4.620 – 1.292</td>
<td>3.405 – 0.698</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>0.310 – 0.180</td>
<td>0.349 – 0.177</td>
<td>0.312 – 0.170</td>
</tr>
<tr>
<td>Mn (mg L⁻¹)</td>
<td>0.070 – 0.074</td>
<td>0.042 – 0.027</td>
<td>0.005 – 0.104</td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>0.540 – 0.330</td>
<td>0.637 – 0.562</td>
<td>0.508 – 0.032</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>0.240 – 0.171</td>
<td>0.300 – 0.138</td>
<td>0.289 – 0.225</td>
</tr>
<tr>
<td>Si (mg L⁻¹)</td>
<td>0.790 – 0.562</td>
<td>0.470 – 0.501</td>
<td>1.237 – 0.188</td>
</tr>
<tr>
<td>Zn (mg L⁻¹)</td>
<td>0.037 – 0.007</td>
<td>0.039 – 0.009</td>
<td>0.039 – 0.006</td>
</tr>
<tr>
<td>TN (mg L⁻¹)</td>
<td>0.550 – 0.740</td>
<td>1.345 – 0.807</td>
<td>0.352 – 0.344</td>
</tr>
<tr>
<td>% Protein-like</td>
<td>74.990 – 7.490</td>
<td>60.990 – 2.744</td>
<td>70.490 – 5.799</td>
</tr>
<tr>
<td>HIX</td>
<td>0.320 – 0.097</td>
<td>0.206 – 0.024</td>
<td>0.353 – 0.076</td>
</tr>
<tr>
<td>BiK</td>
<td>0.460 – 0.153</td>
<td>0.275 – 0.038</td>
<td>0.584 – 0.113</td>
</tr>
<tr>
<td>Pi</td>
<td>1.680 – 1.016</td>
<td>1.586 – 0.128</td>
<td>1.801 – 0.167</td>
</tr>
<tr>
<td>SUVA₂₅₀ (L mg⁻¹ cm⁻¹)</td>
<td>0.020 – 0.009</td>
<td>0.009 – 0.005</td>
<td>0.021 – 0.005</td>
</tr>
<tr>
<td>S₄</td>
<td>1.440 – 0.614</td>
<td>1.091 – 0.433</td>
<td>1.731 – 0.538</td>
</tr>
</tbody>
</table>
Table 2. Physical leaf data and average composite leaf-fitter leachate sample DOC concentration.

<table>
<thead>
<tr>
<th></th>
<th>Leaves area (cm²)</th>
<th>Leaf fresh weight (g)</th>
<th>Leaf dry weight (g)</th>
<th>Water content</th>
<th>Foliar biomass (g cm⁻²)</th>
<th>Average sample DOC (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>MD 30</td>
<td>1171.80</td>
<td>4.22</td>
<td>Missing Data</td>
<td>9.46</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>RI 30</td>
<td>1928.83</td>
<td>12.31</td>
<td>6.66</td>
<td>5.65</td>
<td>0.003</td>
</tr>
<tr>
<td>Fresh</td>
<td>VT 30</td>
<td>1531.34</td>
<td>8.35</td>
<td>4.18</td>
<td>4.17</td>
<td>0.003</td>
</tr>
<tr>
<td>Fresh</td>
<td>NC 30</td>
<td>1394.75</td>
<td>9.00</td>
<td>4.25</td>
<td>5.55</td>
<td>0.004</td>
</tr>
<tr>
<td>Senesced</td>
<td>MD 30</td>
<td>1261.53</td>
<td>6.95</td>
<td>2.83</td>
<td>4.12</td>
<td>0.003</td>
</tr>
<tr>
<td>Senesced</td>
<td>RI 30</td>
<td>1534.85</td>
<td>5.37</td>
<td>4.08</td>
<td>1.29</td>
<td>0.001</td>
</tr>
<tr>
<td>Senesced</td>
<td>VT 30</td>
<td>1727.51</td>
<td>11.14</td>
<td>4.10</td>
<td>7.04</td>
<td>0.002</td>
</tr>
<tr>
<td>Senesced</td>
<td>NC 30</td>
<td>1513.63</td>
<td>12.28</td>
<td>5.29</td>
<td>6.99</td>
<td>0.005</td>
</tr>
<tr>
<td>Fallen</td>
<td>MD 30</td>
<td>1502.00</td>
<td>8.14</td>
<td>4.36</td>
<td>3.78</td>
<td>0.003</td>
</tr>
<tr>
<td>Fallen</td>
<td>RI 30</td>
<td>1506.40</td>
<td>4.96</td>
<td>4.68</td>
<td>0.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Fallen</td>
<td>VT 30</td>
<td>1682.82</td>
<td>10.76</td>
<td>5.44</td>
<td>5.32</td>
<td>0.003</td>
</tr>
<tr>
<td>Fallen</td>
<td>NC 30</td>
<td>1501.13</td>
<td>12.09</td>
<td>7.70</td>
<td>4.39</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Measured fluorescence indices also tended to increase during the senescence phase and plateau at those values (Tab. 1). The HIX showed a shift to longer wavelengths over time due to lower H: C ratios as leaves deteriorated, becoming more aromatic and less bioavailable during the fallen-leaf phase (Senesi, 1990; Ohno, 2002). With few exceptions, the BIX remained below the threshold of 1, indicating that there was little to no biological activity within the samples (Huguet et al., 2009). The OPT threshold between extra-cellular release and plant-terrestrial DOM is above 1.4, and typically, the senesced-leaf samples fell above this threshold, suggesting that cell membrane deterioration is highest at this time, when DOM is also more bioavailable (McKnight et al., 2001). Percent fulvic-like and percent humic-like organic acids increased, while percent protein-like decreased, suggesting that over time there is simply less bioavailable material to be leached (Feldman et al., 2009). The SP followed patterns similar to those of protein and organic acids because it is a measure of the inverse molecular weight, and thus bioavailability. SUVA24 values were generally quite low (0.02 – 0.01 L mg⁻¹ C⁻¹ cm⁻¹) but were within typical values for leachates, between 0.0004 and 0.025 L mg⁻¹ C⁻¹ cm⁻¹ (Smit et al., 2002), indicating low aromaticity and unstable organic compounds, especially during senescence. Many of our measured values are within the range of those found for mixed forest leachates at the same field site in Maryland by Insam et al. (2012).

