The Environmental Microbiome In A Changing World: Microbial Processes And Biogeochemistry

THE ENVIRONMENTAL MICROBIOME IN A CHANGING WORLD: MICROBIAL PROCESSES AND BIOGEOCHEMISTRY

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ABSTRACT

Climate change can alter ecosystem processes and organismal phenology through both long-term, gradual changes and alteration of disturbance regimes. Because microbes mediate decomposition, and therefore the initial stages of nutrient cycling, soil biogeochemical responses to climate change will be driven by microbial responses to changes in temperature, precipitation, and pulsed climatic events. Improving projections of soil ecological and biogeochemical responses to climate change effects therefore requires greater knowledge of microbial contributions to decomposition. This dissertation examines soil microbial and biogeochemical responses to the long-term and punctuated effects of climate change, as well as improvement to decomposition models following addition of microbial parameters.

First, through a climate change mesocosm experiment on two soils, I determined that biogeochemical losses due to warming and snow reduction vary across soil types. Additionally, the length of time with soil microbial activity during plant dormancy increased under warming, and in some cases decreased following snow reduction. Asynchrony length was positively related to carbon and nitrogen loss. Next, I examined soil enzyme activity, carbon and nitrogen biodegradability, and fungal abundance in response to ice storms, an extreme event projected to occur more frequently under climate change in the northeastern United States. Enzyme activity response to ice storm treatments varied by both target nutrient and, for nitrogen, soil horizon. Soil horizons often experienced opposite response of enzyme activity to ice storm treatments, and increasing ice storm frequency also altered the direction of the microbial response. Mid-levels of ice storm treatment additionally increased fungal hyphal abundance. Finally, I added explicit microbial parameters to a global decomposition model that previously incorporated climate and litter quality. The best mass loss model simply added microbial flows between litter quality pools, and addition of a microbial biomass and products pool also improved model performance compared to the traditional implicit microbial model.

Collectively, these results illustrate the importance of soil characteristics to the biogeochemical and microbial response to both gradual climate change effects and extreme events. Furthermore, they show that large-scale decomposition models can be improved by adding microbial parameters. This information is relevant to the effects of climate change and microbial activity on biogeochemical cycles.

DEDICATION

To Jimmy, for his unwavering support.

And to Leo, who would help if he were allowed in the lab.

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CHAPTER 1: INTRODUCTION

1.1. Climate Change Effects in the Northeastern United States

1.1.1. Changes in Temperature and Precipitation

Climate change in the northeastern United States is increasing temperature and growing season length and altering precipitation dynamics, with even greater changes projected by the end of the century (Northeast Climate Impacts Assessment 2006, Hayhoe et al. 2008, Demaria et al. 2016, Janowiak et al. 2018). In the northeastern United States mean annual air temperature has increased by 1.3 °C, with the greatest increases occurring in winter (1901-2011) (Janowiak et al. 2018). These trends are projected to continue, with expected increases in mean annual and minimum winter temperatures of up to 4.2 °C and 5.5 °C, respectively, by the year 2100 (Janowiak et al. 2018). Warmer winter temperatures are expected to decrease snow cover extent, snowpack depth, and the length of the snow season (Northeast Climate Impacts Assessment 2006, Burakowski et al. 2008, Hayhoe et al. 2008, Campbell et al. 2010, Demaria et al. 2016). Indeed, the number of days with snow cover and the average snowpack depth have already decreased across the region (Hodgkins and Dudley 2006), with the snow season beginning later (Peng et al. 2013) and spring starting earlier (Choi et al. 2010, Brown and Robinson 2011). There is also expected to be greater winter and spring precipitation (Horton et al. 2014).

1.1.2. Changes in Extreme Events

In addition to the gradual changes in mean temperature and precipitation occurring with climate change, disturbance regimes such as those characterized by pulsed climatic events are also changing (He et al. 1999). In particular, warming is projected to alter the frequency and severity of extreme weather events (IPCC 2013, Horton et al. 2014), which can have a large impact on species composition and distribution as well as ecosystem processes with potential lasting legacy effects (Jentsch et al. 2007, Arnone et al. 2011). Ice storms are one example of an extreme event that is common in temperate forests worldwide. They are caused by rain falling from a warm air mass through sub-freezing air masses closer to the ground, which super cools the precipitation and causes it to freeze on contact with cold surfaces. However, ice storms are relatively rare, localized events, which makes them and their ecological impacts a challenge to study. Weather conditions leading to ice storms are well understood, and their frequency and severity may increase under future climate change scenarios, as shown by predictions for south-central and eastern Canada (Cheng et al. 2007, Cheng et al. 2011), with the northeastern United States (US) at particularly elevated risk of damage (Dale et al. 2001, Changnon 2003).

1.1.3. Phenological Changes Due to Climate Change

Earlier springs and later snow seasons associated with climate change may also increase the period of time when microbes are active but plants are not (Groffman et al. 2012). These asynchronies in plant and microbial activity could have important implications for carbon (C) and nutrient balances (Groffman et al. 2012, Contosta et al. 2017). When soils are warm, but plants are inactive, microbial activity can lead to nitrogen (N) buildup in soils (Muller and Bormann 1976). During spring melt, in the absence of plant uptake, N accumulated in soil is vulnerable to leaching which reduces forest N retention, plant productivity (Vitousek et al. 1997), and stream water quality (Likens et al. 1996). Similarly, increases in mobile N throughout the growing season following soil freezing have been attributed to temporal mismatch in nutrient availability and uptake (Tierney et al. 2001). Asynchronies in plant and microbial activity are ameliorated when the deciduous canopy expands and plants take up N, transpire, and shade the forest floor. There is evidence that asynchronies have been amplified by climate change (Groffman et al. 2012, Contosta et al. 2017), potentially causing a growing disconnect between plant and microbial activity with implications for C and N retention and loss in northern forests. Further studies are needed to confirm potential links between climate change, plant-microbe asynchronies, and biogeochemical losses of nutrients.

1.2. Interaction Between Soil Characteristics and Climate Change

Differences in ecosystem properties, such as parent material or soil type, could create substantial variation in ecosystem responses to climate change. Because both climate and soil properties regulate resources such as soil moisture (Merz and Plate 1997, Dai et al. 2004) and nutrient availability (Melillo et al. 2011, Sanders-DeMott et al. 2018, Ge et al. 2019) that affect plant and microbial growth and activity (Vitousek and Howarth 1991, Craine et al. 2007, LeBauer and Treseder 2008, Wang et al. 2019), climate and soils will likely interact to affect nutrient cycling and losses. If true, this will have substantial implications for predicting the impacts of climate change on ecosystems such as northeastern forests, which have served as a C sink over the past several decades (Pugh et al. 2019).

Soil properties have the capacity to mitigate or exacerbate many of the impacts of climate change on ecosystem processes. For example, soil texture can impact the nature and extent of soil freezing (Fuss et al. 2016), and soil freezing impacts aggregate stability (Lehrsch et al. 1991) and the amount of water available during dry (Ritchie 1981, Aksakal et al. 2019) or frozen periods (Gray et al. 1985, Tucker 2014). Soil texture also impacts microbial activity and nutrient retention (Silver et al. 2000, Hamarashid et al. 2010, Tahir and Marschner 2017). Phosphorus (P) leaching, for example, has been found to be markedly reduced on finer textured soils (Sims et al. 1998). Conversely, agricultural studies have found greater N loss from finer textured soils (Obcemea et al. 1988).

In addition to physical differences such as texture, the chemical characteristics of soils vary across the region. In particular, northern forests have been subjected to acid deposition, which reduces calcium (Ca) availability in soils (Lawrence et al. 1995). Critical to plant biological function, Ca depletion is associated with increased susceptibility to stressors such as winter injury (Schaberg et al. 2001). In the northern forest, Ca depletion of soils varies depending on pre-acid deposition chemical characteristics. Furthermore, differences in soil texture have been raised as one possible explanation for the variable response of N leaching to soil freezing (Groffman et al. 2011). The mosaic of soil types across the northeast provides the background conditions for climate change in the region, and their compounded effects on forest C and N loss are unknown.

1.3. Climate Change Effects on Soil Biogeochemistry

1.3.1. Effects of Warming

Warming temperatures have important consequences for C and N dynamics in forested systems. In long term studies, soil warming increased soil carbon dioxide (CO_2) efflux and inorganic N loss (Melillo et al. 2002, Melillo et al. 2011). A meta-analysis of warming studies found an average increase in soil respiration of 20%, and increase in net N mineralization of 46% (Rustad et al. 2001). However, net N mineralization and nitrification were found to be slower in warmer zones of a natural climate gradient, presumably due to lower summer soil moisture and greater winter soil freezing (Groffman et al. 2009).

1.3.2. Effects of Snow Reduction

Winter warming may also have specific impacts on nutrient cycling and losses. Snow shapes winter soil conditions by providing insulation from below-freezing air temperatures (Boutin and Robitaille 1995, Hardy et al. 2001, Decker et al. 2003, Henry 2008). This insulation allows microbial processes to continue in relatively warm (i.e., unfrozen) soils and C and nutrients to accumulate (Schimel et al. 2004, Brooks et al. 2011). Without this protection, soils have been shown to be colder or freeze (Groffman et al. 2001a), with decreased availability of liquid soil water (Henry 2008, Öquist et al. 2009). Soil freezing has the potential to affect many belowground processes related to biogeochemical cycling, including fine root and microbial mortality, hydrologic and gaseous N loss, and the acid-base status of drainage water (Sanders-DeMott et al. 2019). Experimental snowpack reduction and associated increases in soil freezing (Groffman et al. 2001a, Campbell et al. 2005, Blankinship and Hart 2012) can damage tree roots

(Tierney et al. 2001, Cleavitt et al. 2008, Comerford et al. 2013), reducing their ability to take up nitrogen during the growing season (Campbell et al. 2014). Soil freezing can also strongly reduce microbial activity due to temperature limitations (Lloyd and Taylor 1994a, Kirschbaum 1995) and the reduced availability of liquid water (Edwards and Cresser 1992, Panikova et al. 2006, Öquist and Laudon 2008, Clark et al. 2009, Brooks et al. 2011). Regardless, soil thaws can increase soil C and nutrient concentrations, likely by disrupting soil aggregates, plant litter, plant roots, and lysing microbial cells (Schimel and Clein 1996, Oztas and Fayetorbay 2003, Campbell et al. 2014, Song et al. 2017). The impacts of repeated freeze thaw cycles are less clear. A recent meta-analysis of lab studies found that multiple freeze-thaw cycles decreased microbial C and dissolved organic nitrogen (DON) and did not change inorganic N or dissolved organic carbon (DOC), but these trends often did not match in-field results (Song et al. 2017).

How these multiple responses to soil freezing will integrate to impact forest C and nutrient cycling and losses remains unclear, but soil frost at the watershed level has been significantly correlated with interannual variability in stream water nitrate (NO_3^-), Ca, and magnesium (Mg) concentrations (Fitzhugh et al. 2003b). Similarly, plot-level snow removal experiments have generally led to increases in leaching of NO_3^-, inorganic P, and base cations with unexplained variation across experiments in the magnitude of the response (Sanders-DeMott et al. 2019). Soil freezing has also been found to have legacy effects throughout the growing season. A meta-analysis of snow manipulation studies found a twofold increase in N loss from soils during the growing season following snow reduction (Blankinship and Hart 2012). The same meta-analysis found that growing season soil CO_2 efflux decreased 35% following snowpack reduction (Blankinship and Hart 2012).

1.3.3. Effects of Ice Storms on Soil Conditions

The effects of ice storms are long-lived, with legacies that can affect tree structure and ecosystem processes for years after the event. Damage caused by individual storms is very variable, ranging anywhere from broken twigs to broken trunks and loss of crowns (Cannell and Morgan 1989, Smith 2000). Root damage can also occur depending on wind and ice conditions (Irland 2000). Following the 1998 ice storm at the Hubbard Brook Experimental Forest (HBEF), moderately to severely damaged stands experienced an increase in root growth compared to undamaged stands (Rhoads et al. 2002). The same storm resulted in a one-third reduction in leaf area, representing a significant loss of photosynthesis, that took 3 years to recover (Rhoads et al. 2002). Damage to the canopy and root system such as this may alter plant C allocation, potentially reducing root exudates that reach the microbial community. Additionally, new soil temperature patterns post-storm could also affect the activity or the composition of the soil microbial community. For instance, during the 3 years following the 1998 ice storm at HBEF, soil temperature at 15 cm depth increased 2 °C on average, and the range of temperatures experienced was amplified (Likens et al. 2004).

Litter deposition resulting from an ice storm represents another alteration to the soil environment that may impact microbial community composition or activity. For example, in a pilot ice storm simulation at the HBEF, the amount of fine litter deposited onto the soil surface immediately following treatment was the equivalent of the amount typically deposited over the course of a year, and coarse litter deposition was ten times the long term mean (Rustad and Campbell 2012). This large pulse of inputs is fundamentally different than the litter deposited in a typical year, since it is dominated by C rich fine and coarse woody debris, as opposed to relatively nutrient-rich leaf litter (Rustad and Campbell 2012).

The damage caused to trees by an ice storm, therefore, has the potential to alter the soil environment in a variety of ways, including by: i) increasing soil temperature average and range; ii) increasing inputs of woody debris; iii) decreasing inputs of root exudates if trees reallocate C use aboveground during recovery; iv) creating belowground hotspots of C input if fine root growth increases during recovery; and v) reducing growing season leaf litter inputs following crown damage. These potential impacts would alter both the soil physical environment and the quality of the substrate available to the microbial community, and it may be expected that community composition or activity would consequently be altered.

Post-ice storm conditions may affect not only microbial activity, but also microbial community composition with important implications for ecosystem C retention or loss. For example, the fungal population is affected by environmental conditions including temperature and moisture (Frey et al. 1999, Staddon et al. 2003, Cregger et al. 2012, McGuire et al. 2012) as well as substrate characteristics (Garrett 1951), all of which can be altered by ice storms. The fungal contribution to ecosystem processes includes improvement of soil fertility by making nutrients available via decomposition and mineralization, and enhancing plant productivity through mycorrhizal associations (Dighton 2016), both of which feed back to ecosystem C and nutrient retention or loss. A meta-analysis of global belowground microbial community traits found that fungal abundance relative to bacterial abundance has been shown to increase with increasing soil C:N (Fierer et al. 2009). Indeed, higher fungal to bacterial ratios have been causally linked to greater soil C storage (Malik et al. 2016), while soil inorganic N availability has been found to be negatively correlated with measures of fungal abundance (Bardgett and McAlister 1999). For these reasons, fungal abundance relative to bacterial abundance may be expected to change under altered C and nutrient conditions post-ice storm, which is of importance due to its implications for global C and nutrient budgets under climate change.

1.4. Microbial Role in Soil Biogeochemistry

1.4.1. Extracellular Enzyme Activity

The breakdown of complex organic material into simpler forms that can be taken up by microbes and plants is primarily carried out by extracellular enzymes (EEs), which are secreted by microorganisms and to a lesser extent, plant roots. This process is the first step of decomposition and nutrient mineralization (Wallenstein and Weintraub 2008). By breaking down the net primary production of ecosystems, microbial metabolism drives biogeochemical cycles of C and nutrients and forms the trophic base for detrital food webs by allowing access to otherwise biologically unavailable resources (Sinsabaugh and Follstad Shah 2012). Additionally, EE activity is critical to the maintenance of soil function and microbial community biodiversity (Wallenstein et al. 2011), and changes may cascade through ecosystem biogeochemical cycles. For example, through the action of microbially produced EEs, decomposition of plant and soil material results in the production DOC in forest soils (Guggenberger et al. 1994, Cory et al. 2011). DOC not only is the result of microbial metabolism, it also fuels the microbial food web (Bott et al. 1984). However, not all compounds classified as DOC are equally biodegradable. Some DOC contains high contents of carbohydrates and organic acids which are easily biodegradable, as opposed to DOC composed of aromatic and hydrophobic structures (Marschner and Kalbitz 2003).

