DISSOLVED ORGANIC NITROGEN (DON) CYCLING ALONG A TEMPERATE FOREST NITROGEN AVAILABILITY GRADIENT

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ABSTRACT

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Nitrogen (N) is essential for ecosystem productivity, yet frequently constrains ecosystem primary production. Previously, the majority of research on N cycling has focused on inorganic N biogeochemistry. More recently, research investigating dissolved organic N (DON) has found it also plays a significant role in N biogeochemistry, both as a vector for N loss from terrestrial ecosystems and as a source of plant-available N, which suggests DON is an important component of the terrestrial N cycle.

This dissertation research investigated the role of DON as an N source for temperate trees and as a vector of N loss, using northen hardwood forests of varying tree species composition and soil properties as a basis for study. First, in Chapter 2 I investigated DON uptake by four tree species that commonly occur in either low or high N availability forests. I grew tree seedlings in a greenhouse and labeled them with ^{15}N-enriched amino acids (organic N source), ^{15}N-ammonium, and ^{15}N-nitrate (inorganic N sources). I found that specific uptake rates of amino acid-N were similar across all tree species. However, high N availability species took up NH_4^+ twice as fast as low N availability species, suggesting amino acid-N was relatively more important to low versus high N availability species. Low N availability species also acquired up to 4 times more total N from amino acids compared to inorganic N sources. These results suggest plant species dominance in a habitat is linked to their ability to use the most available N pool.

Second, in Chapter 3 I investigated dissolved organic matter (DOM) leaching losses from forests that spanned a gradient of N availability and tree species composition. I collected soil solutions for three years with lysimeters and analyzed them for dissolved organic carbon (DOC), dissolved inorganic nitrogen (DIN), and DON composition and fractionated DOC in the solutions into hydrophobic and hydrophilic compounds. I also evaluated the characteristics of soils in the forests. I found that DON losses at 100 cm soil depth were not related to increasing soil N stocks across forests, contrary to my expectations. Surprisingly, DOM losses at this same soil depth demonstrated a unimodal pattern of DOC:DON, with relatively low DOC:DON in DOM losses from low and high N availability forests and relatively high DOC:DON from intermediate N availability forests. These patterns likely resulted from the different source and sink strengths of forest soils for DOM as forest floor composition and soil characteristics changed.

Finally, in Chapter 4 I evaluated how soil characteristics impacted the chemistry of soil waters leaching from soil cores collected from the above forests. I leached 0-10, 0-25, and 0-50 cm deep cores with a common organic matter solution and analyzed the solutions for DOC, DIN, DON, and hydrophobic/hydrophilic fractions. I also measured multiple physical and geochemical characteristics of the soil cores. Soil depth had a stronger impact on DOM chemistry compared to forest differences. DOM concentrations in soil core leachate decreased with soil depth due to the removal of hydrophobic compounds. Noticeably, DON concentrations increased between the input organic matter solution and 10 cm soil depth, which was accompanied by 67-fold increase in the hydrophilic fraction of DON. These results demonstrated that soil has a strong ability to influence the quantity and quality of DOM leaching through forest soils.

DEDICATION

This dissertation is dedicated most deservedly to Craig, my husband, for his unimaginable amount of patience, tolerance, and support while I worked my way through grad school - again. I thoroughly appreciate your willingness to move to Michigan on my urging and, most recently, to take on the second job of babysitter on the weekends. I also dedicate this work to my daughter, Iselin, who multiple times was told “Mommy’s working” when she asked for me and had to do without. Finally, I dedicate this dissertation to my brother, Jon. Of the two of us, you were always more likely to get a Ph.D. Perhaps I am still trying to follow in your footsteps, even though the ground is bare before me.

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CHAPTER 1

INTRODUCTION

Until relatively recently, the majority of research on the terrestrial nitrogen (N) cycle has focused on the production and consumption of inorganic forms of N, namely ammonium (NH_4^+) and nitrate (NO_3^-). It is understood that these N forms are tightly cycled in forest ecosystems where plants and microorganisms strongly compete for N, such as low fertility habitats or ecosystems with rapid biomass accumulation (Vitousek and Reiners 1975). In forests where plant-microbe competition for N is minimized, such as recently disturbed forests that experienced a large-scale destruction of biomass (i.e., clear-cutting, fire) or high fertility forests where rates of N mineralization outweigh the amount of N immobilized by plants and microbes (Vitousek et al. 1979, Aber et al. 1998), N losses ensue. These results demonstrate the ability of plants and microorganisms to exert profound control over the retention of inorganic N in forest ecosystems. They also suggest NH_4^+ and NO_3^- are the primary forms of plant-available N, with net N mineralization rates providing an index of overall N availability to plants.

More recently, research on terrestrial N cycling that also included organic N has demonstrated N loss patterns that call into question our previous understanding of the controls that govern N retention and loss from temperate forests. Unlike inorganic N, dissolved organic N (DON) is lost from terrestrial ecosystems that span a range of N fertilities independent of biotic N limitation (Perakis and Hedin 2002), suggesting there is an N "leak" from temperate forests that is outside of biologic control and has previously been ignored in N cycling models (Hedin et al. 1995, Neff et al. 2003). Moreover, this DON "leak" can account for a substantial fraction of total N losses from terrestrial ecosystems (Campbell et al. 2000, Perakis and Hedin 2002, Möller et al. 2005). For example, across a range of unpolluted temperate forests in Chile, Perakis and

Hedin (2002) found DON losses accounted for 61-97% of total N losses. In streamwater draining forests of New England, USA, DON comprised up to 90% of total N leaching from the ecosystem (Campbell et al. 2000). These DON losses can perpetuate N limitation in terrestrial ecosystems (Neff et al. 2003) and limit the ability of an ecosystem to sequester carbon (C) under rising atmospheric carbon dioxide levels (Rastetter et al. 2005). Additionally, multiple studies have confirmed the ability of plants to take up small DON molecules, such as free amino acids, at rates equivalent to, or exceeding, those of NH_4^+ and NO_3^- (Kielland 1994, Schimel and Chapin 1996, Näsholm et al. 2000, Thornton and Robinson 2005), suggesting organic N is a potentially important pool of plant-available N previously unexplored in studies relating plant productivity with soil N cycling. Taken together, these results indicate DON has a significant role to play in the N biogeochemistry of terrestrial ecosystems.

DON is comprised of a heterogeneous pool of organic compounds that range along a continuum of molecular sizes, solubilities, and reactivities (Qualls and Haines 1991). Despite this diversity, DON has been operationally divided into two broad categories (Neff et al. 2003): 1) low molecular weight, labile molecules, such as amino acids and amino sugars, that are recognized as a potentially important pool of plant-available N in many ecosystems (Lipson and Näsholm 2001, Schimel and Bennett 2004) and 2) higher molecular weight, more recalcitrant molecules too large to be taken up by plants and microbes and therefore most likely to leach from terrestrial ecosystems (Neff et al. 2003). The former category of DON molecules has been the subject of emerging biogeochemical theory that predicts labile DON will dominate pools of plant-available N in low fertility ecosystems but increasingly give way to inorganic forms as N mineralization rates increase (Schimel and Bennett 2004). Specifically, N-pool dominance is expected to shift from amino acid-N in low-N availability habitats, to NH_4^+-N in intermediate N

availability habitats due to increasing N mineralization, and finally NO_3^--N in high N availability habitats where nitrification rates are high. This new model deemphasizes the role of N mineralization as the key rate-limiting process making N available to biota in the soil N cycle in favor of microbial depolymerization of large organic polymers into bioavailable, N-containing monomers. This model also suggests that plants and microorganisms will equally compete for organic N resources in low fertility habitats.

Many of the data behind this new model of N cycling have been collected in high latitude/altitude ecosystems where cold temperatures limit N mineralization, resulting in the accumulation of organic matter rich in amino acids (Kielland 1994, Schimel and Chapin 1996, Raab et al. 1999, Öhlund and Näsholm 2001). This is fundamentally different from temperate regions where N mineralization is frequently constrained by xeric site conditions and poor litter quality (Pastor et al. 1984), making it unclear if the above model of shifting N-pool dominance is transferable to temperate ecosystems. However, there is increasing evidence that amino acids can dominate plant-available N pools in low-fertility temperate forests, and that these pools give way to inorganic N forms as predicted in the above model. Gallet-Budynek et al. (2009) and Rothstein (2009) found decreasing standing pools of free amino acids in favor of NH_4^+ and NO_3^- with increasing site fertility in northeast hardwood forests, USA. Furthermore, there are a growing number of studies that have demonstrated the ability of plants in temperate regions to take up amino acid-N (Bennett and Prescott 2004, Hofmockel et al. 2007, Warren and Adams 2007). For example, Finzi and Berthrong (2005) and Gallet-Budynek et al. (2009) found that roots collected from northeastern forests, USA, with lower N mineralization rates had higher ^{15}N contents compared to more fertile forests after being supplied with ^{15}N-labelled amino acids.

The shift in N-pool dominance between organic and inorganic N forms may have implications for plant species distributions across the landscape. In a study along a boreal forest N-fertility gradient, Nordin et al. (2001) found plant species in low-fertility habitats took up more amino acid-N compared to plant species in high fertility forests; correspondingly, amino acid N was the largest N pool in the low fertility habitat whereas high fertility habitats were dominated by inorganic N forms. These results provide compelling evidence that a species' ability to dominate in a particular ecosystem is related to its ability to access the most available N pool in that ecosystem, regardless of whether it is comprised of organic or inorganic N forms (McKane et al. 2002).

The ability of DON to be leached from terrestrial ecosystems despite N limitation by biota has spurred much research investigating the processes that either restrict or facilitate DON losses across a variety of ecosystems (Qualls et al. 2000, Pregitzer et al. 2004, Lajtha et al. 2005, Brookshire et al. 2007, Sleutel et al. 2009). As stated previously, these DON losses are thought to be dominated by DON molecules that are typically unavailable for biotic uptake because of their high molecular weights and recalcitrant nature (Yu et al. 2002). Therefore, abiotic processes are thought to be paramount in regulating DON losses from terrestrial ecosystems (Qualls 2002, Yano et al. 2005), particularly the ability of soil to either release or adsorb DON as water percolates through the soil. Typically, organic soil horizons are the primary region of DON production (Qualls et al. 2002, Park and Matzner 2003), although canopy throughfall and wet deposition also contribute DON (Seely et al. 1998, Dittman et al. 2007). In organic horizons, DON is generated by organic matter inputs from plant litter (Qualls et al. 2002), microbial exoenzymes produced during decomposition, microbial biomass turnover, faunal N production (reviewed by Kalbitz et al. 2000), and the interaction between NO_3^- and dissolved organic matter

(Perakis and Hedin 2001). Fluxes of DON from the O horizon can range from 10 to 18 kg ha^{-1} yr^{-1} in forest ecosystems, roughly 2 to 9 times greater than inputs from throughfall and up to 66 times greater than DON fluxes from deep soils (Qualls et al. 1991, Qualls et al. 2002, Schwendenmann and Veldkamp 2005, Sleutel et al. 2009). Studies that have manipulated litter inputs to the forest floor found greater DON concentrations in leachate from the O horizon in plots that received increased litter additions, indicating litter quantity can impact DON losses from the O horizon (Park and Matzner 2003, Kalbitz et al. 2007).

In order to fully understand the controls over DON dynamics, it is critical to understand how dissolved organic matter (DOM) overall is regulated as it moves through soils, of which DON is one facet. Multiple studies have demonstrated the strong ability of mineral soil to sorb DOM, which can attenuate DON losses from forest ecosystems (Seely et al. 1998, Qualls et al. 2002, Yano et al. 2004, Möller et al. 2005). Indeed, Qualls et al. (2002) found fluxes of DON from the C horizon of hardwood forests in North Carolina, USA, were only 1.6% of fluxes from the O_a horizon. The ability of a particular soil to adsorb DOM depends in part on its texture; coarse soils retain less DOM than those that are fine textured due to lower surface areas and shorter hydrologic flow paths that reduce adsorption opportunities between percolating solutions and the soil matrix (Seely et al. 1998, Qualls et al. 2002, Asano et al. 2006). Therefore, soils that favor short contact times (i.e. soils with coarse texture, large macropores, and/or high pore connectivity) between the soil solution and matrix, or no contact time in the extreme case of overland flow, are more likely to leach DOM compared to soils that favor long contact times (Michaelson et al. 1998, Möller et al. 2005). Paradoxically, finer textured soils may have better soil structure characterized by preferential flow, which would reduce soil solution exposure to

sorbing materials and increase losses of DOM (Castellano and Kaye 2009). Castellano and Kaye (2009) found soils with 12% clay were most likely to exhibit good structure with preferential flow; soils with clay contents on either side of this figure had poor structure and were dominated by matrix flow. Other factors, such as organic matter content (Lilienfein et al. 2004), the presence of carbonates (Kaiser et al. 1996), and mineral composition (i.e. Fe and Al oxides; Qualls 2000, Lilienfein et al. 2004, Yano et al. 2004) can also influence the ability of a soil to adsorb DOM.

While mineral soil has the capacity to adsorb DOM that leaches from the forest floor, it also has the capacity to release DOM into solution (Kaiser and Zech 1998, Yano et al. 2004, Möller et al. 2005). In a conifer forest in western Oregon, Yano et al. (2004) found concentrations of DON peaked from 0 to 10 cm depth in mineral soil compared with the more typical pattern of DOM reaching maximum concentrations at the bottom of the O horizon (Qualls et al. 2002, Schwendenmann and Veldkamp 2005). They attributed this partly to the thin O horizon of these Douglas-fir-western hemlock forests (*Pseudotsuga menziesii, Tsuga heterophylla*, respectively), suggesting a greater accumulation of litter and humic material in the O horizon was necessary for this layer to be a significant source of DOM to mineral horizons below. Therefore, though the soil may generally be a net sink for DOM, specific horizons may be a net source of these compounds.

Losses of DOM from forest ecosystems fluctuate throughout the year in response to seasonal variability in precipitation (Michalzik and Matzner 1999, Campbell et al. 2000, Fisk et al. 2002). Peak losses of DOM occur during snowmelt and after litterfall in the autumn when sources of soluble organic compounds are expected to be high (Michalzik and Matzner 1999, Qualls et al. 2002, Yano et al. 2004). Interestingly, the concentration of DOM leaving organic

and mineral horizons is frequently unaffected by water fluxes through the soil (Michalzik and Matzner 1999, Campbell et al. 2000, Fisk et al. 2002). Qualls et al. (2002) hypothesized that the fairly constant release of DOM throughout the year was due to sorption phenomena between organic compounds and soils that gradually released compounds from a pool of potentially soluble organic material throughout the year. They suggested hydrogen bonding or Van der Waals forces were the most likely mechanisms for dissolved organic-organic surface sorption in organic horizons while Fe and Al oxyhydroxides were likely the primary compounds interacting with DON in the mineral soil.

In order to investigate how sorption/desorption phenomena between mineral soil and percolating solutions impacts total DOM losses from terrestrial ecosystems, many studies fractionate DOM into hydrophobic and hydrophilic components (Kaiser and Zech 1998, Qualls and Haines 1991, Yano et al. 2004). These studies have found that hydrophobic compounds preferentially sorb with mineral soil relative to hydrophilic compounds (Kaiser and Zech 1998, Yano et al. 2004). Hydrophobic molecules typically have high molecular weights and consist of protein-tannin complexes and amino acids complexed with humic substances; hydrophilic molecules consist of relatively lower molecular weight compounds such as amino acids and free peptides (Guggenberger et al. 1994). The tendency of hydrophobic compounds to preferentially sorb to mineral soil over hydrophilic compounds suggests those ecosystems with soils that favor long contact times between soil solutions and the soil matrix, such as fine-textured soils, would also be more likely to remove a greater proportion of hydrophobic compounds from solution. It also suggests that the proportion of hydrophobic compounds in solution will decrease with increasing soil depth (Yano et al. 2004, Lajtha et al. 2005). Hydrophobic compounds have also been shown to displace previously sorbed hydrophilic substances (Kaiser and Zech 1998),

suggesting these fractions compete for binding sites on soils. The preferential sorption of hydrophobic DOM to mineral soil compared to hydrophilic substances led Yu et al. (2002) to suggest the dominance of DON in the hydrophobic fraction they found in O horizon leachate at two northern California forests would limit the loss of DON from those ecosystems. Instead, the most likely vector for DON losses would be the hydrophilic fraction. In a study of tropical forests in Thailand, Möller et al. (2005) found that the hydrophilic fraction of DON comprised from 54% to roughly 75% of total DON in soil solutions and was the dominant fraction of DON in streams. Therefore, ecosystems with DON inputs that are dominated by the hydrophobic fraction may experience relatively lower total DON losses compared to those ecosystems with a greater proportion of hydrophilic DON in inputs.

Studies that fractionate DON into hydrophobic and hydrophilic compounds, and examine the biodegradability of those fractions, provide results that question the assumption that recalcitrant, biotically unavailable fractions will necessarily dominate DON losses from forests. Soil solutions inoculated with microbes frequently display a positive relationship between the amount of DOM consumed and the concentration of operationally defined hydrophilic compounds in the solution; less DOM consumption is generally observed where hydrophobic compounds dominate (Michaelson et al. 1998, Cleveland et al. 2004, Qualls 2004, Kaushal and Lewis 2005). Kaushal and Lewis (2005) found similar results in two Colorado streams where 15-71% of DON was biodegradable; they attributed the relatively labile nature of the DON pool to the presence of non-humic (i.e. operationally hydrophilic) N compounds. The higher molecular weights, higher aromaticity, and lower acidity (Guo and Chorover 2003) of hydrophobic compounds compared to hydrophilic compounds may contribute to this fraction's decreased biodegradability. However, in a study investigating the biodegradability of extracts from fresh

and senesced foliage, Cleveland et al. (2004) found 60-97% of DOM in the humic fraction was consumed by microbes, similar to the consumption levels of non-humic compounds in their samples. The authors' attributed the high biodegradability of the more recalcitrant DOM pool to the absence of soil in their experiment, suggesting the lack of competing sinks for DOM from soil allowed microorganisms greater access to a wider range of DOM compounds than they experienced in a natural soil environment. Therefore, the potential for microbial interaction with DOM compounds, and thus their bioavailability, may be equally as important as the ability of microorganisms to take up DOM (i.e., biodegradability) for determining how biota use organic compounds (Marschner and Kalbitz 2003).

The inorganic N status of a particular ecosystem may also influence the extent to which DON is used by biota for N nutrition. Dissolved inorganic N (DIN) is preferentially taken up by biota compared to organic N and therefore may out compete DON as a N source where inorganic N concentrations are high (Kaushal and Lewis 2005). Kaushal and Lewis (2005) found this pattern to be true in streams where the biodegradability of DON was inversely related to the concentration of DIN in the stream. These data suggest the biodegradability of DON may change depending on inorganic N status of a particular ecosystem and could vary across the landscape.

Soils not only directly influence DON losses through sorption/dissolution dynamics, but also indirectly through their influence on plant community composition. Edaphic characteristics and plant community composition vary in tandem across the landscape, with coarse-textured soils of low moisture-holding capacity associated with tree species having relatively low litter N while fine-textured soils are associated with high N species (Pastor et al. 1984, Host et al. 1988). As the plant community composition changes across the landscape, so does the nature of the forest floor and the quantity and composition of organic molecules available for dissolution

(Park and Matzner 2003, Dittman et al. 2007). Forests with low C:N in organic horizons have correspondingly larger N pools and therefore a greater quantity of N subject to leaching (Seely et al. 1998, Brookshire et al. 2007).

These complex interactions between soils and plants make it difficult to predict how DON fluxes will vary across ecosystems. However, a growing number of studies investigating DON loss in a variety of ecosystems (Qualls et al. 2000, Pregitzer et al. 2004, Lajtha et al. 2005, Brookshire et al. 2007, Sleutel et al. 2009) seem to suggest DON loss is a function of soil N availability. Total N losses from forests with low N availability are typically dominated by DON but shift towards being dominated by inorganic N forms as the soil N stocks across ecosystems increase (Perakis and Hedin 2002, Pregitzer et al. 2004, Brookshire et al. 2007, Sleutel et al. 2009). Additionally, DON is lost in direct proportion with DOC from ecosystems with low N availability, but disproportionately increases over DOC losses in ecosystems with high N availability, creating a shift in DOM stoichiometry (Pregitzer et al 2004, Brookshire et al. 2007). These patterns suggest that there is a fundamental change in the way DON is cycled as from low to high N availability ecosystems, although the mechanisms for this change are still unclear. Linking fine-scale DON controls with a landscape perspective of DON cycling is necessary to shape a comprehensive understanding of N biogeochemistry.

From the body of research that has investigated DON dynamics in terrestrial N cycling and plant N nutrition, it is clear that DON holds a critical place in our understanding of N biogeochemistry, yet there is still much ambiguity about the controls that regulate DON losses from terrestrial ecosystems and the way DON cycling may impact plant species distributions in temperate environments. For my dissertation research, I sought to add clarity to these information gaps by developing studies that: i) linked local processes that influence DON

cycling with larger ecosystem patterns of DON leaching losses, and ii) investigated whether or not tree species typically found in low-N temperate forest environments use DON differently than those found in high-N temperate forest environments. To do this, I used a combination of field and laboratory studies based in northern hardwood forests of the Manistee National Forest in the northwestern Lower Peninsula of Michigan, USA. These forests represent distinct landform-vegetation associations that reoccur throughout the Lake States region (Host et al. 1988), suggesting the results of my research may be applicable to a large portion of this region. The forests I worked in spanned a gradient of N availabilities (Zak et al. 1986), with potential N mineralization and nitrification rates ranging from 0.61-1.32 µg N g^{-1} day^{-1} and 0.01-1.10 µg N g^{-1} day^{-1}, respectively (Rothstein 2009). The lowest N availability forest occurred on a sandy outwash plain and is dominate by oaks (*Quercus alba, Q. velutina*) with soil that is classified as a Typic Udipsamment. Moderate N availability forests shifted from red oak (*Q. rubra*)/red maple (*Acer rubrum*) dominance to sugar maple (*A. saccharum*)/red oak- dominated communities with soils that range from Entic Haplorthods to Typic Haplorthods. The forests with the highest N availabilities occurred on sugar maple-dominated moraines with soils classified as Typic Haplorthods with clay lamellae. The soils of these five forests represent a spodic developmental series.

My first research chapter, Chapter 2, focuses on the role of DON in the plant N nutrition of four tree species commonly found in northern hardwood forests. I developed a greenhouse study where I grew seedlings of two hardwood tree species that are typically found in low N forests and two hardwood tree species typically found in high N forests and exposed them to uptake solutions containing ^{15}N-enriched amino acids (organic N source), ^{15}N-ammonium, and

^{15}N-nitrate (inorganic N sources). This enabled me to determine whether or not tree species characteristic of low fertility sites would exhibit greater preference for amino acids compared to species characteristic of high fertility sites. My overarching research question for Chapter 2 was: *Are there differences in the way tree species from habitats of either low or high N fertility use organic and inorganic N forms?*

The next two research chapters of my dissertation focus on the patterns of DON cycling across forests and the mechanisms that either facilitate or restrict DON leaching losses. In Chapter 3, I collected soil solutions from five hardwood forests with pan trap and tension lysimeters and analyzed the solutions for DON, dissolved inorganic nitrogen (DIN), and DOC composition in order to understand how nutrient losses varied across forests that spanned an N-availability gradient. I then fractionated DOC in the soil solutions into hydrophobic and hydrophilic constituents and analyzed various properties of the soils near the lysimeters to understand how fine-scale processes control DOM mobility in soils, and thereby contribute to landscape patterns of DOM cycling. My overarching research question for Chapter 3 was: *How do DOM leaching losses from temperate forests change across a gradient of N-availability and soil conditions?*

Finally, in Chapter 4 I collected soil cores from the above forests and leached them with a common organic matter solution in order to investigate the interactions between DON leaching losses and soil properties. I analyzed the soil core leachate for DON and DOC concentrations, fractionated these pools into their hydrophobic/hydrophilic constituents, and analyzed the biodegradability of the solutions. I also analyzed soil characteristics, including texture, iron and aluminum content, and hydraulic conductivity and linked their differences to the DON and DOC chemistry in the leachate. My overarching research question for Chapter 4 was: *How do soil*

characteristics affect the quantity and quality of DON and DOC as soil solutions percolate to depth?

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CHAPTER 2

AMINO ACID UPTAKE BY TEMPERATE TREE SPECIES CHARACTERISTIC OF LOW- AND HIGH-FERTILITY HABITATS

Abstract

The relationship between inorganic-nitrogen (N) cycling and plant productivity is well established. However, recent research has demonstrated the ability of plants to take up low molecular weight organic N compounds (i.e., amino acids) at rates that often rival those of inorganic N forms. In this study, we hypothesize that temperate forest tree species characteristic of low fertility habitats will prefer amino acids over species characteristic of high fertility habitats. We measured the uptake of ^{15}N-labeled amino acids (glycine, glutamine, arginine, serine), ammonium (NH_4^+), and nitrate (NO_3^-) by four tree species that commonly occur in eastern North America where their abundances have been correlated with inorganic-N availability. Specific uptake rates of amino acids were largely similar for all tree species; however, high fertility species took up NH_4^+ at rates more than double those of low fertility species, rendering amino acid-N relatively more important to the N nutrition of low fertility species. Low fertility species acquired over four times more total N from arginine compared to NH_4^+ and NO_3^-; high fertility species acquired the most N from NH_4^+. Arginine had the highest

uptake rates of any amino acid by all species; there were no significant differences in uptake rates of the remaining amino acids. Our results support the idea that the dominant species in a particular habitat are those best able to utilize the N resources most available.

Key words: ammonium, nitrate, organic-N, molecular weight, ^{15}N

Introduction

Nitrogen (N) is a fundamental component of plant tissues and is often considered to be the nutrient that most limits plant growth. As a result, there has been considerable research across a wide range of terrestrial ecosystems devoted to studying how this element is made available to plants. Until relatively recently, the majority of this work has focused on the cycling of inorganic forms of N (i.e., ammonium and nitrate), assuming they represented the form most available to plants (Pastor et al. 1984; Zak et al. 1986; Zak et al. 1989). However, more recent research has demonstrated the ability of plants to take up low molecular weight organic N compounds, primarily free amino acids, at rates that often rival those of ammonium (NH_4^+) and nitrate (NO_3^-) (Kielland 1994; Schimel and Chapin 1996; Näsholm et al. 2000; Näsholm and Persson 2001; Thornton and Robinson 2005). Therefore, organic-N is a potentially important pool of plant-available N that has largely been overlooked in previous studies relating soil N cycling with plant productivity.

In an attempt to incorporate organic-N into conceptual models of soil N cycling, Schimel and Bennett (2004) proposed that the predominant form of plant-available N would shift from organic-N to NH_4^+-N to NO_3^--N as the overall N cycling rate of a habitat increased. This new model emphasizes microbial depolymerization of large organic polymers into bioavailable, N-containing monomers (e.g., amino acids, amino sugars) as the key process in the soil N cycle, rather than N mineralization. The shift in emphasis away from N mineralization assumes that plants can compete successfully with microbes for organic-N when mineralization is constrained. This pattern has largely been born out in high latitude/altitude ecosystems where cold temperatures limit N mineralization and organic matter accumulates in soils (Kielland 1994;

1995; Schimel and Chapin 1996; Raab et al. 1999; Nordin et al. 2001; Öhlund and Näsholm 2001). In these systems, free amino acids dominate pools of potentially-available N in habitats where inorganic N is scarce (Kielland 1994, 1995; Raab et al. 1999; Nordin et al. 2001). Moreover, there are numerous studies documenting the ability of boreal, arctic, and alpine plants to take up free amino acids (Kielland 1994; Schimel and Chapin 1996; Näsholm et al. 1998; Raab et al. 1999). Interestingly, there is evidence of covariation between the availability of amino acid versus inorganic N in soil and the physiology of the dominant plant species in these cold climate ecosystems. For example, the dominance of different plant species in tussock tundra habitats was related to their ability to utilize the largest pool of available N in a particular habitat, be it inorganic or organic (McKane et al. 2002). Nordin et al. (2001) found that plant species growing in a low-fertility habitat along a boreal forest N-fertility gradient had 30% of their total N uptake fulfilled by glycine compared to the high-fertility habitat where glycine uptake was only 10% of total plant N uptake. Correspondingly, the largest N pool at the low-fertility site was comprised of amino acid-N (always at least 70% of the total N pool), whereas amino acid-N was never more than 19% of total N at the high-fertility site. This trend was reversed with NO_3^- where both plant uptake of NO_3^- and pool sizes were largest at the high fertility site. The authors' speculated that the change in plant species composition across the gradient might be a function of particular species' ability to acquire different forms of N.

There has been less work in temperate ecosystems investigating the availability of amino acids across the landscape and the role these compounds may play in plant N nutrition. However, there is growing evidence that amino acid pools are relatively more abundant in temperate forests where N mineralization rates are low (Finzi and Berthrong 2005; Gallet-Budynek et al. 2009; Rothstein 2009), providing a pool of potentially plant-available N in what were previously

considered low-fertility habitats. Also, a few studies have shown that temperate trees have the ability to use amino acid-N (Bennett and Prescott 2004; Hofmockel et al. 2007; Warren and Adams 2007). In an attempt to link plant nutrition with patterns of amino acid versus inorganic N availability, Finzi and Berthrong (2005) and Gallet-Budynek et al. (2009) compared root uptake of ^{15}N-labelled amino acids among several forest ecosystems and found, in general, that roots from sites with slower rates of N mineralization had a higher ^{15}N content than those from more fertile sites. Neither study identified the roots to species, though, so it is unclear how different species of plants varied in their use of amino acid versus inorganic forms of N. However, oak-beech-hemlock forests dominated the low-fertility sites of both studies while a sugar maple-white ash forest dominated the high-fertility sites, suggesting the shift in uptake rates of glycine by roots was at least partly driven by species replacement.