For each state, many of the nutrient variables were significantly different over the three phenophases. For North Carolina leachates, 44% of nutrients varied significantly among phenophases, while 83% of nutrients were significantly different for Maryland, and 89% for Rhode Island and Vermont (Tab. 3). Additionally, the optical properties of the samples from each state were significantly different over the three phenophases for over 87% of the measured variables (Tab. 3). It should therefore be unsurprising that an ANOVA would reveal significant differences among leaf characteristics between sites due to nutritional differences in the soils and genetic populations at each site, and among fluorescence indices and, even more significantly, the nutritional composition between phenophases, due to changes in the breakdown of OM and release of nutrients over time (Tab. 3).

3.1 State-based Discriminant Function Analysis

Forward-stepwise discriminant function analysis of the standardized data identified seven variables with which it was possible to validate a model: sample pH, electrical conductivity (EC), B. P. leaf thickness standard deviation, leaf area, and dry weight. The model was cross-validated by training, using 35 of the 60 cases and 24 cases withheld as a validation sample. All model tests were significant at the = 0.0001 level, and only 4% of the excluded cases were misclassified (one RI fallen leaf was classified as a VT leaf). Results of the DFA by state imply that the P concentration of the leachate samples was the most influential variable for differentiating between states (Tab. 4).
### Table 3: Results of Kruskal-Wallis Test of variance of phenophases by state (DF = 2) and results of analysis of variance of foliar pH and leaf-litter leachates by state (DF = 2) and phenophase (DF = 3). Significant results indicated by bold face.

<table>
<thead>
<tr>
<th>Chemical properties</th>
<th>ANOVA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>ChH</td>
</tr>
<tr>
<td>Sample pH</td>
<td>10.194</td>
</tr>
<tr>
<td>EC (mhos cm⁻¹)</td>
<td>1.916</td>
</tr>
<tr>
<td>Al (mg L⁻¹)</td>
<td>6.007</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>8.840</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>3.340</td>
</tr>
<tr>
<td>Cu (mg L⁻¹)</td>
<td>2.204</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>1.520</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>4.340</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>8.640</td>
</tr>
<tr>
<td>Mn (mg L⁻¹)</td>
<td>7.220</td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>3.025</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>7.740</td>
</tr>
<tr>
<td>S (mg L⁻¹)</td>
<td>7.273</td>
</tr>
<tr>
<td>Total Dissolved C (mg L⁻¹)</td>
<td>5.040</td>
</tr>
<tr>
<td>DIC (mg L⁻¹)</td>
<td>0.720</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>5.680</td>
</tr>
<tr>
<td>Tln (mg L⁻¹)</td>
<td>3.571</td>
</tr>
<tr>
<td>Zn (mg L⁻¹)</td>
<td>1.340</td>
</tr>
</tbody>
</table>