EE activity is controlled by a suite of interacting factors that includes substrate availability, EE concentration, soil physics, temperature, and pH (Wallenstein and Weintraub 2008). Moreover, enzyme production is a C, N, and energy intensive process, so microbes theoretically only produce EEs to obtain a necessary or limited resource (Koch 1985). This causes microbes to reduce EE production for acquisition of nutrients that are readily available in the soil environment, and increase production of EEs for resources that are scarce (Harder and Dijkhuizen 1983, Sinsabaugh and Moorhead 1994, Allison et al. 2011). In fact, both P and N acquiring enzymes (P: phosphatase, N: peptidase, chitinase) are inversely related to environmental concentrations of those elements (Olander and Vitousek 2000, Treseder and Vitousek 2001). Changes in litter deposition are therefore likely to alter microbial EE expression due to the altered quality of the substrate, namely the increased abundance of C rich litter relative to nutrient rich leaf litter, and potential changes in belowground C exudation. For example, as lignin content of litter increases, so does oxidative enzyme expression (Herman et al. 2008, Sinsabaugh 2010). Additionally, EE expression has been observed to shift from P-acquiring to N-acquiring enzymes (Sinsabaugh and Follstad Shah 2011). This is due to more recalcitrant substrates requiring oxidative enzymes to release C, N, and P as opposed to hydrolytic enzymes, which have a lower activation energy due to their specificity and the structure of the molecules they degrade. The increased metabolic effort therefore slows microbial growth rates, which reduces the demand for P and results in the observed shift to expression of N-acquiring enzymes (Frost et al. 2006, Allen and Gillooly 2009, Sinsabaugh and Follstad Shah 2011). Increased organic matter (OM) recalcitrance has also been associated with increased microbial C demand relative to N and P demand, which had the effect of reducing the N and P assimilation efficiency of microbes, and increasing N and P mineralization (Sinsabaugh and Follstad Shah 2011).

1.4.2. Microbial Mediation of Decomposition

Ecosystem productivity relies on the availability of nutrients and energy sources that are released through decomposition, a complex, microbially-mediated ecosystem process. By breaking down complex organic compounds, decomposition releases biologically necessary elements into the environment in forms that are more accessible to plants and microbes. Release of C and N is of particular interest due to the vital importance of these elements to ecosystem function and structure, and because their biogeochemical cycles have been drastically altered by anthropogenic activity in such a way as to change ecosystem and global ecology.

Decomposition is carried out largely by microbial extracellular enzymes, which break complex organic molecules from litter into simpler inorganic and organic products. Factors that affect microbial populations therefore affect decomposition rates. These include climate variables and litter quality (Meentemeyer 1978, Aber et al. 1990), predation (Santos et al. 1981, Bouwman et al. 1994), competition between microbes (Allison 2006), and soil macrofauna activity (Seastedt 1984, Huhta 2006). Site characteristics such as soil physical structure (Allison and Jastrow 2006) and solar radiation (Austin and Vivanco 2006) also affect decomposition rates. Microbial physiology, including such factors as carbon use efficiency (CUE) (Allison et al. 2010), microbial growth efficiency (MGE) (Wieder et al. 2013), and EE activity (EEA) (Allison et al. 2010, Allison 2012) similarly alter decomposition rates. Additional factors include the chemical composition of microbial products (Campbell et al. 2016) and the resilience of the microbial community composition and functioning under changing environmental conditions (Martiny et al. 2016).

1.4.3. Microbial Models of Decomposition

Due to its role in nutrient cycling, productivity, and soil respiration, decomposition is a major regulator of ecosystem functioning. It is also of great importance to global C cycling, and thus global climate change. Decomposition also provides bioavailable C substrates that fuel the heterotrophic soil food web (Sinsabaugh and Follstad Shah 2012), and is the predominant process releasing CO_2 that was previously removed from the atmosphere by photosynthesis. In fact, decomposition releases about ten times more CO_2 to the atmosphere than fossil fuel and industrial sources (Prentice et al. 2001). In this way, decomposition of plant material links the terrestrial and atmospheric C pools (Houghton 2007). Furthermore, soils are the largest terrestrial C reservoir, and contain two to three times the amount of C in the atmosphere (Jobbágy and Jackson 2000). Microbial decomposition is the process that partitions organic C between the atmosphere and longer-term soil C storage, with old soil C comprised nearly entirely of microbial products (Allison 2006). For these reasons, it is of vital importance to gain a clear understanding of the drivers of decomposition, since any alteration in the balance between C storage and CO_2 release from soils could have consequences for global climate dynamics.

Despite the central role of microbes to decomposition, the majority of C cycling models largely rely on empirically derived relationships with litter chemistry and climate to predict decomposition rates (Parton et al. 1994, Gholz et al. 2000, Adair et al. 2008). Although these environmental variables may implicitly account for microbial activity (Schimel 2001), it is increasingly recognized that explicit inclusion of microbial activity or community dynamics into decomposition models may improve model outcomes (Lawrence et al. 2009, Allison 2012, Treseder et al. 2012, Wieder et al. 2013). Due to the importance of projecting soil C dynamics in the future, models of soil C dynamics in particular have been developed to include microbial activity or community composition in an attempt to better predict future C dynamics under changing global climate scenarios. Such models have incorporated many different microbial community or activity parameters, including: microbial biomass pools (Wieder et al. 2014, Xu et al. 2014), microbial product pools (Campbell et al. 2016), turnover of microbial biomass (Wieder et al. 2014), microbial CUE (Allison et al. 2010, Wieder et al. 2014, Campbell et al. 2016), EE activity (Lawrence et al. 2009, Allison et al. 2010, Allison 2012), and microbial growth strategy (i.e., r vs. k) (Wieder et al. 2014). However, the improvement in these models over first order models has been largely unexamined (Treseder et al. 2012).

1.5. Conclusions

Environmental alterations in temperature, precipitation, and organismal phenology associated with climate change are all capable of altering microbial activity and community composition with important consequences for ecosystem biogeochemistry. Due to their central importance to global nutrient cycles and ecosystem function, better representation of microbial contributions to ecosystem processes is necessary to improve environmental projections. In this dissertation, I have investigated the effects of long-term and pulsed climatic changes on microbial activity and biogeochemistry. I also examined improvement to a decomposition model through inclusion of microbial parameters. The projects I describe aim to increase understanding of the effects of global change on microbial ecology and ecosystem nutrients flows. My results can inform models on the importance of soil characteristics and microbial activity for improving projections of future environmental conditions.

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CHAPTER 2: SOIL TEXTURE MODIFIES THE IMPACTS OF WARMING AND SNOW EXCLUSION ON PLANT-MICROBE SYNCHRONY, AND CARBON AND NUTRIENT BIOGEOCHEMISTRY

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2.1. Abstract

The effects of climate change on ecosystem processes are complex, in large part due to the many biophysical interactions that govern basic functions. For example, soil texture and chemistry can alter the availability of limiting resources via water holding capacity and soil nutrient status. In northern climates, climate change is increasing mean temperatures, reducing and delaying snowpack, and changing the length and timing of the growing season. Plant and microbial adjustments to altered seasonal timing may occur at different rates, resulting in an increased period of microbial activity during plant dormancy. Soil nutrients that build up during these plant-microbe asynchronies (i.e., in the absence of plant uptake) are vulnerable to leaching loss, with potentially important consequences for ecosystem productivity. Our objective was to quantify the interacting effects of soil characteristics and climate change, year-round and during plant-microbe asynchronies, on carbon (C), nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and aluminum (Al) losses. To achieve this, we conducted a mesocosm experiment, which imposed replicated warming and snow exclusion treatments on two soil treatments in forest mesocosms. We found that nutrient loss responses to climate treatment often varied by soil treatment. Generally, the coarse textured soil, with its lower soil-water holding capacity, had higher nutrient losses, except in the case of phosphate (PO_4^{3-}), which had consistently higher losses in the finer textured soil. The length of time with soil microbial activity during plant dormancy was increased by warming, in some cases decreased by snow exclusion, and increased on fine vs. coarse soils. The duration of plant-microbe asynchrony resulted in increased losses of C, total dissolved nitrogen (TDN), and nitrate (NO_3^-), but generally decreased losses of PO_4^{3-}, Ca, and Mg. Our results demonstrate that biogeochemical responses to climate change are mediated by soil characteristics, and that microbial activity in the absence of plant uptake generally compounds nutrient losses.

Keywords: climate change, soil freezing, infrared warming, mesocosm, base cations, leachate, soil treatment, plant-microbe asynchrony, vernal window

2.2. Introduction

Over much of the world, climate change is increasing air temperature, extending growing season length, and altering precipitation dynamics (Northeast Climate Impacts Assessment 2006, Hayhoe et al. 2008, Demaria et al. 2016, Janowiak et al. 2018). However, these changes are occurring across heterogeneous landscapes. Differences in ecosystem properties, such as parent material or soil type, could create substantial variation in ecosystem responses to climate change.

Soil texture and composition help control soil biogeochemistry and carbon (C) storage (Silver et al. 2000, Doetterl et al. 2015, González-Domínguez et al. 2019), in part by determining soil water-holding and sorption capacity (Mayes et al. 2012, Weil and Brady 2017). These effects, combined with the fact that soils are a critical component of the terrestrial C cycle (Jobbágy and Jackson 2000, Köchy et al. 2015), has prompted researchers to increasingly call for the inclusion of soil properties in Earth system models to reduce uncertainty and improve C and climate projections (Todd-Brown et al. 2013, Doetterl et al. 2015). Because both climate and soil properties regulate resources, such as soil moisture (Merz and Plate 1997, Dai et al. 2004) and nutrient availability (Melillo et al. 2011, Sanders-DeMott et al. 2018, Ge et al. 2019), that affect plant and microbial growth and activity (Vitousek and Howarth 1991, Craine et al. 2007, LeBauer and Treseder 2008, Wang et al. 2019), climate and soils will likely interact to affect nutrient cycling and losses. If true, this will have substantial implications for predicting the impacts of climate change on ecosystems such as northeastern forests, which have served as an important C sink over the past several decades (Pugh et al. 2019).

Ongoing and projected climate changes will continue to impact nutrient cycling and losses by altering the abiotic drivers of ecosystem processes (e.g., temperature and moisture). Soil warming increases carbon dioxide (CO_2) efflux, inorganic nitrogen (N) loss (Rustad et al. 2001, Melillo et al. 2002, Melillo et al. 2011), and net N mineralization (Rustad et al. 2001), with variations attributed to soil moisture and freezing (Groffman et al. 2009). Warmer winter temperatures decrease snow cover extent, snow pack depth, and the length of the snow season (Northeast Climate Impacts Assessment 2006, Hayhoe et al. 2008, Demaria et al. 2016).

Snow plays an important biogeochemical role by insulating soils from below-freezing air temperatures (Boutin and Robitaille 1995, Hardy et al. 2001, Decker et al. 2003, Henry 2008), maintaining soil temperatures that support microbial processes that lead to nutrient accumulation (Schimel et al. 2004, Brooks et al. 2011). With insufficient snowpack, soils can freeze more deeply (Groffman et al. 2001a), affecting many belowground processes related to biogeochemical cycling. This includes soil respiration (Blankinship and Hart 2012, Reinmann and Templer 2018), N cycling, and hydrologic losses of nitrate (NO_3^-), inorganic phosphorus (P), and base cations (magnesium (Mg) and calcium (Ca)) (Fitzhugh et al. 2003b, Sanders-DeMott et al. 2019), with legacy effects lasting throughout the growing season. Soil freezing can also strongly reduce microbial activity due to temperature limitations (Lloyd and Taylor 1994a, Kirschbaum 1995) and reduced availability of liquid water (Edwards and Cresser 1992, Panikova et al. 2006, Öquist and Laudon 2008, Clark et al. 2009, Brooks et al. 2011). Conversely, soil freeze-thaw cycles can alter soil C and nutrient dynamics, likely by disrupting soil aggregates, plant litter, plant roots, and lysing microbial cells (Schimel and Clein 1996, Oztas and

Fayetorbay 2003, Campbell et al. 2014, Song et al. 2017). How these multiple factors combine to impact forest C and nutrient cycling and losses remains unclear.

Earlier arrival of spring and delayed snow onset due to climate change may increase ecosystem C and nutrient losses by increasing occurrence of soil temperatures that support microbial activity during plant dormancy (i.e., plant-microbe asynchronies) (Groffman et al. 2012). Because soil biological activity is responsive to temperature, delayed onset of cold soil temperatures at the end of the growing season, more frequent freeze-thaw cycles, and earlier snowmelt will likely extend these asynchronies (Campbell et al. 2005, Contosta et al. 2017), leading to belowground buildup of C and other nutrients (Muller and Bormann 1976, Schimel et al. 2004, Brooks et al. 2011) that are vulnerable to leaching loss (Muller and Bormann 1976, Brooks et al. 1998, Tierney et al. 2001, Schmidt et al. 2007). Climate change has altered plant phenology (Polgar and Primack 2011), but if plants are not as responsive to warming as microbes, then the incidence of plant-microbe asynchronies may increase (Groffman et al. 2012, Contosta et al. 2017). It remains uncertain if lengthening periods of plant-microbe asynchrony increases ecosystem losses of C and other nutrients (Groffman et al. 2012, Contosta et al. 2017), and how this may vary across different soils.

The physical and chemical mosaic of soil types across the landscape provides a foundation upon which climate change will act, yet the compounded effects of soils and changing climate on ecosystem C and nutrient losses are largely unknown. Soil texture, for example, determines soil moisture (Cosby et al. 1984, Jawson and Niemann 2007), which can modify the effects of warming on soil temperature (Subin et al. 2013) and regulate microbial activity (Tiwari et al. 1987, Prado and Airoldi 1999). Soil texture itself also influences microbial activity, including decomposition, soil respiration, N mineralization, and denitrification (Silver et al. 2000, Hamarashid et al. 2010, Xu et al. 2016). In winter, soil texture affects the extent of soil freezing (Fuss et al. 2016), which alters aggregate stability (Lehrsch et al. 1991) and water availability during dry (Ritchie 1981, Aksakal et al. 2019) or frozen periods (Gray et al. 1985, Tucker 2014). Many of the basic chemical attributes of soils that shape biological activity also vary across soil textures, including N, P, and base cation concentrations, C:N ratio, and soil organic matter pools (Silver et al. 2000, Hamarashid et al. 2010) as well as nutrient leaching rates (Tahir and Marschner 2017). For example, P and N leaching can vary markedly across soil textures (Obcemea et al. 1988, Sims et al. 1998, Tahir and Marschner 2017). Differences in soil texture provide one possible explanation for the variable response of N leaching to soil freezing (Groffman et al. 2011).

Soils also vary in their chemical properties due to the legacy effects of acid deposition, which reduced soil base cation concentrations across the northeastern United States (US) (Lawrence et al. 1995) and much of the globe. Soil properties therefore have the capacity to mitigate or exacerbate many of the impacts of climate change on ecosystem processes, as well as influence plant and microbial activity, phenology, and the synchrony between them.