A large number of the studies evaluating plant uptake of amino acids have used glycine as their "test" amino acid due to its low molecular weight, relative ease of mobility in the soil compared to other amino acids (Owen and Jones 2001), and poor substrate quality for microbial growth (Lipson et al. 1999). However, soluble N pools in soil contain a wide variety of different amino acids with varying abundances and molecular structures (Kielland 1995; Yu et al. 2002; Rothstein 2009). While glycine is often one of the dominant free amino acids in field soil solutions, ranging from approximately 5-20% of total free amino acids depending on the ecosystem (Kielland 1995; Rothstein 2009), other amino acids can occur in equal or greater concentrations (Senwo and Tabatabai 1998; Raab et al. 1999), suggesting glycine uptake potential alone does not necessarily represent plant access to amino acid-N pools. Plants have been shown to take up a variety of other amino acids (Kielland 1994; Öhlund and Näsholm 2001; Persson and Näsholm 2001), sometimes to a greater extent than glycine (Weigelt et al. 2005),

suggesting studies evaluating amino acid uptake should use multiple amino acids when trying to determine whether or not amino acid-N is important for plant nutrition.

The objective of this study was to test the hypothesis that temperate deciduous forest species characteristic of low-ertility sites would exhibit greater preference for amino acids compared to species characteristic of high-fertility sites. We used tree species that commonly occur in eastern North American forests where it has been well documented that tree species composition is correlated with patterns of inorganic N pools in repeatable assemblages across the landscape (Host et al. 1988; Zak et al. 1986; Zak et al. 1989; Finzi et al. 1998). These predictable associations make this region an ideal study area to investigate whether or not amino acid-N availability might also influence tree species dynamics, especially since recent studies have shown that gradients of free amino acids in soil run opposite to those for inorganic N (Gallet-Budynek et al. 2009; Rothstein 2009). Our research compared the uptake of four amino acids, NH_4^+, and NO_3^- by seedlings of red oak (*Quercus rubra*), American beech (*Fagus grandifolia*), white ash (*Fraxinus americana*), and black cherry (*Prunus serotina*). We hypothesized that red oak and American beech would take up amino acids to a greater extent than black cherry and white ash because the former two species are most often found in habitats with relatively slow rates of N mineralization and abundant pools of amino acids (Host et al. 1988; Zak et al. 1986; Rothstein 2009). Conversely, we hypothesized that black cherry and white ash would prefer inorganic forms of N because they are most abundant in habitats with rapid ammonification and nitrification as well as smaller pools of free amino acids. Finally, we hypothesized that the distribution of N within seedlings would depend on the form in which it was taken up. Specifically, we hypothesized that NO_3^--N would most likely be found in leaves due to its ability to bypass assimilation into organic molecules in the roots (Andrews 1986); that NH_4^+-N would

predominantly reside in roots; and that the distribution of amino acid-derived N would vary depending on the metabolic roles of individual amino acids.

Methods

Plant establishment

In order to determine whether or not there were differences in how northern hardwood trees use amino acids versus inorganic N forms, we germinated seeds of red oak, American beech, white ash, and black cherry obtained from F.W. Schumacher Co., Inc., Sandwich, MA, USA (41.759° N, 70.494° W) and supplied them with ^{15}N-enriched substrates. The seeds were soaked overnight in tap water and stratified at 5° C for 30-90 days, depending on species' requirements. Prior to sowing, all seeds were surface sterilized in a 10% sodium hypochlorite solution for 30 seconds, rinsed in deionized (DI) water, and allowed to air dry. The seeds were sown in a peat, vermiculite, and perlyte growth medium (Faford #2) in 10 cm x 36 cm pots; the potting medium contained a starter supply of nutrients including inorganic N. All pots were placed in a greenhouse at natural light levels. The average high temperature in the greenhouse over the course of the experiment was 26.5˚C, the average low temperature was 18.3˚C. Two weeks after germination, each germinant began receiving 250 ml of a fertilizer solution containing a cocktail of 0.6 mM arginine, 2.5 mM glycine, 2.5 mM serine, and 1.3 mM glutamine twice per week for approximately 19-21 weeks (depending on species); amino acid concentrations were varied so that each supplied an equimolar amount of N to the fertilizer solution. We used amino acids in the fertilizer solution to insure amino acid uptake by the seedlings would not be limited by a predisposition towards inorganic N forms (Henry and Jefferies 2003). The macronutrient concentrations in the solution followed a modified

Hoagland's solution (Hoagland and Arnon 1938): 2.0 mM $CaSO_4$, 1.0 mM $Ca(H_2PO_4)_2$, 2.5 mM K_2SO_4, 2.0 mM $MgSO_4$. The micronutrient makeup was: 46.3 μM H_3BO_3, 9.0 μM $MnSO_4 \cdot H_2O$, 0.8 μM $ZnSO_4 \cdot 7H_2O$, 0.3 μM $CuSO_4 \cdot 5H_2O$, 0.02 μM $(NH_4)Mo_7O_{24} \cdot 4H_2O$, 12.0 μM Fe EDTA. At the time of the labeling experiment, seedling biomass averaged 7.17 g for red oak (range: 2.96 -14.97), 2.45 g for American beech (range: 1.56 – 3.50), 1.93 g for white ash (range: 0.46 – 6.86), and 10.40 g for black cherry (range: 3.78-15.95).

Plant labeling

To conduct the labeling experiment, the tree seedlings were carefully removed from the potting medium, and their roots were gently washed with DI water; a visual inspection of roots after washing suggested mycorrhizal infection was either absent or limited in scope. Once clean, the roots were immediately sterilized by submersion in a 10% sodium hypochlorite solution for 30 sec (Reissinger et al. 2001) followed by a rinse in a 0.5 mM $CaCl_2$ solution and a second rinse in reverse osmosis water. We wanted to measure the specific uptake of N when there was competition between inorganic and amino acid N to capture any inhibitory effects of one N species on another that might influence plant uptake (Thornton and Robinson 2005). Therefore, each seedling's root system was submerged in 750 mL of a solution containing 300 μM L^{-1} of ammonium nitrate (NH_4NO_3), 300 μM $L^{-1}$of one of four amino acids (i.e., glycine, serine, glutamine, or arginine), and 0.5 mM of $CaCl_2$ (to maintain membrane integrity), although only one N species was enriched in ^{15}N per treatment (Table 2.1). We selected the 300 μM L^{-1} concentration for the labeled substrates to ensure N uptake by the seedlings would not be substrate limited over the course of the labeling exercise (Henry and Jefferies 2003). We used L-

isomers instead of D-isomers of each amino acid since they are more prevalent in soils (Lipson and Näsholm 2001). In most cases, each treatment per species had 5 replicate seedlings, although the glutamine and NO_3^- treatments for American beech, and the NO_3^- treatment for black cherry only had 4; white ash had only 4 replicates in each treatment due to lower seedling survival. We included an additional treatment of universally-labeled glycine (U-$^{13}C_2$, ^{15}N-glycine) in order to verify that the experimental setup would allow for the uptake of intact amino acids by the tree species selected; plant tissues enriched in ^{13}C would indicate that the C skeleton of the amino acid was taken up concomitant with the N group (Näsholm et al. 1998). The seedlings were suspended for 50 min in aerated solutions that had been adjusted to pH 5.5 (a typical surface-soil pH for northern hardwood forests in this region) with 0.1 N hydrochloric acid or sodium hydroxide as appropriate. The control solution contained only $CaCl_2$.

After labeling, each seedling's root system was soaked in a 5.0 mM $CaCl_2$ solution for 5 min to remove any ^{15}N adsorbed to the exterior of the roots (Persson and Näsholm 2001). The seedling roots were rinsed thoroughly in reverse osmosis water before the plant was separated into above- and belowground organs, frozen in liquid nitrogen, and stored frozen until further processing. Tissues were later oven dried at 65°C for 48hours (dual-labeled glycine plants were lyophilized) and separated into fine and coarse roots, shoots, and leaves. Dried plant material was weighed, ground first with a mortar and pestle then a ball mill, and analyzed for ^{15}N and ^{13}C content on a Europa Integra continuous flow isotope ratio mass spectrometer at the Stable Isotope Facility, University of California at Davis.

Calculations and Statistical Analyses

Atom% excess of plant organs was determined by subtracting the mean ^{15}N and ^{13}C abundances of unlabeled plants from those supplied with enriched compounds. We determined the quantity of tracer ^{15}N or ^{13}C in each organ by multiplying the atom % excess by the total moles of N or C. These values were summed for each plant and expressed on gram^{-1} of fine root hour^{-1} basis to account for variations in plant size and root:shoot ratios. To examine the partitioning of ^{15}N throughout the seedlings, we calculated the percentage of ^{15}N tracer found in different plant organs (leaves, stems, coarse roots, fine roots) as the amount of tracer ^{15}N in each tissue divided by the whole plant ^{15}N tracer and multiplied by 100.

We used General Linear Models to determine if there were significant differences in the uptake rates of the four amino acids, NH_4^+, and NO_3^- within a tree species. The models consisted of specific uptake rate as the dependent variable and the labeled N species as the independent variable. We used pairwise t-tests with a Bonferroni correction to compare differences in uptake rates between specific N species. The specific uptake rates for all tree species were log transformed to meet assumptions of normality. We used analysis of covariance (ANCOVA) to determine whether or not there were different distribution patterns of ^{15}N tracer in fine roots and leaves across the treatments within each tree species. An ANCOVA approach allowed us to account for the influence of total plant ^{15}N uptake on the ^{15}N tracer found in specific organs (Atchley et al. 1976). We focused on fine roots and leaves because they were the most likely to capture differences in the metabolism and partitioning of the substrates. The

amount of ^{15}N tracer (µg) in each organ was the independent variable, N form was the main effect, and total plant ^{15}N tracer (µg) was the covariate. We initially modeled our data with an interaction term between N form and total plant ^{15}N tracer, but it was never significant; therefore, our final models contained only main effects. ^{15}N tracer of specific organs and total plant ^{15}N tracer were log transformed when appropriate to ensure a normal distribution of residuals. Finally, we compared the ^{13}C of fine roots in tree seedlings labeled with U-$^{13}C_2$, ^{15}N-glycine to the unlabeled controls using t-tests within each species. For black cherry, we used a Welch's t-test because the dual-labeled and unlabeled seedlings had unequal variances. We used linear regressions within each tree species to determine whether plants fed U-$^{13}C_2$, ^{15}N-glycine took up the amino acid intact (Näsholm et al. 2000). Our models consisted of excess ^{15}N (per gram of fine root tissue) as the independent variable and excess ^{13}C (per gram of fine root tissue) as the dependent variable. All of our analyses were accepted as significant at $\alpha = 0.05$ and were conducted using R statistical software (R Development Core Team 2008).

Results

All tree species examined were able to take up the full complement of N forms offered, although there were significant differences in specific uptake rates depending on the N form. Red oak and American beech took up NH_4^+ and arginine at significantly higher rates than the remaining N forms (ANOVA, oak: $F_{5, 24} = 21.39$, $P < 0.001$; beech: $F_{5, 22} = 26.08$, $P < 0.001$; Figure 2.1a). There were no significant differences in specific uptake rates among glutamine,

serine, glycine, or NO_3^-. White ash and black cherry had significantly faster specific uptake rates for NH_4^+ compared to any other N form (ANOVA, ash: $F_{5, 18} = 22.30$, $P < 0.001$; cherry: $F_{5, 23} = 22.32$, $P < 0.001$). There were no significant differences in specific uptake rates among the amino acids or NO_3^- for black cherry, although white ash took up arginine significantly faster than NO_3^-. Overall, our high fertility species had specific uptake rates for NH_4^+ that were over 5 times faster than for our low fertility species. When we evaluated the total amount of N contributed by each ^{15}N-enriched N form to our seedlings, arginine provided red oak and American beech with significantly more N than any other N form (ANOVA, red oak: $F_{5, 24} = 55.82$, $P < 0.001$; beech: $F_{5, 22} = 56.68$, $P < 0.001$; Figure 2.1b). NH_4^+-N still provided white ash and black cherry with their greatest source of N, although the contrast between NH_4^+ and arginine was not statistically significant for black cherry (ANOVA, ash: $F_{5, 18} = 129.24$, $P < 0.001$; cherry: $F_{5, 23} = 26.61$, $P < 0.001$). Among the remaining amino acids, glutamine-N supplied our seedlings with the most N, although the differences were not always significant. Across all tree species, the average specific uptake rates decreased as molecular weight increased for glycine, serine, and glutamine, but then increased to the highest specific uptake rates for arginine, the heaviest amino acid (Figure 2.2).

The distribution of ^{15}N between fine roots and leaves varied significantly across our treatments; however, not all species demonstrated the same ^{15}N distribution patterns. There were no significant differences in leaf ^{15}N across all N forms for red oak (ANCOVA, $F_{5, 23} = 0.42$, P

= 0.83) or American beech (ANCOVA, $F_{5, 21} = 0.22$, $P = 0.95$), and only 11% and 3% of total ^{15}N were found in the leaves of these species, respectively (Figure 2.3a, b). However, white ash and black cherry had significantly more ^{15}N in leaves from the $^{15}NO_3^-$ treatment compared to most other treatments (ANCOVA, ash: $F_{5, 17} = 4.73$, $P = 0.01$; cherry: $F_{5, 22} = 13.40$, $P < 0.001$), with an average of 38% and 33% of total ^{15}N found in leaves, respectively (Figure 2.3c, d). In fine roots, the ^{15}N-arginine and $^{15}NH_4^+$ treatments had significantly more ^{15}N compared to most other N forms for all species except black cherry, where only the $^{15}NH_4^+$ treatment was significantly higher than the rest (ANCOVA, oak: $F_{5, 23} = 31.27$, $P < 0.001$; beech: $F_{5, 21} = 113.27$, $P < 0.001$; ash: $F_{5, 17} = 36.85$, $P < 0.001$; cherry: $F_{5, 22} = 43.76$, $P < 0.001$). The distribution of the ^{15}N tracer between above- and belowground organs was the most disparate for the two inorganic N forms I supplied the seedlings: in the $^{15}NO_3^-$ treatment, roughly 20%, 16%, 62%, and 45% of the ^{15}N tracer was found in aboveground organs for red oak, American beech, white ash, and black cherry, respectively, while only 3%, 5%, 9%, and 2% of the ^{15}N tracer was found in aboveground tissues for the $^{15}NH_4^+$ treatment, respectively. The percentages of ^{15}N in aboveground organs from the amino acid treatments were generally intermediate between the $^{15}NO_3^-$ and $^{15}NH_4^+$ treatments and decreased from ^{15}N-glutamine > ^{15}N-glycine = ^{15}N-serine > ^{15}N-arginine in all tree species.

The fine roots of red oak, American beech, white ash, and black cherry seedlings supplied with U-$^{13}C_2$, ^{15}N-glycine were significantly enriched in ^{13}C compared to unlabeled seedlings (oak: $t = 4.08$, $df = 11$, $P < 0.01$; beech: $t = 5.42$, $df = 12$, $P < 0.01$; ash: $t = 5.05$, $df = 10$, $P < 0.01$; cherry: $t = 7.00$, $df = 9.58$, $P < 0.01$). Linear regressions of ^{15}N and ^{13}C for whole plants were not significant (data not shown). However, when the ^{15}N content of fine roots was regressed against ^{13}C content of fine roots, red oak ($t = 3.69$, $df = 6$, $P < 0.05$) and American beech ($t = 4.53$, $df = 8$, $P < 0.01$) had significant linear relationships that corresponded to a minimum of 78% and 51% of glycine taken up intact, respectively (Figure 2.4a, b). White ash and black cherry had weak linear relationships between ^{15}N and ^{13}C content (ash: $t = 1.52$, $df = 5$, $P = 0.19$; cherry: $t = 1.33$, $df = 7$, $P = 0.23$; Figure 2.4c, d).

Discussion

In this study, we evaluated the role amino acids played in the N nutrition of temperate hardwood tree species characteristic of habitats with either low- or high- inorganic N availability. We hypothesized that the uptake of amino acids by each tree species would reflect the N fertility of the habitats in which they were typically found, with low fertility species (i.e., red oak and American beech) taking up amino acid-N at greater rates than high fertility species (i.e., white ash and black cherry) which would prefer inorganic-N. Contrary to our hypothesis, specific uptake rates of amino acids were largely similar for all tree species (Figure 2.1a). However, patterns of NH_4^+ uptake were consistent with our hypothesis; high fertility species took up NH_4^+ at rates more than double those of the low fertility species. The disparity between the rates of NH_4^+ uptake between our low- and high-fertility species rendered amino acid-N relatively more

important to the N nutrition of low-fertility species. Amino acid uptake was between 27 – 97% (red oak) and 19 – 81% (American beech) of their respective NH_4^+ uptake rates for the low-fertility species whereas it was between 8 – 19% (white ash) and 11 – 17% (black cherry) for high fertility species. When we evaluated the total amount of N taken up from each N-form, our results provided additional evidence supporting a greater role for amino acids in the N nutrition of low-fertility species. Red oak and American beech acquired over four times more N from arginine compared to NH_4^+ and NO_3^-; white ash and black cherry acquired the most N from the NH_4^+ treatment, although there was no significant difference between arginine-N and NH_4^+-N for white ash (Figure 2.1b). Additionally, glutamine supplied an equal amount of N to red oak as NH_4^+. All of our tree species were supplied with identical labeling solutions within a treatment, so our results reflect inherent species differences rather than uptake patterns based on the availability of substrate. Therefore, the similar patterns of N-form uptake between species from low inorganic-N habitats and similar patterns from those in high inorganic-N habitats suggest they were physiologically adapted to access the more prevalent pool of plant-available N in their respective habitats, in agreement with studies from other ecosystems (McKane et al. 2002; Nordin et al. 2001; Weigelt et al. 2005). These patterns of N-form uptake by our trees support Schimel and Bennett's (2004) model of N biogeochemistry, which predicts that plants will be more likely to access amino acid pools in habitats where N mineralization is low and free amino acid pools are high. While this model has largely been based on data from cold climates, our study suggests it is also applicable to temperate ecosystems. The ability of the low-fertility species to take up amino acids (Figures 2.1, 2.4), combined with the presence of large pools of amino acids in low-fertility stands (Gallet-Budynek et al. 2009; Rothstein 2009), suggest some

habitats may not be as depleted in plant-available N as previously thought based solely on measures of inorganic N. In Northern Michigan, free amino acid-N comprised 31% of the total pool of plant-available N in forest stands where red oak and American beech dominate, providing an N source that is generally lacking in stands where N mineralization rates are high (free amino acid-N = 2% of total N pool in high-fertility stands; Rothstein 2009). Conversely, N mineralization rates in stands where white ash and black cherry occur are close to 127.8 µg N g^{-1}: roughly two and a half times faster than stands where red oak and American beech are prevalent (Zak et al. 1989). The greater availability of NH_4^+ in these stands, and paucity of amino acids, would make NH_4^+ a more available source of N for white ash and black cherry to access.

For three of the four amino acids we supplied to our seedlings, there was a decreasing trend of uptake rate with amino acid molecular weight (Figure 2.2). This relationship was realized among the neutral amino acids with glycine uptake > serine uptake > glutamine uptake. However, arginine, a basic amino acid, was taken up at the highest rates by all tree species, despite being the heaviest amino acid. The basic nature of arginine may have contributed to its distinct uptake pattern in two ways: first, the positive charge on arginine's side chain could have increased its attraction to negatively charged root surfaces (Haynes 1980) compared to the other amino acids, thereby facilitating its uptake. This mechanism may also explain why NH_4^+ was taken up at such high rates by all tree species. Second, different genes regulate membrane transporters for basic amino acids than neutral amino acids (Tanner and Caspari 1996; Näsholm and Persson 2001), with the lysine histidine transporter 1 (LHT1) and amino acid permease 1 (AAP1) responsible for neutral and acidic amino acid transport and amino acid permease 5

(AAP5) responsible for basic amino acid transport (reviewed by Näsholm et al. 2009). Membrane transporters for neutral amino acids have broad substrate affinities that synchronously regulate the transport of amino acids across membranes (Näsholm and Persson 2001), which may explain the non-significant differences in specific uptake rates of glycine, serine, and glutamine (Figure 2.1). In this instance, bioenergetics would support lighter molecules being preferentially transferred across membranes resulting in higher specific uptake rates for smaller molecules, in agreement with our average uptake pattern for neutral amino acids (Figure 2.2). This interpretation is consistent with the results of Harrison et al. (2007), who found low molecular weight compounds were taken up at greater rates by grassland plants due to their relative ease of transport across cellular membranes.

The relatively high specific uptake rates of arginine by all species, combined with the high N content of arginine (i.e., 4 N atoms per molecule), suggest it may be an important source of amino acid-N for plants. However, most studies evaluating plant uptake of amino acids use glycine to determine whether or not amino acid-N is important to plant nutrition (i.e., Näsholm et al. 2000; Weigelt et al. 2005; Finzi and Berthrong 2005; Gallet-Budynek et al. 2009). Based on our results, glycine uptake rates alone could underestimate the overall importance of amino acid-N to plants. All of our tree species had the highest specific uptake rates for arginine, sometimes taking it up 3.5 times faster than glycine (i.e., red oak; Figure 2.1a). Also, the total amount of N contributed to our trees by arginine ranged between 4 – 14 times greater than the amount of N supplied by glycine (Figure 2.1b). Persson and Näsholm (2001) also found significantly higher rates of arginine uptake compared to glycine by *Pinus sylvestris* in an experiment similar to ours. Whether or not the patterns we found between glycine and arginine uptake are realized in the field is unclear. In a natural environment, trees must compete for amino acids with

microorganisms and abiotic soil sorption processes (Jones and Hodge 1999; Rothstein 2010). Glycine diffuses relatively easily through soil to root surfaces because of its low molecular weight and neutral charge (Owen and Jones 2001); it is also a poor substrate for microbial growth (Lipson et al. 1999), making it an ideal amino acid for plant uptake. Arginine, however, has slower diffusion rates due to its relatively high molecular weight and positive charge, which increases its propensity to sorb to negatively charged soil colloids (Owen and Jones 2001; Weigelt et al. 2005), although Öhlund and Näsholm (2001) suggested this same phenomenon might reduce potential losses. Arginine is also readily mineralized by a variety of microorganisms in forest soil (Alef and Kleiner 1986; Lin and Brookes 1999). These characteristics may reduce plant access to arginine in the field in favor of glycine.

The uptake rates of amino acids and inorganic N forms measured in our study represent gross uptakes by our tree species and do not account for the potential efflux of these compounds from roots. As a result, the uptake rates found in this study may overestimate the importance of an N form in plant N nutrition if efflux rates are high. In particular, glycine and serine have demonstrated relatively high rates of efflux compared to influx in several agriculturally important plant species (Lesuffleur et al. 2007). Plant species vary in their tendency to exude different N forms (Kronzucker et al. 2003, Lesuffleur et al. 2007), suggesting the different uptake patterns of N forms across our species may change when efflux is considered. However, in a literature review that addressed this question, Näsholm et al. (2009) found that efflux of amino acids did not significantly detract from conclusions of amino acid absorption by plants in gross labeling studies.

At the end of the labeling period, the distribution of ^{15}N in the seedlings followed our predictions, with all species partitioning the most ^{15}N into aboveground organs in the $^{15}NO_3^-$

treatment, the least in the $^{15}NH_4^+$ treatment, and intermediary levels in the ^{15}N-amino acid treatments (Figure 2.3). Other studies evaluating plant uptake of inorganic and amino acid N-forms have also found a greater distribution of NO_3-N in leaves compared to NH_4-N and amino acid-N, citing a faster transport of NO_3^- out of roots compared to amino acids or NH_4^+ (Persson et al. 2006). NO_3^- can be transported directly from roots to other plant organs before being assimilated into organic compounds via nitrate reductase and the glutamine synthetase/glutamate synthase (GS/GOGAT) system (Andrews 1986). Conversely, NH_4^+ must be assimilated in roots via the GS/GOGAT system into glutamine before it can be transported throughout a plant.

In contrast to NH_4^+ and NO_3^-, amino acids are already in a form immediately usable by plants and therefore are not necessarily metabolized into other compounds before being transported within a plant. For example, glutamine acts as a vector for transporting N assimilated in roots to N sinks, such as leaves, and is prevalent in phloem and xylem (Lam et al. 1996; Miller and Cramer 2004). Its primary role in N transport from roots to aboveground organs was evident in our data by the relatively high percentage of ^{15}N in aboveground organs in this treatment for all tree species. Arginine, however, is primarily a storage amino acid (Rosnitschek-Schimmel 1985; Staswick 1994), which may explain the prevalence of ^{15}N in roots and near absence in leaves in this treatment compared with the others. Additionally, our labeling period occurred in late summer, when the seedlings may have begun storing reserves for their winter dormancy, also contributing to the predominance of arginine in belowground organs. Glycine and serine were the most similar of the amino acids we examined, both being small, hydrophilic molecules with low C:N (glycine, 2:1; serine, 3:1). They are involved in similar metabolic processes and

are readily interconvertible (Cossins and Sinha 1966), which may explain their similar ^{15}N distributions in all species. Our study was limited to examining only short-term distribution patterns of N forms within seedlings. Therefore, it is unclear whether or not the differences we found in N form distribution will be maintained over longer periods of time.

Although the overall distribution patterns of ^{15}N in our seedlings from the various N forms were similar for all species, there were distinct differences in the proportion of ^{15}N in above- versus belowground organs from the $^{15}NO_3^-$ treatment between our species from high- versus low-N habitats. White ash and black cherry had approximately 62% and 45% of ^{15}N from the NO_3^- treatment in aboveground organs, respectively, compared to only 20% for red oak and 16% for American beech (Figure 2.3). Plant species from high-N environments tend to assimilate most of their NO_3^- in aboveground organs while those from low-N environments assimilate NO_3^- in belowground organs (Andrews 1986), in agreement with our results. These differences would likely be greater in the field where NO_3^- assimilation would also be dependent on substrate availability; greater external NO_3^- concentrations can result in an increase in shoot NO_3^- assimilation (Gebauer et al. 1988).

Because our experiment was conducted with only ^{15}N-enriched substrates, we cannot conclusively rule out the possibility that amino acids were mineralized prior to plant uptake (and therefore not taken up as intact molecules). However, based on several lines of evidence, we feel confident that our data represent intact amino acid uptake. First, we took efforts with the

experimental design to limit opportunities for microbial mineralization to occur; prior to labeling with ^{15}N-enriched substrates, the roots of all tree seedlings were thoroughly washed with reverse osmosis water to remove any attached potting medium and were then sterilized with bleach to reduce the microbial population adhering to roots. We also used a hydroponic method to supply seedlings with labeled compounds, so we did not contend with the potential mineralization of amino acids that might have occurred if we had labeled plants grown in soils. Second, the different patterns of ^{15}N distribution in our trees for the amino acids compared to NH_4^+ suggests our amino acids were not mineralized to NH_4^+ prior to uptake (Figure 2.3). If ^{15}N uptake in our amino acid treatments was dominated by uptake of mineralized $^{15}NH_4^+$, we would have expected there to be no significant differences in ^{15}N distribution patterns in fine roots between the amino acids and $^{15}NH_4^+$. Similarly, NH_4^+ specific uptake rates were significantly different than those of the amino acids we examined in most cases (Figure 2.1a). Again, if the amino acids were mineralized prior to uptake we would have expected there to be no significant differences in specific uptake rates of the different substrates. Third, other studies that have used amino acids enriched in both ^{13}C and ^{15}N have documented the uptake of arginine (Öhlund and Näsholm 2001; Persson and Näsholm 2001), glycine (Nordin et al. 2001; Rains and Bledsoe 2007), and serine (Weigelt et al. 2005; Harrison et al. 2007) as intact molecules, demonstrating that it is physiologically possible for plants to do so. Finally, we tested whether glycine was taken up intact on a subset of our seedlings by supplying them with universally labeled glycine (U-$^{13}C_2$, ^{15}N-glycine) and using regressions of ^{13}C-excess on ^{15}N-excess in fine roots to determine the minimum proportion of glycine taken up intact (Näsholm et al. 1998; Näsholm et

al. 2000; Öhlund and Näsholm 2001). This method compared the slope of the regression with the ratio of C:N in the amino acid of interest (2:1 in the case of glycine) with 100% uptake occurring when the C:N ratio of the amino acid was equal to the slope of the regression line (Näsholm et al. 1998). All of our tree species had slopes less than 2, suggesting some amount of C was lost either prior to uptake via mineralization or nitrification (Quastel and Scholefield 1949), or after uptake by the metabolism of glycine which results in the loss of C to CO_2 (Näsholm et al. 1998). Because our experimental design sought to limit microbial influences, we assume most C was lost by plant respiration. Only red oak and American beech had statistically significant regressions, which suggested roughly 78% and 51% of glycine was taken up intact by our seedlings, respectively (Figure 2.4). We were unable to determine the fraction of glycine taken up intact by white ash and black cherry for two possible reasons. First, white ash had the lowest level of ^{13}C excess of any of the species, which can make detecting the amount of amino acid taken up intact difficult (Näsholm and Persson 2001). Second, black cherry had the highest variation in levels of ^{13}C excess of any of our species (SE = 0.79 compared to 0.10 for oak, 0.03 for beech, and 0.04 for ash), which may have obscured our ability to detect a linear relationship between ^{13}C and ^{15}N excess. However, the fine roots of all our species were significantly enriched in ^{13}C compared to control plants, suggesting that some portion of glycine was taken up intact by all our species. Based on the above reasons, we conclude that ^{15}N uptake in the amino acid treatments was dominated by intact amino acid uptake by our trees.

Conclusion

The results of our study demonstrate the ability of four temperate tree species to take up amino acids in direct competition with inorganic N. We also demonstrated that trees from low-fertility habitats acquired more N from amino acids compared to inorganic forms. These results support the idea that the dominant species in a particular habitat are those best able to utilize the N resources most available, be they inorganic or organic (McKane et al. 2002). All of the amino acids we investigated are commonly found in soil amino acid pools in a variety of ecosystems (Kielland 1995; Nordin et al. 2001; Senwo and Tabatabai 1998), including hardwood forests in eastern North America where our tree species occur (Rothstein 2009). Therefore, it is plausible free amino acids could provide a source of N to trees in this region that has previously been overlooked, especially in low fertility habitats. Evaluating amino acid uptake by red oak, American beech, white ash, and black cherry in the field is the next logical step in understanding the role amino acid-N plays in the N nutrition of these temperate trees.

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Table 2.1 Labeling solution compositions and the chemical formulae of the labeled N forms. Labeled compounds are in bold.