### Optical Properties

| % Fv/Chl | 9.050 | 0.011 | 8.720 | 0.013 | 11.580 | 0.003 | 10.140 | 0.006 | 1.673 | 0.368 | 15.828 | <0.0001 |
| % Hematocrit | 9.620 | 0.008 | 8.340 | 0.016 | 12.500 | 0.002 | 11.580 | 0.003 | 2.918 | 0.042 | 48.703 | <0.0001 |
| % Protein | 10.020 | 0.005 | 9.920 | 0.007 | 12.500 | 0.002 | 11.580 | 0.003 | 1.613 | 0.197 | 30.200 | <0.0001 |
| Bx | 9.420 | 0.009 | 12.500 | 0.002 | 9.620 | 0.008 | 9.380 | 0.009 | 2.209 | 0.097 | 75.978 | <0.0001 |
| Fl | 9.920 | 0.007 | 9.380 | 0.009 | 9.780 | 0.008 | 7.340 | 0.026 | 0.891 | 0.451 | 18.615 | <0.0001 |
| Hx | 12.500 | 0.002 | 9.500 | 0.009 | 9.420 | 0.009 | 10.220 | 0.006 | 2.958 | 0.041 | 66.723 | <0.0001 |
| Tw | 10.000 | 0.005 | 2.958 | 0.145 | 12.500 | 0.002 | 8.890 | 0.012 | 3.692 | 0.012 | 7.198 | 0.001 |
| SI/VAmax (L mg⁻¹ C⁻¹ cm⁻¹) | 11.520 | 0.003 | 7.468 | 0.024 | 11.180 | 0.004 | 12.500 | 0.002 | 0.880 | 0.457 | 45.130 | <0.0001 |

### Leaf Characteristics

| Average leaf thickness (cm) | 4.528 | 0.007 | 3.248 | 0.046 |
| Leaf thickness standard deviation | 8.085 | 0.000 | 1.001 | 0.374 |
| Dry weight (g) | 4.353 | 0.008 | 0.680 | 0.511 |
| Fresh weight (g) | 9.237 | 0.000 | 1.643 | 0.202 |
| Leaf area (cm²) | 1.501 | 0.140 | 1.955 | 0.151 |

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Table 4: Discriminant weights of each variable to the state's function, absolute value reflects relative contributions to the function. Highest contributions are shown in bold. In gray, discriminant loadings for the states, showing the variance that the variables have within the discriminant function, to be interpreted in factor-loading fashion.

<table>
<thead>
<tr>
<th></th>
<th>MD</th>
<th>NC</th>
<th>RI</th>
<th>VT</th>
<th>Canon1</th>
<th>Canon2</th>
<th>Canon3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-4.467</td>
<td>-7.598</td>
<td>7.029</td>
<td>12.900</td>
<td>-0.695</td>
<td>-0.159</td>
<td>0.626</td>
</tr>
<tr>
<td>EC (mmhos cm⁻¹)</td>
<td>-1.488</td>
<td>-6.292</td>
<td>8.083</td>
<td>0.301</td>
<td>-0.281</td>
<td>-0.289</td>
<td>0.122</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>-3.988</td>
<td>2.268</td>
<td>1.042</td>
<td>3.151</td>
<td>-0.122</td>
<td>0.347</td>
<td>0.059</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>9.896</td>
<td>6.974</td>
<td>-14.962</td>
<td>-4.485</td>
<td>0.792</td>
<td>0.098</td>
<td>0.183</td>
</tr>
<tr>
<td>Leaf thickness std dev</td>
<td>8.244</td>
<td>1.459</td>
<td>-5.315</td>
<td>-8.404</td>
<td>0.201</td>
<td>-0.281</td>
<td>0.443</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>7.088</td>
<td>0.195</td>
<td>-4.877</td>
<td>-2.923</td>
<td>0.525</td>
<td>0.255</td>
<td>0.296</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>-4.919</td>
<td>1.794</td>
<td>0.282</td>
<td>0.320</td>
<td>-0.165</td>
<td>0.601</td>
<td>0.305</td>
</tr>
<tr>
<td>Constant</td>
<td>11.573</td>
<td>6.711</td>
<td>16.197</td>
<td>11.221</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This is especially true for the first canonical function, which discriminates between Maryland-North Carolina in the positive direction, and Vermont-Rhode Island in the negative direction. Here, P also had a negative relationship with sample pH, which is unsurprising given that the P availability is quite sensitive to pH (Marschner, 1986). The second canonical function, which appeared to discriminate between North Carolina-Vermont and Rhode Island-Maryland, was weighted almost exclusively by leaf dry weight, which had relatively large contributions to Vermont and Maryland functions, albeit in the opposite direction (Tab. 4). The third canonical function was almost exclusively sample pH, for which North Carolina and Vermont had the highest function contribution, and it appeared to discriminate between the North Carolina-Rhode Island groups and the Maryland-Vermont groups (Tab. 4). In a previous study by Rosson et al. (2014), specific leaf area (leaf area: dry weight ratio) was one of two best parameters for predicting growth in field conditions. Here again, leaf area and dry weight were suggested indicators for predicting leaf-litter decomposition. For the four states studied, while the variables P, pH, and dry weight carried much of the discriminatory power (Tab. 4), removal of the additional, and seemingly unnecessary variables such as B which showed no significant difference between states weakened the discriminatory power of the functions greatly, and confirmed that this reduced set of variables was likely the best of those measured for discriminating between these states.