Here we quantified the interacting effects of soil characteristics and climate change, year-round and during plant-microbe asynchronies, on C, N, P, Ca, Mg, and Al losses. In order to quantify main and interactive effects, we examined these dynamics in a replicated climate change mesocosm experiment that imposed above ground warming and snow exclusion treatments on two soils that differed in texture and chemical composition. The mesocosm approach was chosen to reduce heterogeneity and allow quantification of mechanistic responses to main effects and interactions of treatments. We determined the effect of year-round warming and delayed snowpack formation on losses of C, N, P, and cations prone to leaching following snow exclusion and during soil acidification (Ca, Mg, aluminum (Al)). We hypothesized that the direct effects of warming and snow exclusion would increase C and nutrient losses compared to control conditions (H1), and that losses would be greatest from coarse-textured soils (H2). We also expected that climate treatment effects would vary between different soils (soil × treatment interactions, H3). Additionally, we sought to determine the impact of warming and snow exclusion on the length of plant-microbial activity mismatches, and the consequences for C and other nutrient loss. Here, we expected warming to lengthen plant-microbial asynchronies, and snow exclusion to increase soil freezing thereby delaying spring thaw and shortening asynchrony (H4). We expected longer asynchronies (i.e., in the warming treatment) would lead to greater loss of C and other nutrients (H5), with variation by soil treatment. Finally, we expected responses to treatments to differ across years due to natural climatic variation. New evidence that soil characteristics interact with climate treatments to modify nutrient losses would provide novel details of the nuances of how ecosystems respond to altered climate and influence C and other nutrient losses. Additionally, if increased periods of asynchrony in plant and microbial activity amplify C and nutrient losses, this could represent a positive feedback to climate change.

2.3. Methods

2.3.1. Site description and climate treatments

We examined interactions among climate treatments, seasonal asynchronies, and soil characteristics in a replicated climate change mesocosm experiment at the George D. Aiken Forestry Sciences Laboratory in South Burlington, VT, USA (44°27′ N, 73°12′ W, 60 m elevation). Mean annual temperature in South Burlington is 7.2 °C, and mean annual precipitation is 889 mm. Using a factorial design, we imposed control, warming, and snow exclusion treatments on two soil types across 24 mesocosms, resulting in four replicates of each soil type-climate treatment combination (e.g., fine soil control, fine soil warming, fine soil snow exclusion, coarse soil control, coarse soil warming, coarse soil snow exclusion).

Mesocosms were initially installed in 1995. Tank construction was described in detail in Beard et al. (2005). Briefly, the polyethylene mesocosms had a diameter of 2.4 m, 1 m soil depth, and a closed leachate drainage system from which water was removed from the bottom of the tank using a vacuum extraction system (Fig. 1). All tanks were buried below ground with a 20 cm above ground rim and were filled with one of two mineral soils that were distinct in physical and chemical properties (Table S1). The “coarse” soil was a sandy clay loam with twice the fine gravel content (34%) and significantly higher Ca availability than the “fine” soil, a loamy sand with lower fine gravel content (17%) and Ca availability two orders of magnitude less than the coarse soil. For simplicity, the two soil treatments were labeled “coarse” and “fine,” but we recognize that these soils varied by multiple characteristics. The gravel content of the coarse soil reduced its water holding capacity, while its higher Ca availability increased its buffering capacity relative to the fine soil. Overall, the coarse soil had higher cation exchange capacity, clay, and organic matter content, which are all associated with reduced leachate losses. Soil types were randomly assigned to the mesocosms when the site was established.

Mesocosms were prepared for planting in spring 2013 by tilling the top 5 cm of soil and adding 0.5 L Osmocote fertilizer (16-5-10; The Scotts Company, Marysville, OH, USA) to aid seedling establishment. All mesocosms were planted with four deciduous tree species (20 seedlings of each species in each mesocosm): paper birch (*Betula papyrifera* Marshall), quaking aspen (*Populus tremuloides* Michx.), American chestnut (*Castanea dentate* (Marshall) Borkh.), and black cherry (*Prunus seronita* Ehrh). Planting locations in the mesocosms were equally spaced, but randomly distributed, resulting in an inter-planted deciduous mix. Tree species were chosen to represent both different rooting depths, and different geographic ranges to examine possible effects of future climate conditions on species range shifts (Table S2). American chestnut seedlings were grown from nuts originating in Haun, PA, USA, and were obtained from the American Chestnut Foundation (Asheville, NC, USA). For all other species, 1+ year old seedlings were purchased from a commercial tree nursery (Porcupine Hollow Farm, Central Lake, MI, USA). For six months following planting, seedlings were watered as necessary to encourage establishment before initiating experimental climate treatments. During this period, quaking aspen developed Shepherd's crook fungus (*Venturia tremulae*), which was treated using copper sulfate fungicide at a rate of 35 mL L^{-1} every two weeks throughout the summer (June - August). In fall 2013, a forest floor (2.2 cm depth) was simulated in the mesocosms by applying air-dried and chopped freshly fallen leaves (collected in litter traps) from local mature trees of the four species. During the experiment, all plants other than the four planted species were weeded out and left on the soil surface within mesocosms.

Climate treatments began in December 2013 following the seedling establishment period, and were based on low CO_2 emissions scenario model projections for the northeastern US in the year 2100 (Frumhoff et al. 2007). Treatments consisted of control, infrared (IR) warming of 2 °C above ambient, and snow exclusion at the beginning of winter, each with four replicates per soil treatment. We randomly assigned treatments to mesocosms. Infrared warming was achieved with a modified version of the methods of Kimball et al. (2008) using suspended ceramic IR warming elements (Mor Electric Heating, Comstock Park, MI, USA, FTE-1000-240-0-L6-WH-0 240V 1000W) cased within aluminum extrusion reflectors (Mor Electric Heating). We covered the heaters with aluminum rain gutters, and sealed the seams with silicon caulk. Each mesocosm had 4 heaters installed on the perimeter on 5 cm diameter aluminum posts. Heaters were hung 1.5 m above the soil surface at a 45 ° angle to achieve spatially uniform warming (Kimball et al. 2008), which we confirmed with thermal imaging in preliminary tests. In the IR-warmed and control mesocosms, surface temperature was measured in the center with a radiometer (Apogee Instruments, Logan, UT; SI-111) controlled by a CR1000 datalogger (Campbell Scientific, Logan, UT, USA). Radiometers were scanned every 30 or 60 seconds in year 1 and year 2, respectively, and used to maintain IR-warmed mesocosms at 2 °C warmer than their paired control tank. Surface temperature means were logged every 5 minutes, and used to calculate hourly average surface temperatures for analysis. Un-warmed mesocosms had identical non-functional heater assemblies as the warmed mesocosms to standardize any potential infrastructure effects. To minimize wind interference with the warming treatment during the winter and spring, all mesocosm perimeters were enclosed within 0.6 m tall clear plastic sheeting (December to June). We excluded snow by covering mesocosms with tarps during snow events for six weeks following the first snow storm of the year. This began on 14 December 2013 and 9 December 2014 for winters 2013/2014 and 2014/2015, respectively. Prior to initiation of snow exclusion, we allowed the first two inches of snow to accumulate to maintain consistent albedo across treatments.

2.3.2. Environmental measurements

Soil temperature in each mesocosm was measured at 1, 5, 10, 30, and 60 cm depths using type T (copper/constantan) thermocouples (Omega Engineering, Inc., Stamford, CT, USA, #FF-T-24-TWSH-SLE-1000). Temperature values from all thermocouples and radiometers were scanned every 30 or 60 seconds in winters 2013/2014 and 2014/2015, respectively. Mean values were recorded every 5 minutes with a CR1000 datalogger and used to calculate hourly average temperatures for analysis.

During the snow-free period, we measured soil moisture weekly in each mesocosm in 8 (2014) or 6 (2015) locations using a FieldScout Time Domain Reflectometer (TDR) 300 Soil Moisture Meter (Spectrum Technologies, Aurora, IL, USA). We calibrated TDR measurements with gravimetric soil moisture, which was determined by oven drying a soil sample previously measured with the TDR at 60 °C to constant mass.

From the first snowfall in winter until snowmelt in spring, we measured soil frost depth weekly, and snow depth three times weekly. Soil frost depth was determined using frost tubes (Iwata et al. 2012), which consisted of tygon tubing filled with 0.03% methylene blue solution and inserted into a PVC pipe previously installed vertically into the soil to 60 cm depth. Soil freezing around the tube caused the water in the methylene blue solution to freeze, and the dye to be pushed into the unfrozen liquid portion. We measured the resulting clear frozen segment to determine soil frost depth. Meter sticks affixed to the above-ground portion of the frost tube were used for weekly snow depth measurements. Snow depth and soil freezing depth were examined by calculating the area under the curve (AUC) using the trapz command in the R pracma package (version 2.1-4, Borchers 2018) with date and depth as the independent and dependent variables, respectively. This provided an integrated metric of the depth and duration of snow and frost depth (Durán et al. 2014).

2.3.3. Sapling phenology and identifying asynchronies

Spring plant phenology was assessed with a numerical rating system modified from West and Wein (1971) in which each stage is carefully defined (Table S4) to allow for quantitative comparisons. In spring 2014, bud and leaf development of all saplings were visually assessed weekly until the completion of foliar expansion. In spring 2015, the same protocol was used on a subset of four saplings of each species per mesocosm due to over-winter mortality. In all cases, the most advanced bud expansion phenological stage on the plant was identified as well as the percentage of the plant that had reached that stage. Mesocosm-level average plant phenological stage by date was then calculated for analysis.

Fall leaf phenology was measured in 2014 by digital assessment of vegetation color. From the onset of fall until leaf drop, weekly photos were taken of each mesocosm from the exact same location and angle from four vantage points that aligned with the cardinal directions (4 photos/mesocosm/day). Using ImageJ software (Schneider et al. 2012), pixel color (green, yellow, or red) was identified according to the unique spectral range of each color. The color thresholder function in ImageJ was used to quantify green and yellow pixels according to the hue, saturation, and brightness (HSB) color space, which defines a color in terms of those HSB components. Threshold ranges of HSB for the analysis had no overlap between green and yellow, and were defined as follows: green (H: 50-141, S: 0-255, B: 0-255), yellow (H: 30-49, S: 0-255, B: 110-255), and background (H: 0-255, S: 0-255, B: 0-255). The L*a*b* color space model function was used to define the red color class to solve the complication caused by the presence of red objects in the images that were not foliage (e.g., stems and instruments). This model represents colors in three-dimensional space, with one axis for luminance (L) and two for colors (a and b). The threshold ranges used for red were L (122-209), a (126-255), and b (163-201). There was unavoidable minor overlap between the definitions of red and yellow due to the complexity of the red color class. A mesocosm-level mean for each color on each date was calculated by averaging the values from the four images. The sum of green, yellow, and red pixels represented all the foliage in each mesocosm, and the green pixels represented potentially active (photosynthesizing and transpiring) foliage.

Soil temperature data were cross-referenced with fall and spring phenology data to identify plant-microbe activity asynchronies as times when soil temperature allowed biological (microbial) activity but plant metrics indicated dormancy. In practice, this could occur anytime between the diminution of plant activity in autumn through leaf expansion in spring (i.e., the overwinter period), depending on soil temperatures. Although belowground biological processes do occur under the snowpack (Brooks et al. 2011), the rate is relatively low until soils reach 4 °C, at which point rapid biological activity is thought to begin (Groffman et al. 2012). Therefore, soil temperature of at least 4 °C at 5 cm depth was considered indicative of biologically active soils.

Because active foliage drives bulk transpiration and nutrient uptake, leaf condition provides evidence of water and nutrient flow from soils through the plant. Much prior work has shown that nutrients are more tightly held by ecosystems during the growing season when plant and microbial activity levels are high (e.g., Mitchell et al. 1992, Mitchell et al. 1996). The potential for asynchronies was therefore considered to begin in the fall on the vegetation downturn day (DD), as defined by the mesocosm-level fall phenology curve, which marks the day on which canopy photosynthetic capacity begins to decline (Gu et al. 2009). For each mesocosm, the curve of percent green pixels during fall 2014 was fit by a method proposed by Klosterman et al. (2014) and the DD was identified using the greenProcess function in R phenopix package (Filippa et al. 2017). The potential for asynchronies was considered to end when plants reached the stabilization date (SD) in spring, which marks the end of the rapid leaf recovery and expansion phase of spring leaf out (Gu et al. 2009). The SD was identified for each mesocosm by fitting the phenology curve and identifying phenophases as described by

Gu et al. (2009) using the greenProcess function in R package phenopix (Filippa et al. 2017). Prior to analysis, spring and fall phenology trajectories were mirrored to achieve a U shape that met the mathematical requirement of the curve fit equations. Because climate treatments began in December 2013, that date, as opposed to the DD, was considered the onset of potential asynchronies for the first year of the experiment.

During plant dormancy, asynchrony length was calculated as the number of days with mean daily soil temperature above 4 °C at 5 cm depth during daylight hours. Total asynchrony length was assessed during the entire period of plant dormancy, from the DD in fall (for year 1, 12/3/2013 when treatments started) until the SD in spring. Asynchrony length was additionally calculated for each year between dates when the mesocosm leachate was pumped during plant dormancy (winter 1: 2/15/2014 - 5/8/2014; winter 2: 11/10/2014 - 5/7/2015). This allowed us to assess the statistical significance of asynchrony length on soil water C and nutrient losses. Finally, seasonal effects were assessed by determining asynchrony length during the following periods: (1) fall: from the DD until the last day soils were above 4 °C; (2) winter: from the last day soils were above 4 °C in fall until the first day they were above 4 °C in spring; (3) spring: from the first day soils were above 4 °C until the SD.

2.3.4. Leachate collection and analyses

Water level in each mesocosm was quantified weekly during the snow-free period by inserting a measurement rod into the center tube (Fig. 1), which reached to the bottom of the leachate drainage area. To prevent artificial soil saturation, leachate was pumped out when the water level rose to the height of the leachate drainage area. The water volume removed was measured by a totalizer and a sample was collected, filtered using 0.45 µm nylon filters (Fisher Scientific, Hampton, NH, cat. no. 09-719-008), and frozen until analysis.

Leachate samples were analyzed for inorganic N (Ammonium (NH_4^+) and nitrate-nitrite (NO_3^- + NO_2^-, hereafter referred to as NO_3^-)), P (PO_4^{3-}), dissolved organic C (DOC), total dissolved nitrogen (TDN), and cations prone to loss following soil freezing and soil acidification (Ca, Mg, Al). Nitrate and PO_4^{3-} were quantified colorimetrically using a Lachat QuikChem 8000 flow-injection analyzer (Lachat Instruments, Hach Company, Loveland, CO, USA). Ammonium was quantified using a salicylate method modified from Weatherburn (1967) and analyzed with a Synergy HT Microplate Reader (BioTek Instruments, Winooski, VT, USA). Dissolved organic C and TDN measurements were done using a Total Organic C Analyzer (Shimadzu TOC-L with TNM-L, Columbia, MD, USA) by sample combustion followed by infrared gas analysis and chemiluminescence for DOC and TDN, respectively. Lastly, Ca, Mg, and Al were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) on an Optima 3000DV (Perkin Elmer, Inc., Boston, MA, USA). Concentration of each nutrient species was multiplied by the volume of leachate pumped on each day to calculate total losses on each sampling day. Daily losses were summed by year and asynchrony period to express totals lost during the course of each experimental interval.

2.3.5. In situ N mineralization and nitrification

In situ net N-mineralization and nitrification were quantified using an intact core method (Durán et al. 2012) during three periods spanning from November 2014 - July

2015: overwinter (11/16/2014-4/23/2015), in early spring (4/22/2015-6/3/2015), and during the growing season (6/3/2015-7/6/2015). In this method, two soil cores were collected from each mesocosm, one of which was enclosed in a polyethylene bag and returned to the soil to incubate. The other core was sieved to remove particles greater than 2 mm, subsampled, and extracted with 2 M potassium chloride (KCl) in a 1:10 soil:KCl ratio. Concentrations of NH_4^+ and NO_3^- were quantified using a salicylate method modified from Weatherburn (1967), and the vanadium method of Doane and Horwáth (2003), respectively, and analyzed on a Synergy HT Microplate Reader. For each field incubation, potential N mineralization was calculated as the accumulation of total inorganic N (NH_4^+ + NO_3^- + NO_2^-) and potential net nitrification was calculated as the accumulation of nitrate (NO_3^-). Ammonium levels were below analytical detection limits, so we only present results for nitrification.