Solution number	Solution name	Inorganic N	Organic N	Molecular formula
1	Ammonium	**$^{15}NH_4$**NO_3 (98% ^{15}N)	Glycine	NH_4
2	Nitrate	NH_4**$^{15}NO_3$** (98% ^{15}N)	Glycine	NO_3
3	Glycine	NH_4NO_3	**^{15}N-glycine** (98% ^{15}N)	$C_2H_5NO_2$
4	Glutamine	NH_4NO_3	**^{15}N-glutamine** (98% amide ^{15}N)	$C_5H_{10}N_2O_3$
5	Arginine	NH_4NO_3	**^{15}N-arginine** (98% guanido ^{15}N)	$C_6H_{14}N_4O_2$
6	Serine	NH_4NO_3	**^{15}N-serine** (98% ^{15}N)	$C_3H_7NO_3$
7	Dual-glycine	NH_4NO_3	**U-$^{13}C_2$, ^{15}N-glycine** (98% ^{13}C, ^{15}N)	$C_2H_5NO_2$

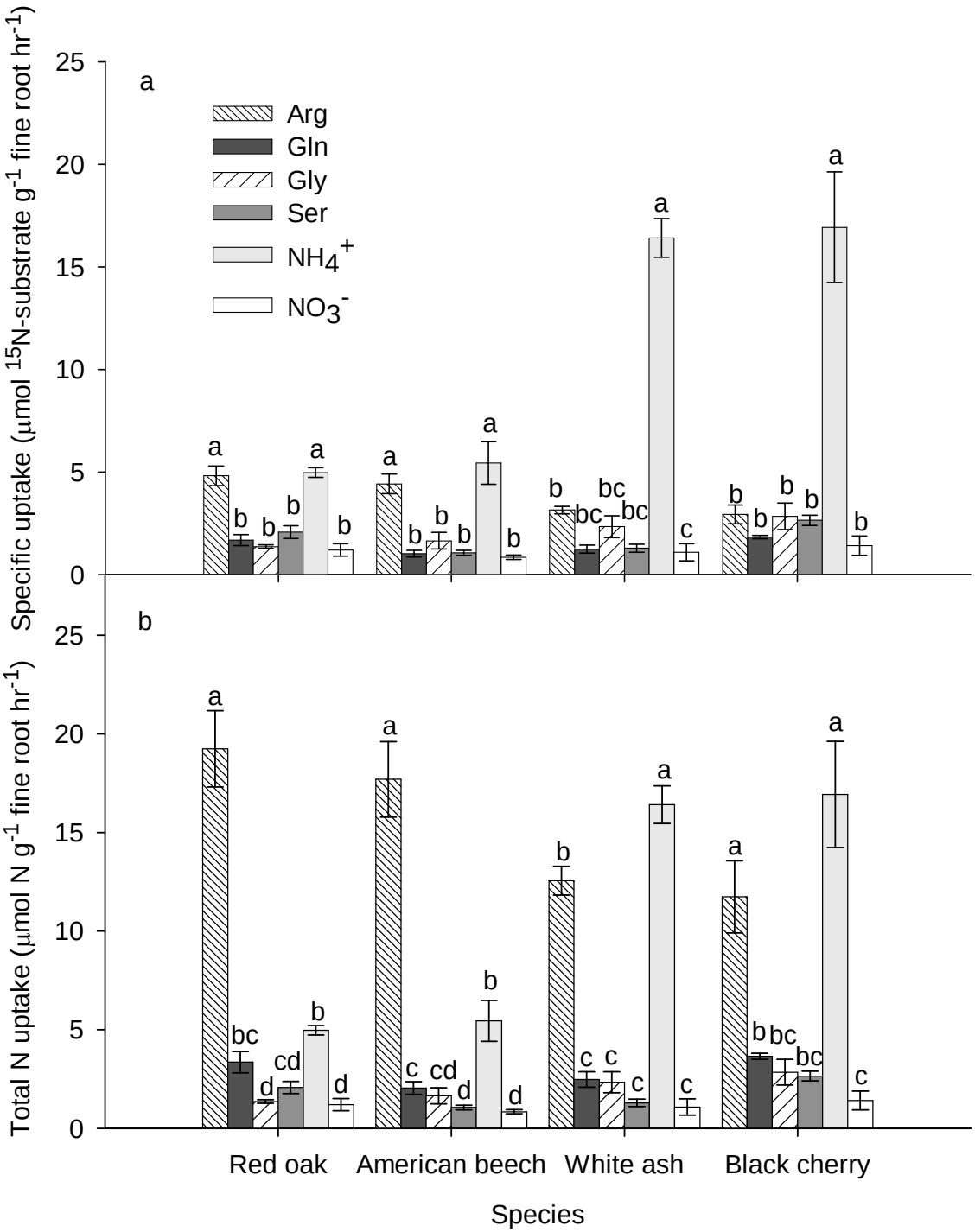

Figure 2.1 (a) Specific uptake rates (mean ± 1 SE) and (b) total N uptake rates (mean ± 1 SE) of ^{15}N-enriched amino acids, $^{15}NH_4^+$, and $^{15}NO_3^-$ for red oak (Quercus rubra, n=30), American beech (Fagus grandifolia, n=28), white ash (Fraxinus americana, n=24), and black cherry (Prunus serotina, n=29). Bars with the same letter(s) are not significantly different from each other. Arg = arginine, Gln = glutamine, Gly = glycine, Ser = serine, NH_4^+ = ammonium, NO_3^- = nitrate.

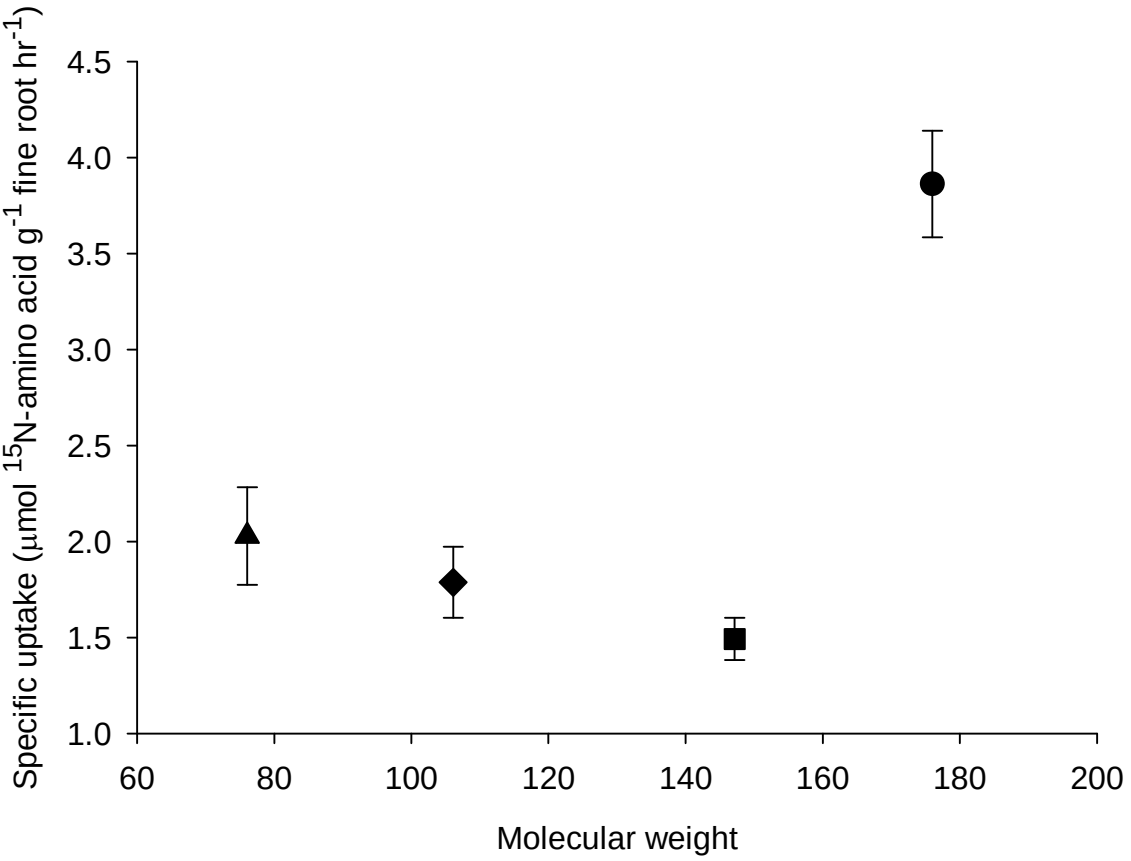

Figure 2.2 Specific uptake rates (mean ± 1 SE, n=75) of glycine (triangle), serine (diamond), glutamine (square), and arginine (circle) averaged across species as a function of amino acid molecular weight.

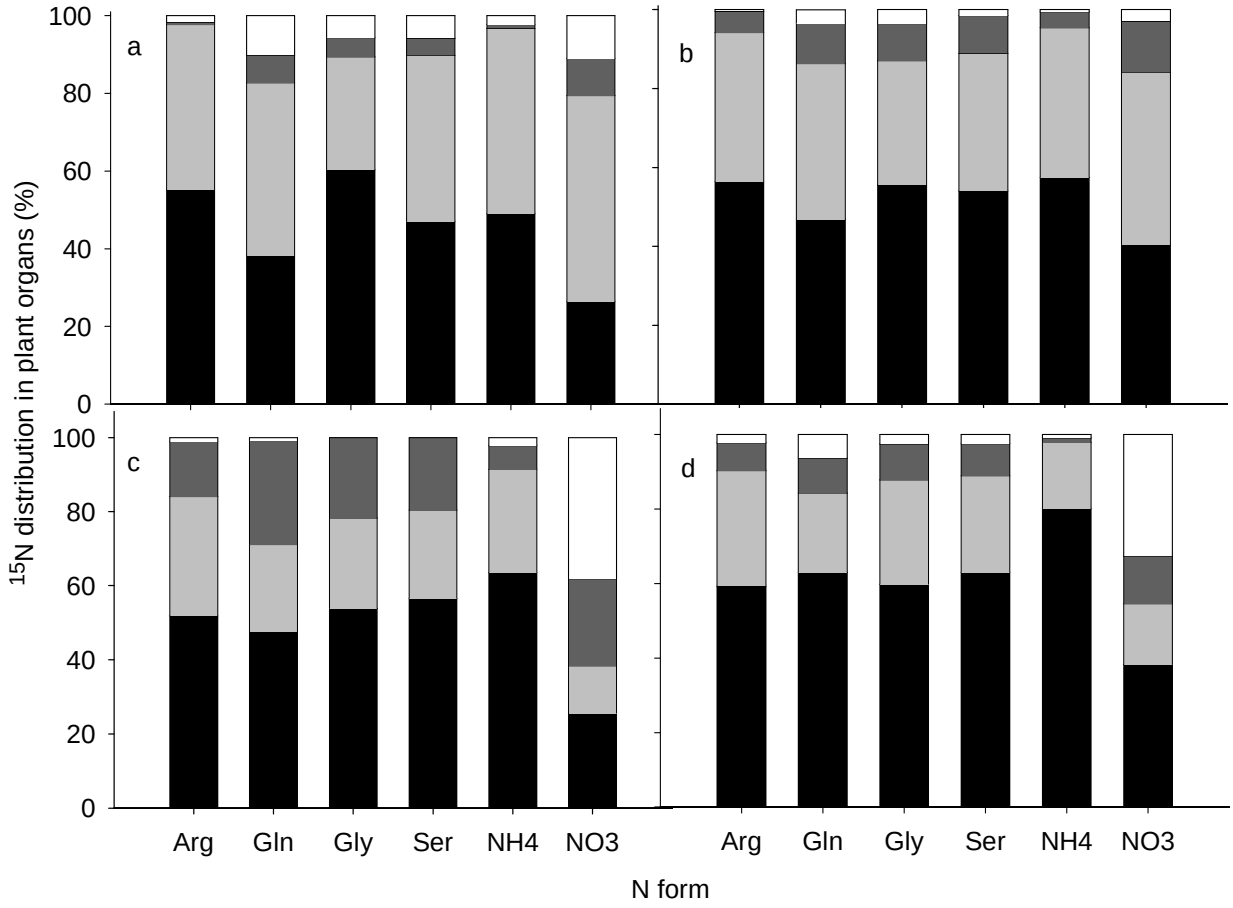

Figure 2.3 The proportion of ^{15}N tracer found in coarse roots (black bars), fine roots (light gray bars), stems (dark gray bars), and leaves (white bars) for each N-form treatment within a species. (a) Red oak, (b) American beech, (c) white ash, (d) black cherry. See Figure 2.1 for abbreviations.

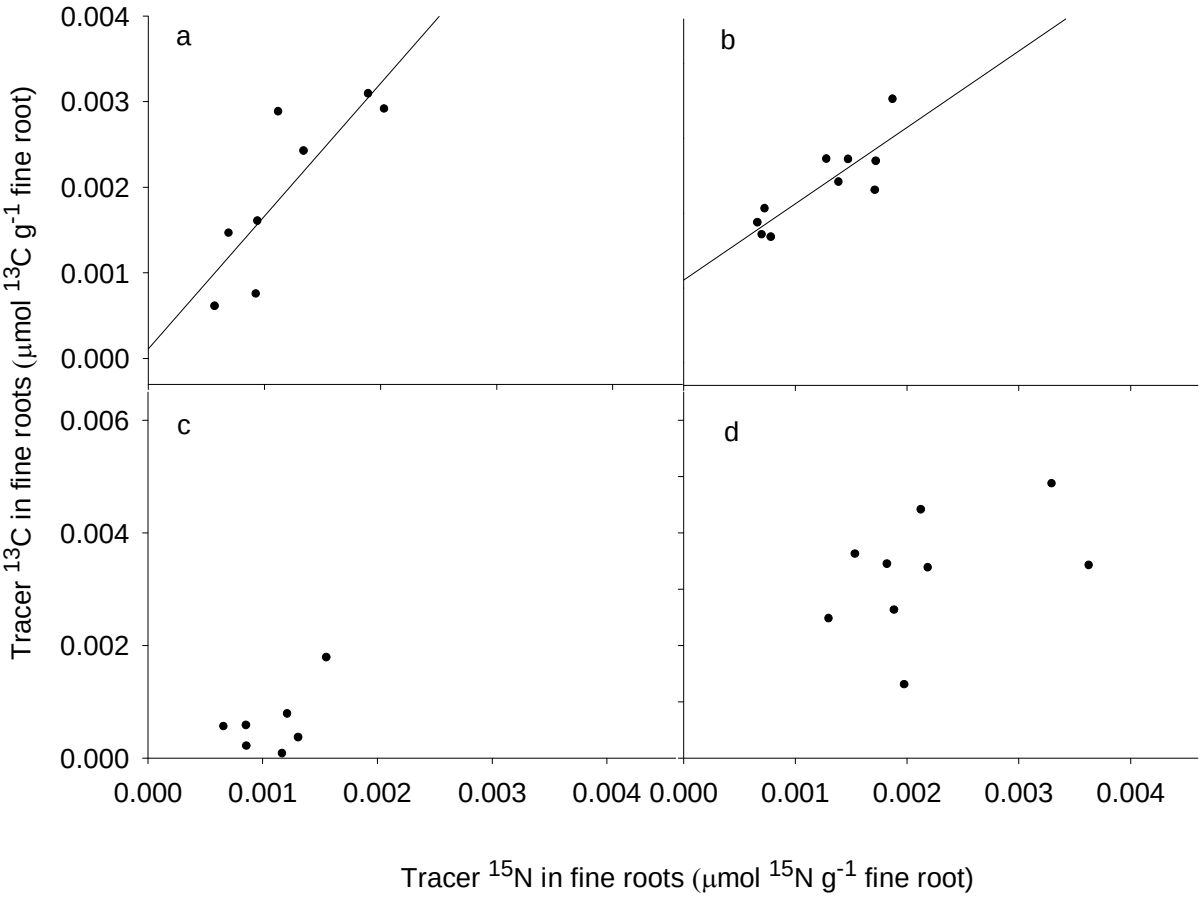

Figure 2.4 Linear regressions of fine root tracer ^{15}N on fine root tracer ^{13}C for (a) red oak, ($y=1.55x + 0.0001$, $r^2=0.72$, n=8), (b) American beech ($y=1.02x + 0.0011$, $r^2=0.72$, n=10), (c) white ash, and (d) black cherry. Regressions for white ash and black cherry were not statistically significant.

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CHAPTER 3

PATTERNS OF DON AND DOC LEACHING LOSSES ACROSS A NATURAL N-AVAILABILITY GRADIENT IN TEMPERATE HARDWOOD FORESTS

Abstract

Dissolved organic nitrogen (DON) has increasingly become recognized as an important component of terrestrial N cycling, yet we understand little of the processes that regulate its cycling across terrestrial ecosystems. Recent reseach investigating DON dynamics has found that DON losses are the greatest from forests with relatively high ecosystem N availability, suggesting ecosystem N availability is a primary driver of DON production and retention across ecosystems. However, the mechanisms responsible for these patterns are not well studied. In this chapter, I investigated the patterns of DON loss and DOM chemistry in five northern hardwood forests that span a natural gradient in N availability to examine how local processes of DON production and retention serve to shape landscape patterns of DON loss. I collected solutions from beneath the forest floor and from two soil depths along with corresponding forest floor and mineral soil samples. I predicted that: i) DON losses would increase across the N availability gradient, ii) DON would comprise a decreasing proportion of total N losses from low to high N availability forests, and iii) DOM losses would be stoichiometrically enriched in DON in high versus low N availability forests. I also proposed two alternative hypotheses to explain the mechanisms behind the predicted patterns. I hypothesized that DOM chemistry and losses were either a direct function of DOM forest floor inputs or, alternatively, were a product of soil sorption phenomena. DON losses were largely unrelated to soil N stocks, although they did comprise a decreasing proportion of total N losses across the N availability gradient.

Surprisingly, the DOC:DON by mass of deep soil losses demonstrated a unimodal pattern, with relatively low DOC:DON leaching from both the lowest and highest N availability forests. Neither alternative hypothesis could fully account for these DON loss and DOM chemistry patterns. Instead, these patterns were best understood after using a comprehensive approach that allowed for the interactive effects of forest floor litter composition and soil characteristics. This approach suggested DON losses and DOM chemistry depended on the combined ability of a particular forest to produce DOM from the forest floor and retain DOM by mineral soils. The results of this study emphasize the need to understand how fine-scale processes can interact to shape ecosystem patterns of DON losses and DOM chemistry.

Introduction

Dissolved organic nitrogen (DON) is increasingly acknowledged as a vehicle for N loss from terrestrial ecosystems that can perpetuate N limitation and constrain net primary productivity (Campbell et al. 2000, Perakis and Hedin 2002, Neff et al. 2003). Despite the importance of DON in terrestrial N biogeochemistry, it has been difficult to determine the controls over its production, retention, and loss in terrestrial ecosystems. The heterogeneous nature of DON compounds, which range along a continuum of molecular sizes, reactivities, and solubilities (Qualls and Haines 1991, Smolander and Kitunen 2002), allows for a variety of mechanisms to regulate different fractions of DON depending on the specific chemistry of the fraction in question (Kaiser and Zech 1998). Also, many studies on DON dynamics focus on either a single ecosystem (Lajtha et al. 2005, Yano et al. 2005) or evaluate patterns at coarse scales (Perakis and Hedin 2002, Brookshire et al. 2007) and have yet to link fine-scale DON controls with a landscape understanding of DON cycling. Reducing this knowledge gap will be critical for producing a broad understanding of DON biogeochemistry.

Attention to DON dynamics gained relevance when it was discovered that DON losses occurred despite N limitation in terrestrial environments (reviewed by Neff et al. 2003). This was contrary to previous models of N cycling where N losses were restricted to high N ecosystems where N availability exceeded biotic demand (Aber et al. 1998). As a result, hydrologic fluxes of DON were characterized as an "N leak" that was outside of biological control (Hedin et al. 1995, Perakis and Hedin 2002). Much of the subsequent DON research has focused on understanding the mechanisms that either facilitate or restrict DON losses from a variety of ecosystems (Qualls et al. 2002, Pregitzer et al. 2004, Lajtha et al. 2005, Brookshire et al. 2007, Sleutel et al. 2009). From these studies, we are beginning to see that patterns of DON loss depend on the N content

of a particular ecosystem. Low N ecosystems typically lose most of their N as DON, which is lost in direct proportion with dissolved organic carbon (DOC; Perakis and Hedin 2002, Pregitzer et al. 2004, Sleutel et al. 2009). Across ecosystems with increasing N content, DON losses disproportionately increase over DOC losses, creating a shift in dissolved organic matter (DOM) stoichiometry (Pregitzer et al. 2004, Brookshire et al. 2007). DON also occupies a decreasing percentage of total N losses in favor of inorganic N (i.e., nitrate; Perakis and Hedin 2002, Pregitzer et al. 2004, Brookshire et al. 2007). These trends suggest that there are fundamental differences in the way DON is cycled among ecosystems that vary in N content. However, the mechanisms for this change are still unclear. Pregitzer et al. (2004) suggested the increased DON losses they found in forests receiving NO_3^- additions resulted from either altered availability of the substrates that formed DON, altered processing of organic matter by soil microbes, or some combination of both. In a watershed study comparing DON and DOC losses from forests that experienced a gradient of atmospheric N deposition, Brookshire et al. (2007) concluded that the N-rich DOM fluxes from high deposition sites resulted from the direct enrichment of DOM pools from atmospheric N inputs. This conclusion was based on decreasing stream DOC:DON in high N catchments relative to the mineral soil C:N of the catchment that the stream drained. While this mechanism may be correct, Brookshire et al. (2007) did not explicitly test this hypothesis with their data; their study compared stream chemistry with only surface soil organic matter (SOM) pools (i.e., upper 10 cm), which may, or may not, be representative of total SOM pools. An alternative explanation for their result is that SOM becomes more enriched in N with depth due to the preferential sorption of hydrophobic, high C:N compounds to surface soils (Kaiser and Zech 1998), which is reflected in DOM by decreased DOC:DON. Linking DON and DOC

directly with corresponding SOM pools would provide clearer evidence of the mechanisms shaping DOM dynamics as water moves through terrestrial ecosystems.

Understanding DON biogeochemistry in a landscape context is important because of the tight association between ecosystem characteristics and the production and retention of DON. For example, the forest floor is the primary region of DON production (Qualls et al. 2002, Park and Matzner 2003) due to organic matter inputs from plant litter (Qualls et al. 2002), microbial exoenzymes produced during decomposition, microbial biomass turnover, and faunal N production (reviewed by Kalbitz et al. 2000). As forest community composition changes across the landscape, so does the nature of litter inputs to the forest floor and the type and amount of organic molecules available for dissolution (Pastor et al. 1984, Park and Matzner 2003, Dittman et al. 2007). Concomitant with changes in forest community composition are shifts in moisture-edaphic conditions (Pastor et al. 1984, Host et al. 1988), which can strongly impact an ecosystem's ability to retain DON (Seely et al. 1998, Qualls et al. 2002, Yano et al. 2004, Möller et al. 2005). The underlying mineral soil is the dominant sink for DON leached from the litter layer (Sleutel et al. 2009), but the strength of its sorption ability depends on its texture (Seely et al. 1998, Qualls et al. 2002), structure (Castellano and Kaye 2009), organic matter content (Lilienfein et al. 2004, Kleber et al. 2007) and mineral composition (Qualls 2000, Lilienfein et al. 2004, Yano et al. 2004).

The complex interactions between soil and vegetation make predicting how DON will move through ecosystems challenging. For example, forest species associated with coarse-textured soils have relatively high litter C:N (Pastor et al. 1984, Host et al. 1988), which can limit DON production and, subsequently, the amount of DON subject to loss (Seely et al. 1998). However, the limited capacity of coarse-textured soils to retain DON, due to lower surface areas

that reduce sorption opportunities between percolating solutions and the soil matrix (Seely et al. 1998, Qualls et al. 2002), could potentially facilitate DON loss despite its limited production. Alternatively, fine-textured soils are often associated with forest species that produce leaf litter with relatively low C:N (Pastor et al. 1984, Host et al. 1988), providing a scenario for both greater DON production from forest litter and greater retention by soils with high sorption capacity. Using a fine-scale approach of directly comparing DON sources and sinks in soils across a variety of ecosystems of varying N N availability will help explain how these processes interact to produce the landscape patterns of DON loss previously described.

In order to link the local processes that influence DON cycling with larger ecosystem patterns of DON loss, I developed a study that investigated DON production and retention in five hardwood forests of northern Lower Michigan that spanned a natural gradient of N N availability (Zak et al. 1986, Rothstein 2009). This approach allowed me to examine how the fine-scale processes that produce and retain DON in soil profiles within a forest landscape impact broader patterns of DON cycling. In each forest, I collected soil water from beneath the forest floor and at two soil depths to determine where the predominant sources and sinks for DON occurred in each forest type. I also collected and analyzed soil samples at the same depths that soil solutions were collected to relate dissolved organic matter pools with the soil characteristics of their immediate environment. My first objective was to investigate whether or not these ecosystems followed the DON and DOC loss patterns described in the literature. I predicted that: i) DON losses from these ecosystems would increase concomitantly with ecosystem N content; ii) DON would comprise a decreasing proportion of total N losses in favor of inorganic N as ecosystem N content increased; and iii) DON losses would increase disproportionately over DOC losses resulting in a decrease in the DOC:DON of soil waters as ecosystem N content increased. My

second objective was to investigate the mechanisms behind the patterns I found in objective 1. To do this, I evaluated two alternative hypotheses. First, I hypothesized that the chemistry of DOM losses would be directly related to the chemistry of DOM inputs from the forest floor. The changes in forest species composition, and subsequently forest floor composition, across the N availability gradient would be the dominant mechanism shaping the quality of leachate chemistry rather than soil dynamics. The decreasing C:N of leaf litterfall across the ecosystem gradient (Rothstein 2009) could explain the expected patterns of increasing DON losses and decreasing leachate DOC:DON across these forests. Alternatively, I hypothesized that that chemistry of DOM losses would be a function of sorption/dissolution phenomena between soils and percolating waters. The ability of soils to alter DOM chemistry through the preferential sorption of hydrophobic, high C:N compounds could explain the stoichiometric enrichment of solutions in N across the N availability gradient and higher DON losses; soil textures become finer from low to high N availability forests suggesting the ability of soil to adsorb N-poor hydrophobic compounds would increase across these ecosystems and favor losses of N-rich hydrophilic compounds.

Methods

Study area

I conducted my study in northern hardwood forests in the Manistee National Forest in the northwestern Lower Peninsula of Michigan, USA (44°48′N, 85°48′W). In this region, long-term precipitation averages roughly 81 cm per annum and is evenly distributed throughout the year; mean annual temperature is 7.2° C (Albert 1995). Elevation ranges from 213-369 m above sea level. The geomorphology of the region reflects the last major glacial advance, which ended

ca. 12,000 years BP, leaving behind a landscape mosaic of sandy outwash plains, ice-contact hills, and moraines. Soils are predominantly classified as Spodosols and were all formed in glacial drift. Rates of inorganic N wet deposition at the nearby Wellston National Atmospheric Deposition Program station were 6.7, 5.2, and 6.6 kg ha^{-1} for 2006, 2007, and 2008, respectively (National Atmospheric Deposition Program (NRSP-3)/National Trends Network [2010]).

Five forest stands that spanned a gradient of potential N mineralization and nitrification rates (Zak et al. 1986, Rothstein 2009) were selected from a pool of stands previously classified by Host et al. (1988) into ecological land units based on vegetation composition, landform, and soil classification (Table 3.1). These stands are characteristic of landscape-vegetation associations that reoccur throughout the Great Lakes region (Host et al. 1988). The low N stand is an oak-dominated (*Quercus spp.*) outwash plain, moderate N availability stands are dominated by red oak, and N-rich stands are sugar maple-dominated (*Acer saccharum*) moraines. All sites were within 32 km of each other (average distance = 14 km), therefore I assumed that they experienced the same weather.

Sample collection

Soil water collectors were installed in August and September 2005 in each forest stand and allowed to equilibrate with the soil for at least 6 months before samples were collected. I sampled solutions at five sampling stations per forest that were located along a 100 x 40 m transect, with the stations at stratified random points. Forest floor water prior to infilttation into the mineral soil laers was collected in segments of plastic gutters fitted in the soil beneath the Oa horizon. Glass wool was placed on a screen on top of the gutter to help filter out particulate matter from percolating solutions. Prior to installing the traps, the litter layer was carefully removed, and was later returned to its original position in the O horizon on top of the glass

wool. Each trap drained its contents passively via Tygon tubing into an HDPE plastic bottle situated in a bucket buried below the depth of the trap. Soil solutions were collected with PTFE (Teflon) suction lysimeters (PRENART Equipment ApS, Frederiksberg, Denmark) at 15- and 100-cm depths at each sampling station. The lysimeters were installed at a 45˚ angle to limit disturbance of the soil above the lysimeter. I bathed the lysimeters in a silica-flour slurry prior to installation to ensure continuous contact with the surrounding soil and backfilled the installation holes with the previously removed soil by horizon. The lysimeters at each depth were at right angles to each other so that the column of soil above each lysimeter was undisturbed. For sample collection, each lysimeter was placed under vacuum (70 kilopascals) with a hand pump for 48 h. Soil water was collected in glass Erlenmeyer flasks and immediately transported to the lab after collection.

All water samples were filtered through GF/A Whatman glass microfibre filters before being stored frozen until analysis. I determined the total dissolved N (TDN) and total organic C (TOC), after acidification and purging of dissolved inorganic C, by oxidative combustion-chemiluminescence and oxidative combustion-infrared analysis, respectively (Shimadzu Corp., Kyoto, Japan). I determined the NH_4^+ concentration calorimetrically after Sinsabaugh et al. (2000) by reacting 50 or100 µl of soil core leachate with 40 µl of an ammonia salicylate reagent (Hach, Loveland, CO) followed 3 minutes later by 40 µl of an ammonia cyanurate reagent (Hach, Loveland, CO). The higher volume of leachate was used when NH_4^+ concentrations were expected to be low. Color development lasted for 20 minutes, and samples were analyzed on an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT) at 595 nm. I similarly determined the NO_3^- concentration of soil leachate by reacting 25, 50, or 100 µl of core

leachate (depending on expected NO_3^- concentration) with 160, 140, or 100 µl, respectively, of a vanadium (III) chloride reagent for 5 to 16 h (to allow color development to occur; Doane and Horwath (2003)). Samples were read on the microplate absorbance reader at 540 nm. DIN was calculated as the sum of the NH_4^+ and NO_3^-; DON was calculated by subtracting DIN from the total N of each sample.