3.2 Phenophase-based Discriminant Function Analysis

Forward-stepwise discriminant function analysis of the standardized data identified eight variables with which it was possible to validate a model: K, Mg, Al, B, percent humic-like, ELQ, FI, and SIVATAX. The model was cross-validated using 30% of the 60 cases for training and 24 cases withheld as a validation sample. All model tests were significant at the < 0.01% level. One hundred percent of the training cases were correctly classified and only 16.0% of the excluded cases were misclassified, one fresh leaf was classified as senescing, one senesced leaf was classified as fallen, and two fallen leaves were classified as senescing. While the ANOVA suggested that there were no significant differences in the variances for B between phenophases (Tab. 3), it stood out as the highest relative contribution for all three leaf phenophases (Tab. 5). The first canonical function of the DFA suggested that phenophases were being discriminated primarily by the amount of extra-cellular release. All variables of the first canonical function (K, Mg, Al, B) were directly related to cell-membrane structure, biosynthesis, and secondary metabolic functions, suggesting that as growth ends, leaf functionality slows, and breakdown of the leaf tissues progresses over time, these nutrients are most representative of the change in activity and growth (Tab. 5). As the biological and fluorescence indices increased in the canonical function, there was a negative relationship with the mobile micronutrients essential for enzymatic and biosynthetic activity (Marschner, 1986; Taz and Zieger, 2002). This second canonical function was loaded almost entirely by “percent humic-like”, suggesting the decomposition stage of the OM may be the final necessary variable needed to discriminate between the phenophases (Tab. 5). These results are similar to those of Feilman et al. (2013), who found significant correlations between increasing leaf-litter age and increasing bioavailability of DOM in leaf-litter leachates. The result of this DFA should therefore be unsurprising given that decomposition stage is essentially a proxy for leaf age, and thus phenophase.

Surprisingly, the addition of a dummy variable for genetic population did not provide enough discriminatory power to be entered into either the state or phenophase analysis and we found no significant latitudinal or genetic differences in intra-specific DOM quality for P. grandifolia.

4 Conclusions

DOM quality indices were useful for discriminating among leaf phenophases, while sample chemistry, rather than quality indices, was more useful for discriminating among the geographic states. The results of this study indicate that it is highly likely that geographically or genetically separate populations of the same species do not experience senescence in the same way. This has implications for (1) phenological timing of nutrient and DOM input to watersheds and subse-
Table 5: Discriminant weights of each variable to the phase’s function; absolute value reflects relative contribution to the function. Highest contributions are shown in bold. In gray, discriminant loadings for the phases, showing the variance that the variables have within the discriminant function, to be interpreted in factor-loading fashion.

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Senescence</th>
<th>Fallen</th>
<th>Canon1</th>
<th>Canon2</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (mg L⁻¹)</td>
<td>-3.733</td>
<td>-1.356</td>
<td>4.021</td>
<td>-0.838</td>
<td>-0.461</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>5.985</td>
<td>-1.902</td>
<td>-5.516</td>
<td>-0.146</td>
<td>0.042</td>
</tr>
<tr>
<td>Al (mg L⁻¹)</td>
<td>-9.421</td>
<td>5.903</td>
<td>3.571</td>
<td>-0.836</td>
<td>0.136</td>
</tr>
<tr>
<td>B (mg L⁻¹)</td>
<td>-12.722</td>
<td>11.605</td>
<td>8.018</td>
<td>-0.847</td>
<td>0.120</td>
</tr>
<tr>
<td>% Humic</td>
<td>2.450</td>
<td>3.336</td>
<td>-7.066</td>
<td>0.581</td>
<td>0.721</td>
</tr>
<tr>
<td>BS</td>
<td>7.313</td>
<td>-5.748</td>
<td>-3.655</td>
<td>0.833</td>
<td>-0.217</td>
</tr>
<tr>
<td>FL</td>
<td>6.172</td>
<td>-3.005</td>
<td>0.500</td>
<td>0.859</td>
<td>-0.143</td>
</tr>
<tr>
<td>SUVA₂₅₄ (L mg⁻¹ cm⁻¹)</td>
<td>12.163</td>
<td>-3.935</td>
<td>-5.038</td>
<td>0.897</td>
<td>0.030</td>
</tr>
<tr>
<td>Constant</td>
<td>26.435</td>
<td>9.488</td>
<td>11.385</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgments

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cence induced patterns in leaf litter leachate using parallel factor

analysis modeling (PARAFAC) and self-organizing maps. J.


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### Appendix C

**LIST OF SAMPLES COLLECTED FOR MICROBIAL STUDY**

List of samples collected for the microbial study

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Figure C1: Biplot of throughfall to soil concentrations of K, Ca, Mg, and Mn for all four states. There is no noticeable relationship between throughfall concentration and soil concentration.
Figure C2: Biplot of throughfall to soil concentrations of Zn, Cu, B, Fe, and Al for all four states. There is no noticeable relationship between throughfall concentration and soil concentration.
Appendix E

SOIL NUTRIENT CONCENTRATIONS

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<th>B (mg/kg)</th>
<th>S (mg/kg)</th>
<th>Al (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermont</td>
<td>5.4</td>
<td>7.61</td>
<td>6.8</td>
<td>7.95</td>
<td>23.03</td>
<td>825.01</td>
<td>101.30</td>
<td>62.23</td>
<td>3.19</td>
<td>3.29</td>
<td>217.45</td>
<td>1.33</td>
<td>8.33</td>
<td>1052.94</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>3.9</td>
<td>6.24</td>
<td>29.0</td>
<td>21.55</td>
<td>70.95</td>
<td>110.33</td>
<td>43.32</td>
<td>4.26</td>
<td>2.47</td>
<td>1.26</td>
<td>339.31</td>
<td>2.04</td>
<td>20.04</td>
<td>1294.65</td>
</tr>
<tr>
<td>Maryland</td>
<td>3.8</td>
<td>7.25</td>
<td>3.3</td>
<td>5.15</td>
<td>26.91</td>
<td>43.27</td>
<td>18.33</td>
<td>6.72</td>
<td>0.81</td>
<td>0.88</td>
<td>147.38</td>
<td>0.90</td>
<td>18.57</td>
<td>1035.42</td>
</tr>
</tbody>
</table>

Soil Na data was not collected for this study. Calcium and pH values for RI are between VT and MD. K and CEC are higher for RI than the other two states (70 mg/kg compared to mid-20’s). Fe is also high for RI in both soil and Tf; according to Skudlark paper - Fe and Al are often localized).
Appendix F

FORECAST MAPS

Public domain forecast map data comes from The Daily Weather Map, which is published by the U.S. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA) National Weather Service, National Centers for Environmental Prediction.

Figure F1: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #1, 20 June 2015
Figure F2: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #2, 20 August 2015.
Figure F3: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #3, 29 Sep 2015.
Figure F4: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #4, 9 October 2015
Figure F5: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #5, 28 October 2015.
Figure F6: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #6, 5 June 2016.
Figure F7: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #7, 21 August 2016.
Figure F8: Forecast map for coordinated, accumulated precipitation and HYSPLIT Model reverse trajectory showing antecedent deposition sources for event #8, 6 July 2017.
Appendix G

NADP DEPOSITION TIME SERIES PLOTS

NADP deposition time series plots from the National Atmospheric Deposition Program (NRSP-3). 2019. NADP Program Office, Wisconsin State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706.

Figure G1: The NADP deposition time series plot for the station nearest the Maryland sampling location. The green circle surrounds annual totals of Na deposition, which correspond to the time of our study, and Na values are below 2.5 kg/ha.
Figure G2: The NADP deposition time series plot for the station nearest the Vermont sampling location. The green circle surrounds annual totals of Na deposition, which correspond to the time of our study, and Na values are below 0.55 kg/ha.
Figure G3: The NADP deposition time series plot for the station nearest the Rhode Island sampling location. The green circle surrounds annual totals of Na deposition which correspond to the time of our study, and Na values are quite high, between 10 and 26 kg/ha, greater than 4 times higher the amount of deposition in MD.