2.3.6. Soil CO_2 efflux

Soil CO_2 efflux was measured biweekly during the snow free period using a Li-Cor 8100A Automated Soil CO_2 Flux System (Lincoln, Nebraska, USA). In each mesocosm, measurements were taken from the same two permanently-installed collars to reduce variability across measurements. Linear fluxes were calculated using SoilFluxPro software (version 4.0, LI-COR Biosciences, Inc, Lincoln, Nebraska, USA). Soil CO_2 efflux from the two collars were averaged to calculate one mean soil respiration value per mesocosm per day. Yearly cumulative soil CO_2 flux was calculated using the trapz command in the R pracma package (R Core Team 2017, Borchers 2018), with date and CO_2 flux as the independent and dependent axes, respectively.

2.3.7. Statistical analyses

All statistical analyses were performed in R (R Core Team 2017). Effects of experimental climate treatment and soil treatment on surface and soil temperatures and soil moisture were determined using linear mixed effects models in the R nlme package (version 3.1-131, Pinheiro et al. 2017) with mesocosm as a random effect to account for non-independence due to repeated measures (Zuur et al. 2009). Day of year (doy) and a quadratic day of year term (doy^2) were included in the surface and soil temperature models to account for nonlinearity in temperature by day relationships.

Snow and soil freezing AUC, cumulative CO_2 flux, nitrification rates, and leachate loss of C (DOC) and other nutrients (TDN, NO_3^- , NH_4^+, PO_4^{3-}, Ca, Mg, Al), both for the full year and during seasonal asynchronies, in response to soil treatment and climate treatment were determined using generalized least squares (gls) models in the R package nlme (Pinheiro et al. 2017). Despite its potential importance to water and nutrient dynamics, plant biomass could not be included as a model covariate due to collinearity with soil treatment; fine soils supported 25% more biomass relative to the coarse soils (X^2 = 7.2, p = 0.007, R^2 = 0.26). Asynchrony length and climate treatments were also highly collinear (variance inflation factor, VIF > 10), so leachate losses of C and nutrients during asynchronies were examined as a function of soil treatment and climate treatment, and soil treatment and asynchrony length in separate models. In all cases, significance of model terms was determined with type 3 (partial) Analysis of Deviance models conducted in the R car package (Fox and Weisberg 2011).

For all models, assumptions of constant variance and normality were assessed by visual inspection of residual plots. When necessary, variance structures were constructed for categorical and continuous variables using the varIdent and varPower functions, respectively, in the nlme package (Pinheiro et al. 2017), and power transformations were applied to non-normal data. Results were considered significant at $p < 0.05$. Unless otherwise noted, all reported values are means plus or minus one standard error of the mean.

2.4. Results

2.4.1. Treatment effectiveness

Climate treatments significantly altered mean surface and soil temperatures year-round, and treatment and soil treatment both influenced water dynamics. Infrared warming achieved an average increase in surface temperature of 2.04 °C ± 0.001 °C (X_1^2 =223.2, $p < 0.0001$, $R^2 = 0.66$). Relative to control mesocosms, snow exclusion significantly decreased and warming significantly increased mean soil temperature to a depth of 60 cm ($p < 0.05$; Fig. 2; Table S3). There was no difference associated with soil treatment in surface temperature or soil temperatures. In all cases, both doy and doy^2 had significant effects on surface and soil temperature ($p < 0.0001$). In 2014, the fine soil held twice as much moisture as the coarse soil ($X_1^2 = 72.6$, $p < 0.0001$, $R^2 = 0.16$), a difference that increased to 2.25 times in 2015 ($X_1^2 = 107.8$, $p < 0.0001$, $R^2 = 0.34$). Both warming and snow exclusion reduced soil moisture by ~20% in 2015 ($X_1^2 = 7.1$, $p = 0.03$), as opposed to 2014 which did not have any significant treatment effects on soil moisture. Volume of leachate collected similarly varied by both soil treatment and climate treatment. In 2014, 10% more leachate volume was collected from the coarse soils than fine soils ($X_1^2 = 8.7$, $p = 0.003$, $R^2 = 0.52$), and warming reduced leachate volume by 14% relative to control ($X_2^2 = 10.5$, $p = 0.005$). In 2015, soil treatment and climate treatment interacted such that on both coarse and fine soils, warming reduced leachate volume by ~20%, and on fine soils snow exclusion reduced leachate volume by 30% (X_2^2= 6.3, p = 0.04, R^2 = 0.36).

Warming and snow exclusion significantly altered winter snow and soil freezing dynamics. Both climate treatments reduced snow pack AUC compared to controls. Patterns were consistent between years, with the warmed treatment having the smallest snowpack followed by snow exclusion and control mesocosms having the largest snow AUC (Table S3, 2014: X_2^2 = 399.1, p < 0.0001, R^2 = 0.94; 2015: X_2^2 =294.7, p < 0.0001, R^2 = 0.94). Snow exclusion significantly increased soil freezing AUC during both winters of the study, as did the warming treatment, but only in 2014 (Table S3, 2014: X_2^2 =187.6, p < 0.0001, R^2 = 0.89; 2015: X_2^2 =9.2, p = 0.01, R^2 =0.36). As compared to control, soil frost AUC was more than doubled by snow exclusion in 2014. That year, warming also increased soil frost AUC by 18% relative to control. Delayed onset of snowfall in 2015 in all mesocosms followed by below freezing temperatures allowed a deep soil freezing layer to establish in all mesocosms prior to onset of the snow exclusion treatment (Fig. S1). This led to reduced differences in soil freezing depth and duration among treatments as compared to 2014, with 2015 soil frost AUC 10% greater in snow exclusion than control, which was comparable to the warming treatment.

2.4.2. Carbon loss

Treatment effects on C losses (CO_2 efflux and leachate DOC) varied across time. In 2014, warming increased soil CO_2 efflux 30% over control, which was comparable to snow exclusion (X_2^2 = 6.1, p = 0.048, R^2 =0.20). Soil CO_2 efflux did not vary by soil or climate treatment in 2015. Conversely, climate treatment and soil treatment interacted to alter DOC leaching only in the second year of the experiment (Table 1, Fig. S2). That year, DOC loss from coarse soil control (908 mg ± 121) and warmed (990 mg ± 27) treatments increased 50% relative to coarse soil snow exclusion (601 mg ± 10), which was comparable in magnitude to the lower losses measured in all treatments of the fine soil (control: 548 mg ± 88; warmed: 645 mg ± 133; snow exclusion: 640 mg ±10).

2.4.3. Nitrification and nitrogen loss

Effects of soil and climate treatment on nitrification rates varied by sampling period. During the 2014/2015 winter nitrification rates were 26 times higher in warmed coarse soils compared to the other soil-treatment combinations (significant soil × treatment interaction; X_2^2 = 16.3, p = 0.0003, R^2 = 0.68; Fig. S3a). In the spring, nitrification in warmed mesocosms was nearly twice that of controls, which were comparable to snow exclusion mesocosms (significant treatment effect; X_2^2 = 7.2, p = 0.03, R^2 = 0.16; Fig. S3b). Finally, in the summer, coarse soil nitrification rates were 56% higher than in fine soils (significant soil effect; X_1^2 =4.0, p = 0.046, R^2 = 0.27; Fig. S3c).

Similar patterns emerged in TDN and NO_3^- losses, which significantly varied across climate treatments by soil treatment, or by soil alone in 2015 (Table 1). In 2014 (Fig. S4a), TDN loss from fine soils was 110% higher from snow exclusion (935 mg ± 75) and 75% higher from warmed (781 mg ± 104) than controls (446 mg ± 32). TDN loss from coarse soils did not vary by treatment (control: 938 mg ± 209; warmed: 768 mg ± 130; snow exclusion: 721 mg ± 153). In 2015 (Fig. S4b), TDN loss from coarse soils (503 mg ± 135) was roughly twelve times as great as the loss from fine soils (41 mg ± 8).

The interaction of soil treatment with climate treatment maintained similar patterns as in 2014, such that in fine soils TDN loss from snow exclusion (74 mg ± 6) and warmed (34 mg ± 5) mesocosms increased 463% and 161%, respectively, relative to control (13 mg ± 7). In coarse soils, warmed (704 mg ± 259) and control TDN losses (537 mg ± 302) were roughly twice as great as from snow exclusion (267 mg ± 95; soil × treatment interaction).

Significant NO_3^- loss dynamics were similar to those observed in TDN (Table 1, Fig. 3). In 2014, the effect of climate treatment on NO_3^- loss varied by soils (soil × treatment interaction, Fig. 3a). On fine soils, loss of NO_3^- from snow exclusion (1029 mg ± 90) and warmed (482 mg ± 110) mesocosms increased 440% and 150%, respectively, compared to control (191 mg ± 52). On coarse soil, NO_3^- loss from warmed mesocosms (573 mg ± 124) was elevated 50% relative to snow exclusion (388 mg ± 63), with losses from control mesocosms (467 mg ± 177) falling between the two experimental treatments. Much like TDN, NO_3^- loss from coarse soils (507 mg ± 125) in 2015 was ~17 times the loss from fine soils (30 mg ± 9), and climate treatment effects varied by soil treatment (soil × treatment interaction, Fig. 3b). In 2015, coarse soil NO_3^- loss from warmed (723 mg ± 224) and control mesocosms (516 mg ± 290) was elevated over snow exclusion (281 mg ± 63). Conversely, snow exclusion (55 mg ± 18) on fine soils in 2015 resulted in nearly four times as much NO_3^- loss as from controls (15 mg ± 5), which experienced similar losses as the warmed mesocosms (21 mg ± 14). Unlike TDN and NO_3^-, NH_4^+ dynamics did not vary by soil or climate treatment either year of the experiment.

2.4.4. Phosphorus and cation loss

Both PO_4^{3-} and Ca losses varied significantly by soil treatment, and Ca additionally varied by climate treatment in 2015 (Table 1). Fine soils (2014: 39 mg ± 3; 2015: 37 mg ± 3) experienced 23% and 60% higher PO_4^{3-} losses than coarse soils (2014: 32 mg ± 1.7; 2015: 23 mg ± 1) in 2014 and 2015, respectively. Conversely, Ca losses were roughly twice as high from coarse (2014: 66 g ± 3; 2015: 73 g ± 2) as from fine soils (2014: 33 g ± 2; 2015: 43 g ± 2) both years of the experiment. Additionally, in 2015 Ca losses from control mesocosms (65 g ± 6) were higher than from both warmed (55 g ± 5) and snow exclusion (55 g ± 7). Effects of soil treatment and climate treatment on Mg and Al varied across years with no consistent patterns (Table 1, Supplementary Results).

2.4.5. Plant-microbe asynchrony length

Warming lengthened asynchronies during the entire period of plant dormancy, and the effect of snow removal varied between years and soil treatments. In 2014, warmed and snow exclusion mesocosms experienced on average three more and three fewer asynchrony days than controls, respectively (Fig. 4a, X_2^2 = 6.9, p = 0.03, R^2 = 0.39). In 2015, warming increased asynchrony length by 18 days relative to control and snow exclusion, which had comparable asynchrony lengths (Fig. 4b, X_2^2 = 19.3, p < 0.0001, R^2 = 0.55). Asynchrony length measured between pumping dates for comparison with leachate chemistry also varied significantly by climate treatment and soil treatment. In the first year, warming increased asynchrony length by an average of three and a half days over controls, which were similar to snow exclusion (X_2^2 = 17.2, p = 0.0002, R^2 = 0.66). In the second year's period between pumping dates, treatment had a significant effect (X_2^2 = 119.7, p < 0.0001, R^2 = 0.91) but it varied across soils (soil × treatment interaction, X_2^2 = 13.8, p = 0.001). On coarse soils, warming increased and snow exclusion decreased asynchrony length by an average of eight and four days, respectively. On fine soils, warming increased asynchrony length by an average of 17.5 days over controls, which were comparable to snow exclusion.

Effects of climate treatment and soil treatment on asynchrony length varied by season. Warmed mesocosms experienced on average one additional day of asynchrony as compared to control in the first winter (Fig. S5a, X_2^2 = 6.0, p = 0.05, R^2 = 0.39), and treatment did not alter winter asynchrony in the second winter (Fig. S5b). Spring asynchrony length varied by both climate treatment and soil treatment, with effects differing between years. In 2014, warmed mesocosm soils reached a daily mean temperature of 4 °C during daylight hours four days earlier than control or snow exclusion soils (Fig. S6a, X_2^2 = 13.3, p = 0.001, R^2 = 0.61). Climate treatments also altered plant stabilization date (SD, the end of the rapid leaf recovery and expansion phase of spring leaf out) in 2014, with the effect varying across soils (soil × treatment interaction, X_2^2 = 7.2, p = 0.03, R^2 = 0.25). The plant mix growing on coarse soils under warming or snow exclusion reached its SD on average two days earlier than controls. However, on fine soils, the plants that experienced warming or snow exclusion reached their SD on average two days later than controls. Overall in 2014, the spring asynchrony in warmed mesocosms was on average three days longer than in controls, and in snow exclusion it was five days shorter (Fig. S5c, X_2^2 = 8.2, p = 0.02, R^2 = 0.30). Warming accelerated spring soil warming in 2015 as well (X_2^2 = 32.4, p < 0.0001, R^2 = 0.78), but the magnitude of the effect varied by soil (Fig. S6b, soil × treatment interaction, X_2^2 = 8.5,

p = 0.01). Warmed coarse soils reached 4 °C on average three days earlier than controls, and warmed fine soils reached 4 °C on average 11 days earlier than controls. That year, the plant mix SD did not respond to climate treatment or soil treatment. The overall effect of asynchrony length measured in spring 2015 varied by soil (Fig S4d, soil × treatment interaction; X_2^2 = 10.9, p = 0.004, R^2 = 0.82). On coarse soils, warming increased asynchrony length by an average of three days, and snow exclusion decreased it by an average of nearly three days relative to controls. However, on fine soils warming increased asynchronies by ten days as compared to control, which was comparable to snow exclusion. Finally, fall asynchrony was unaffected by climate treatment or soil treatment (Fig. S5e). Neither plant DD, the last day soils were above 4 °C, nor the asynchrony length were significantly altered.

2.4.6. Carbon and nutrient loss during asynchronies

Unlike our year-round findings, DOC loss during asynchronies was significantly impacted by climate treatment and effects varied across years (Table 2). Warming increased asynchrony DOC losses by 50% in 2014 (2,230 mg ± 298) relative to controls (1,494 mg ± 277), which had similar loss as from snow exclusion (1,410 mg ± 187). In 2015, warmed mesocosm DOC losses (618 mg ± 68) were significantly higher than from snow exclusion (434 mg ± 44), and control losses (494 mg ± 91) were intermediate between the two. Conversely, that year the dominant effect was a soil × treatment interaction for the year-round data. As opposed to the significant soil × treatment effect on TDN loss in 2014, there were no significant effects observed during the asynchrony that year. Finally, climate treatment significantly affected NO_3^- loss during asynchronies, but the dominant effect was the soil × treatment interaction, as throughout the rest of the year (Table 2). All other significant effects observed during asynchronies were the same as those previously described year-round (Supplementary Results).