Sampling frequency

I collected forest floor leachate and soil water at 15- and 100-cm depths from April – November in 2006 and 2007. Forest floor leachate and soil waters were collected during intensive sampling periods after storm events several times each season (i.e., spring, summer, fall). Precipitation in the summer of 2007 was markedly less than in 2006 (14.4 cm versus 24.2 cm, respectively, from July through September) resulting in poor collection volumes and ecosystem representation during this period. Therefore, I confined my analyses of N fluxes to the spring collection times (April - June) and added an additional sampling season in the spring of 2008 (soil water only). I collected forest floor solutions continuously during one week periods immediately preceding my collection of soil waters with tension lysimeters. Soils waters were collected continuously for 48 h after storm events.

Estimating nutrient fluxes

Water fluxes through the forest floor were assumed to be 2% less than total precipitation during a collection interval (Helvey 1964, Qualls et al. 1991). I estimated water fluxes through the upper 15 cm of soil and at 100 cm depth using the BROOK90 water balance model (Federer 2002). I supplied the model with precipitation data collected by the Wellston NADP station (National Atmospheric Deposition Program (NRSP-3)/National Trends Network [2010]), which

was centrally located to all ecosystems, and daily maximum and minimum temperatures (˚C) and total solar flux densities recorded at the Bear Lake station in the Enviro-weather Automated Weather Station Network (www.agweather.geo.msu.edu/mawn/). I used the parameter files included with the BROOK90 model for Watershed 6 of the Hubbard Brook Experimental Forest in New Hampshire to estimate environmental parameters, such as canopy cover and soil characteristics. To determine the nutrient fluxes at each sampling interval, I summed the volume of water passing through each depth over the 48 hour period when the lysimeters were under vacuum extraction and multiplied that by the concentration of DON, DOC, or DIN of my samples. Because I was interested in relating the local soil environment with DOM chemistry, I averaged DON, DOC, and DIN fluxes across spring sampling dates (2006-2008) for each sampling station; this gave me roughly five estimates of soil and leachate chemistry per ecosystem (some lysimeters at certain sampling stations never collected soil water, so some sites had less then 5 repetitions).

Soil characteristics

Samples of forest floor litter were collected on one day in November of 2008 at each sampling station from all study sites. I collected all organic matter down to mineral soil within a 31.2 x 31.2 cm metal sampling frame, placed the contents into paper bags, and air-dried them to constant weight before recording the forest floor litter layer mass (g). Forest floor samples were subsampled, pulverized, and analyzed for C and N by gas chromatography on an elemental combustion system (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA).

Soils were collected at each sampling station with a bucket auger in the summer of 2008 from three depth ranges: 0 -15 cm, 15 – 57 cm, and 57 – 100 cm. Soils within a depth range were homogenized and subsampled before transport to the lab where they were air dried and sieved

through a 2 mm mesh. I determined the particle size distribution of my soils using the pipette method after removing organic matter with 30% hydrogen peroxide (McKeague 1978, Gee and Bauder 1986). I also determined the C and N content of subsamples of soils by gas chromatography on an elemental combustion system (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA).

I investigated the ability of soils across the N availability gradient to serve as net sources or sinks of DOM with batch equilibrium experiments to produce adsorption isotherms. Adsorption isotherms can be used to summarily explore the affects of soil texture, organic matter content, and mineral composition on soil sorption by describing the simple partitioning of organic substances between the solid and liquid phases of the soil-water system (Nodvin et al. 1986). Soils from three of the five sampling stations located at each forest were randomly chosen for this portion of my research. I created an organic matter stock solution by mixing equal parts of ground leaf litter collected at all five sampling stations at each study site with E-pure deionized (DI) water to produce a solution with 500 mg L^{-1} of DOC. The stock solution was diluted with DI water to produce additional solutions with the concentrations of 250, 100, 50, 25, and 10 mg DOC L^{-1}; DI water was used for my 0 mg DOC L^{-1} trial. I selected this range of DOC concentrations to examine how my soils would respond to solution concentrations they experience in a natural setting (7.9 -76.7 mg L^{-1}, median = 26.1 mg L^{-1} across all forest floor leachate collection dates) as well as to test the limits of their ability to adsorb DOM. Twenty-five mL of DOM solution were added to 5 g of soil and shaken horizontally for 24 h. Immediately after shaking, the samples were centrifuged for 15 – 20 minutes at 3000 rpm and syringe filtered through sterile Millex-Ha 0.45µm filters (Millipore, Co. Cork, Ireland). DOC, TN, DIN, and

DON were determined as described above. The amount of DOM retained or released by the soils was evaluated using the Initial Mass (IM) Isotherm after Nodvin et al. (1986). This relationship describes the sorption of DOM in soils where the release of native organic matter needs to be considered and, according to Neff and Asner (2001), best represents DOM sorption reactions. The IM isotherm is represented by:

$$RE = mX_i - b$$

where *RE* is the amount of DOM released into or removed from solution, *m* (the slope) is the partition coefficient of the IM isotherm and provides a measure of the affinity of a substance (i.e., DOM) for the sorbent (i.e., soil), X_i is the initial concentration of DOM in the supplied solution with respect to the mass of soil, and *b* (the intercept) indicates the amount of DOM released when the initial DOM solution concentration is zero (i.e. $X_i = 0$). These data can then be used to calculate the reactive soil pool (RSP), which is the amount of organic matter sorbed to soil that can readily exchange with organic matter in solution under the experimental conditions (Nodvin et al. 1986). The RSP is calculated by:

$$RSP = b/(1 - m)$$

Soil water fractionation

Soil solutions were fractionated into operationally defined hydrophilic acids (Hy) and hydrophobic organic neutral matter (HON) using DAX-8 exchange resins (Supelco, Bellefonte, PA) following a modified batch procedure of Van Zomeren and Comans (2007). HON sorb to DAX-8 resins whereas Hy remain in solution. DAX-8 resins were thoroughly cleaned by soaking them in 0.1M HCl for 5 days (HCl was exchanged every 24 h) followed by similar soakings in 0.1M NaOH for an additional 5 days. Next, resins were Soxhlet extracted in 55 g batches for 24 h with 200 mL of acetonitrile at 300˚C followed by 24 h with 200 ml of methanol at 250˚C.

Resins were stored refrigerated in methanol until use. Twenty-four hours prior to use, resins were rinsed 10 times in DI water with each rinse consisting of a 6:1 ratio of water to resin (v/v). After each rinse, the water was vacuum-extracted off the resins using a Buchner funnel fitted with a #41 Whatman filter. These rinses were followed by 10 rinses in 0.1M HCl in a 3:1 acid/resin ratio (v/v), with each rinse vacuum-extracted off the resins. A subsample of solution from the last HCl rinse was analyzed on a TOC/TN analyzer to verify that < 2.0 mg L^{-1} of C bled from the resins.

In order to meet volume requirements for fractionation, I combined soil solutions collected during the April – June sampling intervals within a year; in some cases, I diluted soil solutions with DI water to meet the necessary volume. Composite samples were analyzed on the Shimadzu TOC analyzer for initial DOC concentrations. Twenty mL of soil solution were poured into a 50 mL plastic centrifuge tube and adjusted to pH 2 with 3 M HCl. I added roughly 4 g of DAX-8 resins to each sample before it was continuously tumbled on a rotary shaker for 1 h. Soil solutions were carefully transferred into new vials without resins for measurement on the TOC/TN analyzer to determine the amount of DOC in the Hy component; HON was determined by subtraction between the initial and final DOC concentrations. I ran a blank using 0.1M HCl to correct for C loss off the resins during the fractionation procedure. The moisture content of the resins was also determined to correct for the amount of moisture added to the sample by the resins. To do this, I dried a subsample of cleaned resins for 24 h at 105˚C; the moisture content was 72%.

Statistical analyses

I used mixed-effects analysis of covariance (ANCOVA) models to compare the relationship between DON fluxes, DIN fluxes, and the DOC:DON of DOM in soil waters

leaching from 100 cm soil depth across forest stands with the soil N content (i.e., the total N content of litter and soil to 100 cm depth) of each stand. DON flux, DIN flux, or DOC:DON at 100 cm was the dependent variable, and soil N content and forest stand were the fixed-effects. I included data from individual sampling stations within a forest stand in these analyses because each sampling station represented a unique relationship between soil N content and leachate chemistry. Therefore, to account for the multiple samples within a forest stand in these analyses, I included the random effect of "sampling station" in the models. For the DOC:DON analysis, I also included a quadratic term in the model to account for the non-linear relationship of the data. Preliminary analyses fitting splines to the data suggested that addition of the quadratic term adequately accounted for the curvature of the data. I removed one data point from the DOC:DON analysis because it had a Cook's distance >0.5; the data point was from ecosystem 2 and had a DOC:DON of 87 and a soil N content of 304 g m^{-2}. I also used mixed-effect ANCOVA models to investigate whether or not soil leachate chemistry at 100 cm was directly related to that of forest floor leachate, as well as to compare how DOM fractions (i.e., hydrophobic or hydrophilic DOM) changed across forest stands at both soil depths. For the former comparison, leachate chemistry at 100 cm soil depth was the dependent variable, leachate chemistry from the forest floor and forest stand were the fixed-effects. Again I used data from individual sampling stations within a forest stand for these analyses, so each model contained "sampling station" as a random effect. For the latter comparison, the concentration of hydrophobic DOM at either 15 or 100 cm was the dependent variable and total DOM concentration at 15 or 100 cm and forest stand were the fixed-effects; samping station was the random effect. Using ANCOVA for this particular analysis allowed me to account for the influence of total DOM concentration on the hydrophobic

DOM fraction without the concerns associated with statistically analyzing ratio variables (i.e., % hydrophobic DOM; Atchley et al. 1976).

I analyzed forest stand differences in absolute concentrations of hydrophobic and hydrophilic DOM, partition coefficients ("m"), IM isotherm intercepts ("b"), and RSP at either 15 or 100 cm soil depths with mixed-effect analysis of variance (ANOVA) models; hydrophobic DOM, hydrophilic DOM, m, b, or RSP was the dependent variable, forest stand was the fixed effect, and sampling station or soil repetition was the random effect. I log transformed variables when necessary to meet assumptions of normality. I specified Type III sums of squares in all the analyses so that all terms in the models were considered simultaneously. I used Tukey contrasts for post-hoc comparisons when categorical variables in the model were significant. All analyses were accepted as significant at ⟨=0.05 and were conducted using R statistical software (R Development Core Team 2011).

Results

Ecosystem DOM and DIN losses as a function of soil N content

DON leaching losses measured at 100 cm soil depth increased subtly across forest stands with progressivly higher soil N content, although the trend was not statistically significant after I accounted for the fact that I used multiple samples within a forest stand in this analysis (i.e., "sampling station"; mixed-effects ANCOVA: $F_{1,13}$=0.14, p=0.72; Figure 3.1a). Interestingly, when I just compared the amount of DON leaching from forest stands, there were significant differences across stands ($F_{4,13}$=4.37, p=0.019) but not in the manner I had predicted; DON losses were highest from the intermediate (stand 3) and highest (stand 5) N availability forest stands, with stand 3 having significantly higher DON losses than the next lowest (stand 2) and

highest (stand 4) N availability forests (Tukey contrasts: stands 3-2, z=3.45, p=0.005; stands 4-3, z=-3.11, p=0.014; Table 3.2). I had expected DON losses to steadily increase across the N availability gradient. This unanticipated trend occurred despite a steady increase in soil N content in deep (i.e., 10-100 cm soil increment) soils across the forest gradient (Table 3.2). DIN leaching losses at the 100 cm soil depth also tended to increase across forest stands with higher soil N content, although the relationship was not statistically significant after accounting for forest (mixed-effects ANCOVA: $F_{1,13}$=0.91, p=0.36; Figure 3.1b). However, unlike DON leaching losses, average DIN leaching losses from individual forest stands increased steadily across the N availability gradient ($F_{4,13}$=4.90, p=0.013) with stands 4 and 5 having significantly higher leaching losses than the lower N availability forests (Tukey contrasts: stands 5-1, z=3.68, p=.002; 5-2, z=3.85, p=<0.001; 5-3, z=3.91, p=<0.001; 4-1, z=3.07, p=0.016; 4-2, z=2.70, p=0.048). Indeed, DIN losses were on average 18 times greater from the highest N availability ecosystem compared to the lowest, whereas DON losses were only 4 times greater. As a result, DIN losses occupied a greater proportion of total N losses in high N availability forests compared to those forests where N was less available, which was demonstrated by a corresponding trend of increasing DIN:DON along the N availability gradient.

DOM losses at 100 cm soil depth from individual sampling stations became progressively more enriched in N (i.e., decreasing DOC:DON) as the soil N content of forest stands increased ,but only from intermediate to high soil N contents (Figure 3.1c). Surprisingly, the forest with the lowest soil N content (i.e., stand 1) had DOM leaching losses that were actually more enriched in N compared to intermediate N forests, giving the overall relationship between the DOC:DON of soil leachate and soil N content a unimodal appearance. Indeed, the significant differences in the average DOC:DON of DOM leaching from 100 cm across forest

stands ($F_{4,11}$=4.25, p=0.025) supported this unimodal trend, with stand 5 having significantly lower DOC:DON compared to stands 2, 3, and 4 (Tukey contrasts: 5-2, z=-3.30, p=0.008; 5-3, z=-3.27, p=0.008; 5-4, z=-4.01, p<0.001) but similar DOC:DON to stand 1. However, neither the relationship between the DOC:DON of 100 cm leachate losses and soil N content (mixed-effects ANCOVA: $F_{1,11}$=1.86, p=0.20), nor the quadratic relationship between these variables ($F_{1,11}$=1.09, p=0.32) was statistically significant after the effect of multiple sampling stations within a forest stand was accounted for. The unimodal pattern contradicted my prediction of a linear, negative relationship between the DOC:DON of DOM leaching from 100 cm soil depth and increasing soil N content.

Forest floor litter composition as a driver of DOM chemistry

The amount of DON lost at 100 cm depth was unrelated to inputs of DON from the forest floor to the soil profile after accounting for the effect of including multiple sampling stations within a forest stand in the model (mixed-effects ANCOVA: $F_{1,12}$=0.07, p=0.79). The average DON fluxes from the forest floor steadily increased across the N availability gradient from 26.7 to 45.7 mg m^{-2}, a pattern not mimicked by the average DON fluxes at 100 cm soil depth, which were highest from forest stands 3 and 5 (Table 3.2). There was also no relationship between the DOC:DON of forest floor solutions and that in leachate from 100 cm ($F_{1,12}$=1.05, p=0.32). The average DOC:DON of forest floor leachate decreased from low to high N availability forests (33.4 to21.8) while the average DOC:DON of fluxes at 100 cm soil depth were lowest at those forests where N was least and most available.

Soil sorption phenomena as drivers of DOM chemistry

Hydrophobic compounds dominated soil solution fluxes from 15 and 100 cm soils across almost all forests. Stand 1 was the only stand where the proportion of hydrophobic DOC in fluxes leaving 100 cm soils dropped below 50%, indicating hydrophilic DOC dominated total DOC losses from this forest. There was no evidence that hydrophobic compounds were removed to a greater extent in high versus low N availability forests (mixed-effects ANCOVA: 15 cm soils, $F_{4,5}$=0.65, p=0.65; 100 cm soils, $F_{4,10}$=0.85, p=0.52; Figure 3.2a). The proportion of hydrophobic DOC in solutions collected from 15 and 100 cm depths averaged 65 and 55% across all forest stands, respectively. In general, the percentage of hydrophobic compounds in DOC fluxes from 100 cm soil depth were less than those from 15 cm except for stand 5, which demonstrated an increase in the proportion of hydrophobic DOC between 15 and 100 cm soil depths, although the difference was slight (54 vs 57%, respectively).

While there were no significant differences in the proportion of hydrophobic DOC in leachate from 15 or 100 cm soil depths across forest stands, there were significant differences in the absolute concentrations of DOC fractions. Hydrophobic DOC concentrations leaving 15 cm soils were significantly different across forests (mixed-effects ANOVA: $F_{4,6}$=6.55, p=0.022), with intermediate N availability forests leaching higher concentrations of hydrophobic compounds compared to the lowest and highest N availability forests at this soil depth (Tukey contrasts: 3-1, z=4.21, p<0.001; 4-1, z=2.84, p=0.035; 5-3, z=-4.19, p<0.001; 5-4, z=-2.82, p=0.037; Figure 3.2b). Stand 3 had the highest concentration of hydrophobic DOC (30.3 mg L^{-1}) of any forest stand, leaching roughly 4.6 times more hydrophobic DOC than the lowest and highest N availability forests. In general, intermediate N availability forests leached significantly

more hydrophobic DOC compared to the lowest and highest N availability forests. Hydrophilic DOC concentrations in leachate from 15 cm soil depth had similar leaching patterns as hydrophobic compounds, although their overall concentrations were less in every forest. Intermediate N availability forests had the highest losses of hydrophilic DOC compared to the lowest and highest N availability forests ($F_{4,6}$=5.22, p=0.037; Tukey contrasts: 3-1, z=4.25, p<0.001; 5-3, z=-3.50, p=0.004).

DOC leaching from 100 cm soil depth also had significant differences in the absolute concentrations of hydrophobic compounds across forest stands (mixed-effects ANOVA: $F_{4,11}$=8.53, p=0.002; Figure 3.2c). The concentration of hydrophobic DOC from stand 1 was significantly less than all other forest stand except stand 4 (Tukey contrasts: 2-1, z=3.13, p=0.015; 3-1, z=5.72, p<0.001; 5-1, z=2.87, p=0.034). Additionally, the concentration of hydrophobic compounds in 100 cm leachate from stand 4 was significantly less than that of stand 3 (z=-3.37, p=0.007). The intermediate N availability forest, forest stand 3, had the highest average concentration of hydrophobic DOC fluxes of any forest (5.1 mg L^{-1}), whereas stand 1 had the lowest (1.6 mg L^{-1}). Unlike hydrophobic compounds, there were no significant differences in the hydrophilic concentration of DOC in 100 cm fluxes across forests (mixed-effects ANOVA: $F_{4,11}$=2.09, p=0.15).

In general, soils across the forest gradient demonstrated similar propensities to sorb DOC, based on batch equilibrium experiments (mixed-effects ANOVA: $F_{4,6}$=1.60, p=0.289; Table 3.3). The partition coefficients (i.e., “m”) of soils collectred from 0-15 cm soil depth at forests 1-4 indicated that between 28-32% of DOC in the supplied organic matter solution sorbed to soils.

Stand 5 had the highest partition coefficient of any forest stand, which indicated roughly 43% of DOC sorbed to soils. For soils collected at 15-57 cm soil depth, all forest soils sorbed between 28-34% of DOC (mixed-effects ANOVA: $F_{4,6}$=0.17, p=0.947). Higher N availability forests also tended to have lower coarse sand contents, and higher fine sand, clay, and silt contents in both 0-15 and 15-57 cm soil increments compared to lower N availability forests.

Soils collected at 0-15 cm depth across the forest gradient also had a similar tendency to release organic matter into solution when soils were leached with DI water (mixed-effects ANOVA: $F_{4,6}$=0.43, p=0.784; Table 3.3). The intercepts of the IM isotherms (i.e., "b") were -13.4 to -16.9 mg kg^{-1} across forests, with stand 1 releasing the least amount of organic matter into solution and stand 3 releasing the most organic matter into solution. In soils collected at 15-57 cm depth, the tendency of soils to release organic matter into solution increased significantly from low to high N availability forests ($F_{4,6}$=4.89, p=0.043). Stands 3, 4, and 5 all released significantly more organic matter into solution compared to stand 1 (Tukey constrasts: 3-1, z=3.98, p<0.001; 4-1, z=2.94, p=0.028; 5-1, z=3.25, p=0.010). Overall, soils from 15-57 cm depth released less organic matter into solutions than soils collected at 0-15 cm depth. Soils from higher N availability forests also had higher amounts of organic matter that was readily exchangeable with organic matter in solution compared to soils from lower N availability forests. The RSP of organic matter increased in soils collected from both depth increments from soils collected from low to high N availability forests, with soils from 0-15 cm depth having larger reactive pools compared to soils from 15-57 cm depth. The largest differences in RSP among forests was found in soils from 15-57 cm depth, where stands 3-5 had roughly 3 times the RSP compared to stand 1. However, these differences were not statistically significant (mixed-effects

ANOVA: $F_{4,6}$=2.77, p=0.127). Neither were there any statistically significant differences in RSP in soils from 0-15 cm depth ($F_{4,6}$=0.28, p=0.878).

Discussion

Ecosystem DOM and DIN losses as a function of soil N content

Previous research on the terrestrial N cycle has focused on the production and consumption of inorganic N, which is known to be tightly cycled in forest ecosystems except where N supply exceeds biotic demand (Pastor et al. 1984, Aber et al. 1998, Perakis and Hedin 2001). Our knowledge of DON cycling is less certain but no less important due to its potential to dominate total N losses from forests and perpetuate N limitation in terrestrial ecosystems (Neff et al. 2003). The growing body of work investigating DON dynamics across ecosystems with different N availabilities suggests that DON losses will be higher from ecosystems with higher N availability relative to demand, that DON will account for a decreasing percentage of total N losses in favor of DIN, and that DON losses will increase to a greater extent than DOC losses (McDowell et al. 2004, Pregitzer et al. 2004, Brookshire et al. 2007, Sleutel et al. 2009). In order to investigate these patterns further, I examined DON, DIN, and DOC losses across 5 forests that demonstrated a natural gradient in N availability. I had predicted that DON leaching losses from these forests would conform to the patterns mentioned above. Contrary to my first prediction, DON leaching losses at 100 cm soil depth were not directly related to soil N content (Figure 3.1). Instead, DON losses from intermediate N availability forests were as high, or higher, than those from high N availability forests despite steadily increasing soil N and N mineralization rates across the gradient (Tables 3.1, 3.2). The data supported my second prediction comparing DON and DIN losses across the N availability gradient. The average DIN losses across forests steadily

increased from low to high N availability forests (Table 3.2) so that DON generally comprised a decreasing proportion of total N losses. Finally, I had predicted that DON losses would increase disproportionately over DOC losses as forest N availability increased reltive to demand (Brookshire et al. 2007). Instead, DOM losses demonstrated a unimodal pattern, where the DOC:DON of DOM leaching from 100 cm soil depth at the lowest and highest N availability forest stands was lower than that of the intermediate N availability forests, which had relatively high DOC:DON in leachate collected from soils at 100 cm depth (Figure 3.2). This result is puzzling because I had predicted that DOM losses from the lowest N availability forest would be the least enriched in DON of any forest in this study due to its depauperate N status.

Forest floor litter composition as a driver of DOM chemistry

In order to better understand the patterns of DON losses and DOM chemistry characterized in the first portion of my research, I proposed two alternative hypotheses that investigated some of the potential mechanisms that may regulate DOM across these forests. First, I hypothesized that DON losses and DOM chemistry were a direct product of the forest floor litter composition and reflected changes in tree species composition across the N availability gradient. The lack of a relationship between DON inputs from the forest floor and losses from soils at 100 cm depth does not support the idea that the forest floor litter composition is the primary driver of DON losses from these soils. While forest floor leachate demonstrated the expected results of increasing DON inputs to mineral soil and decreasing DOC:DON from low to high N availability forests, DOM leaving soils at 100 cm depth exhibited neither of these trends; DON losses from these soils were idiosyncratic along the N availability gradient (Table 3.2) and their DOC:DON was lowest in the forests at opposite ends of the N availability gradient (Figure 3.2). Consequently, the chemistry of forest floor leachate failed to explain DON loss

patterns across forests and the unexpected result of relatively low DOC:DON leaching from 100 cm deep soils from both the lowest and highest N availability forests. Yano et al. (2005) also found that the chemistry of DOM leaching from deep soils was similar regardless of whether a plot experienced regular, reduced, or increased inputs of plant litter in a litter manipulation study in old-growth Douglas-fir forests of the Pacific Northwest. Instead, they cited the strong ability of soil to sorb DOM compounds as the primary mechanism controlling DOM retention in this ecosystem rather than input chemistry.

Soil sorption phenomena as drivers of DOM chemistry

The disconnect between DOM inputs and outputs across these forest soils suggests soil waters underwent a substantial alteration in chemistry as they percolated through mineral soil to depth. However, the mechanism proposed in the alternative hypothesis regarding the role of soil sorption in regulating DOM chemistry also failed to completely explain the patterns I found across forests. I had proposed that the increasingly fine texture of soils from low to high N availability forests would favor the preferential sorption of hydrophobic, relatively high C:N compounds from solution, resulting in decreasing DOC:DON of DOM leaching losses and increasing DON leaching losses across the N availability gradient. Instead, I found no evidence that hydrophobic compounds were preferentially removed from solutions leaving surface or deep soils despite decreasing coarse sand contents and increasing fine sand and clay contents across the gradient (Figure 3.2a, Table 3.3). Moreover, if soil sorption potential really increased with increasing N availability, I would have expected the DOC:DON of soil leachate from the forest with the most coarse-textured soil, forest stand 1, to be the highest along the gradient rather than equally as low as stand 5. These results suggest soil sorption phenomena alone are not able to fully explain the patterns of DON loss and DOM chemistry found across these forests.

A comprehensive, ecosystem approach to DOM dynamics

The inability of either of my alternative hypotheses to fully account for the DOM loss patterns experienced by these forests suggests it is necessary to have a more comprehensive approach when investigating DOM dynamics that allows for interactions among forest components. For example, the ability of the forest floor composition to influence the chemistry of DOM inputs to mineral soils (Park and Matzner 2003, Lajtha et al. 2005) may interact with changes in soil sorption abilities across forests to either exacerbate or ameliorate DOM losses and alter DOM chemistry. By taking this comprehensive approach, it becomes more apparent how forest characteristics could interact to produce the DON loss patterns and DOM chemistry found in this study. In the case of the lowest N availability forest, the interaction between strongly sorbing DOM inputs with highly sorptive soils may have resulted in the relatively low DOC:DON leaching from this forest. First, the low N mineralization rates (Table 3.1), the high C:N of the forest floor in this oak-dominated forest (Table 3.2), and the relatively high DOC:DON in forest floor leachate indicate DOM inputs are largely recalcitrant in this forest and likely contain a greater proportion of hydrophobic versus hydrophilic compounds (Lajtha et al. 2005). This idea was reinforced by hydrophobic compounds dominating fluxes at 10 cm soil depth (Figure 3.2a, b). It is unlikely hydrophobic compounds were added to solutions to any great extent as they percolated through the upper 10 cm of mineral soil due to their high soil sorption potential (Kaiser and Zech 1998). Therefore, solutions entering mineral soil would have relatively high sorption potential based on the preponderance of hydrophobic compounds in solution. Second, despite the coarse texture of this soil, the low reactive soil pool in soils collected from both 0-15 and 15-57 cm depths, combined with the low tendency of this soil to release organic matter when leached with water (i.e., "-b"), indicated that this soil was more likely to retain rather than release organic matter into solution (Table 3.3, Appendix). The

interaction between solutions dominated by highly sorptive hydrophobic compounds with a highly retentive soil would serve to preferentially remove C-rich, hydrophobic compounds from percolating solutions and result in an overall decrease in the DOC:DON of soil solutions with depth. This was substantiated by the shift in DOM chemistry from being dominated by hydrophobic to more N-rich hydrophilic compounds (Figure 3.2), as well as the decrease in DOC:DON from 33.4 - 16.9 with increasing soil depth at stand 1. Additionally, the relatively low DON fluxes into mineral soil from the forest floor with little potential release of organic matter into soil solutions as waters percolated to depth supports the overall low DON leaching losses I found from this forest.

In intermediate N availability forests, an increase in DOM production coupled with a reduced ability of soils to sorb DOM could explain the increase in DOC:DON in deep soils from these forests. First, these forests shifted from being dominated by black and white oaks to species with more labile leaf litter, such as red maple, red oak, and sugar maple. With this shift are concomitant increases in the basal area and leaf litter fall compared to the lowest N availability forest (Zak et al. 1989, Rothstein et al. 2009), indicating overall greater biomass production in these forests. As a result, these forests have higher soil C and N stocks compared to stand 1, which may limit the number of available sorption sites on these fairly coarse textured soils (Kaiser et al. 1996, Kaiser and Zech 1998, Seely et al. 1998; Table 3.2). This is evident in the tendency of these soils to release more organic matter into solutions, to have higher reactive soil pools, and to leach up to 4 times more DOC from soils at 100 cm depth despite similar inputs from the forest floor compared to forest stand 1 (Tables 3.2, 3.3). Kaiser et al. (1996) found a negative relationship between DOC sorption and the organic C content of soil, suggesting soil organic matter hinders DOM sorption by covering binding sites on mineral soil. These author's

also found that the sorption of hydrophobic compounds was less affected by high soil organic C contents compared to hydrophilic compounds; they suggested hydrophobic compounds may hinder the sorption of hydrophilic compounds to soils when binding sites are limited, possibly through the displacment of previously sorbed hydrophilic compounds. In this study, the concentration of hydrophobic compounds decreased by 3-7 times between 15- and 100-cm soil depths at intermediate N-availablity forests compared to only a 3-4 fold decrease in the concentration of hydrophilic compounds, supporting the idea that the soil organic matter content of soils will limit the sorption of hydrophilic compounds more so than hydrophobic compounds. However, leaching losses of DOC at 100 cm soil depth were still dominated by hydrophobic compounds in intermediate N-availablity forests (Figure 3.2b, c). High DOC leaching losses from these forests that are dominated by C-rich hydrophobic compounds would result in relatively higher DOC:DON of DOM leaching losses, in agreement with the patterns I found of DOM leaching losses at 100 cm soil depth from intermediate N-availability forests.