Carbon and nutrients leached during asynchronies showed significant relationships to both soil and length of asynchrony (Table 3, Fig. 5). In 2014, DOC did not vary significantly with asynchrony length. However, in 2015, longer asynchronies resulted in higher losses of DOC. TDN loss also did not vary with asynchrony length in 2014, but in 2015 coarse soils leached more TDN during longer asynchronies, whereas fine soils did not. Nitrate losses in 2014 were increased by longer asynchronies, and in 2015 the effect varied by soil in the same way as TDN, such that NO_3^- losses from coarse soils, but not fine soils, were increased during longer asynchronies. In 2014, average PO_4^{3-} loss during asynchronies was higher from fine soils than coarse soils. That year on fine soils, PO_4^{3-} loss decreased slightly during longer asynchronies, while on coarse soils there was no relationship between PO_4^{3-} loss and asynchrony length. In contrast, in 2015 soil treatment was not significantly related to PO_4^{3-} loss, and asynchrony length had a negligible effect on loss from fine soils, but longer asynchronies resulted in lower PO_4^{3-} loss from coarse soils. Calcium loss during asynchronies was on average higher from coarse than fine soils both years, but in 2014 longer asynchronies tended to reduce Ca losses on coarse soils, while on fine soils the effect was negligible (no significant length x soil interaction in 2015). Ammonium and Al losses did not vary by soil treatment or asynchrony length, and the effect of soil treatment and asynchrony length on Mg loss varied across years (Supplementary Results and Fig. S7).

2.5. Discussion

We found that the impacts of warming and snow removal varied with soil treatment. In general, the coarse (Ca-rich) soil experienced higher losses of C and most nutrients (except PO_4^{3-}) than fine soils. Additionally, fine snow exclusion soils experienced elevated NO_3^- losses (vs. controls), but coarse soils did not. Warming increased plant-microbe asynchronies, leading to higher losses of C and N, and lower losses of PO_4^{3-} and Ca. Soil type also modified asynchrony length, and the relationship between asynchrony length and nutrient loss. Overall, our results provide evidence that climate treatment interactions with soil properties are extremely prevalent and an important determinant of the magnitude of the effects of climate change on plant microbe synchrony and biogeochemistry.

2.5.1. Treatment impacts on abiotic factors

Warming and snow exclusion achieved their intended effects, which respectively were to raise surface temperatures by an average of 2 °C and induce winter time deep soil freezing that is associated with a diminished snowpack. As a consequence, the warmed treatment increased soil temperatures to 60 cm and reduced snowpack depth. Snow exclusion decreased soil temperatures to 60 cm and reduced snowpack depth. However, between-year variation in winter air temperatures and onset of snowpack altered wintertime treatment impacts. This natural variation resulted in significant differences in treatment impacts between winters. In 2014, warming reduced early winter snowpack, causing soils to freeze deeper than in controls (Fig. S1). Conversely, in 2015 warmed mesocosms experienced overall less soil freezing than controls due to faster soil thaw in the spring (Fig. S1). Importantly, late snowpack development in 2015 allowed deep soil freezing in all mesocosms that persisted under the insulating snowpack as seen in previous studies (Goodrich 1982, Hardy et al. 2001).

Notably, soil treatment and climate treatment contributed to variation in soil moisture throughout the experiment, with the coarse soil consistently drier and soils in the warming and snow exclusion treatments drier relative to controls in the second year. Although fine soils were twice as moist as coarse soils, the leachate drained from coarse soils was only 10% higher than from fine soils. Variation in evapotranspiration by soil treatment likely explains the remaining difference in soil moisture. The trajectory of plant growth and survival throughout the experiment also likely played a role in differential response to treatments between the two years. From 2014 to 2015, overall plant biomass increased (Kosiba 2017), increasing water and nutrient uptake.

2.5.2. (H1) Warming and snow exclusion did not have strong impacts on C and nutrient loss that were independent of soil treatment.

Warming and snow exclusion increased C and nutrient losses compared to control conditions, but in most cases soil treatment strongly interacted with these direct effects. The few independent effects of warming on C and nutrient losses occurred inconsistently throughout the experiment, likely due to reduced soil moisture in that treatment in 2015. Warming increased growing season soil CO_2 efflux and asynchrony DOC loss in 2014, as well as early spring nitrification in 2015. The lack of a soil CO_2 efflux and DOC response to warming in 2015 could be related to the drying impact of warming that same year. Soil moisture limitations under warming can reduce microbial activity and associated soil respiration (Liu et al. 2009). In the early spring, warming increased net nitrification by 50%, slightly higher than the average increase of 32% attributed to experimental warming (Bai et al. 2013). The lack of a summertime nitrification response to warming may have also been related to reduced soil moisture in warming treatments during the summer, when plant activity dominates terrestrial water movement (Jasechko et al. 2013). Thus, early spring water availability during plant dormancy combined with warmer temperatures in the warmed treatment could explain the ephemeral nature of the nitrification response to warming.

Finally, Ca loss from controls exceeded that from snow exclusion during the asynchrony, and from both climate treatments during the second year. Conversely, watershed studies found Ca loss to increase following soil freezing events (Fitzhugh et al. 2003b), however on the plot scale the effect depended on the dominant tree species (Fitzhugh et al. 2003a). In those cases, Ca leaching accompanied acidification of soil solution by mobilized NO_3^- in sugar maple, but not yellow birch, stands. Although fine soils under snow exclusion experienced elevated NO_3^- losses in our experiment, it was not accompanied by elevated Ca leaching as seen in previous studies.

2.5.3. (H2) Coarse textured soil experienced greater C and nutrient leaching, except for phosphate

In general, cation exchange capacity, clay content, and organic matter content all correlate with reduced leachate losses. In our study, the coarse soil exceeded the fine soil in each of these metrics (Table S1). Despite this fact, in all cases with a significant soil effect, except for PO_4^{3-}, leachate losses were higher from the coarse soil. It is likely that the high gravel content of the coarse soil reduced its water holding capacity compared to the fine soil, thereby diminishing its storage capacity for cations and nutrients (Dudley et al. 2008). The nutrients that experienced significant losses across years by soil treatment were Ca, which had higher losses from coarse soils, and PO_4^{3-}, which had higher losses from fine soils. The effect of soil on losses of all other nutrients varied across time, but in all significant cases, coarse textured soils experienced higher losses. The higher loss of PO_4^{3-} from fine textured soils, contrary to our expectations, could be due to its higher sand content (Table S1), which correlates with increased P leaching (Glæsner et al. 2011). Additionally, although both soil treatments had low clay contents, the high gravel content coarse soil had 20% more clay, which can bind to PO_4^{3-} and reduce leachate losses (Frossard et al. 1995, Yaghi and Hartikainen 2013, Tahir and Marschner 2017). Throughout the experiment, one of the largest differences we observed was the change in the magnitude of N loss (TDN and NO_3^-) across years. The coarse soil experienced roughly the same N loss both years, whereas N loss from the fine soil dropped to extremely low levels in 2015 (Fig. 3), despite no associated decrease in leachate volume, and causing a significant soil effect during the asynchrony and the year as a whole. Greater N uptake due to the higher plant biomass supported by the fine soil treatment provides one possible explanation for the large reduction in leachate loss. Furthermore, greater nitrification rates were measured on the coarse soil during the growing season, which could have created a pool of nutrients vulnerable to leaching given the low water holding capacity and larger volume of water leached from this soil.

2.5.4. (H3) Climate treatment effects varied by soil treatment.

The effects of climate treatments year-round and during asynchronies varied by soil treatment in losses of DOC, TDN, NO_3^-, Mg, and Al during one or both years, but not PO_4^{3-} or Ca. Nitrate loss demonstrated the most consistent pattern, varying by soil and climate treatment overall and during asynchronies in both years (Fig. 3). In all cases, fine soil snow exclusion NO_3^- loss exceeded control, whereas on coarse soil, snow exclusion losses were either equal to or lower than control. Increased NO_3^- loss following soil freezing has been well-documented (Mitchell et al. 1996, Fitzhugh et al. 2001, Groffman et al. 2001b, Campbell et al. 2014), although with variability (Groffman et al. 2011, Judd et al. 2011), and is attributed to root mortality (Tierney et al. 2001) and decreased root nutrient uptake (Campbell et al. 2014). Our results indicate that soil differences could additionally account for variability in the leaching response of NO_3^- to soil freezing. Warming also elevated NO_3^- loss from the fine soil both years and during the 2014 asynchrony, a response not observed in the coarse soil, perhaps due to differences in moisture availability. Effects of warming and snow exclusion on loss of DOC, TDN, Mg, and Al also differed by soil treatment, but the occurrence and patterns varied across time and period (year-round vs. during asynchronies).

2.5.5. (H4) Warming lengthened plant-microbe asynchronies, snow exclusion shortened vernal asynchronies, and asynchrony length varied with soil texture

Warming lengthened plant-microbial asynchronies, with the largest effect in the spring, and snow exclusion shortened vernal asynchrony with variation by year and soil treatment. Over the period of plant dormancy (fall to spring), warming consistently increased asynchrony length, with a much larger effect in 2015 (Fig. 4, 18 additional asynchrony days in 2015 vs 3 additional in 2014). Snow exclusion shortened asynchrony length only in 2014 (Fig. 4a), likely due to increased soil freezing depth and duration relative to other treatments that winter (all mesocosm soils were deeply frozen in 2015; Fig. S1). Most asynchrony days occurred in the spring (Figs. S4c and S4d), coinciding with regional evidence of lengthened vernal asynchronies (Groffman et al. 2012, Contosta et al. 2017), which can be protracted following warm winters with low snowpack (Contosta et al. 2017). In our experiment, the warming treatment had the shallowest snowpack and its soils thawed first, as compared to control and snow exclusion, likely explaining why warming prolonged asynchronies and snow exclusion occasionally shortened them. In 2015, spring asynchrony length also varied by soil treatment, such that the fine soil warming treatment had longer asynchronies than coarse soil warming treatment, and reductions of asynchronies with snow exclusion only occurred in the coarse soil treatment (Fig. S5d). Although the soil treatments did not significantly differ in soil frost AUC, the nature of soil frost can vary by soil texture and moisture (unsaturated vs. concrete frost; Gray and Granger 1986, Aksakal et al. 2019) and consequently thaw at different rates. In this case, the coarse soil snow exclusion thaw occurred later than control, shortening the vernal asynchrony on only coarse soils under snow exclusion. Climate treatments did not alter fall asynchrony length (Fig. S5e), as opposed to evidence that climate change delays fall phenology (Jeong et al. 2011), albeit with response heterogeneity (Dragoni and Rahman 2012). Finally, warming lengthened winter (vs. spring and fall) asynchrony only in 2014 (Fig. S5a), and the effect was very small (1 additional asynchrony day). The deep soil frost in all treatment soils in 2015 likely explains the lack of wintertime response that year.

2.5.6. (H5) Longer asynchronies correlated with greater C and N loss, and the response varied by soil treatment

Carbon and N losses often increased during longer asynchronies, with more consistent impacts in 2015 (Fig. 5). Loss of NO_3^- in 2014, and DOC in 2015 correlated with asynchrony length regardless of soil treatment. However, in 2015 the effect of asynchrony length on N (TDN and NO_3^-) loss varied by soil treatment, such that N losses on coarse soils increased with asynchrony length, but there was no effect on fine soils (Fig. 5), which experienced very low N losses that year overall (Fig. 3). Our finding that longer asynchronies correlate with greater C and N loss aligns with prior evidence that the timing of plant and microbial phenology critically control ecosystem nutrient loss (Muller and Bormann 1976, Brooks et al. 1998, Schmidt et al. 2007). And the variation in nutrient losses we observed by soil treatment coincides with previous findings that soil characteristics alter snow melt chemistry (Fahey 1979) and may explain the variable response of N leaching to soil freezing (Groffman et al. 2011). In addition to augmented nutrient losses during protracted asynchronies, longer vernal asynchronies can have lagged effects throughout the growing season, including reduced peak photosynthesis (Ouimette et al. 2018). Although the mechanisms driving these reductions in photosynthesis are currently unclear, the increased nutrient loss we observed under longer asynchronies provides a potential explanation.

As opposed to C and N, Ca losses (Fig. 5) declined with asynchrony length, and PO_4^{3-}(Fig. 5) and Mg (Fig S7) losses varied greatly by year and soil treatment. Reduced nutrient losses during longer asynchronies could reflect enhanced microbial uptake with augmented soil activity.

2.6. Conclusion

Our work provides evidence of the importance of soil properties in modifying the effects of climate change on ecosystem biogeochemistry. Our replicated climate change experiment on two soils clearly demonstrated that the occurrence and magnitude of biogeochemical losses depends on the interaction of climate treatment with soil. Furthermore, soil characteristics contributed to variation in the length of plant-microbe asynchronies, as well as the incidence and extent of C and nutrient loss experienced as a function of the length of the asynchrony. Our finding that warming increased asynchrony length provides more evidence that temporal shifts due to climate change may decouple plant and microbial activity. We found this decoupling to lead to increased loss of C and N, which could impact plant productivity, ecosystem level C storage, and ultimately represent a terrestrial ecosystem feedback to the climate system.

2.7. Acknowledgements

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2.8 Tables and Figures

2.8.1. Tables

Table 2.1. Analysis of deviance results (X^2, degrees of freedom (df), and *p* values) for 2014 and 2015 models of soil water leachate carbon and nutrients as a function of soil × treatment. Asterisks (*) indicate $p < 0.05$.

		2014				2015			
	(df):	Soil (1)	Trt (2)	Soil × Trt (2)	R^2	Soil (1)	Trt (2)	Soil × Trt (2)	R^2
DOC	X^2:	3.7	4.6	3.2	0.36	8.6	5.5	7.2	0.49
	p:	0.06	0.10	0.20		0.003*	0.07	0.03*	
TDN	X^2:	0.6	2.3	9.0	0.31	43.4	3.9	12.4	0.31
	p:	0.43	0.32	0.01*		<0.0001*	0.14	0.002*	
NO_3^-	X^2:	2.2	1.8	24.9	0.62	32.7	1.7	6.8	0.47
	p:	0.13	0.41	<0.0001*		<0.0001*	0.43	0.03*	
NH_4^+	X^2:	0.0	1.3	1.6	0.05	0	0.7	1.6	0.08
	p:	0.85	0.53	0.45		1.00	0.70	0.44	
PO_4^{3-}	X^2:	5.6	3.0	0.1	0.29	5.6	3.0	0.1	0.59
	p:	0.02*	0.22	0.96		0.02*	0.22	0.96	
Ca	X^2:	85.2	3.2	2.9	0.83	181.3	27.7	2.1	0.91
	p:	<0.0001*	0.21	0.24		<0.0001*	<0.0001*	0.35	
Mg	X^2:	3.8	4.1	4.9	0.31	150.8	18.0	23.8	0.91
	p:	0.05	0.13	0.09		<0.0001*	0.0001*	<0.0001*	
Al	X^2:	12.6	0.2	0.8	0.38	2.8	2.5	6.9	0.32
	p:	0.0004*	0.90	0.68		0.10	0.28	0.03*	

DOC: dissolved organic carbon, TDN: total dissolved nitrogen, NO_3^-: nitrate, NH_4^+: ammonium, PO_4^{3-}: phosphate, Ca: calcium, Mg: magnesium, Al: aluminum.

Table 2.2. Plant-microbe asynchrony analysis of deviance results (X^2, degrees of freedom (df), and *p* values) for 2014 and 2015 models of soil water leachate carbon and nutrients as a function of soil × treatment. Asterisks (*) indicate $p < 0.05$. Bold *p* values indicate different significance than during the entire year (Table 1). Note that although climate treatment was significant for $\mathbf{NO_3^-}$, the dominant effect remains the soil × treatment interaction, which was observed in the full year data as well.

		2014				2015			
	(df):	Soil (1)	Trt (2)	Soil × Trt (2)	R^2	Soil (1)	Trt (2)	Soil × Trt (2)	R^2
DOC	X^2:	2.0	6.1	4.2	0.37	4.1	6.5	5.8	0.39
	p:	0.17	**0.047***	0.12		0.04*	**0.04***	**0.06**	
TDN	X^2:	0.6	1.4	3.7	0.17	34.5	3.2	10.8	0.29
	p:	0.46	0.49	**0.15**		<0.0001*	0.20	0.004*	
NO_3^-	X^2:	2.7	14.2	10.8	0.59	79.2	6.9	11.9	0.37
	p:	0.10	**0.0008***	0.005*		<0.0001*	**0.03***	0.003*	
NH_4^+	X^2:	0.3	1.0	0.1	0	2.0	0.1	1.1	0.11
	p:	0.58	0.62	0.94		0.16	0.94	0.58	
PO_4^{3-}	X^2:	13.0	1.5	0.2	0.26	15.2	5.9	3.5	0.49
	p:	0.0003*	0.48	0.91		<0.0001*	0.05	0.17	
Ca	X^2:	32.6	0.9	3.7	0.66	93.8	32.7	3.1	0.86
	p:	<0.0001*	0.65	0.16		<0.0001*	<0.0001*	0.22	
Mg	X^2:	0.2	2.9	7.1	0.25	40.5	42.8	10.2	0.81
	p:	0.67	0.23	**0.03***		<0.0001*	<0.0001*	0.006*	
Al	X^2:	3.9	1.7	5.2	0.28	0.8	3.4	7.7	0.23
	p:	0.049*	0.43	0.07		0.39	0.18	0.02*	

DOC: dissolved organic carbon, TDN: total dissolved nitrogen, NO_3^-: nitrate, NH_4^+: ammonium, PO_4^{3-}: phosphate, Ca: calcium, Mg: magnesium, Al: aluminum.

Table 2.3. Analysis of deviance results (X^2, degrees of freedom (df), and *p* values) for 2014 and 2015 models of soil water leachate carbon and nutrients during plant-microbe phenological asynchronies as a function of soil × asynchrony length. To evaluate asynchrony length effects on leachate chemistry, we defined the length as the number of days with mean daylight soil temperatures over 4 °C at 5 cm depth between dates when mesocosms were pumped. Asterisks (*) indicate $p < 0.05$.

		2014				2015			
	(df):	Soil (1)	Length (2)	Soil × Length (2)	R^2	Soil (1)	Length (2)	Soil × Length (2)	R^2
DOC	X^2:	0.1	0.6	0.1	0.14	2.7	9.4	3.7	0.40
	p:	0.70	0.46	0.76		0.10	0.002*	0.05	
TDN	X^2:	1.0	0.6	1.1	0.16	2.3	4.5	4.9	0.58
	p:	0.31	0.46	0.30		0.13	0.03*	0.03*	
NO_3^-	X^2:	0.1	4.9	0.1	0.13	1.9	0.1	6.9	0.51
	p:	0.74	0.03*	0.75		0.17	0.72	0.009*	
NH_4^+	X^2:	0.0	0.3	0.0	0.12	0.5	0.2	0.4	0.06
	p:	0.97	0.59	0.99		0.47	0.69	0.51	
PO_4^{3-}	X^2:	5.3	0.6	4.1	0.71	1.9	2.4	4.0	0.24
	p:	0.02*	0.43	0.04*		0.17	0.12	0.046*	
Ca	X^2:	8.0	41.2	13.5	0.63	5.9	0.3	2.5	0.69
	p:	0.005*	<0.0001*	0.0002*		0.02*	0.61	0.12	
Mg	X^2:	0.2	3.9	0.0	0.63	9.4	0.6	6.8	0.56
	p:	0.6	0.048*	0.8		0.002*	0.43	0.009*	
Al	X^2:	0.1	0.0	0.1	0.08	0.0	3.8	0.2	0.1
	p:	0.75	0.94	0.81		0.84	0.05	0.69	

DOC: dissolved organic carbon, TDN: total dissolved nitrogen, NO_3^-: nitrate, NH_4^+: ammonium, PO_4^{3-}: phosphate, Ca: calcium, Mg: magnesium, Al: aluminum.

2.8.2. Figures

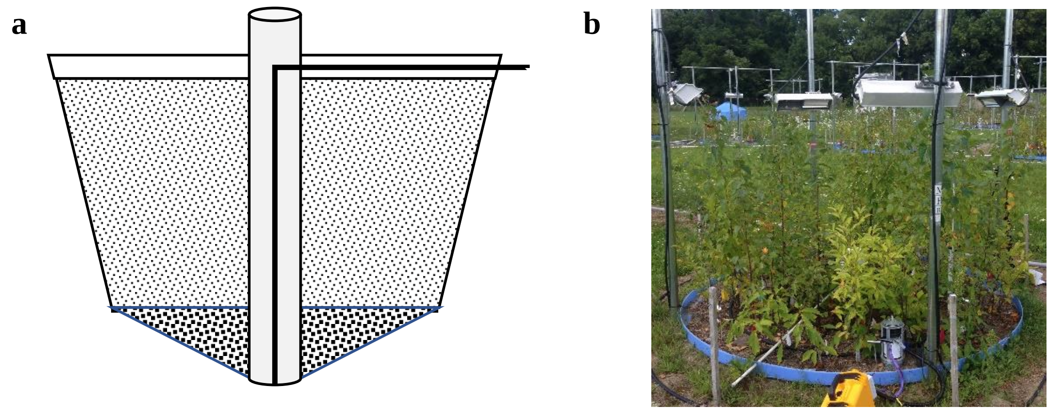

Fig. 2.1. (a) Cross section diagram of the mesocosms used for the NForM experiment. Coarse fill at the bottom represents gravel in the leachate drainage area, and the fine fill above it represents soil. The two layers were divided by landscape cloth. The top of the soil corresponds to the surrounding ground level. The dark line entering horizontally and bending 90 degrees through a center tube to the bottom of the mesocosm represents the tubing that allowed for leachate removal by pumping. (b) Photo of an installed mesocosm with the planted sapling community.

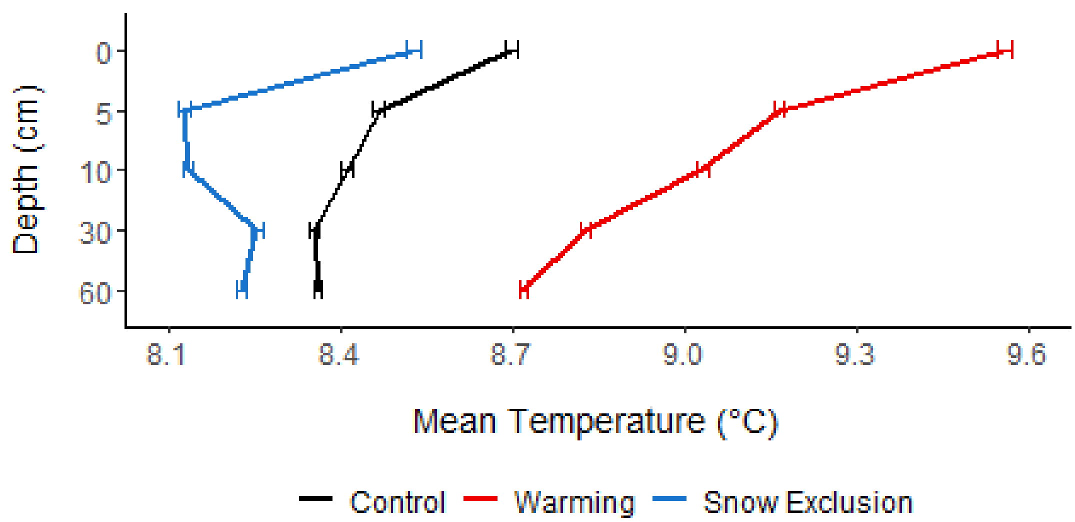

Fig. 2.2. Mean soil temperatures by depth for the duration of the experiment. Error bars are ±1 standard error.

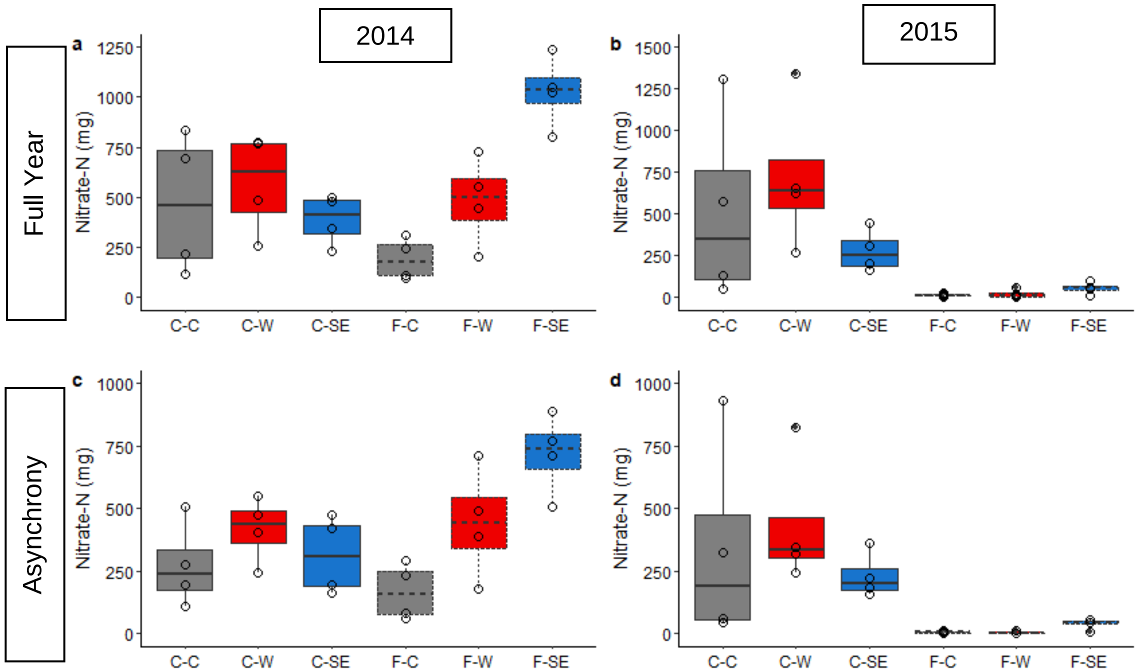

Fig. 2.3. Nitrate-N leachate loss from mesocosms in 2014 and 2015, both overall and during plant-microbe activity asynchronies: (a) 2014 $\mathbf{NO_3^-}$ leachate loss; (b) 2015 $\mathbf{NO_3^-}$ leachate loss; (c) 2014 asynchrony $\mathbf{NO_3^-}$ leachate loss; (d) 2015 asynchrony $\mathbf{NO_3^-}$ leachate loss. X axis codes are soil (C = coarse soil, solid lines or F = fine soil, dashed lines) followed by treatment (C= control (gray), W = warming (red), SE = snow exclusion (blue)). Note the different y axis limits in the panels. Open circles represent data points and filled circles represent outliers.

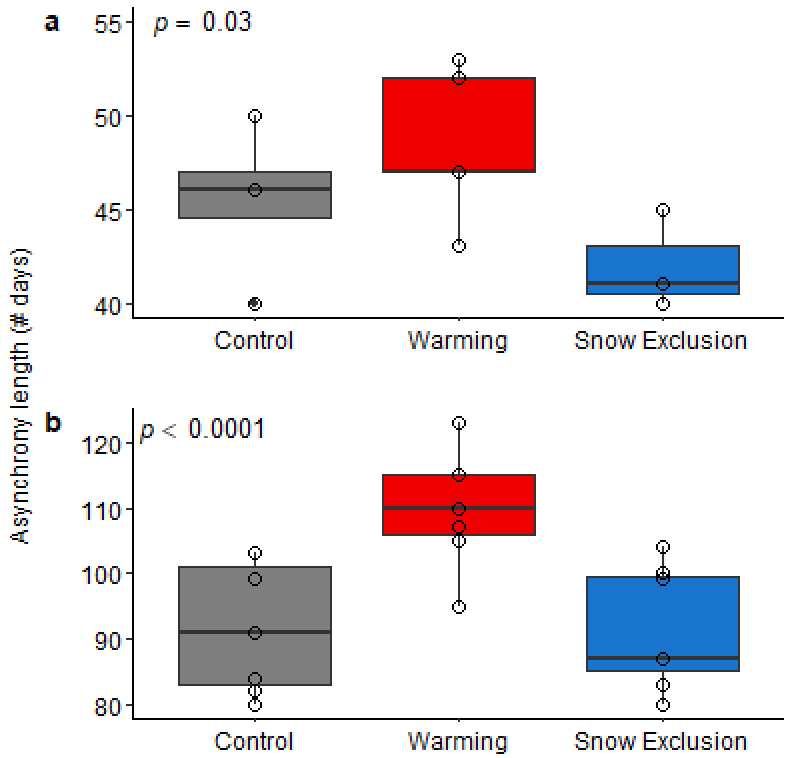

Fig. 2.4. Asynchrony length in (a) 2014 and (b) 2015. Asynchrony length was calculated as the number of days with daytime soil temperatures > 4 °C at 5 cm depth while plants were dormant. Note the varying y axis limits in each panel. Open circles represent data points and filled circles represent outliers.

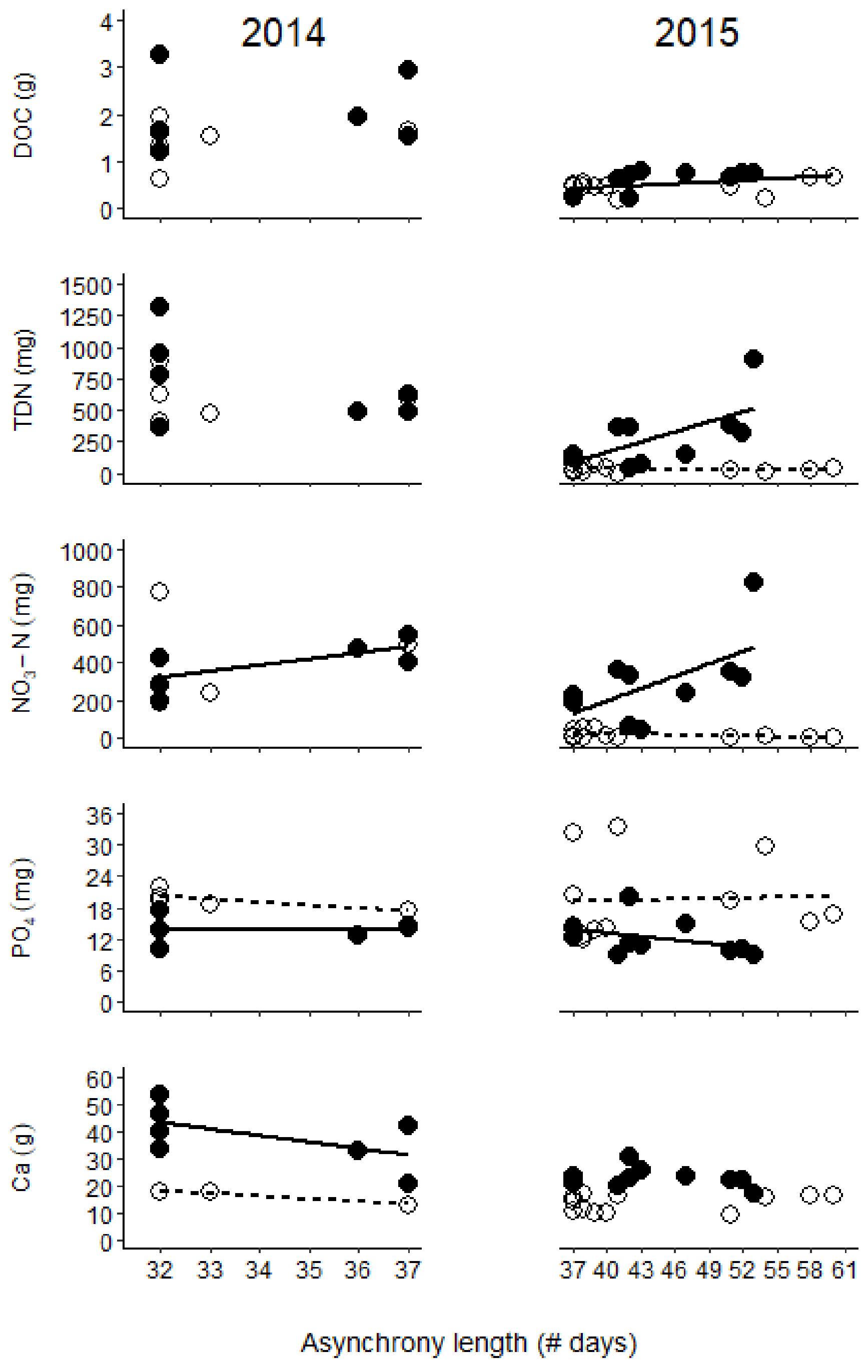

Fig. 2.5. Relationships between plant-microbe activity asynchrony length and leachate loss of carbon and other nutrients during plant-microbe asynchronies and soil type in 2014 (left column) and 2015 (right column). Asynchrony length was calculated as the number of days with daytime soil temperatures > 4 °C at 5 cm depth while plants were dormant. Regression lines are shown for significant relationships. One regression line indicates losses varied significantly with asynchrony length, and two regression lines indicate that the relationship of analyte loss to asynchrony length varied by soil type. Solid circles and lines represent the coarse soil, open circles and dashed lines represent the fine soil.