Finally, in high N availability forests the decreased DOC:DON in leachate from 100 cm soil depth, and similar levels of DON leaching losses as those in intermediate N availability forests, could result from an increase in the sink strength for DOM by both biotic and abiotic mechanisms. As N availability increases across the landscape, a larger fraction of the microbial community in relatively high N forests acquires N directly from organic sources rather than inorganic N diffusing from N-rich microsites, mineralizing N once their N needs have been met (Schimel and Bennett 2004). Consequently, total N fluxes leaving biologically active soil horizons would be dominated by inorganic forms in these high N ecosystems. Forest stand 5 had the highest rates of N mineralization and nitrification along the gradient (Rothstein 2009) and was the only forest to demonstrate an increase in DIN leaching from soils at 10 cm depth relative

to forest floor inputs (by 59%; Table 3.2) supporting the idea that microorganisms removed a greater proportion of DON from this forest compared to other forests by converting it to inorganic forms. This would serve to constrain DON losses from this forest despite having the highest DON inputs from the forest floor of any forest along the gradient. Previously, Rothstein (2009) demonstrated that stand 5 had the smallest free amino acid pools, largest inorganic N pools, and the highest potential for microbes to mineralize amino acids, adding credence to the idea of a strong biotic sink for N in this forest. The high N mineralization in stand 5 suggests the microbial community is C rather than N limited, which may account for the dramatic decrease in DOC fluxes through the upper 10 cm of soil in this forest (95% reduction); intermediate N availability forests had only a 70-76% reduction in DOC in this same horizon. This mechanism could help explain the low DOC:DON in DOM losses from this forest. Additionally, stand 5 had a relatively low coarse sand content and relatively high clay and silt contents compared to the other forests, especially in surface soils, which could have resulted in a greater abiotic sorptive ability in this forest (Seely et al. 1998). Indeed, the partition coefficient for DOM in surface soils of stand 5 was the highest of any forest examined (Table 3.3) as was the C and N content in surface soils. However, unlike in most intermediate N availability forests, the high organic matter content of surface soil in stand 5 did not result in an equally high tendency to release organic matter when leached with water, suggesting the overall sorption capacity of this soil for organic matter was greater than that of intermediate N availability forests. This strong sorptive ability would limit overall DON losses from this forest and decouple the expected relationship of increased DON losses from high N forests that I had initially predicted. Interestingly, although the DOC:DON of soil solution decreased with increasing depth, the proportion of hydrophobic compounds in solution was fairly consistent across depths (Figure 3.2), making it unclear if the

preferential removal of C-rich, hydrophobic compounds was the mechanism behind the DOC:DON change.

While low DON inputs from the forest floor to mineral soil in the lowest N availability forest and high DON retention by biotic and abiotic processes in the highest N availability forest offers one explanation for the lack of a strong relationship between DON losses and soil N content across these forests, it is also possible that DON losses at 100 cm soil depth in these forests were not directly linked to actively cycling soil N pools (Hedin et al. 1995, Perakis et al. 2005). Instead, DON losses may be under the control of soil organic matter dissolution and transport processes and follow similar loss patterns as DOC (Hedin et al. 1995, Brookshire et al. 2007). Indeed, my forests with the highest DON losses had correspondingly high DOC losses compared to the remaining forests, in support of this idea (Table 3.2, Appendix). However, despite the lack of a discernible trend between DONleaching losses from deep soils and soil N content, the average DON leaching losses from 15 cm soils steadily increased from 2.7 to 22.5 mg m^{-2} across the N availability gradient, suggesting that, at least in surface soils, DON leaching losses are not wholly independent of soil N (Table 3.2). A similar trend was found by Sleutel et al. (2009), where differences in DON fluxes among 3 forests experiencing either low or high N inputs were more pronounced below the E horizon compared to those below the BC horizon. The strong ability of mineral soil to sorb DON from percolating solutions (Qualls et al. 2002) could have obscured the relationship between DON leaching losses at 100 cm soil depth and soil N found in the surface soils by the time they leached from deep soils (McDowell et al. 2004).

Alternatively, the lack of relationship between DON losses and soil N content may be a function of the range of N availabilities in the forests I evaluated. Other studies that have demonstrated increased DON losses with increasing ecosystem N have either artificially

increased N inputs via N fertilization (McDowell et al. 2004, Pregitzer et al. 2004), or evaluated ecosystems experiencing rates of N deposition that were roughly 1.3-7.4 times higher than the forests in my study (Brookshire et al. 2007, Sleutel et al. 2009). For example, Pregitzer et al. (2004) found that NO_3^- additions of 3 g m^{-2} yr^{-1} for 8 years increased DON losses over 6 times those found in control plots in an N fertilization study in northern hardwood forests similar to the those in this study. They also found that forests with higher initial N availability lost greater amounts of DON, and in a shorter period of time, than those forests with lower initial N availability. Brookshire et al. (2007) found their highest DON leaching losses occurred in watersheds that experienced atmospheric N inputs of 7.7- 45.0 kg ha^{-1} y^{-1}, whereas the forests in this study experienced an average of 6.1 kg ha^{-1} yr^{-1} of atmospheric N inputs over the study period. They also evaluated DON leaching losses from watersheds that largely had higher surface soil N contents compared to those than in this study (95 to 190 g m^{-2} in 0-10 cm deep soils versus 58 to 163 g m^{-2} in 0-15 cm deep soils in this study). Both of these studies evaluated ecosystems that were considered saturated in N (Aber et al. 1989), which may be a requirement before ecosystems begin losing increasing amounts of DON.

The results of this study are based on spring patterns of DOM leaching losses from these forests and may differ in other seasons. However, DON and DOC leaching losses from deep soils and streamwater DOM concentrations generally show little variation througout the year (Campbell et al. 2000, Qualls et al. 2002, Yano et al. 2004), suggesting the patterns I found in leaching from 100 cm soils are more a product of the forests I examined rather than a seasonal phenomenon. DOM leaching losses from organic horizons, though, are more variable than deep soil leachate across seasons, and reflect seasonal differences in hydrologic and leaf litter inputs,

with peak DOM losses occuring in the fall after leaf drop and in the spring as snow melts (Qualls and Haines 1991, Michalzik and Matzner 1999, Yano et al. 2004). These leaching losses from organic horizons also vary in their hydrophobic and hydrophilic DOM composition in different seasons, which in turn impacts the kinds of molecules leaching from mineral soils (Qualls and Haines 1991, Yano et al. 2004). Therefore, although total DOM leaching losses remain fairly consistent throughout the year, the chemical composition of those losses (i.e., hydrophobic versus hydrophilic compounds) may differ during other seasons from the patterns I found. This also suggests the mechanisms for DOM retention, such as soil sorption/desorption and microbial uptake, may play different roles than those I proposed depending on the particular season being evaluated.

Conclusions

The results of this study demonstrated that DON losses and DOM chemistry from 5 northern hardwood forests were not a simple function of soil N content, nor were they the product of individual forest parameters, such as forest floor litter composition or soil texture. Instead, DON losses and DOM chemistry across these forests were best explained when multiple forest parameters were considered simultaneously. In the lowest N availability forest, the interaction between the low production of C-rich DOM compounds combined with highly retentive, yet coarse textured, soils relatively low in organic matter served to limit overall DON losses at 100 cm soil depth while simultaneously enriching them in N-rich compounds (Figure 3.3). In intermediate N availability forests, high DOM production coupled with soils of relatively limited sorption capacity increased overall DON and DOC losses at 100 cm soil depth so that the DOM stoichiometry of solutions leaving these forests actually became less enriched in N compared to the lowest N availability forest. Finally, at the highest N availability forest, the

combination of high DOM retention by both abiotic and biotic mechanisms resulted in DON losses at 100 cm soil depth similar to those from intermediate N availability forests as well as DOM stoichiometry that matched that of the lowest N availability forest. The complexity of ecosystem interactions demonstrated in this study emphasizes how fine-scale controls over DOM dynamics can shape landscape level patterns of DON and DOC losses in unexpected ways. This was especially apparent in the unimodal pattern of DOC:DON losses across the N availability gradient found in this study.

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Table 3.1 Forest stand characteristics. Data in parentheses represent ranges.

Stand	1	2	3	4	5
Location (North latitude, West longitude)	44.2, 85.9	44.3, 85.9	44.2, 85.7	44.3, 85.8	44.2, 85.7
Stand age (2005)[a]	74	85	83	104	97
Stand basal area (m^2 ha^{-1})[a]	21.2	29.7	32.6	33.5	36.1
% Oak (*Q. velutina, Q. alba, Q. rubra*)[a]	99	83	54	15	0
% Maple (*A. saccharum, A. rubrum*)[a]	0	17	46	41	66
N mineralization (µg N g^{-1} day^{-1})[a]	0.61	0.7	0.6	1.04	1.32
Nitrification (µg N g^{-1} day^{-1})[a]	0.01	0.01	0.04	0.16	1.1
Soil N content (g m^{-2})	258 (220-295)	339 (304-369)	439 (359-515)	415 (352-457)	524 (462-625)
Leaf litterfall N (g m^{-2})[a]	2.4	3.1	4.6	4.5	4.7
Microbial biomass C (µg g^{-1})[a]	400	580	671	1037	1169
Soil Classification	Typic Udipsamment	Entic Haplorthod	Typic Haplorthod	Typic Haplorthod (clay lamellae)	Typic Haplorthod (clay lamellae)

[a]Data from Rothstein 2009

Table 3.2 Dissolved organic carbon (DOC) and nitrogen (DON) and dissolved inroganic nitrogen (DIN) in leaching losses of soil waters collected beneath the forest floor litter layer (0), at 15 cm soil depth, and 100 cm soil depth. Also listed are the average C and N contents and the C:N ratio (by mass) of the forest floor and soil. For 100 cm soils, the C and N contents are a weighted average of soils from 15-57 cm and 57-100 cm depth increments. Leaching losses are the averages of spring samples from 2006, 2007, and 2008 for all sampling stations within a forest stand. Data in italics represent 1 SE. Table continued on next page.

Depth (cm)		DOC ($mg\ m^{-2}$)		DON ($mg\ m^{-2}$)		DIN ($mg\ m^{-2}$)		DOC:DON		DIN:DON	
0											
	1	883.3	*95.8*	26.7	*2.7*	18.9	*2.7*	33.4	*2.5*	0.7	*0.1*
	2	916.7	*184.6*	33.0	*6.7*	46.8	*22.0*	29.5	*4.4*	1.2	*0.3*
	3	815.9	*93.5*	37.7	*6.9*	20.0	*6.9*	22.9	*2.3*	0.5	*0.1*
	4	849.1	*120.0*	38.7	*4.8*	10.6	*2.2*	21.9	*1.1*	0.3	*0.1*
	5	958.2	*27.1*	45.7	*4.0*	22.2	*11.7*	21.8	*2.3*	0.5	*0.2*
15											
	1	52.2	*12.4*	2.7	*1.0*	0.5	*0.1*	22.8	*2.2*	0.2	*0.1*
	2	88.4	*65.8*	3.5	*3.3*	1.0	*0.0*	66.8	*44.3*	2.5	*2.3*
	3	242.0	*97.3*	9.7	*3.9*	3.6	*1.6*	25.1	*2.2*	0.3	*0.1*
	4	206.5	*84.4*	9.2	*2.7*	2.7	*1.1*	21.2	*4.1*	0.4	*0.2*
	5	148.8	*46.6*	22.5	*9.5*	53.7	*15.3*	19.2	*10.3*	7.4	*4.7*
100											
	1	13.2	*0.6*	0.8	*0.1*	0.6	*0.1*	16.9	*2.1*	0.7	*0.2*
	2	22.0	*6.3*	0.7	*0.4*	1.2	*0.7*	41.1	*15.6*	3.1	*2.2*
	3	55.3	*5.9*	2.4	*0.2*	1.9	*1.0*	24.2	*4.2*	1.0	*0.6*
	4	25.1	*5.7*	0.9	*0.2*	2.8	*0.2*	25.2	*4.3*	3.6	*1.1*
	5	32.9	*8.7*	3.3	*1.1*	9.9	*2.0*	12.7	*3.2*	4.6	*1.9*

Table 3.2 (cont'd)

C content ($g\ m^{-2}$)		N content ($g\ m^{-2}$)		C:N	
599.1	*40.9*	12.4	*1.2*	49.2	*2.8*
702.3	*70.7*	15.9	*2.0*	44.7	*1.4*
927.0	*66.9*	20.1	*2.1*	46.7	*2.1*
745.7	*69.1*	16.9	*1.4*	44.2	*2.0*
409.3	*71.5*	10.6	*1.8*	38.4	*0.9*
1124.4	*101.3*	57.7	*4.3*	19.4	*0.5*
1749.6	*112.6*	88.8	*14.0*	20.0	*1.9*
1589.7	*315.4*	97.6	*17.2*	16.1	*0.8*
1159.0	*134.6*	80.5	*10.4*	14.5	*0.4*
2020.9	*263.4*	163.7	*22.7*	12.4	*0.3*
1209.9	*108.3*	93.4	*7.7*	13.1	*1.0*
1863.0	*97.4*	121.8	*7.0*	15.4	*0.9*
2485.1	*259.1*	159.5	*9.1*	15.4	*0.8*
2152.3	*316.7*	159.2	*12.4*	13.3	*1.1*
2201.7	*162.0*	173.8	*7.6*	12.6	*0.4*

Table 3.3 Soil characteristics of each forest stand. RSP is the reactive soil pool, b is the intercept of the IM isotherm and represents the amount of organic matter released from a soil when it is leached with water, and m is the slope of the IM isotherm and represents the partition coefficient. b, m, and RSP were measured on 0-15 (i.e., surface soil) and 15-57 cm (i.e., deep soil) soil depth increments and represent the averages of three subsamples per forest stand. Soil textures listed are for 0-15 (i.e., surface soil) and 15-100 cm (i.e., deep soil) increments. The deep soil textures are the weighted average of 15-57 and 57-100 cm depth increments. Data in italics represent 1 SE.

Site	RSP ($mg\ kg^{-1}$)		b ($mg\ kg^{-1}$)		m		% Coarse sand		% Fine sand		% Clay		%Silt	
Surface soils														
1	20.3	*4.7*	-13.4	*2.3*	0.3	*0.1*	57.0	*3.1*	32.8	*2.9*	3.7	*0.2*	6.5	*0.4*
2	24.7	*4.6*	-16.4	*1.8*	0.3	*0.1*	55.9	*2.1*	30.6	*1.9*	3.6	*0.1*	9.9	*0.5*
3	24.8	*7.1*	-16.9	*3.1*	0.3	*0.1*	47.6	*3.3*	44.2	*3.0*	2.7	*0.1*	5.6	*0.3*
4	21.8	*4.6*	-14.6	*2.7*	0.3	*0.0*	35.3	*2.9*	53.0	*2.1*	3.2	*0.5*	8.5	*0.4*
5	26.5	*3.0*	-15.0	*1.4*	0.4	*0.0*	37.6	*3.1*	47.7	*2.3*	5.2	*0.4*	9.5	*1.3*
Deep soils														
1	3.9	*2.0*	-2.5	*1.3*	0.3	*0.1*	58.6	*1.6*	37.3	*1.6*	4.2	*0.1*	0.5	*0.2*
2	8.2	*2.7*	-5.2	*1.3*	0.3	*0.1*	55.2	*3.6*	35.3	*2.6*	9.5	*1.2*	1.5	*0.1*
3	11.8	*1.7*	-8.0	*0.9*	0.3	*0.0*	50.1	*3.4*	46.8	*3.3*	3.1	*0.2*	0.9	*0.1*
4	10.5	*3.5*	-6.6	*1.7*	0.3	*0.1*	35.7	*1.6*	57.0	*1.3*	7.3	*0.8*	1.2	*0.2*
5	11.5	*1.5*	-7.8	*0.6*	0.3	*0.0*	42.1	*4.9*	50.6	*3.3*	7.4	*1.9*	1.2	*0.2*

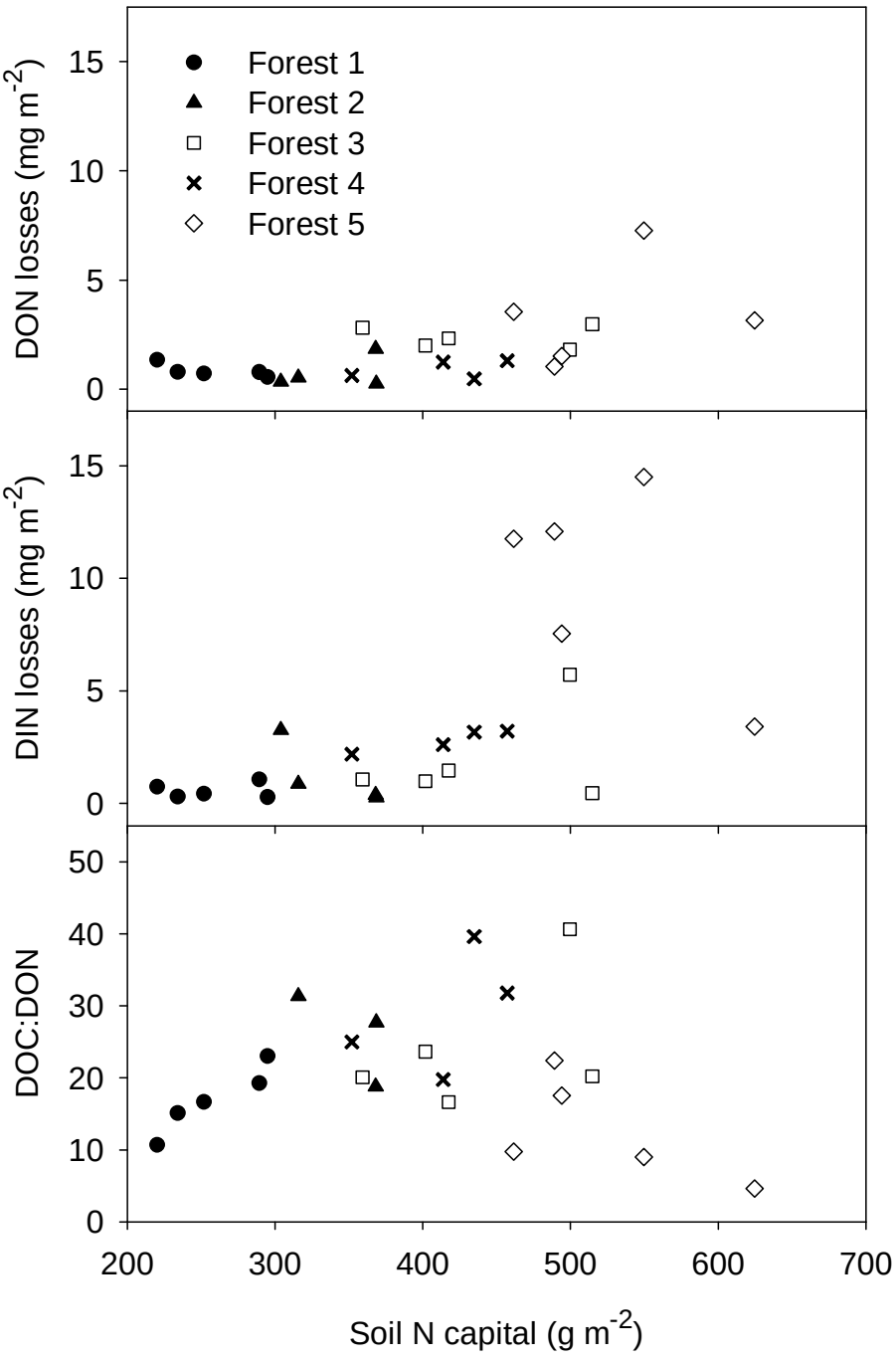

Figure 3.1 The relationship between total forest floor litter layer and soil N content with the (a) DON, (b) DIN, and (c) the DOC:DON (by mass) of soil leaching losses at 100 cm soil depth. Data points represent the average DON, DIN, or DOC:DON leaching losses across spring collections in 2006, 2007, and 2008 for each collection station. Replicate samples within an individual forest are represented by specific symbols as indicated in the figure legend.

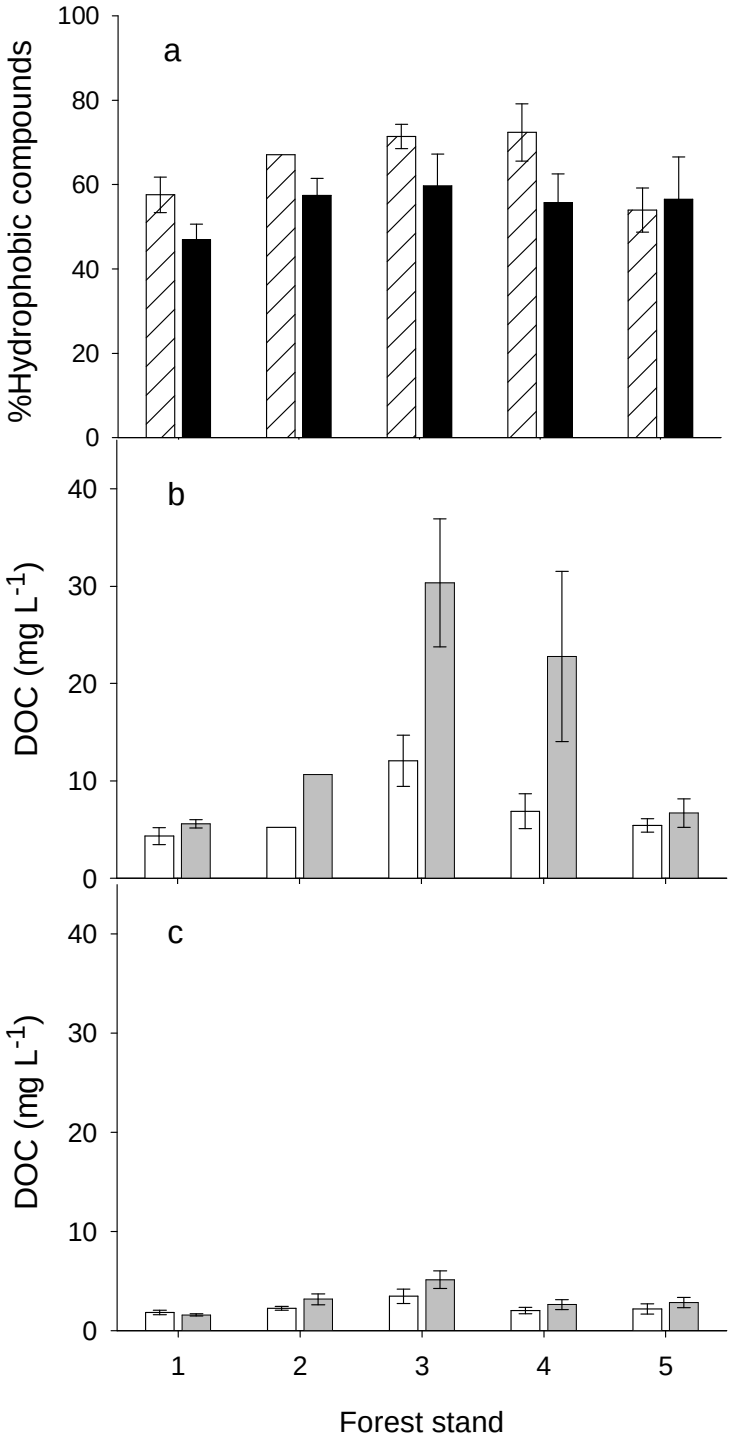

Figure 3.2 (a) The %hydrophobic (±1 SE) DOC in solutions collected at 15 (*striped bars*) and 100 (*black bars*) cm soil depths and the absolute concentrations (±1 SE) of hydrophilic (*white bars*) and hydrophobic (*gray bars*) DOC in solutions collected at (b) 15 and (c) 100 cm soil depths. Data are average soil solution DOC concentrations of spring composite samples in 2006, 2007, and 2008 for all sampling stations in a forest stand.

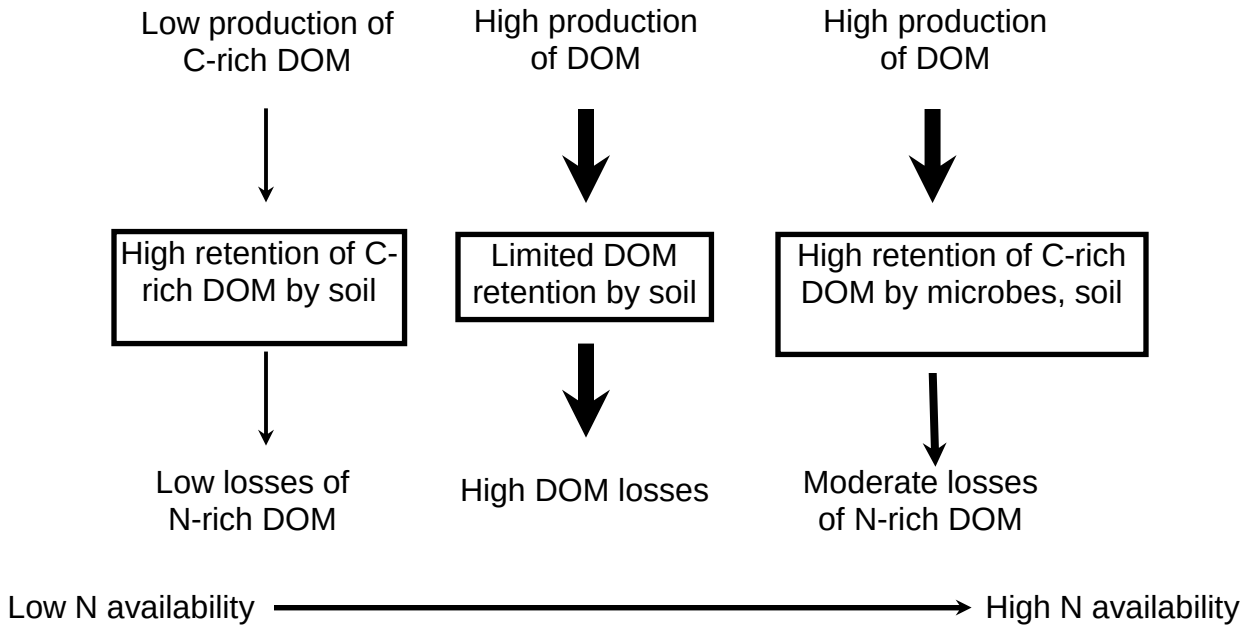

Figure 3.3 Conceptual model of DOM production, retention, and leaching losses across forest stands that span a gradient of N availability. Arrow size represents the approximate size of DOM fluxes. Boxes represent 0-100 cm soil profiles.

APPENDIX

Appendix: Initial mass sorption isotherms and the relationship between DOC and DON leaching losses

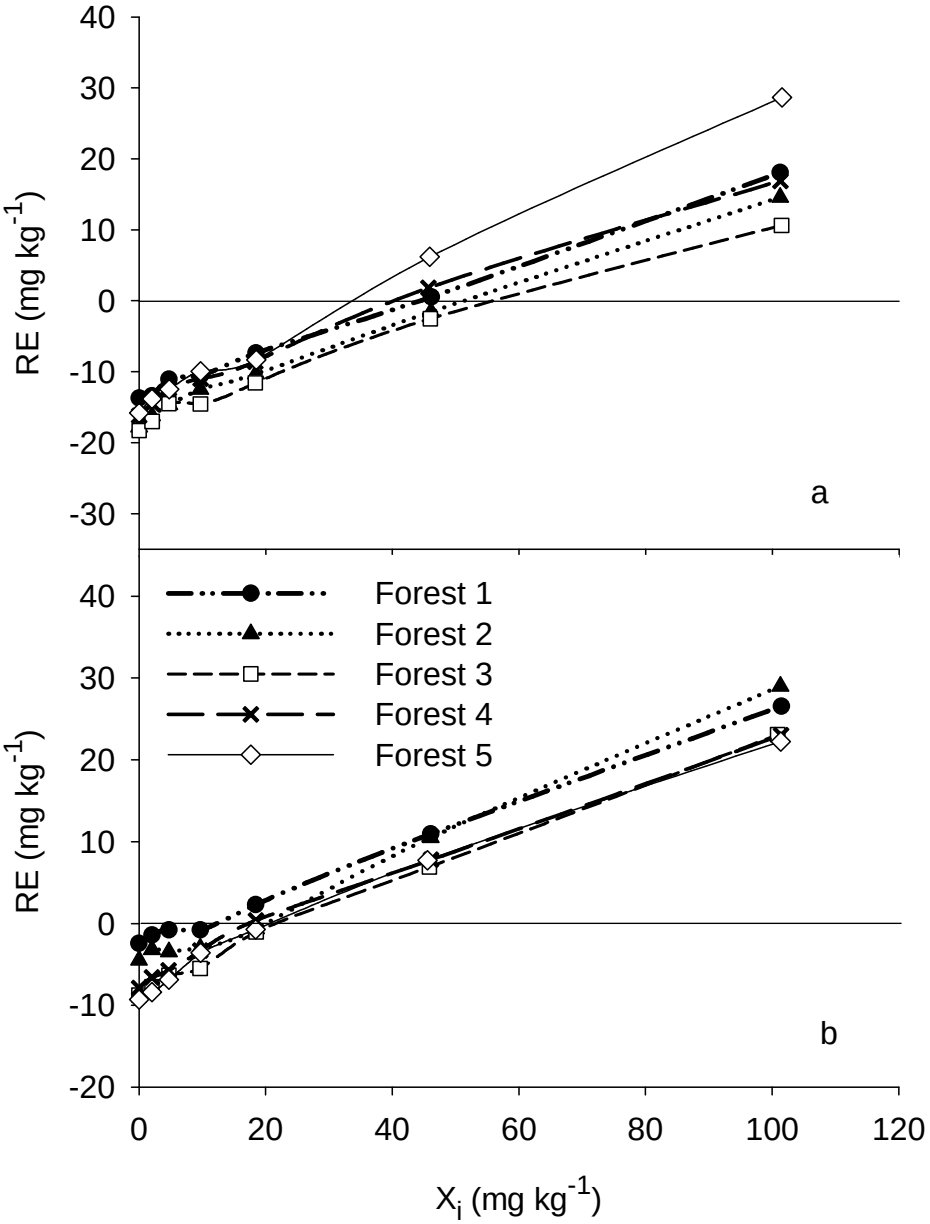

Figure A3.4 Initial mass isotherms from batch soil sorption experiments of (a) 0-15 and (b) 15-57 cm soil increments from each forest stand. RE is the amount of DOC removed from or released to the solutions with respect to the soil mass. X_i is the initial amount of DOC added to the solution with respect to the soil mass. Each point represents the average value of 3 soil samples collected from the sampling satations within each forest stand.