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CHAPTER 3: SIMULATED ICE STORMS ALTER SOIL FUNGAL ABUNDANCE, MICROBIAL ACTIVITY, AND CARBON AND NITROGEN CHARACTERISTICS WITH VARIATION BY SOIL HORIZON

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3.1. Abstract

As the climate changes, there is evidence that periodic, extreme weather events such as ice storms will increase in frequency and severity in the northeastern United States. Although rare, such extreme events may have an equal or greater impact on ecosystem structure and function than the gradual changes in temperature and precipitation associated with climate change. Ice storm damage can cause changes to forest structure that may cascade through the ecosystem to alter microclimate and impact the soil microbial community and soil processes. Here, we examined post-ice storm fungal abundance, extracellular enzyme activity (EEA), and the availability and chemical characteristics of soil carbon (C) and nitrogen (N). We examined these responses in a simulated ice storm experiment at the Hubbard Brook Experimental Forest (HBEF) that applied four levels of ice storm intensity (control, low, mid, high) and one level of increased frequency (mid × 2) to forested plots. Mid-levels of ice storm treatment increased fungal hyphal abundance. While C acquiring EEAs were not affected by ice storm frequency or intensity, ice storm treatments impacted both N and phosphorus (P) acquiring EEA. P EEA was highest in mid-levels of ice storm treatment, while the direction and response of N EEA to ice storms depended on soil horizon (organic or mineral). This provides evidence of variable elemental responses to extreme events associated with climate change. Additionally, the mid and mid × 2 treatments had opposite directional effects on enzyme stoichiometric ratios, suggesting that increased ice storm frequency can alter microbial response. Finally, ice accretion in some cases increased dissolved organic matter (DOM) aromaticity and decreased its humification, evidence of altered soil chemical composition following ice storms. Overall, our results demonstrate that ice storms can alter soil fungal abundance, microbial activity, and C and nutrient cycles, and that effects differ by soil horizon and element of interest (C, N, or P).

Keywords: Extracellular enzyme activity, fungal hyphal abundance, DOC, TDN, DOM biodegradability, climate change, extreme event

3.2. Introduction

Ice storms have potentially large impacts on ecological processes (Jentsch et al. 2007, Arnone et al. 2011) and are expected to increase in frequency and severity under climate change (Cheng et al. 2007, Cheng et al. 2011). However, ice storms are inherently difficult to study. It is therefore a challenge to understand how these events shape ecosystem processes and biogeochemistry and very little is known about the belowground effects of ice storms. Altered soil temperature, litter deposition, photosynthetic capacity, root growth, and carbon (C) allocation following ice storms impact the soil environment, which shapes both fungal abundance and microbial activity, with consequences for soil nutrient cycling.

Ice storms directly impact forest structure with potentially large impacts on belowground processes. Ice storms can result in broken twigs and trunks, loss of crowns, and damage to root systems (Cannell and Morgan 1989, Irland 2000, Smith 2000). By creating canopy gaps, altering litter deposition, and changing tree C allocation, ice storms alter soil microclimate and litter quality, with implications for microbial community composition and activity. Canopy gaps resulting from crown damage create new soil temperature regimes, both increasing temperatures and expanding temperature range (Rhoads et al. 2002, Likens et al. 2004, Rustad and Campbell 2012). Because temperature drives microbial activity and ecosystem processes, ice storm damage could result in faster biogeochemical cycling in affected stands. Crown damage additionally increases woody debris and decreases leafy litter, reducing leaf area and photosynthetic capacity (Rhoads et al. 2002, Rustad and Campbell 2012). This shift in litter quality results in more recalcitrant material (Rustad and Campbell 2012). Such changes in soil temperature and substrate characteristics could affect fungal abundance in addition to microbial activity (Garrett 1951, Frey et al. 1999, Staddon et al. 2003, Cregger et al. 2012, McGuire et al. 2012). For example, a meta-analysis of global belowground microbial community traits found fungal abundance increased relative to bacterial abundance with increasing soil carbon:nitrogen (C:N) (Fierer et al. 2009). Moreover, in the trajectory of recovery, plant growth and C allocation could vary and alter rhizosphere dynamics. Stands experiencing moderate to severe damage in the 1998 ice storm at the Hubbard Brook Experimental Forest (HBEF) had increased root growth compared to undamaged stands (Rhoads et al. 2002). Root traits influence microbial activity and community structure (Bardgett et al. 2014), as does C exudation through plant root systems (De Nobili et al. 2001, Ekblad and Nordgren 2002, Drake et al. 2013). Plants may respond to ice storm damage by increasing root activity and exudation to stimulate the microbial community and acquire more nutrients through its activity (Bengtson et al. 2012). Alternatively, plants experiencing reduced C assimilation due to reduced photosynthetic capacity could mobilize C and nutrients for canopy recovery (Millard and Grelet 2010), potentially reducing belowground microbial activity. In sum, ice storm damage alters soil temperature, quality of litter, and potentially plant C allocation, all of which may alter microbial activity and community composition.

Microbial activity drives ecosystem biogeochemistry, and changes to community composition or activity levels following ice storms could alter ecosystem C and nutrient cycling. For example, higher fungal to bacterial ratios cause greater soil C storage (Malik et al. 2016), while increased fungal abundance may decrease soil inorganic N availability (Bardgett and McAlister 1999). If altered litter chemistry following ice storms favors fungi, soil C storage could increase, and N availability decrease, as a result. Microbial activity also drives ecosystem biogeochemistry. Specifically, the breakdown of complex organic material into simpler forms by microbially-produced extracellular enzymes (EEs) is the first step of decomposition and mineralization (Wallenstein and Weintraub 2008), processes that drive biogeochemical cycling and availability of nutrients, form the base of soil food webs, and critically control soil biodiversity and function (Wallenstein et al. 2011, Sinsabaugh and Follstad Shah 2012). Substrate availability, EE concentration, soil physics, temperature, and pH all interact to determine extracellular enzyme activity (EEA) (Wallenstein and Weintraub 2008). For example, increasing substrate recalcitrance can lead to increased oxidative enzyme expression (Herman et al. 2008, Sinsabaugh 2010), a shift from phosphorus (P) acquiring to N acquiring enzymes (Frost et al. 2006, Allen and Gillooly 2009, Sinsabaugh and Follstad Shah 2011), and increased microbial C demand relative to N and P demand (Sinsabaugh and Follstad Shah 2011). Changes in litter deposition following an ice storm could therefore alter microbial EE expression by changing litter quality, namely increasing C richness of litter relative to nutrients, and potentially changing belowground C exudation. Substrate induced changes to EEA can alter the quantity and quality of C and other nutrients available for biological activity. For example, abiotic factors (i.e., temperature, moisture) and microbial activity produce dissolved organic carbon (DOC) (Guggenberger et al. 1994, Marschner and Kalbitz 2003, Cory et al. 2011), which fuels the microbial food web (Bott et al. 1984). However, dissolved organic matter (DOM) composition varies from easily biodegradable carbohydrates and organic acids to difficult to decompose aromatic and hydrophobic structures (Marschner and Kalbitz 2003). Because DOM contains organically bound nutrients, its biodegradability impacts nutrient availability and mobility (Kalbitz et al. 2000). Changes in litter quality following ice storms could, in sum, change microbial community composition and activity, which could alter nutrient biodegradability and cycling.

Here, we quantified the effect of ice storms of varying intensity and frequency on fungal abundance, microbial activity, and soil C and N. We sought to determine how ice storms of varying intensity and frequency: (1) alter fungal abundance; (2) affect microbial activity (i.e., EEA); and (3) impact the amount of soil C and N, and the biodegradability of DOM. We studied these dynamics in an experiment that simulated four intensities and two frequencies of ice storms at the HBEF. Evidence that ice storm severity or frequency alters microbial activity, fungal abundance, or soil C and N would provide new understanding of how soil ecology and biogeochemistry respond to extreme events and ecosystem disturbances projected to increase under climate change. Because soil communities and EEA are key drivers of soil C storage and biogeochemical cycles (Gougoulias et al. 2014, Luo et al. 2017), their modification following ice storms could cascade through ecosystem biogeochemistry with relevance for future plant productivity, soil activity, and C cycling.

3.3. Methods

3.3.1. Site Description and Experimental Design

We examined the effect of ice storms on microbial activity and biogeochemistry at the HBEF in the White Mountains of New Hampshire (43°56’N, 71°45’W). The climate is cool, humid, and continental with monthly mean air temperature ranging from -9 °C in January to 18 °C in July. Average annual precipitation is 1400 mm, one third to one quarter of which falls as snow. Snow cover generally persists from late December until mid-April (Durán et al. 2014). Soils are well-drained spodosols (coarse, loamy, mixed, frigid, typic haplorthods) formed from glacial till with sandy loam to loamy sand texture. Thick organic horizons overlie bouldery mineral soil. Fallen trees and boulders create the characteristic pit and mound topography (Bormann et al. 1970).

The Ice Storm Experiment (ISE) (full details in: Rustad et al. *in prep*) was established near the main branch of the Hubbard Brook within a 70-100-year-old mixed hardwood stand dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), and American beech (*Fagus grandifolia*). In summer 2015, ten rectangular (20 x 30 m each) plots were established, with the dimensions designed to minimize disturbance by maximizing access to the plot interior from the perimeter. Plots were divided into a 1 m buffer zone where no sampling occurred, and 5 × 5 m subplots, four of which were designated for intensive sampling, including soil collection. Plots were randomly assigned to one of 5 experimental icing treatments: (i) control (no icing); (ii) low icing (6.4 mm); (iii) mid icing (12.7 mm); (iv) high icing (19 mm); and (v) mid icing (12.7 mm) for two consecutive years (mid × 2). The ice storm was simulated by spraying stream water from the main branch of the Hubbard Brook above the canopy on below freezing winter nights in 2016 (all icing treatments; 18 January, 27-29 January, and 11 February) and 2017 (moderate × 2 treatment only; 14 January) so that the descending mist froze upon contact. Ice accretion on wooden dowel ornaments hung in the canopy was measured using calipers until targeted ice thickness was achieved (Rustad and Campbell 2012). Ice accretion approximated targeted thicknesses, and resulted in a general stepwise increase in ice accretion from low to high treatments. Full details of the treatment effects on ice accretion, fine woody debris, and coarse woody debris can be found in (Rustad et al. *in prep*).

3.3.2. Soil Collection

We sampled soils in spring, summer, and fall of 2016 (28 April, 24 August, 18 October) and 2017 (10 May, 28 August, 1 November). On each sampling date, we collected 2 - 15 cm deep soil cores from 3 subplots within each plot using a 5 cm diameter PVC split core. We separated each core into organic (Oe and Oa) and mineral horizons (A, E, B), and composited the soils from each subplot by horizon to have 3 composite samples of each soil horizon (organic or mineral) for each plot. We stored the soils on ice following collection and transported them to the laboratory where we homogenized them by removing roots and rocks. We subsampled to measure gravimetric soil moisture, soil C/N, fungal hyphal abundance, EEA, DOC, total dissolved nitrogen (TDN), and DOM biodegradability. Subsamples for EEA and DOC/TDN/DOM were frozen at -80 °C until analysis. We determined gravimetric soil moisture by oven drying a 5 g subsample at 60 °C until constant mass.

3.3.3. Fungal Hyphal Abundance

We quantified fungal hyphal abundance using a modified filtration-gridline method (Sylvia 1992). Briefly, air dried subsamples (10 g) of field moist soil were ground to break up aggregates then stirred vigorously for 30 minutes in 500 mL sodium metaphosphate ($(NaPO_3)_6$, 0.158%). Subsequently, a 1:4 slurry dilution was stirred for 5 minutes and two 5 mL replicates were filtered through a 0.2 µm nylon filter (EMD

Millipore, Billerica, MA, USA). We used acid fuchsin (0.01 %) to stain the filters before mounting them on glass slides using polyvinyl lactic acid (PVLG) and drying them at 60 °C overnight. We determined hyphal abundance using the gridline intercept method (Newman 1966, Tennant 1975, Giovannetti and Mosse 1980) at 200X magnification using a Nikon Eclipse E600 (Nikon Instruments, Melville, New York, USA) and a 1 mm microscope grid.

3.3.4. Extracellular Enzyme Activity

We examined the potential activity of seven hydrolytic EEs (German et al. 2011, Bell et al. 2013), four of which degrade C compounds. These included α-glucosidase (AG) which targets starch, β-1,4-glucosidase (BG) and cellobiohydrolase (CBH) which degrade cellulose, and β-xylosidase (BX) which targets hemicellulose. We also measured two EEs that catalyze reactions to release N from complex molecules: β-N-acetylglucosaminidase (NAG), which breaks down chitin, and leucine aminopeptidase (LAP), which degrades peptides and amino acids. Finally, we measured acid phosphatase (AP) activity, which acts on phospho-monoesters to release P. Using Michaelis-Menten kinetics (Michaelis and Menten 1913), we determined saturating concentrations for the substrates to measure each of the 7 hydrolytic EE activities for HBEF soils (AG - 400 μM, BG - 400 μM, CBH - 350 μM, BX - 400 μM, NAG - 400 μM, LAP - 800 μM, AP - 800 μM). We followed a modified version of a deep-well plate method (Bell et al. 2013) that requires simultaneous preparation and measurement of sample activity with standards and controls (German et al. 2011). Controls include substrate controls, homogenate controls, standard controls, and quench controls (German et al. 2011). Soil homogenate was prepared by blending 2.75 g field moist soil in 91 mL 50 mM sodium acetate buffer at high speed using a Waring Commercial Lab Blender 7010S (model WF2211217, Waring Laboratory Science, Torrington, CT, USA) then passing it through a 1 mm stainless steel strainer and stirring continuously while 800 μL aliquots were pipetted into deep 96 well plates. Standard (4-methylumbelliferone (MUB) for AG, BG, CBH, BX, NAG, and AP and 7-amino-4-methylcoumarin (AMC) for LAP) or substrate (200 μL) was added to each aliquot, and unquenched standards and substrates were prepared simultaneously in a black 96 well microplate. Deep well plates were sealed with plate mats, thoroughly mixed, and incubated for 2 hours at 20 °C. Following incubation, deep well plates were centrifuged for 6 minutes at 1500 rpm, and 250 μL of supernatant from each well was pipetted into a black 96 well microplate. All sample plates and the unquenched plate were read in a microplate reader (BioTek Synergy HT, Winooski, VT, USA) at 365 nm excitation/450 nm emission. Assay fluorescence was corrected for controls and quenching, and enzyme activity was calculated as outlined by German et al. (2011) and German et al. (2012).