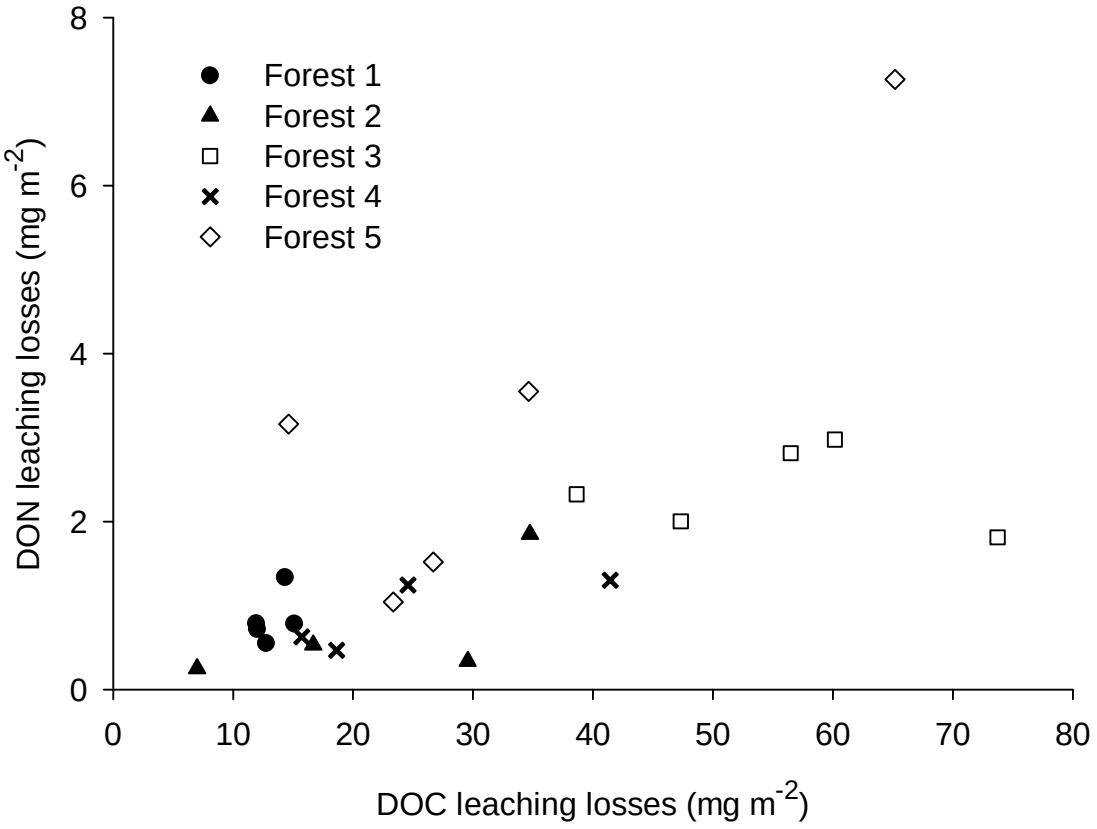

Figure A3.5 The relationship between DOC and DON leaching losses at 100 cm soil depth from five forests that span a gradient of N availability. Forest 1 has the lowest N availability and Forest 5 has the highest N availability. Each point represents the average DOC or DON of leaching losses collected in the spring of 2006, 2007, and 2008 from each sampling station within a forest.

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CHAPTER 4

THE IMPACTS OF SOIL PHYSICAL AND CHEMICAL PROPERTIES ON LEACHING LOSSES OF DISSOLVED ORGANIC MATTER IN NOTHERN HARDWOOD FORESTS

Abstract

Dissolved organic nitrogen (DON) is increasingly recognized as an important component of the terrestrial N cycle, in part due to its ability to "leak" from forests despite biotic N-limitation. These "leaks" are thought to consist primarily of recalcitrant DON compounds that are less for biotic consumption. However, soils preferentially sorb large, recalcitrant molecules over small, more labile molecules as soil waters percolate to depth, suggesting DON lost to leaching is not as recalcitrant as previously thought. In order to investigate how soil sorption dynamics can impact both the quantity and quality of DON losses, as well as dissolved organic carbon (DOC) losses, I collected soil cores of 0-10, 0-25, and 0-50 cm depths from six forests that differed in soil characteristics and leached them with a common organic matter solution. I then fractionated the soil core leachate into hydrophilic and hydrophobic compounds as a measure of leachate quality. I also analyzed the biodegradability of soil core leachate to determine if dissolved organic matter carried through soils in percolating waters was as recalcitrant as currently thought. While there were few differences in the quantity and quality of DON and DOC leaching across forests, there were significant differences across soil depths. DOC concentrations were reduced by 76% in leachate from 50 cm cores compared to the input organic matter solution, mainly due to the removal of hydrophobic compounds, which was consistent with the idea that hydrophobic compounds preferentially sorb to mineral soils. DON concentrations were also reduced from the input organic matter solution to leachate at 50 cm

(~42%), but only after first increasing by 18% in the upper 10 cm of soil. The increase in DON concentration was accompanied by a67-fold increase in the concentration of hydrophilic DON, likely due to displacement from surface soil sorption sites by incoming hydrophobic compounds. Soil core leachate from deep soils had similar levels of C consumed as surface soils by the end of the incubation period, although the C consumption curves of surface and deep soils suggested they differed chemically in way that might impact the longer-term biodegradability of soil solutions at different depths. These results support the strong ability of soils to affect the quantity and quality of DON and DOC losses from forests and suggest DON “leakage” may be more a function of soil sorption phenomena rather than the inability of biota to degrade recalcitrant compounds before they reach to deep soils in percolating water.

Introduction

Dissolved organic nitrogen (DON) has increasingly become recognized as an important component of terrestrial N cycling for its ability to comprise the majority of total N losses from forests (Hedin et al. 1995, Seely et al. 1998, Perakis and Hedin 2002, Qualls et al. 2002, Möller et al. 2005). Indeed, DON losses have been implicated in perpetuating N limitation in forest ecosystems and have been described as an "N leak" that is outside of biologic control (Hedin et al. 1995, Neff et al. 2003). This is contrary to our previous understanding of the N cycle where high biological demand for inorganic N forms (i.e., NH_4^+ and NO_3^-) restricts N losses in N-limited ecosystems (Vitousek et al. 1982); significant DON losses can occur from forests despite N limitation of the terrestrial biota (Perakis and Hedin 2002, Neff et al. 2003). Multiple studies have confirmed the ability of plants to take up small organic N molecules for their N nutrition (Kielland 1994, Turnbull et al. 1996, Öhlund and Näsholm 2001), as well as intense plant-microbe competition for this N source (Lipson and Näsholm 2001, Jones et al. 2005), suggesting that the fraction of DON leaching from ecosystems is relatively recalcitrant in nature and less available for biological uptake (Hedin et al. 1995, Neff et al. 2003). This idea is supported by the macromolecular structure of the majority of DON fractions (Yu et al. 2002), which inhibits the ready uptake of these compounds by microbes and roots. As a result, it has been hypothesized that DON losses are regulated by dissolved organic matter (DOM) dissolution and transport processes and should behave similarly to dissolved organic carbon (DOC) (Hedin et al. 1995, Brookshire et al. 2007). While DON losses have been positively correlated with DOC losses from a variety of ecosystems (Hedin et al. 1995, Perakis and Hedin 2002, Qualls et al. 2002), studies that have evaluated DON losses from ecosystems experiencing high N inputs have demonstrated a breakdown in the tight relationship between DON and DOC losses (McDowell et

al. 2004, Pregitzer et al. 2004, Brookshire et al. 2007), suggesting there are still gaps in our knowledge about the mechanisms that regulate DON and DOC losses from terrestrial ecosystems.

The consistent decrease of total DON and DOC in solutions percolating through the soil demonstrates the strong capacity of mineral soil to adsorb DON and DOC and to play a key role in regulating losses (Seely et al. 1998, Qualls et al. 2002, Möller et al. 2005). In a study by Qualls et al. (2002), 98.4% of DON and 99.3% of DOC leaching from the forest floor of hardwood forests in North Carolina, USA, was removed from solution by the time it left the C horizon as a result of adsorption to mineral soil. These authors also suggested the fairly constant losses of DON and DOC throughout the year resulted from the strong sorption capacity of soil, which helped attenuate losses during periods of peak litterfall (hence peak leaching potential). The ability of a soil to adsorb DON and DOC is a function of many factors, such as the texture (Seely et al. 1998), structure (Asano et al. 2006), organic matter content (Lilienfein et al. 2004), surface area (Kaiser et al. 1996), and mineral composition (i.e. Fe and Al oxides; Qualls 2000, Lilienfein et al. 2004, Yano et al. 2004) of a soil. For example, high soil organic matter concentrations can inhibit DON and DOC adsorption by limiting available binding sites on soil colloids (Kaiser and Zech 1998, Lilienfein et al. 2004). Conversely, increasing concentrations of Fe and Al oxides can facilitate DON and DOC sorption, possibly through ligand exchange (Qualls 2000, Lilienfein et al. 2004).

Mineral soil can also impact the quality, as well as the quantity, of DON and DOC percolating through the soil by influencing the relative amount of hydrophobic and hydrophilic fractions in soil solution. Hydrophobic compounds are typically humic substances that have relatively high molecular weights, are polymeric/aromatic, have a relatively low ratio of carboxyl

functional groups to C content (Qualls and Haines 1991) and, as a result, readily sorb to mineral soil (Kaiser and Zech 1998). In contrast, hydrophilic compounds generally exhibit low molecular weights, are often aliphatic, have high carboxyl-to-C ratios, and tend to remain in solution. Therefore, it is expected that soils with high sorptive strength (i.e., soils with fine textures, micropores, high Fe and Al content) will remove a greater proportion of hydrophobic compounds from solution compared to soils with low sorptive strength (i.e., soils with coarse texture, large macropores, and/or high pore connectivity). It has also been demonstrated that the proportion of hydrophobic compounds in solution decreases with increasing soil depth due to hydrophobic compounds being preferentially removed from solutions as they percolate through the soil (Yano et al. 2004, Lajtha et al. 2005). Indeed, Kaiser and Zech (1998) found hydrophobic constituents actually displaced hydrophilic substances previously sorbed to the soil as solutions percolated to depth. This implies competition between the stronger-binding hydrophobic substances and the relatively weaker-binding hydrophilic compounds for binding sites on soils (Kaiser and Zech 1998).

The amount of hydrophobic compounds relative to hydrophilic compounds comprising DOM has implications for its biodegradability. For example, soil solutions inoculated with microbes frequently display a positive relationship between the amount of DOM consumed and the concentration of operationally-defined hydrophilic compounds in solution; less DOM uptake is generally observed where hydrophobic compounds dominate (Michaelson et al. 1998, Cleveland et al. 2004, Qualls 2004, Kaushal and Lewis 2005), likely due to the higher molecular weight, higher aromaticity, and lower acidity of hydrophobic compared to hydrophilic compounds (Guo and Chorover 2003, Kaushal and Lewis 2005). Therefore, solutions dominated by hydrophilic compounds, such as those found in deep soils after hydrophobic compounds have

been preferentially removed, may also be relatively available for biotic consumption. This idea contradicts our ecological understanding of DON cycling, which postulates that DON leaching losses must be dominated by recalcitrant compounds because they occur despite N-limitation by biota (Hedin et al. 1995). Therefore, in order to resolve this inconsistency between geochemical and ecological linesof thinking, it will be important to evaluate the biodegradability of DOM as it percolates through soil.

The objective of this study was to investigate how soil characteristics and soil depth impact the quantity and quality of DON and DOC as water percolates through the upper 50 cm of soil. To do this, I collected soil cores of three different depths from six forests in the Manistee National Forest, Michigan. Five of the forests were previously studied in Chapter 3, while one forest was added specifically for this study.These forests spanned a range of soil characteristics (i.e., soil texture, organic matter content). I conducted a leaching experiment of the soil cores with a common organic matter solution, which allowed me to isolate the effects of soil characteristics on leachate chemistry. Across the six forests, I hypothesized that the quantity of DOC and DON in soil core leachate would decrease as forest soils increased their ability to sorb organic matter. I also hypothesized that the quality of DOC and DON, as measured by the proprotion of hydrophilic, would increase as forest soils increased their ability to sorb organic matter due to an increasingly higher affinity for the sorption of hydrophobic fractions with mineral soil. Across soil depths, I hypothesized that the quantity of DON and DOC in soil core leachate would decrease with increasing soil depth due to greater opportunities for organic matter sorption with longer exposure to mineral soil. I also hypothesized the hydrophilic content of DON and DOC would be greater in soil solutions leaching from deep versus surface soils due to the preferential removal of hydrophobic compounds from percolating solutions. As a result, I

predicted that the %C consumed in my soil solutions by a common microbial inoculum would be greater in leachate from deep soils compared to surface soils. Similarly, I expected the %C consumed in soil leachate to be greater in soil core leachate from ecosystems with higher sorption tendencies.

Methods

Study area

This study was conducted in the Manistee National Forest in the northwestern Lower Peninsula of Michigan, USA (44°48´N, 85°48´W). Precipitation is evenly distributed throughout the year with an annual average of roughly 81 cm; mean annual temperature is 7.2° C (Albert, 1995). Elevation ranges from 213-369 m above sea level. The retreat of the last major glacial advance ca. 12,000 years BP shaped the current landscape, resulting in a mosaic of sandy outwash plains, ice-contact hills, and moraines.

Six forest stands were selected from a pool of stands previously classified into ecosystem units based on floristic composition, soil properties, and physiography (Host et al. 1988). The forests selected spanned a range of ecosystem classifications from low N fertility, white oak (*Quercus alba*)-black oak (*Q. velutina*)-dominated outwash plains to N-rich, sugar maple (*Acer saccharum*)-dominated moraines (Host et al. 1988, Zak et al. 1989). Forest 1 has the lowest fertility of the gradient with soils classified as Typic Udipsamments; soils in forest 2 are slightly more developed and are classified as Entic Haplorthods. Forests 3, 4, and 5 are all Typic Haplorthods with forests 4 and 5 having clay lamellae. These five ecosystems represent a spodic developmental sequence. Forest 6 is an Alfisol with substrata of sandy clay loam and is classified

as either a Typic Eutroboralf or Typic Hapludalf (Cleland et al 1993). All sites were within 32 kilometers of each other and experienced the same weather.

Soil core collection

I collected soil cores from 0-10, 0-25, and 0-50 cm depth increments at 3 randomly selected positions along a 10 m transect in each forest for a total of 9 soil cores per forest. After first removing the O horizon, I pounded sharpened polyvinyl chloride (PVC) pipes approximately 11 cm in diameter into the soil and carefully removed them to maintain the integrity of the soil column. Cheesecloth was affixed to the bottom of the core and covered in plastic for transport from the field to the lab. Cores were refrigerated for 2-4 weeks after collection to equilibrate after the disturbance of removing the cores from the sites.

Soil core leaching

I determined the hydraulic conductivity on intact cores by the constant head method (Klute 1986) prior to soil core leaching, in part to remove post-disturbance effects that may have resulted from extracting cores from the field. Briefly, the method involved saturating the cores with reverse osmosis (RO) water for 48 h before establishing a constant influx/efflux rate of water flow through the core. Glass wool was placed on top of the cores to distribute incoming water evenly across the core surface. Effluent was collected for 60 sec, weighed, and used to calculate the hydraulic conductivity of a particular core by:

$$K_{sat} = Q/A*L/(H_1-H_2)$$

where K_{sat} is the hydraulic conductivity, Q is the quantity of water that flowed through the core ($cm^3\ sec^{-1}$), A is the cross-sectional area of the core (cm^2), L is the length of the soil core, and H_1-H_2 is the vertical distance from the upper and lower water levels of the core.

Next, I flushed the soil cores with 1 pore volume of a manufactured O horizon solution to displace the RO water in soil pores. To generate the O horizon solution, I used leaf litter that was collected in litter traps from each forest during fall senescence that was part of another experiment. I extracted equal parts of ground leaf litter from all ecosystems in E-pure deionized (DI) water for 24 h to produce a concentrated, O horizon stock solution, which was filtered through glass wool to remove large particulates. I made working solutions daily during the experiment such that final solution concentrations averaged 138.2 mg L^{-1} (SE 2.7) of DOC and 1.0 mg L^{-1} (SE 0.1) of DON. After the cores had drained, I slowly poured the same O horizon solution used above in 100 mL increments until I had collected at least 200 mL of soil solution in a flask at the bottom each core. Solutions were immediately filtered through 0.2µm Whatman Nuclepore Track-Etch membrane filters and frozen.

Soil solution fractionation

I fractionated soil solutions into humic acids (HA), fulvic acids (FA), hydrophilic acids (Hy), and hydrophobic organic neutral matter (HON) in batches using DAX-8 exchange resins (Supelco, Bellefonte, PA) following a modified procedure of Van Zomeren and Comans (2007). Prior to beginning fractionation, the resins were thoroughly cleaned by first extracting them 5 times in 0.1 M hydrochloric acid (HCl) followed by similar extractions in 0.1 M sodium hydroxide (NaOH); each extraction took 24 h. Next, I extracted the resins for 24 h in acetonitrile using a Soxhlet extractor followed by another 24 h extraction in methanol. The resins were stored refrigerated in methanol until use. Twenty-four hours prior to use, I rinsed the resins 5-7 times in DI water, with each rinse a 6:1 ratio of water to resin volume. The water was vacuum-extracted off the resins after each rinse using a Buchner funnel fitted with a #41 Whatman filter (Whatman International Ltd, England). These rinses were followed by 5-7 rinses in 0.1M HCl in

a 3:1 acid/resin ratio and vacuum extracted as for the water rinses. A subsample of solution from the last HCl rinse was analyzed by oxidative combustion-chemiluminescence and oxidative combustion-infrared analysis for TOC and total N (TN), respectively (TOC/TN analyzer; Shimadzu Corp., Kyoto, Japan) to make sure the resins were thoroughly cleaned and bled little C and N. TOC bleeding off cleaned resins averaged 1.1 mg L^{-1} (range: 0.4 to 1.5); total N bleeding averaged 0.4 mg L^{-1} (range: 0.2 to 0.6).

To begin the fractionation procedure, soil solutions from each core were thawed and analyzed for their initial TN and TOC concentrations on the TOC/TN analyzer. I also determined their initial NH_4^+ concentration after Sinsabaugh et al. (2000) by reacting 50 or 100 µL of soil core leachate with 40 µL of an ammonia salicylate reagent (Hach, Loveland, CO) followed 3 minutes later by 40 µL of an ammonia cyanurate reagent (Hach, Loveland, CO). The amount of leachate reacted with the reagents depended on the expected NH_4^+ concentration of a sample, with lower concentrations using the higher amount. Samples were allowed to develop color for 20 minutes before being read on an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT) at 595 nm. I determined the initial NO_3^- concentration after Doane and Horwath (2003) by reacting 25, 50, or 100 µL of core leachate (depending on expected NO_3^- concentration) with 160, 140, or 100 µL, respectively, of a vanadium (III) chloride reagent for 5 to 16 h (to allow color development to occur). Samples were read on a microplate absorbance reader at 540 nm. DON was calculated by subtracting the NH_4^+ and NO_3^- concentrations from the TN concentration of each sample after each fractionation step.

The solutions were next fractionated by first acidifying 40 mL of the solution to a pH of <1 with 6 M HCl. The samples were allowed to sit overnight to precipitate dissolved humic acids then centrifuged for 10 min at 3000 g. Solutions were decanted into new tubes, and a subsample was removed for TOC/TN analysis (the difference between the initial TOC/TN concentrations and this one is the HA content of the sample). Approximately 10 g of DAX-8 resins were added to each sample before continuous tumbling on a rotary shaker for 1 h. Solutions were filtered through monopolyester mesh (#86, Ernst Dorn Co, Santa Clara, CA) fitted to test tube lids to prevent loss of resins and saved for TOC/TN analysis (to provide a measure of Hy content). To desorb FA on the resins, 20 mL of 0.1 M potassium hydroxide (KOH) was poured through the mesh, washing resins back into solution, and equilibrated for 1 h (pH >11) before refiltration through the mesh. This extraction procedure was repeated 3 additional times, and the eluents from each extraction were saved and combined for TOC/TN analysis (to determine the FA composition of the sample).

The HON composition was determined as the difference between the TOC/TN content of the soil solution prior to the addition of DAX-8 resins and the total TOC/TN concentrations of FA and Hy. The concentration of FA in all samples was low (<0.01 mg L^{-1}), so I combined this fraction with the HON fraction for one “hydrophobic” fraction. Humic acids were also negligible and not included in subsequent analyses. As a result, my data analyses contained only a “hydrophobic” fraction, composed of FA + HON, and a “hydrophilic” fraction containing Hy. As a blank, a vial of 0.1 M HCl was subjected to the same steps as soil leachate and used to adjust sample values for any C or N bleeding from the resins. The moisture content of the resins was determined by drying a subsample of cleaned resins for 24 h at 105 ˚C; the moisture content was 71%. The summed DOC concentration of both fractions for each sample was within an average

of 5% of the pre-fractionated DOC concentration. Summed DON concentrations of both fractions were within an average of 27% of pre-fractionated DON concentrations. The greater disagreement between summed and initial DON concentrations compared to DOC likely results from the additional compounding of measurement error while determining the NH_4^+ and NO_3^- concentrations of the samples during each fractionation step.

Soil characteristics

Soil was removed from the cores 24 h after the leaching experiment in 0-10, 10-25, and 25-50 cm depth increments as appropriate (i.e., only 50 cm cores had all three segments). Soils were passed through a 4 mm mesh sieve to remove rocks and homogenize soils. Particle size distributions of each soil segment were determined by the pipette method (McKeague 1978, Klute et al. 1986) after first removing organic matter with 30% hydrogen peroxide. Bulk density was determined for each segment by dividing the oven dry weight of the total soil segment (calculated from a subsample of dried soil) by the volume of the soil segment. Total porosity was calculated as the ratio of bulk density to particle density (2.65 Mg m^{-3}) subtracted from unity. Soil pH was measured in DI water at a soil:water ratio of 1:2 (w/v). The C and N contents were determined by flash combustion/chromatographic separation using a Costech Elemental Combustion System 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA) on soil that had been oven dried at 105˚C for 24 h and ground to a fine powder.

I determined the content of soluble iron and aluminum hydrous oxides (Fe_d, Al_d) by the citrate-dithionite method (McKeague 1978, Klute et al. 1986). Briefly, air-dried soils were sieved through a 100-mesh sieve. I extracted approximately 0.5 g of soil in 30 mL of sodium citrate solution, gradually added 0.5 g of sodium dithionite, and shook the samples for 12-18 h. After

shaking, I added 3 drops of 0.2% Superfloc solution to each tube, agitated the tubes, and centrifuged them for 10 min at 1500 rpm. Roughly 10 mL of the soil extractant was decanted and filtered through a 0.45 µm Millex HA syringe filter unit (Millipore, Carrigtwohill, Ireland). Solutions were analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES) using an Optima 2100DV optical emission spectrometer (PerkinElmer, Inc., Shelton, CT) after a 1:15 dilution with DI water.

I determined the amount of noncrystalline (amorphous) aluminosilicates and hydrous oxides of iron and aluminum (Fe_o, Al_o) using the acid ammonium oxalate dark reaction (McKeague 1978, Klute et al. 1986). I added 10 mL of acid oxalate solution to approximately 0.25 g of soil previously sieved through a 100-mesh sieve. The samples were capped tightly and placed on a rotisserie shaker in a dark room for at least 4 h. After shaking, 2 drops of 0.2% Superfloc solution were added to each sample, the sample shaken vigorously, and then centrifuged for 5 min at 1500 rpm. Soil solutions were carefully decanted and filtered through a 0.45 µm Millex HA syringe filter unit. Samples were analyzed on an ICP-OES (PerkinElmer, Inc., Shelton, CT) after a 1:5 dilution with DI water.

The microbial biomass of each soil segment was determined by chloroform fumigation for 24 h and extraction immediately after each soil core was harvested. Fumigated and unfumigated soils were extracted for 1 h in 0.5 M potassium sulfate (K_2SO_4) before filtration through pre-leached #1 Whatman filters. Total dissolved nitrogen and TOC were determined by TOC/TN analysis. The difference between fumigated and non-fumigated extracts in C content is the chloroform-labile C pool (EC), which is proportional to microbial biomass C following:

$$\text{Microbial biomass C} = EC/kEC$$

where kEC is estimated as 0.45 (Beck et al. 1997). Microbial biomass N is calculated similarly to C except kEN is 0.54 (Brookes et al. 1985).

The soil characteristics listed in Table 4.1 represent the amounts found within a core segment (i.e., 0-10, 10-25, 25-50 cm) in order to illustrate how characteristics changed with depth. Because all cores had 0-10 cm segments, I averaged all of these values when calculating the values in this core increment for a total of 9 soil core increments per ecosystem. For the 10-25 cm core increment, I averaged the increment values in 0-25 and 0-50 cm cores for a total of 6 core increments per ecosystem. The 25-50 cm core increment was found only in 0-50 cm cores for a total of 3 core increments per ecosystem.

Biodegradability assay

I analyzed the biodegradability of soil core leachate by inoculating leachate samples with a standard inoculum and incubating the samples for up to 14 days. To do this, soil leachate was first filtered through a 0.22 µm Millex GS syringe filter (Millipore, Carrigtwohill, Ireland) to remove microbial cells already present in the solution. Additionally, I diluted each solution with varying amounts of DI water so that all samples had an initial mean C concentration of approximately 13.4 mg L^{-1} (range: 11.7 to 16.6 mg L^{-1}) in order to more accurately compare the biodegradability of solutions from different forests and soil depths. The final volume of soil solution and water was approximately 10 mL. I added 6 mL of a nutrient solution containing 0.1% ammonium nitrate (NH_4NO_3) and 0.1% potassium phosphate (K_2HPO_4) to ensure that only C quality limited the microbes and not nutrient limitation. One 6 mm disc punched from a Whatman GF/A glass microfiber filter was added to each vial as a physical substrate for microbial growth. I pipetted 0.2 mL of a standard inoculum (BI-CHEM BOD Seed, Novozymes Biologicals, Inc., Salem, VA) into each vial, agitated the samples, and placed them in an

incubator at 25˚C; time 0 samples were not inoculated. Every other day after inoculation, samples were inverted 5 times, uncapped, and vented for 1.5 min to prevent the build up of excessive amounts of carbon dioxide. Vials were harvested at 0, 2, 7, and 14 days after inoculation. Harvesting involved mixing the samples thoroughly before filtering the solutions through 0.22 µm Millex GS syringe filters and immediately analyzing them for total DOC on a TOC analyzer. I used inoculated DI water plus the nutrient solution as a blank to account for any DOC contributed by the microbes during the assay. The %C consumed was calculated as:

$$\%C \text{ consumed} = 1 - (\text{Adjusted } C_{Tfinal} / \text{Adjusted } C_{Tinitial}) * 100$$

where adjusted C_{Tfinal} is the difference between the DOC of the soil leachate at a particular harvest time and the DOC of the water blank, and adjusted $C_{Tinitial}$ is the DOC concentration at time = 0.

Statistical analyses

I evaluated the effects of forest ecosystem and soil depth on DOC and DON chemistry with mixed-effects analysis of variance (ANOVA) models. DOC or DON chemistry was the dependent variable, forest ecosystem and soil depth were the fixed effects, and soil core replicate was the random effect. For each model, I tested an interaction term between forest ecosystem and soil depth, but it was never significant so I excluded it from the final models. I used Tukey contrasts for post-hoc comparisons to evaluate the significant terms in the models. Data were log transformed when necessary to meet assumptions of normality.

I evaluated the relationship between the hydrophobic and hydrophilic fractions of DOC or DON with soil depth after accounting for total DOC or DON using mixed-effects analysis of covariance (ANCOVA) models. I used an ANCOVA approach to account for the effect of total

DOC or DON on their individual fractions instead of analyzing each fraction as a proportion of total DOC or DON because ratio variables can lead to spurious correlations during statistical analyses (Atchley et al. 1976). The DOC or DON fraction was the response variable, total DOC or DON the covariate, and soil depth the fixed effect. Forest and soil core replicate were the random effects, with replicate nested within forest.

In the biodegradability assay, I used ANCOVA models to evaluate forest and depth differences on the first (day 2) and last (day 14) days of the incubation period to determine how C pools differed through time. The models consisted of the C concentration at either day 2 or day 14 as the dependent variable, the initial C content as the covariate, and forest and soil depth as the fixed effects; replicate was the random effect. In all of the statistical models, I used Type III sums of squares so that all terms were considered simultaneously during the analyses. All analyses were conducted using R statistical software (R Development Core Team 2011) and results were accepted as significant at $\langle = 0.5$.

Because multiple soil characteristics simultaneously influence soil solution chemistry, I conducted multivariate analyses using non-metric multidimensional scaling (NMDS) to explore how multiple measures of a soil's sorption ability may have acted in concert to produce the DON and DOC chemistry in soil core leachate found across the forests. A multivariate approach has the potential to reveal a broader understanding of ecosystem controls over soil solution chemistry compared to a bivariate approach, which seeks to analyze relationships between individual parameters. I first constructed ordinations of individual soil cores from all six forests based on the soil characteristics of the cores (i.e., Matrix 1; Table 4.2), then overlaid the DON and DOC concentrations in core leachate on the ordination (i.e., Matrix 2) to determine if leachate chemistry was correlated with the ordination axes and the arrangement of soil cores. I conducted

separate analyses for 0-10, 0-25, and 0-50 cm cores to prevent the geochemical influences of the soil variables from being confounded by the differences that arose among core increments from the increasing core volume with depth. For 25 and 50 cm cores, I calculated weighted averages for each variable except hydraulic conductivity based on measurements taken on the 0-10, 10-25, and 25-50 cm core segments (the latter segment for 50 cm core only) to generate total core values. Each forest had 3 replicate cores per depth that were included in the analyses. Prior to analysis, the variables in Matrix 1 were transformed with Wisconsin double standardization. A distance matrix was calculated using a Bray-Curtis distance measure, and NMDS was run from random starts. These analyses were conducted using the metaMDS function in the vegan library of R statistical software (R Development Core Team 2011).

Results

Soil characteristics

The hydraulic conductivity of forest soils (K_{sat}) was generally highest in soil from forest 1 and lowest in soil from forest 6 (Table 4.1). In 0-50 cm cores, which represent the total hydraulic conductivity over all the depth increments I examined, forests 4 and 5 had some of the fastest hydraulic conductivities despite having finer textured soils compared to the preceding forests along the gradient. Indeed, the coarse sand content of all soil cores decreased from roughly 60% in forest 1 to roughly 45% in forests 4 and 5. Conversely, the amount of fine sand, clay, and silt generally increased in cores from forest 1 to forest 6. Notably, forest 6 had over 2 times more silt in all soil core increments compared to the remaining forests. The bulk density of soils increased from surface to deeper soils, while the porosity of soils decreased with depth at all forests.

Soil pH in 0-10 cm cores ranged from 4.5 in forest 3 to 5.5 in forest 6, with surface soils always slightly more acidic compared to deeper soils (Table 4.1). Forest 3 had some of the highest soil C in all cores compared to the remaining forests; however, this was not the case for soil N, which was consistently higher in forests 4, 5, and 6 compared to the other forests. Therefore, forests 4, 5, and 6 generally had lower C:N (by mass) ratios compared to forests 1, 2, and 3. In most forests, the concentration of soluble iron and aluminum hydrous oxides (Fe_d, Al_d) and noncrystalline aluminosilicates and hydrous oxides of iron and aluminum (Fe_o, Al_o) increased from surface to deeper soils, with forest 1 having the highest concentrations of both forms across forests. Microbial biomass C and N generally increased from forest 1 to forest 6 in 0-10 cm cores (17 to 21 $\mu g\ g^{-1}$ and 1.5 to 2.2 $\mu g\ g^{-1}$, respectively).

DOC and DON chemistry across forests

Soil cores from all six forests showed net retention of DOC relative to the input organic matter solution (DOC concentration: 138 mg L^{-1}), while net retention of DON relative to the input organic matter solutions (1.0 mg L^{-1}) was evident at only 4 of the six forests. However, the amount of DOC or DON retained by soils varied significantly across forests after accounting for depth, although these differences were constrained to only a few forests and were not straightforward across the soil texture gradient (mixed-effects ANOVA; DOC, $F_{5,44}$=3.63, p=0.008; DON, $F_{5,44}$=4.90, p=0.001; Figure 4.1a). For example, forest 5 had the highest DOC concentration in soil core leachate of any site (mean, 53.3 mg L^{-1}), while forests on either side of forest 5 along the soil texture gradient (i.e., forests 3 and 6) had some of the lowest average DOC concentrations (Tukey contrasts; forests 5 and 3, z=3.33, p=0.01; forests 6 and 5, z=-3.14,

p=0.02). Additionally, the DOC concentration of solution leaching from forest 5 was not significantly different from that of forests 1 and 2, which were at the opposite end of the soil texture gradient.

DON leaching patterns across forests were similar to those of DOC, with forest 5 having the highest average DON concentration in soil core leachate (mean, 1.2 mg L^{-1}), while forest 6 had the lowest DON concentration (0.6 mg L^{-1}; Tukey contrasts: forests 6 and 5, z=-4.07, p<0.001; Figure 4.1b). Forest 4 had a similar DON concentration as forest 5 and was also significantly greater than forest 6 (z=-3.68, p=0.003). Unlike DOC, forest 2 had a significantly lower average DON concentration compared to forest 5 (z=2.85, p=0.05).

The concentrations of hydrophobic and hydrophilic DOC generally tracked those of bulk DOC, with significant differences among forests occurring independent of the soil texture gradient (mixed-effects ANOVA: hydrophobic DOC, $F_{5,43}$=3.09, p=0.018; hydrophilic DOC, $F_{5,43}$=4.36, p=0.003; Figure 4.1a). The concentration of hydrophobic DOC was highest in leachate from forest 5 and lowest from forest 3 (Tukey contrasts: z=3.21, p=0.02), corresponding to bulk DOC patterns. Additionally, forest 4 had significantly higher hydrophobic DON concentrations compared to forest 3 (z=3.05, p=0.03). For hydrophilic DOC, forests 4 and 5 again had the highest concentrations while forests 3 and 6 had the lowest. (Tukey contrasts: forests 5 and 3, z=3.11, p=0.023; forests 6 and 4, z=-3.28, p=0.013; and forests 6 and 5, z=-3.95, p=0.001). Contrary to DOC fractions, both hydrophobic and hydrophilic DON fractions were indistinguishable across forests (mixed-effects ANOVA: hydrophobic, $F_{5,42}$=2.21, p=0.07; hydrophilic, $F_{5,42}$=1.11, p=0.37; Figure 4.1b). When the concentrations of hydrophobic and

hydrophilic DON were summed, they overestimated bulk DON in soil core leachate at all forests. This is likely due to the analytical limitations involved with working at low DON concentrations and from calculating DON by difference (TN - (NH_4^++$NO3^-$)) during each fractionation step. Therefore, these data should be interpreted with some caution.

Multivariate analyses of soil characteristics and leachate chemistry

NMDS analysis of soil characteristics in 0-10 cm soil cores produced a 3-axis solution with a final stress of 8.79**;** subsequent axes minimally reduced stress. Based on Clarke's rule of thumb, a final stress of 5-10 has "no real risk of drawing false inferences" (McCune and Grace 2002). Two convergent solutions were found after thirteen runs with these data. While there were 4 noticeable groupings of forests in the ordination produced by the first two axes, there was no clear grouping structure of forests on the ordination of axes 2 and 3. Therefore, only patterns on the first two axes will be discussed. The replicates from forests 1, 2, and 6 formed three distinct groups, while replicates from forests 3, 4, and 5 overlapped, suggesting they should be grouped together (Figure 4.2). All soil characteristics examined were significantly correlated with the ordination produced by the first two axes except microbial C, soil C, bulk density, and % porosity (Table 4.3). The soil characteristics most strongly related to axis 1 were the Fe_o+Al_o content, the Fe_d+Al_d content, pH, and soil N content of soils, suggesting this axis predominantly described differences among forests in their geochemistry. The Fe_o+Al_o and Fe_d+Al_d contents of soils had the highest negative scores with axis 1 of any variable examined (=-0.88 and -0.89, respectively), which corresponded to forests having relatively high soil Fe and Al contents (Table 4.1). The pH of 0-10 cm soils also had a high negative score with axis 1 (-0.77), although the reason for this is less certain as there was no clear pattern of pH in these soils across forests.

Soil N had the highest positive score (0.72) with axis 1, which was reflected in fairly high soil N contents in forests positively related to axis 1 (i.e., 3, 4, and 5) and low soil N contents in forests negatively associated with axis 1 (i.e., forests 1 and 2). Forests were separated along axis 2 based primarily on soil texture, due to the high silt (-0.89), clay (-0.74), and fine sand (0.62) scores with this axis. Forest 6 had the highest silt and clay contents of any forest while forests 3, 4, and 5 tended to have high fine sand contents. When I overlaid the DON and DOC chemistry leaching from 10 cm cores on the ordination of soil characteristics, neither was significantly correlated with either axis (DON: r^2=0.06, p=0.64; DOC: r^2=0.03, p=0.79).

For 0-25 cm soil cores, NMDS analysis produced a similar grouping structure as was found for 0-10 cm cores, except forests 1 and 2 formed one versus two groups. The final solution had 3 axes that were reached after five runs with these data with a final stress of 6.82. Similar to the previous ordination, there was no clear grouping structure on the ordination of axes 2 and 3, so only patterns on the first two axes are discussed. In 0-25 cm cores, pH was no longer significantly related to the ordination (Table 4.3). Neither were the Fe_o+Al_o or Fe_d+Al_d contents of soils as strongly correlated with the first axis (-0.50 and -0.48, respectively). Instead, the coarse sand content and hydraulic conductivity of soil cores had the highest negative scores with axis 1 (-0.77 and -0.78, respectively) and were most important for explaining the grouping of forests 1 and 2. These forests generally had the highest coarse sand contents and highest hydraulic conductivity rates of any forest examined (Table 4.1). Soil N content was still strongly, and positively, related to axis 1 (0.84). The grouping structure of forests on axis 2 was most influenced by measures of soil texture in a manner similar to 0-10 cm cores. Again, silt and clay contents had high negative scores with axis 2 (-0.79, -0.72, respectively) while fine sand content was positively related to axis 2 (0.70). The Fe_o+Al_o and Fe_d+Al_d contents also had relatively

high scores with the second axis for 25 cm cores (-0.67 and -0.73, respectively). When I overlaid DOC and DON leachate chemistry on the ordination, only DON was marginally significant with the ordination (DON: r^2=0.29, p=0.07; DOC: r^2=0.24, p=0.13). DON was positively related to axis 2 (0.52), which indicated the forests 3, 4, and 5 had some of the highest DON losses from 0-25 cm cores.

For 0-50 cm cores, there was no defined grouping structure of the forests except for forest 1 on the first two axes. Although the final solution had 3 axes, there was no defined grouping structure of the forests when the second and third axes were considered. The final solution had a stress of 7.45, and a convergent solution was found after 1 run. The forest 1 group was most explained by the coarse sand content and hydraulic conductivity rate of these soils, which were both significantly, and positively related to axis 1 (scores 0.71 and 0.61, respectively; Table 4.3). Neither DON nor DOC were significantly related to the ordination (DON: r^2=0.17, p=0.27; DOC: r^2=0.04, p=0.72).

DOC and DON chemistry across soil depths

DOC concentrations in soil core leachate decreased significantly from surface to deep soils after accounting for ecosystem differences (mixed-effects ANOVA; DOC depth: $F_{2,44}$=26.68, p<0.001; Figure 4.3a). The largest decrease occurred in the upper 10 cm of soil where 60% of DOC was removed from solution. DOC concentrations were further reduced by 18% as solutions percolated between 10 and 25 cm depths, and by 28% as solutions percolated between 25 and 50 cm depths (Tukey contrasts: 10-25, z=-3.25, p=0.003; 25-50, z=-4.04, p<0.001). Interestingly, the average DON concentration actually increased between the input organic matter solution and 10 cm soil depth (from 1.0 to 1.2 mg L^{-1}) before steadily decreasing

by 26% between 10 and 25 cm cores (DON depth: $F_{2,44}$=27.44, p<0.001; z=-3.05, p=0.007) and 35% between 25 and 50 cm cores (z=-4.32, p<0.001). Overall, soil cores retained less DON compared to DOC, with DOC concentrations decreasing by 76% between the input organic matter solution and deep soils versus only a 42% decrease in DON concentrations.

The hydrophobic and hydrophilic fractions of DOC behaved similarly to bulk DOC trends, demonstrating a decrease in concentration as solutions percolated from surface to deep soils (Figure 4.3). As with bulk DOC, the largest decrease in both fractions occurred in the upper 10 cm of soil, where there was a 59% decrease in hydrophobic compounds and 50% decrease in hydrophilic compounds compared to the input organic matter solution. Hydrophobic DOC concentrations continued to decrease significantly with soil depth (mixed-effects ANOVA: $F_{2,43}$=34.11, p<0.0001), and were further reduced by 25% between 10 and 25 cm (Tukey contrasts: z=-3.43, p=0.002) and 35% between 25 and 50 cm (z=-4.85, p<0.0001). Unlike hydrophobic compounds, hydrophilic DOC demonstrated a less consistent decrease in concentration as solutions percolated into deep soils ($F_{2,42}$=14.48, p<0.0001). Indeed, hydrophilic DOC concentrations in core leachate from 10 and 25 cm cores were indistinguishable (z=0.74, p=0.74). Instead, hydrophilic DOC concentrations only decreased between 25 and 50 cm depths (24%; z=-4.11, p<0.001).

While DON concentrations of both fractions also decreased significantly as solutions percolated into deeper soils (mixed-effects ANOVA: hydrophobic, $F_{2,42}$=8.94, p=0.0006; hydrophilic, $F_{2,42}$=5.07, p=0.01), there was one noticeable exception: hydrophilic DON had a 70-fold increase in concentration between the input organic matter solution and 10 cm depth, increasing from 0.01 to 0.7 mg L^{-1}. Below 10 cm, hydrophilic DON decreased by 23% between

10 and 25 cm and 33% between 25 and 50 cm, but only the latter decrease was statistically significant (10-25: z=-.08, p=0.70; 25-50, z=-2.31, p=0.05). Average hydrophobic DON concentrations consistently decreased as solutions percolated into deeper soils, with 31% of hydrophobic DON removed between 10 and 25 cm (Tukey contrasts: z=-2.54, p=0.03) and 31% between 25 and 50 cm, although this latter decrease was not statistically significant (z=-1.73, p=0.20).

Both hydrophobic and hydrophilic fractions of DOC were positively related to the total DOC concentration of soil leachate (mixed-effects ANCOVA: hydrophobic, $F_{1,32}$=256.17, p<0.0001; hydrophilic, $F_{1,32}$=148.66, p<0.0001), but the nature of the relationship depended significantly on depth (hydrophobic depth, $F_{2,32}$=4.24, p=0.02; hydrophilic depth, $F_{2,32}$=18.11, p<0.0001). For example, the proportion of hydrophobic DOC relative to total DOC decreased significantly with increasing soil depth (Figure 4.4a). Conversely, the proportion of hydrophilic DOC relative to total DOC increased with increasing soil depth (Figure 4.4b). Hydrophobic and hydrophilic fractions of DON were positively related to total DON concentration regardless of depth (mixed-effects ANCOVA: hydrophobic DON, $F_{1,31}$=14.66, p<0.001, depth, $F_{2,31}$=0.37, p=0.69; hydrophilic DON, $F_{1,31}$=4.26, p=0.05, depth, $F_{2,31}$=0.13, p=0.88).

Biodegradability assay

In the biodegradability assay, the total % of DOC consumed in soil core leachate from all soil depths across forests averaged near 60% after 14 days of incubation (Figure 4.5). However, the shapes of the C consumption curves were different depending on soil depth. After 2 days of incubation, the average amount of C consumed from 10 cm soil core leachate was less than that consumed from deep soils (i.e., 27% for 10 cm cores compared to 37% and 40% for 25 and 50

cm cores, respectively). However, by 7 days of incubation, the average %C consumed in deeper soils began to asymptote near 60%, whereas the average %C consumption in surface soil leachate (i.e., 10 cm) was still increasing, and continued to increase over the remainder of the incubation period. These differences in C consumption from 10- versus 25- and 50-cm core leachates were similarly expressed in the first-order rate constants of changes in DOC concentrations over time: 10 cm soil core leachate had a rate constant of -0.05 (r^2= 0.9), while 25- and 50-cm core leachates had rate constants of -0.6 (r^2 of 0.8 and 0.8, respectively). When I analyzed the absolute amount of C remaining in leachate after 2 days of incubation, there were significant differences across forests (ANCOVA: $F_{5,43}$=4.0, p=0.005) and soil depths ($F_{2,43}$=14.65, p<0.0001), after accounting for initial C concentration. Across forests, significantly more C was consumed in leachate from forests 4, 5, and 6 compared to forest 3 (Tukey contrasts: forests 4 and 3, z=-2.95, p=0.04; 5 and 3, z=-3.15, p=0.02; 6 and 3, z=-3.83, p=0.002). Across soil depths, significantly more C was consumed in leachate from 25 and 50 cm core leachate compared to 10 cm core leachate (25 and 10, z=-4.03, p<0.001; 50 and 10; z=-5.16, p<0.001). After 14 days of incubation, the differences across forests disappeared ($F_{5,43}$=0.09, p=0.99), but differences across soil depths persisted ($F_{2,43}$=6.75, p=0.003). Leachate from deep soil cores had significantly more C consumed compared to 10 cm soil core leachate (Tukey contrasts: 25 and 10, z=-2.59, p=0.03; 50 and 10, z=-3.56, p=0.001).

Discussion

In this study, I explored how the quantity and quality of DOC and DON changed in percolating soil waters across soils collected from six northern deciduous forests. Because of the

different soil characteristics across these forests, I expected changes in the quantity and quality of DOC and DON to be equally as different despite the fact that all soils were supplied with the same input organic matter solution. Surprisingly, the quantity and quality of DOC and DON in soil core leachates were remarkably similar across forests despite their differences in soil characteristics (Figure 4.1). Indeed, the highest DOC and DON concentrations in soil core leachates were from forests with two of the finest textured soils (i.e., forests 4 and 5), contradicting my hypothesis that predicted a continual increase in DOC and DON retention as soils became finer textured. Moreover, there was no evidence that hydrophobic compounds were removed to a greater extent from leachates in finer textured soils in favor of hydrophilic compounds, as I had predicted.

These results suggest that soil DOM retention across these six forests was not a straightforward consequence of geochemical changes as soils became finer textured (Appendix). This was reflected in the multivariate analyses, in which neither DON nor DOC chemistry was related to the ordination axes at any soil depth despite distinct forest groupings based on differences in soil parameters (Figure 4.2). Instead, unmeasured soil structural changes that influence hydrologic controls, such as pore connectivity and soil aggregation, may have affected the ability of these soils to retain DOM and alter its quality (Kalbitz et al. 2000, Qualls 2000, Castellano and Kaye 2009). Forests 4 and 5 had some of the highest rates of hydraulic conductivity in 50 cm cores compared to other forests (Table 4.1). The faster movement of soil solutions through these cores would decrease sorption opportunities between soil waters and the soil matrix, resulting in higher concentrations of DOC and DON in core leachate, as well as the greater proportion of hydrophobic compounds, that I found in these forests (Seely et al. 1998, Kalbitz et al. 2000, Qualls 2000).

Equilibrium conditions are necessary for maximum sorption of DOM to occur (Qualls 2000), so it is possible forests 4 and 5 would retain more DOM, as well as more hydrophobic compounds, under hydraulic conditions that increased the exposure time between soil solutions and the soil matrix (i.e., not under saturated flow as in this experiment), especially since these forests generally had finer soil textures than the preceding forests along the gradient (Table 4.1). In the field, where soil waters were collected over a longer time frame, these forests had lower DOC and DON leaching losses compared to forest 3, supporting this idea (Chapter 3). The high N content, and equally high C content, of these soils demonstrate their ability to retain organic matter under natural conditions as well as, or better than, the other forests in this experiment. DOC sorption assays conducted on soils from forests 4 and 5 in another experiment demonstrated that at DOC concentrations of 100 mg L^{-1}, similar to the input organic matter concentration in this experiment (132 mg L^{-1}), both soils were still linearly sorbing DOC, suggesting sorption sites were still unsaturated (Chapter 3). However, these same assays also demonstrated that 50 cm deep soils from these forests had some of the highest reactive soil pools along the forest gradient, suggesting the sorption of organic matter to these soils is somewhat temporary. The ease with which organic matter can be desorbed back into solution from soils in forests 4 and 5 could also explain their high DOM leaching losses.

The soils from forests 4 and 5 may also have a more aggregated structure that favored preferential flow, resulting in the higher hydraulic conductivity rates of these soils. Conversely, forest 6 had the slowest hydraulic conductivity of any ecosystem in this experiment despite also being a well-developed soil. In this instance, the slow hydraulic conductivity of this soil may have resulted from its relatively high silt content (25 to 27%) compared to that of the remaining ecosystems (≤11%). The slower movement of solutions through soil would facilitate greater

opportunities for DOM sorption to occur, as demonstrated by the relatively low DOC and DON concentrations in soil core leachate found in this forest. Forests 1 and 2 had some of the fastest hydraulic conductivities, likely because of their high coarse sand content (weighted average of 60 and 64% over all depths, respectively), yet DOC and DON concentrations in leachate were similar to other forests with slower hydraulic conductivity rates and finer textures. The relatively high Fe_o+Al_o and Fe_d+Al_d content in soil from these forests may have moderated the DOC and DON concentrations in soil leachate by their strong ability to adsorb organic compounds (Kaiser and Zech 2000, Kothawala et al. 2009).

DON and DOC concentrations in soil core leachate decreased with increasing soil depth in all forests, in agreement with other studies from numerous ecosystems (McDowell and Likens 1988, Qualls and Haines 1991, Lajtha et al. 2005, Möller et. al 2005, Sleutel et al. 2009; Figure 4.3). The total decrease in DOC and DON concentrations between the input organic matter solution and soil core leachate at 50 cm was 76 and 42%, respectively, noticeably less than the roughly 99% reduction in DOC and 97% reduction in DON concentrations leaving the C horizon found by Qualls et al. (2002) in a study of field soils. The lower retention of DOC and DON in this study may partly result from my experimental design, which collected leachate under conditions of saturated flow. Longer soil water residence times where saturated flow is not occurring would favor greater DOM sorption to mineral soil due to the increased exposure of DOM to sorptive surfaces on mineral soil (Qualls 2000).

The reduction in DOC concentrations as waters percolated through soil cores was dominated by the removal of hydrophobic compounds, which is consistent with the idea that they preferentially sorb to mineral soil (Kaiser and Zech 1998, Yano et al. 2004**,** Möller et al. 2005; Figure 4.3). As a result, hydrophilic compounds comprised an increasing proportion of total

DOC in leachate from surface to deep soils (Figure 4.4). Similar trends have been found in other studies investigating soil water chemistry at different soil depths (Qualls and Haines 1991, Yano et al. 2004, Möller et al. 2005), leading Qualls and Haines (1991) to speculate that the net removal of hydrophobic compounds as soil waters percolate to depth was a common phenomenon in forest soils, whereas the patterns of hydrophilic DOC were more difficult to predict. The largest decrease in DOC concentration occurred in the upper 10 cm of soil (60%), despite being the smallest depth increment I investigated and having the highest starting concentration of soil C (Table 4.1). Most of the DOC removed in this increment was again from the hydrophobic fraction, which decreased by 59%. Because hydrophobic compounds preferentially sorb to mineral soils, and are relatively immune to biotic decomposition (Qualls 2004), abiotic sorption to mineral soil seems the most likely explanation for the dramatic decrease in DOC from 0-10 cm (Qualls et al. 2002). Yano et al. (2005) similarly concluded that abiotic rather than biotic mechanisms were primarily responsible for DOM retention on soils due to the low presence of biodegradable hydrophilic compounds (<2%) in their solutions. Some combination of the favorable entropy changes that occur when hydrophobic compounds are removed from solution and ligand exchange between the acidic functional groups of DOM and hydroxyl groups of Fe and Al hydrous oxides (Qualls 2000, Yano et al. 2004) are the most likely mechanisms for the strong removal of DOC in this soil depth increment. The higher molecular weight of hydrophobic compounds compared to hydrophilic compounds may also facilitate their stronger binding to soils by being enriched in strong-binding ligands (Gu et al. 1995).

While DOC had the largest decrease in concentration in the upper 10 cm of soil, DON concentrations increased by an average of 18% in this same depth increment, suggesting soil organic matter provided a reservoir of potentially soluble N that was mobilized when soil cores

were leached. Soil core leachate became more concentrated in DON only in the upper 10 cm of mineral soil, the depth increment with the highest soil N and C concentrations of any soil increment examined (Figure 4.3b, Table 4.1). Moreover, this increase in DON concentration occurred despite previously flushing the soil cores with RO water (to determine their hydraulic conductivity) and the input organic matter solution (to replace water from the hydraulic conductivity determination), indicating a pool of potentially soluble soil organic N large enough to accommodate two previous leaching events and still release DON in the final portion of my experiment. This idea is in agreement with Qualls et al. (2002) who suggested that the pool of potentially soluble organic matter in mineral soils was much larger than the amount dissolved by any particular leaching event. The increase in DON concentration between the organic matter solution and 10 cm soil depth was accompanied by a large increase in hydrophilic compounds, which became 67 times more concentrated in 10 cm leachate compared to the input organic matter solution. Conversely, hydrophobic concentrations decreased by 25%. The decrease in hydrophobic DON and increase in hydrophilic DON may result from the displacement of previously sorbed hydrophilic compounds by hydrophobic compounds from the input solution (Kaiser and Zech 1998, Kalbitz et al. 2000). This is supported by the near absence of hydrophilic compounds in the input organic matter solution, which is the only other potential source of hydrophilic N in this experiment. Hydrophilic compounds are generally more N-rich than hydrophobic compounds (Qualls and Haines 1991, Lajtha et al. 2005), which would account for the large increase in DON found in this fraction between the input solution and 10 cm soil depth. Also, hydrophilic compounds are frequently derived from microbial processes (Guggenberger et al. 1994), which were likely most active in surface soils due to the high microbial C and N content found in this increment (Table 4.1).

Few other studies have documented a similar increase in hydrophilic DON in soil solutions percolating through surface soils. Möller et al. (2005) found an increase in hydrophilic DON concentrations between forest throughfall and 5 cm soil depth at three forests in northern Thailand, but it is unclear if this increase was the result of water passing through the forest floor or through the upper 5 cm of mineral soil. Yano et al. (2004) found a 133% increase in the hydrophilic neutral fraction of DOC between 0 and 10 cm soil depths in conifer forests of the Pacific Northwest. They did not investigate DON fractions in this part to their research, although they did note that the 0-10 soil increment was an especially important source of DON compared to the O horizon. Therefore, in order to evaluate this pattern more explicitly, I conducted additional analyses that examined DON chemistry in leachate from the 10 cm soil cores across forests. To do this, I used mixed-effect ANOVA models with either total DON or hydrophilic DON concentration as the dependent variable, forest as the fixed effect, and leachate replicate as the random effect. For both models, there were no significant differences in either total or hydrophilic DON concentrations across forests (total DON: $F_{5,10}$=0.65, p=0.67; hydrophilic DON: $F_{5,9}$=1.79, p=0.21), suggesting this was a common trend across forests. Indeed, all forests demonstrated a minimum increase in hydrophilic DON concentration of 97% in 10 cm leachate relative to the input organic matter solution.

The proportionate decrease in hydrophobic compounds and proportionate increase in hydrophilic compounds as solutions percolate through soils may indicate that DOM quality was higher in deep versus surface soils. This was partially confirmed in my biodegradability assay where roughly 10% more C was consumed in leachate from deep versus surface soils after 2 days of incubation (Figure 4.5). Additionally, by the end of the incubation period, leachate from 25 and 50 cm soils had significantly more C consumed compared to 10 cm soil leachate.

However, the nature of the C consumption curves in deep soils suggested the presence of two pools of biodegradable compounds (Qualls 2004); one labile pool that was quickly degraded in the first 2 days of the experiment, removing between 30 to 45% of the available C, and a relatively slower degrading pool that removed an additional 20% of the C over the remainder of the experiment. This two-phase pattern may result from the rapid degradation of the relatively larger pool of the labile hydrophilic fraction, which would serve to remove the majority of biodegradable compounds, followed by biodegradation of the remaining recalcitrant hydrophobic fraction with a slower rate of C consumption.

The greater proportion of hydrophobic DOC compounds in 10 cm leachate may explain the lower C consumption in the first 2 days of the experiment (19-32% across ecosystems). However, while the % DOC consumed in deep soils had reached a maximum near 60% by the end of the experiment, the % DOC consumption in 10 cm leachate was still increasing, suggesting surface soils ultimately had a higher potential for total DOC biodegradation despite having a higher proportion of more recalcitrant compounds. It is possible there were important chemical differences between surface and deep soil solutions not captured by the broad hydrophilic/hydrophobic classification that influenced their biodegradability. For example, hydrophobic and hydrophilic compounds can be further fractioned into acidic, neutral, and basic fractions, all of which have different levels of biodegradability (Guggenberger et al. 1994, Qualls 2004) and vary in their contribution to total DOC depending on soil depth (Qualls and Haines 1991).

Conclusions

The results of this study demonstrated the strong capacity of soils from a range of northern forests to influence the quantity and quality of DOC and DON percolating to depth.

Soils not only mitigate DOM losses from terrestrial ecosystems by retaining organic compounds on mineral soil, they influence the chemical composition of percolating solutions through the preferential sorption of hydrophobic compounds and, in the case of DON especially, the release of less strongly sorbing hydrophilic compounds. This increase in DOM quality with increasing soil depth is particularly relevant to our understanding of DON losses from terrestrial ecosystems, which have previously been described as an "N leak" from temperate forests (Hedin et al. 1995, Neff et al. 2003, Perakis and Hedin 2002). As a part of the "leak" hypothesis, DON losses were expected to consist of relatively refractory compounds that were resisted biotic decomposition over the time scale of leaching. However, the steady removal of refractory hydrophobic compounds from percolating solutions in favor of labile, hydrophilic compounds, coupled with the potential for over half of the DOM in deep soils to be readily degradable by microbes, calls this assumption into question. Instead, DON "leaking" from terrestrial ecosystems may be more a happenstance of soil sorption phenomena and hydrologic controls and less the inability of biota to process relativley recalcitrant compounds before they percolate to deep soils. Indeed, multiple studies have cited soil sorption dynamics as the primary mechanism regulating DOM chemistry over biotic controls (Qualls et al. 2002, Yano et al. 2005). This is in contrast to our understanding of inorganic N cycling, which is tightly controlled by microbial mineralization and immobilization (Schimel and Bennett 2004).

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Table 4.1 Soil characteristics for individual core segments (depth ranges in cm) except for hydraulic conductivity, which was measured on intact cores. Data in italics are 1 SE.

	K_{sat} (cm s^{-1})		% Coarse sand (2.0-0.25mm)		% Fine sand (0.25-0.05mm)		% Silt (0.05-0.002mm)		% Clay (<0.002)		Bulk density (g cm^{-3})		Porosity (%)		pH	
Forest 1																
0-10	1.5	*0.1*	65.7	*1.7*	25.0	*1.6*	5.7	*0.3*	3.6	*0.2*	0.8	*0.1*	70.7	*3.6*	5.0	*0.2*
10-25	1.4	*0.1*	59.6	*2.6*	33.5	*2.4*	6.0	*0.5*	1.2	*0.5*	1.4	*0.0*	46.0	*1.8*	5.6	*0.4*
25-50	1.3	*0.2*	59.2	*3.6*	35.3	*3.2*	4.7	*0.7*	0.7	*0.0*	1.5	*0.0*	42.6	*1.5*	5.8	*0.6*
Forest 2																
0-10	0.9	*0.1*	67.0	*1.3*	20.2	*1.1*	10.8	*0.6*	2.0	*0.6*	0.8	*0.1*	71.2	*3.4*	5.0	*0.1*
10-25	1.3	*0.1*	63.8	*1.5*	24.9	*1.2*	10.4	*0.3*	1.0	*0.3*	1.3	*0.1*	51.9	*2.0*	5.2	*0.1*
25-50	0.8	*0.1*	63.3	*2.8*	26.5	*2.1*	10.9	*0.6*	0.1	*0.1*	1.6	*0.1*	38.4	*4.8*	5.4	*0.1*
Forest 3																
0-10	0.4	*0.1*	39.0	*3.0*	50.0	*2.2*	8.9	*0.8*	2.1	*0.3*	0.8	*0.1*	69.4	*2.0*	4.5	*0.2*
10-25	0.7	*0.0*	37.4	*3.5*	53.0	*2.4*	8.8	*1.1*	1.1	*0.4*	1.4	*0.0*	48.3	*1.0*	4.8	*0.1*
25-50	0.5	*0.1*	37.0	*6.0*	56.1	*4.4*	6.4	*1.8*	0.5	*0.2*	1.4	*0.0*	46.4	*0.6*	5.1	*0.2*
Forest 4																
0-10	0.8	*0.3*	44.3	*1.3*	44.8	*1.3*	7.9	*0.5*	3.0	*0.4*	0.7	*0.1*	73.9	*2.6*	5.2	*0.2*
10-25	0.6	*0.2*	41.9	*0.9*	49.6	*1.5*	7.4	*0.9*	1.3	*0.5*	1.2	*0.2*	54.3	*6.8*	5.4	*0.2*
25-50	0.9	*0.2*	41.3	*0.7*	53.4	*2.4*	4.7	*1.6*	0.6	*0.3*	1.5	*0.0*	43.5	*0.9*	5.5	*0.3*
Forest 5																
0-10	1.0	*0.4*	50.6	*1.4*	36.4	*1.4*	10.1	*0.3*	2.9	*0.5*	0.9	*0.1*	67.2	*4.5*	4.8	*0.2*
10-25	0.6	*0.2*	47.0	*1.7*	41.8	*1.4*	9.5	*0.5*	1.7	*0.3*	1.4	*0.0*	47.0	*1.7*	5.1	*0.2*
25-50	0.6	*0.2*	44.5	*1.7*	45.3	*2.6*	10.7	*0.4*	0.5	*0.5*	1.5	*0.1*	42.9	*2.0*	5.4	*0.3*
Forest 6																
0-10	1.3	*0.3*	36.4	*0.7*	31.6	*0.3*	26.9	*0.8*	5.2	*0.4*	0.8	*0.1*	68.6	*3.2*	5.5	*0.2*
10-25	0.6	*0.2*	35.5	*0.7*	34.2	*0.5*	27.1	*1.1*	3.3	*0.6*	1.2	*0.1*	54.1	*2.7*	5.8	*0.2*
25-50	0.3	*0.1*	37.5	*2.2*	37.3	*0.1*	24.8	*2.0*	0.4	*0.2*	1.6	*0.2*	39.7	*7.0*	6.0	*0.2*

Table 4.1 (cont'd)

Soil C ($g\ kg^{-1}$)		Soil N ($g\ kg^{-1}$)		C:N		Fe_d+Al_d ($g\ kg^{-1}$)		Fe_o+Al_o ($g\ kg^{-1}$)		Microbial C ($\mu g\ g^{-1}$)		Microbial N ($\mu g\ g^{-1}$)	
22.8	*2.1*	0.3	*0.1*	79.6	*8.7*	8.9	*0.5*	4.9	*0.3*	16.8	*1.6*	1.5	*0.3*
6.6	*0.6*	0.1	*0.0*	72.1	*7.0*	11.6	*0.7*	7.5	*0.4*	4.5	*0.7*	0.2	*0.0*
2.3	*0.3*	0.2	*0.0*	10.1	*0.5*	10.6	*1.4*	6.3	*1.0*	4.3	*0.3*	0.0	*0.0*
19.6	*3.1*	0.8	*0.2*	33.8	*8.6*	5.0	*0.5*	2.9	*0.4*	16.0	*1.7*	1.7	*0.3*
6.9	*0.5*	0.1	*0.0*	63.7	*2.4*	10.6	*0.8*	9.1	*0.7*	5.8	*0.8*	0.4	*0.1*
2.3	*0.7*	0.2	*0.0*	9.7	*1.6*	10.0	*0.6*	6.8	*0.8*	3.6	*0.2*	0.0	*0.0*
24.7	*4.8*	1.2	*0.2*	20.1	*1.5*	2.9	*0.4*	1.4	*0.3*	13.8	*1.4*	1.2	*0.2*
7.3	*0.7*	0.1	*0.0*	63.4	*3.2*	5.1	*0.7*	3.6	*0.7*	6.4	*0.5*	0.3	*0.1*
6.3	*0.4*	0.4	*0.0*	14.6	*0.3*	7.9	*1.4*	7.8	*2.3*	5.8	*0.2*	0.2	*0.1*
21.8	*3.4*	1.5	*0.2*	14.8	*0.6*	3.6	*0.5*	2.2	*0.5*	16.3	*2.0*	1.9	*0.3*
5.5	*0.8*	0.3	*0.1*	34.4	*10.7*	5.2	*0.5*	4.0	*0.6*	4.9	*0.3*	0.3	*0.1*
4.2	*0.8*	0.3	*0.0*	12.3	*1.1*	6.7	*0.8*	6.1	*0.6*	4.4	*0.2*	0.1	*0.0*
23.6	*5.1*	1.8	*0.3*	12.3	*0.6*	4.7	*0.3*	2.0	*0.4*	18.3	*2.3*	1.7	*0.3*
4.5	*0.3*	0.5	*0.0*	9.0	*0.4*	6.5	*0.8*	3.4	*0.9*	4.5	*0.3*	0.2	*0.1*
4.7	*0.3*	0.4	*0.0*	11.7	*0.8*	9.7	*1.0*	6.8	*0.2*	5.3	*0.7*	0.1	*0.0*
25.2	*4.7*	1.4	*0.3*	28.6	*6.7*	4.9	*0.4*	2.7	*0.3*	20.5	*2.5*	2.2	*0.4*
6.9	*1.0*	0.6	*0.0*	10.9	*0.8*	9.6	*0.4*	6.6	*0.6*	8.1	*0.9*	0.5	*0.1*
3.4	*1.1*	0.3	*0.1*	9.7	*1.7*	8.3	*1.5*	6.0	*2.0*	5.3	*0.5*	0.1	*0.0*

Table 4.2 Matrices, sample units, and variables used in non-metric multidimensional scaling multivariate analyses.

	Sample units (SU)	Number of SU	Variables	Number of variables
Matrix 1	Individual cores	18	Fe_o+Al_o, Fe_d+Al_d, hydraulic conductivity, soil C, soil N, coarse sand content, fine sand content, clay content, silt content, microbial biomass C, microbial biomass N, pH, bulk density, % porosity	14
Matrix 2	Individual cores	18	DON concentration, DOC concentration	2

Table 4.3 Fits of soil characteristic vectors with axes 1 and 2 on NMDS ordinations. Numbers in bold are significant.

Soil characteristics	0-10 cm cores		0-25 cm cores		0-50 cm cores	
	r^2	P	r^2	P	r^2	P
Microbial C	0.31	0.079	**0.58**	**0.002**	**0.68**	**0.001**
Microbial N	**0.58**	**0.001**	**0.71**	**0.001**	**0.69**	**0.001**
pH	**0.61**	**0.001**	0.08	0.521	0.07	0.595
$Fe_o + Al_o$	**0.77**	**0.001**	**0.72**	**0.001**	0.23	0.146
$Fe_d + Al_d$	**0.80**	**0.001**	**0.77**	**0.001**	0.23	0.159
Soil N	**0.55**	**0.004**	**0.71**	**0.001**	**0.73**	**0.001**
Soil C	0.09	0.452	0.26	0.106	**0.68**	**0.001**
Bulk density	0.06	0.593	**0.34**	**0.013**	**0.54**	**0.003**
% porosity	0.06	0.593	**0.35**	**0.012**	**0.54**	**0.003**
K_{sat}	**0.46**	**0.016**	**0.64**	**0.003**	**0.84**	**0.001**
Coarse sand	**0.54**	**0.003**	**0.60**	**0.005**	**0.62**	**0.003**
Fine sand	**0.68**	**0.001**	**0.61**	**0.002**	0.20	0.224
Clay	**0.62**	**0.001**	**0.85**	**0.001**	0.33	0.055
Silt	**0.82**	**0.001**	**0.75**	**0.001**	**0.56**	**0.004**

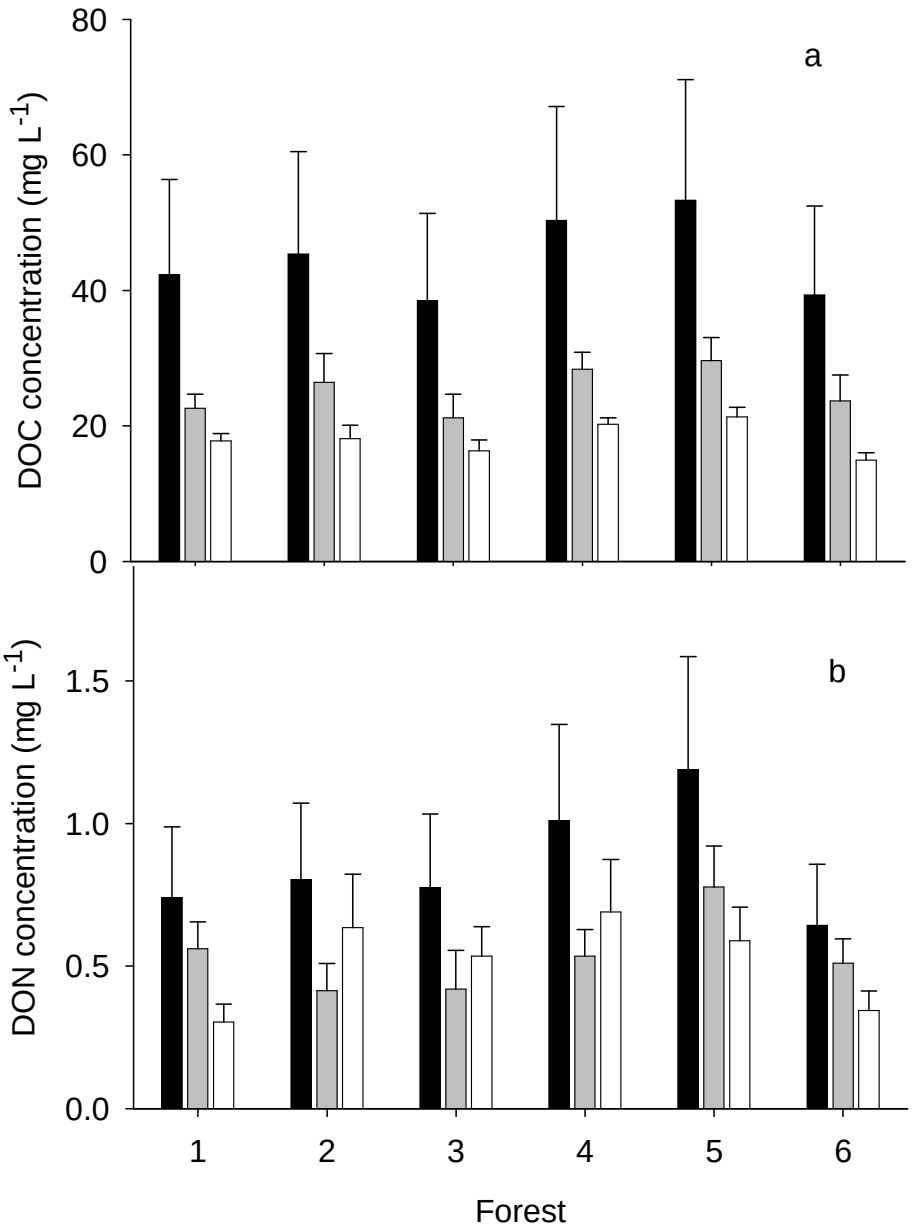

Figure 4.1 Total (*black bars*), hydrophobic (*gray bars*), and hydrophilic (*white bars*) concentrations (± 1 SE) of (a) DOC and (b) DON in soil core leachate collected across the forest gradient. Data are the DOC or DON concentrations across all soil depths.

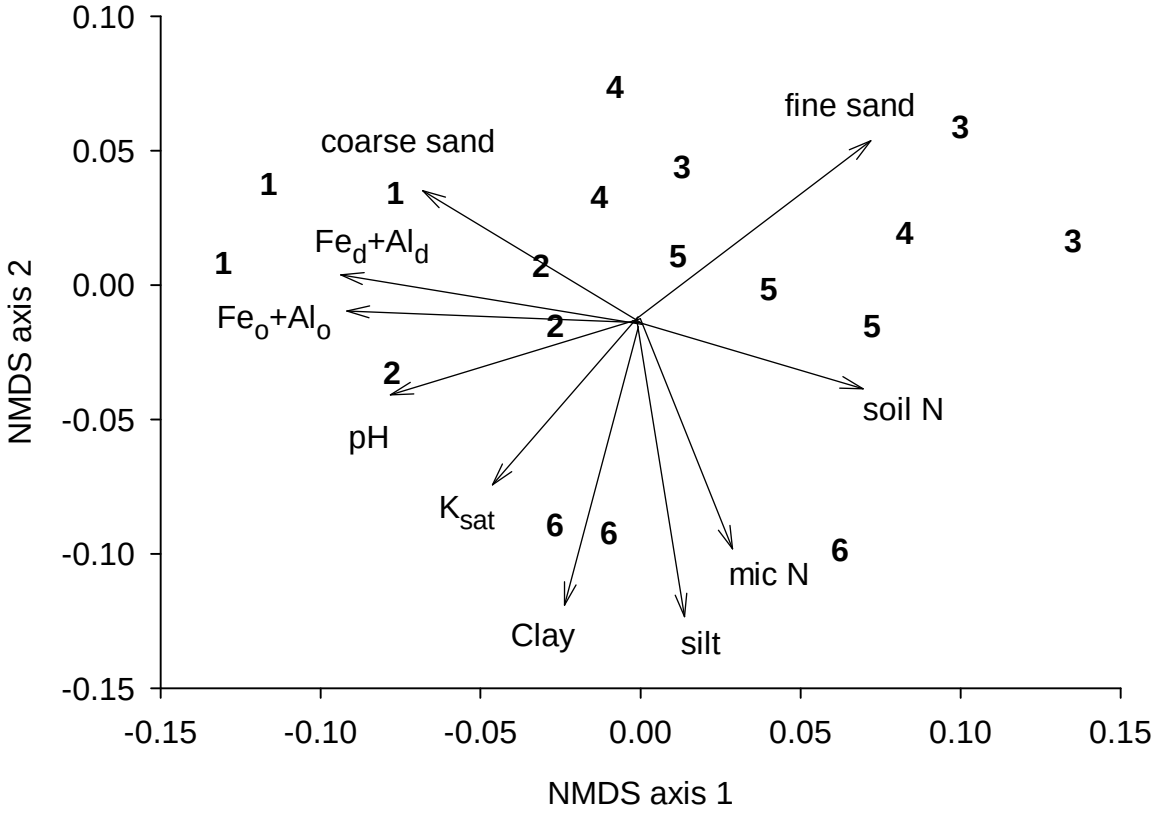

Figure 4.2 Non-metric multidimensional scaling (NMDS) ordination of the first two axes for 10 cm cores. Numbers represent 3 core replicates from each forest. Arrows are significant vectors of soil characteristics overlain on the ordination.

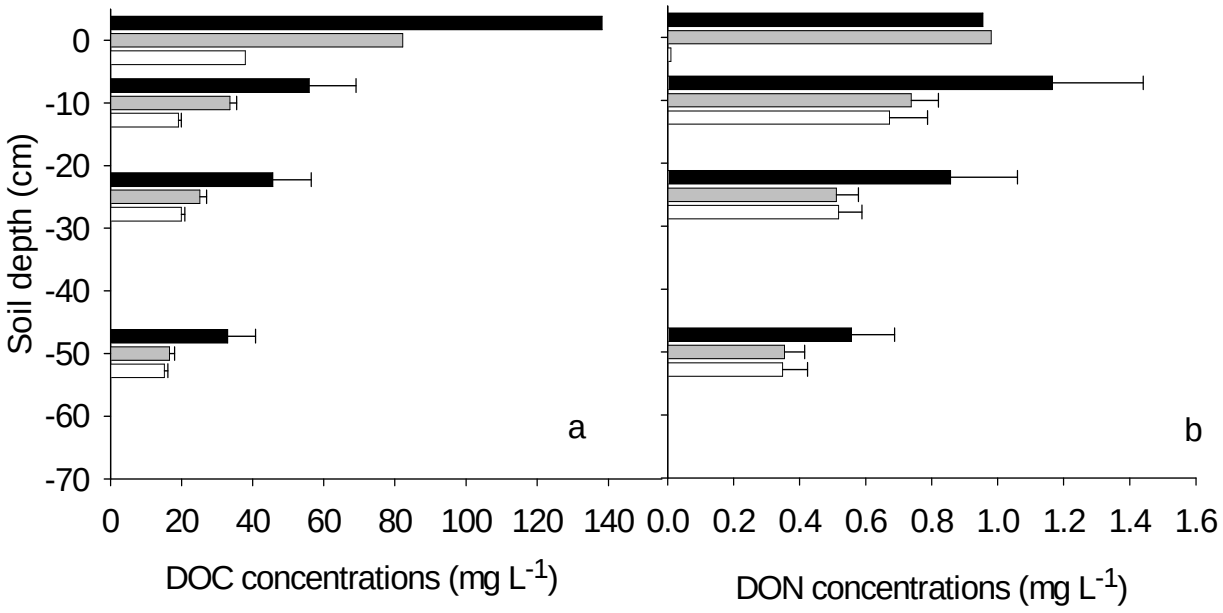

Figure 4.3 Total (*black bars*), hydrophobic (*gray bars*), and hydrophilic (*white bars*) concentrations (±1 SE) of (a) DOC and (b) DON by soil depth. "0" values represent the input organic matter solution. Data represent the average DOC and DON across all forests.

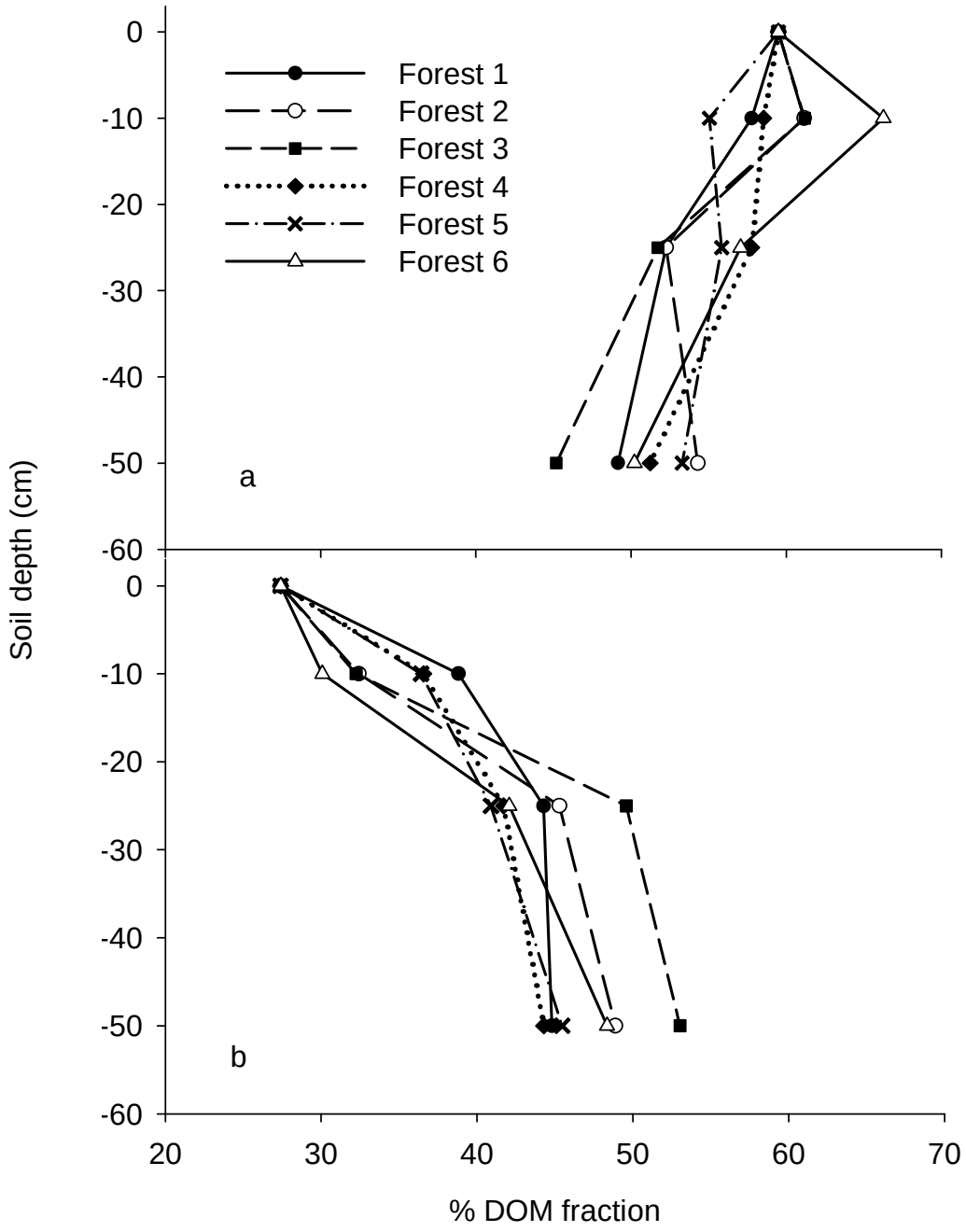

Figure 4.4 The (a) %hydrophobic and (b) %hydrophilic DOC in the organic matter input solution ("0") and at 10, 25, and 50 cm soil depths for each forest. Data represent forest stand averages of leachate from individual soil cores.

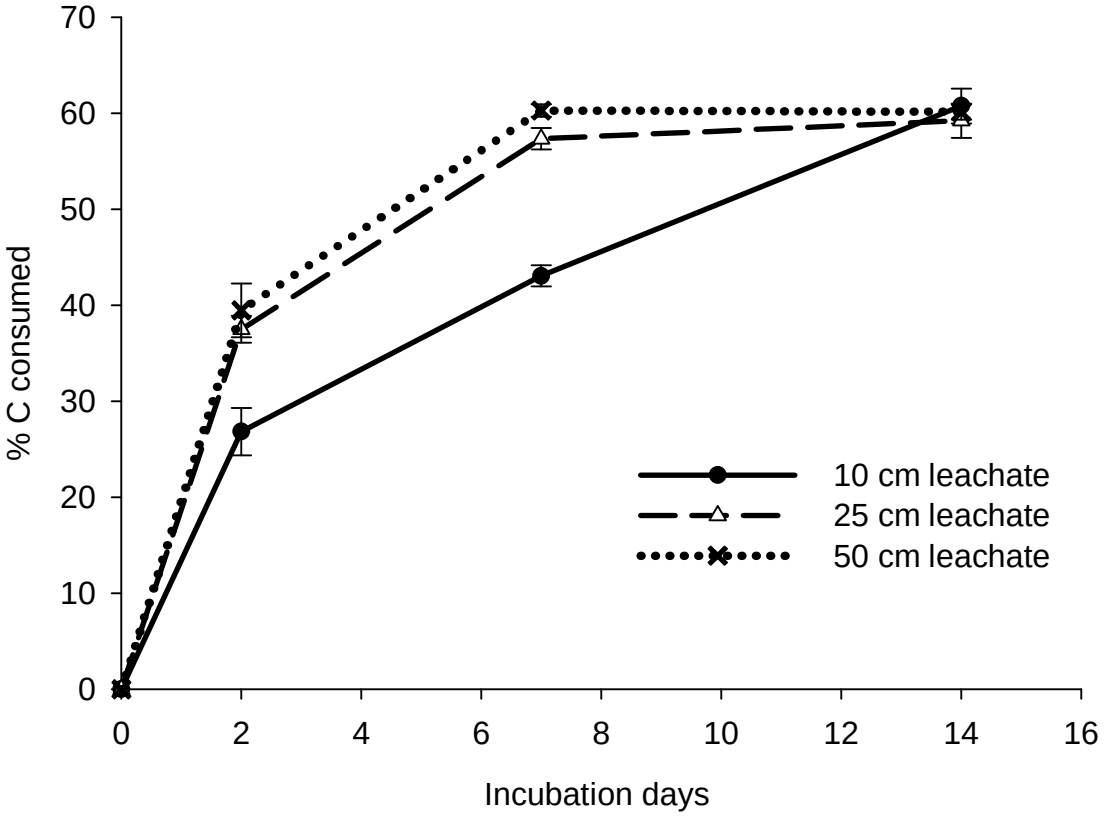

Figure 4.5 The average %DOC consumed by microorganisms over a 14 day period in soil leachate collected from 0-10, 0-25, and 0-50 cm soil cores that were inoculated with microbes. Data are averages across forests (± 1 SE).

APPENDIX

Appendix: Table of bivariate comparisons.

Table A4.4 Mixed-effects analysis of covariance results from bivariate comparisons between DON or DOC concentrations (mg L^{-1}) of 0-10, 0-25, and 0-50 cm soil core leachate and the soil texture classes of the cores. DON or DOC concentration was the dependent variable, each soil texture class was the independent variable, and soil core repetition was the random effect. For 0-25 and 0-50 cm cores, the soil texture values used were weighted averages of 0-10, 10-25, and 25-50 soil core increments as described in the text. Values in bold are significant. DF is the degrees of freedom of each analysis (numerator, denominator). All analyses were accepted as significant at α=0.05.

	DON			DOC		
	DF	F-value	p-value	DF	F-value	p-value
0-10 cm						
Coarse sand	1, 12	0.81	0.39	1, 12	2.56	0.14
Fine sand	1, 12	0.10	0.76	1, 12	1.87	0.20
Silt	1, 12	0.35	0.56	1, 12	0.52	0.48
Clay	1, 12	0.12	0.73	1, 12	1.06	0.32
0-25 cm						
Coarse sand	1, 14	0.01	0.91	1, 14	0.09	0.77
Fine sand	1, 14	1.87	0.19	1, 14	0.61	0.45
Silt	1, 14	3.62	0.08	1, 14	2.69	0.12
Clay	1, 14	0.16	0.70	1, 14	1.66	0.22
0-50 cm						
Coarse sand	1, 13	0.75	0.40	1, 13	0.04	0.84
Fine sand	1, 13	**5.15**	**0.04**	1, 13	0.18	0.68
Silt	1, 13	2.02	0.18	1, 13	0.97	0.34
Clay	1, 13	0.23	0.64	1, 13	0.53	0.48

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CHAPTER 5

CONCLUSIONS

It is becoming clear that DON has a critical role to play in our understanding of terrestrial N biogeochemistry, both as a source of N for plants and as a vector for N loss. In this dissertation, I investigated both of these facets of DON biogeochemistry to determine whether or not DON could serve as a potential source of plant-available N in temperate forests and to explore how DON retention and leaching losses varied across forests that spanned a gradient of N availability. The main conclusions of my research are summarized as follows:

In Chapter 2, my results demonstrated that four temperate tree species were physiologically capable of taking up DON in the form of free amino acids, with tree species that are typically relagated to lower N availability habitats acquiring relatively more N from organic sources compared to tree species typically found in habitats with higher N availability. However, in order to more fully understand how organic N pools contribute to plant N nutrition in temperate forests it will be imperative to evaluate tree amino acid uptake in a field setting. The prevalence of the particular amino acids I investigated in soil DON pools of northern hardwood forests indicates that they are potentially available for plant uptake by these species, although microbial uptake and/or mineralization of these amino acids and soil sorption phenomena could restrict their availability for plant uptake and limit their usefulness as a source of plant-available N. Experiments that evaluate amino acid uptake by plants despite these competitive forces will be necessary to determine the exact role of amino acid N in the nutrition of temperate tree species. Also, evaluating amino acid uptake in a wider range of temperate tree species will be necessary to determine how N-pool dominance by either organic or inorganic forms can influence plant species composition across the landscape in temperate forests.

In Chapters 3 and 4, I found that DON leaching losses and DOM chemistry from northern hardwood forests were not a simple function of soil N content or changes in soil textures, but were best understood in the context of a variety of ecosystem parameters that interacted to produce the DOM patterns I found. In chapter 3, DON leaching losses and DOM chemistry across the N-availability gradient were shaped by low rates of DOM production of C-rich compounds and high rates of DOM retention in the lowest N availability forest, high DOM production but limited DOM retention in intermediate N availability forests, and high DOM production coupled with high DOM retention by both biotic and abiotic mechanisms in the forest with the highest N availability. In chapter 4, soil characteristics that affected the structure of soil were likely responsible for the DOM leaching patterns I found. These complex interactions between DOM sources and sinks emphasize how important fine-scale controls over DOM dynamics can be for shaping landscape level patterns of DON loss and DOM chemistry. In these studies, there was no replication of individual forest types, so it is unclear if the patterns I found will be repeated by similar northern hardwood forests. Additional research investigating multiple forest stands within and ecosystem type would strengthen the results I found, especially the unimodal pattern of DOC:DON in leaching losses from deep soils in Chapter 3. This pattern was largely driven by the forest with the lowest N availability, so greater repetition would clarify whether or not relatively low DOC:DON in deep soil leaching losses was a common phenomenon in low N availability forests or specific to the forest in this study. It will also be important to conduct similar studies in other types of forests with different tree species compositions and soil types in order to create an overarching model of DON biogeochemistry that explains what factors control DON leaching losses from temperate ecosystems.

The research in chapters 3 and 4 also demonstrated that the quality of DOM can have an important bearing on the retention of DOM by both abiotic and biotic processes as well as the tendency of organic matter to desorb from mineral soil and become subject to leaching losses. In both chapters, DOC retention by soils was primarily due to the sorption of hydrophobic compounds to soil. In Chapter 4, DON losses actually increased in leachate from surface soils due to the release of less strongly sorbing hydrophilic compounds. These findings emphasize the importance of considering the complex nature of DOM compounds when trying to understand DOM cycling in forest ecosystems. The relatively higher proportion of hydrophilic DOM in leachate from deep soils compared to surface soils is particularly relevant to our understanding of DON losses from terrestrial ecosystems, which have previously been described as an "N leak" from temperate forests comprised of recalcitrant compounds that were relatively unavailable to biota. However, the removal of refractory hydrophobic compounds from percolating soil solutions in favor of labile, hydrophilic compounds calls this assumption into question, as does the ability of microbes to consume over half of the C in deep soil leachate. Instead, DON losses may be more a function of soil sorption dynamics and hydrology rather than the inability of biota to degrade recalcitrant compounds before they are transported away from surface horizons. This contrasts with inorganic N cycling, which is tightly controlled by biological processes.