Using the same soil slurry prepared for hydrolytic enzyme activity, we assayed the activity of phenol oxidase (PO) and peroxidase (PER), two oxidative enzymes associated with the breakdown of lignin. We pipetted three replicates of 1.4 mL homogenate into 2 mL centrifuge tubes then added either buffer (0.35 mL) for sample controls, L-DOPA (L-3,4-dihydroxyphenylalanine, 0.35 mL) for PO activity, or L-DOPA (0.35 mL) and hydrogen peroxide (H_2O_2, 0.07 mL) for PER activity. Tubes were inverted to thoroughly mix contents and incubated for 20 – 24 hours at 20 °C before centrifuging at 3,600 RPM for 5 minutes. Finally, we pipetted four replicates of the supernatant (250 µL) of each tube into clear 96 well microplates and read them colorimetrically on a microplate reader at 460 nm absorbance. Assay absorbance was corrected for sample controls and enzyme activity was calculated according to German et al. (2011) using the extinction coefficient of 7.9 μmol^{-1} described by Bach et al. (2013).

We summed EEA for labile C (AG + BG + BX + CBH), recalcitrant C (PPO + PER), N (NAG + LAP) and P (AP) acquisition. Next, we calculated stoichiometric ratios for C:N EEA, N:P EEA, and C_{labile}:$C_{recalcitrant}$ to determine the relative changes in C and nutrient demand across ice storm severities and frequency.

3.3.5. DOC, TDN, and DOM Biodegradability

We extracted soils to measure DOC and TDN, and DOM biodegradability using a 5:1, deionized water:soil ratio. Slurries were shaken vigorously for 1 hour, then centrifuged at 3,000 rpm for 30 minutes before being vacuum filtered through a previously combusted 0.7 µm glass fiber filter (Whatman GF/F, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). Extract aliquots were analyzed for DOC/TDN and DOM. DOC and TDN were determined using a Total Organic C Analyzer (Shimadzu TOC-L with TNM-L, Columbia, MD, USA) by catalytic oxidation of samples at 720 °C followed by infrared detection of carbon dioxide (CO_2) and chemiluminescence for DOC and TDN, respectively. We characterized DOM with a Horiba Aqualog Fluorescence Spectrometer (Horiba, Irvine CA, USA). Data were corrected for inner filter effects, Rayleigh scatter, and normalized for Raman intensity. Corrected data were used to calculate three indices that characterize DOM. First, the absorbance at 254 nm was normalized to the DOC concentration to calculate specific ultraviolet absorbance

($SUVA_{254}$), a measure of DOM aromaticity (Weishaar et al. 2003). The fluorescence index (FI) was calculated as the emission intensity at 470 nm divided by that at 520 nm, each at excitation 370 nm, and provided an indication as to whether the source of the DOM was microbial or terrestrial (McKnight et al. 2001, Cory and McKnight 2005). Finally, the humification index (HIX), which provided a measure of DOM humification, was calculated as the area under emission 435-380 nm divided by the area at 300-345 nm, each at excitation 254 nm (Zsolnay et al. 1999). The HIX value represents the relative contributions of humified organic matter from the soil matrix and microbially or plant-sourced DOM, which is not humified, to the DOM pool (Zsolnay et al. 1999).

3.3.6. Statistical Analyses

All statistical analyses were performed in R (R Core Team 2019). Effects of simulated ice storm treatment and soil horizon on fungal hyphal length, DOC, TDN, indices of DOM biodegradability, and EEA were determined using linear mixed effects models in the R nlme package (Pinheiro et al. 2019) with plot as a random effect, and month of experiment nested within plot as the time step in a compound symmetry temporal autocorrelation structure (corCompSymm) to account for repeated measures. Significance of model terms was determined with type 3 (partial) Analysis of Deviance (ANODE) models conducted in the R car package (Fox and Weisberg 2019). For all models, assumptions of constant variance and normality were assessed by visual inspection of residual plots. When necessary, variance structures were constructed for categorical variables using the varIdent function in the nlme package (Pinheiro et al. 2019), and non-normal data were log transformed. Results were considered significant at

$p < 0.05$. Unless otherwise noted, all reported values are means plus or minus one standard error of the mean.

3.4. Results

3.4.1. Fungal Hyphal Abundance

Fungal hyphal abundance in the mid ice treatment (49.8 ± 6.5 cm g^{-1}) was roughly twice that observed in all other treatments (Fig. 1; control: 21.9 ± 3.8 cm g^{-1}; low: 29.5 ± 4.5 cm g^{-1}; high: 23.9 ± 3.6 cm g^{-1}; mid × 2: 21.3 ± 3.9 cm g^{-1}; X_4^2 = 10.3, p = 0.04, R^2 = 0.40). Additionally, fungal hyphal length in the organic horizon (47 ± 4 cm g^{-1}) was 147% greater than in the mineral horizon (19 ± 2 cm g^{-1}; X_1^2 = 80.7, $p < 0.0001$).

3.4.2. Extracellular Enzyme Activity

Carbon, N, and P acquiring enzymes all varied by soil horizon, and P additionally varied by treatment while N varied by the interaction of ice treatment with soil horizon (Fig. 2). Labile C EEA activity (Fig. 2A) of the organic horizon (482.4 ± 14.7 nmol g^{-1} h^{-1}) was 370% higher than that observed in the mineral horizon (101.8 ± 4.5 nmol g^{-1} h^{-1}; X_1^2 = 1304.4, $p < 0.0001$, R^2 = 0.74). Conversely, recalcitrant C EEA (Fig. 2B) was 65% higher in mineral (56.1 ± 1.7 nmol g^{-1} h^{-1}) than organic soil (34.6 ± 1.2 nmol g^{-1} h^{-1}; X_1^2 = 128.8, $p < 0.0001$, R^2 = 0.27). N acquiring EEA in organic horizon soils (269.7 ± 12.4 nmol g^{-1} h^{-1}) exceeded that of mineral soils (46.1 ± 4.7 nmol g^{-1} h^{-1}) by nearly 500% (X_1^2 = 1067.6, $p < 0.0001$, R^2 = 0.82). Additionally, the effect of the ice treatment on N acquiring EEA varied across soil horizons (Fig. 2C; X_4^2 = 14.5, p = 0.006). In organic soils, mid icing (219.3 ± 13.9 nmol g^{-1} h^{-1}) had less N acquiring EEA than control (271.5 ± 18.1 nmol g^{-1} h^{-1}) and low icing (333.1 ± 45.2 nmol g^{-1} h^{-1}). High (265.2 ± 21.1) and mid × 2 (259.3 ± 26.0) were additionally lower in activity than low icing. In mineral soils, mid × 2 icing (27.4 ± 3.1 nmol g^{-1} h^{-1}) had significantly lower N acquiring EEA than all other treatments (control: 35.3 ± 4.8 nmol g^{-1} h^{-1}; low: 58.9 ± 14.9 nmol g^{-1} h^{-1}; mid: 56.6 ± 8.0 nmol g^{-1} h^{-1}; high: 52.3 ± 14.6 nmol g^{-1} h^{-1}). N acquiring EEA in all other icing treatments were higher than the control (Fig. 2C; low, mid, and high). Phosphorus acquiring EEA (Fig. 2D) in the mid × 2 treatment (1002.6 ± 94.2 nmol g^{-1} h^{-1}) was lower than that of low (1265.4 ± 116.4 nmol g^{-1} h^{-1}), mid (1320.0 ± 108.1 nmol g^{-1} h^{-1}), and high (1252.9 ± 109.5 nmol g^{-1} h^{-1}). Additionally, control (1103.6 ± 104.1 nmol g^{-1} h^{-1}) was lower than mid (X_4^2 = 10.0, p = 0.04, R^2 = 0.87). P acquiring EEA was also 300% higher in the organic horizon (1928.5 ± 47 nmol g^{-1} h^{-1}) than the mineral horizon (462.2 ± 31.2 nmol g^{-1} h^{-1}; X_1^2 = 1045.4, p < 0.0001), and there was no interaction between soil horizon and ice treatment.

Enzyme stoichiometry varied significantly by soil horizon in all cases, and for C:N and N:P the effects of ice treatment varied across soil horizons (Fig. 3). C:N EEA was more than twice as high in the mineral (5.4 ± 0.2) than in the organic soil (2.2 ± 0.01; X_1^2 = 285.5, p < 0.0001, R^2 = 0.65). Additionally, the effect of ice treatment on C:N of EEA (Fig. 3B) varied by soil horizon (X_4^2 = 14.7, p = 0.005). In organic soil, C:N EEA of high (2.4 ± 0.1) was greater than control (2.1 ± 0.1), low (2.0 ± 0.2), and mid × 2 (2.1 ± 0.1). In mineral soil, low and mid icing C:N EEA (4.8 ± 0.5 and 4.4 ± 0.4, respectively) were lower than high (5.8 ± 0.5) and mid × 2 (6.5 ± 0.4). Mid icing C:N EEA was also lower than the control. N:P EEA was 55% higher in organic (0.14 ± 0.005) than mineral soil (0.1 ± 0.004; X_1^2 = 98.3, p < 0.0001, R^2 = 0.30). The effect of ice treatment on N:P of EEA varied by soil horizon (Fig. 3C; X_4^2 = 12.3, p = 0.02). In organic soil, mid ice N:P

EEA (0.11 ± 0.01) was lower than both control (0.14 ± 0.01) and high (0.14 ± 0.01), which were both lower than low ice (0.16 ± 0.01) and mid × 2 (0.16 ± 0.01). In mineral soil, the high ice treatment N:P EEA (0.09 ± 0.01) was lower than that of the low treatment (0.11 ± 0.01), with no significant differences between any other treatments (control: 0.1 ± 0.01; mid: 0.1 ± 0.01; mid × 2: 0.09 ± 0.0). Finally, C_{labile}:$C_{recalcitrant}$ EEA was 660% greater in organic (16.8 ± 0.8) than mineral soil (2.2 ± 0.2), with no significant differences associated with treatment (X_1^2 = 1220.1, p < 0.0001, R^2 = 0.75).

3.4.3. Dissolved Organic Carbon

Overall, 238% more DOC was extracted from the organic (1191.0 ± 37.6 mg kg^{-1}) than the mineral horizon (352.0 ± 16.9 mg kg^{-1}; X_1^2 = 922.7, p < 0.0001, R^2 = 0.76). The effect of icing treatment on DOC varied by soil horizon (Fig. 4A; X_4^2 = 18.3, p = 0.001). In the organic soil, the high treatment (1049.4 ± 77.1 mg kg^{-1}) had less extractable DOC than the low (1241.2 ± 79.4 mg kg^{-1}) and mid × 2 treatment (1306.6 ± 94.7 mg kg^{-1}), with control (1196.8 ± 85.0 mg kg^{-1}) and mid icing (1170.7 ± 83.4 mg kg^{-1}) DOC overlapping with all other treatments. On the mineral soil, both low (381.4 ± 32.4 mg kg^{-1}) and mid (448.9 ± 57.3 mg kg^{-1}) icing increased extractable DOC relative to control (311.4 ± 24.9 mg kg^{-1}), high icing (343.7 ± 35.4 mg kg^{-1}) resulted in lower extractable DOC than mid, and mid × 2 (272.6 ± 23.7 mg kg^{-1}) was lower than all treatments except control.

3.4.4. Total Dissolved Nitrogen

Extractable TDN was 388% greater from organic (127.7 ± 3.1 mg kg^{-1}) than mineral (26.1 ± 1.1 mg kg^{-1}) soil (X_1^2 = 1884.5, p < 0.0001, R^2 = 0.88). The effect of icing treatment on extractable TDN varied across soil horizons (Fig. 4B; X_4^2 = 11.9, p = 0.02). In the organic soil, mid (115.4 ± 6.5 mg kg^{-1}) and high (118.4 ± 6.0 mg kg^{-1}) icing reduced extractable TDN compared to control (137.7 ±8.6 mg kg^{-1}), low (137.9 ± 6.6 mg kg^{-1}), and mid × 2 (129.6 ± 6.5 mg kg^{-1}). In mineral soil, low (29.1 ± 2.7 mg kg^{-1}) and mid (30.0 ± 3.4 mg kg^{-1}) icing extractable TDN was elevated over control (23.9 ± 1.8 mg kg^{-1}) and mid × 2 (22.2 ± 2.4 mg kg^{-1}). High icing extractable TDN (25.3 ± 2.2 mg kg^{-1}) overlapped with all other treatments.

3.4.5. Dissolved organic matter biodegradability

SUVA$_{254}$ was 68% higher in the mineral (0.62 ± 0.02) than the organic ((0.37 ± 0.01) soil (X_1^2 = 194.2, p < 0.0001, R^2 = 0.28), indicating a higher degree of aromaticity in mineral soils. Additionally, the effect of icing treatment on SUVA$_{254}$ varied by soil horizon (Fig. 4C; X_4^2 = 16.7, p = 0.002). In organic soils, SUVA$_{254}$ was higher in low (0.38 ± 0.02) and high (0.42 ± 0.03) icing treatments than in control (0.33 ± 0.02), with mid (0.37 ± 0.03) and mid × 2 (0.35 ± 0.02) SUVA$_{254}$ intermediate to all other treatments. Conversely, in mineral soils SUVA$_{254}$ was elevated in mid × 2 soils (0.77 ± 0.06) over all other treatments (control: 0.58 ± 0.04; low: 0.55 ± 0.04; mid: 0.60 ± 0.06; high: 0.61 ± 0.04).

FI had no significant relationships to soil horizon or icing treatment.

HIX of mineral soil (11.9 ± 1.0) was 88% higher than that of organic (6.3 ± 0.3) soil (X_1^2 = 37.0, p < 0.0001, R^2 = 0.23), indicating more humified organic matter in mineral soils, and the effect of icing treatment on HIX varied across soil horizons (Fig. 4D; X_4^2 = 12.8, p =0.01). In organic soil, the HIX of the mid icing treatment (5.1 ± 0.4) was lower than all other treatments (control: 6.4 ± 0.4; low: 6.1 ± 0.3; high: 6.9 ± 0.9; mid × 2: 7.1 ± 0.7). In mineral soil, there was no difference in HIX across icing treatments (control: 10.4 ± 2.3; low: 13.9 ± 2.6; mid: 11.6 ± 1.7; high: 13.7 ± 2.3; mid × 2: 10.0 ± 2.7).

3.5. Discussion

We found that ice storms altered fungal abundance, microbial activity, DOC and TDN availability, and DOM biodegradability, with variation across soil horizons. Ice storm treatments directly caused significant variation in fungal hyphal abundance and P EEA, C EEA varied by soil horizon, and N EEA response to ice storms depended on soil horizon (treatment × horizon interaction). In several cases, the direction of EEA response changed depending on soil horizon, as did the response of TDN availability. Similarly, ice storms only altered soil DOC patterns in mineral soil. Organic soils exceeded mineral soils in fungal abundance, labile C, N, and P EEA, N:P EEA, C_{labile}:$C_{recalcitrant}$ EEA, DOC, and TDN. Mineral soils had higher recalcitrant C EEA, C:N EEA, $SUVA_{254}$ and HIX.

The abundance of fungal hyphae in the mid ice storm treatment exceeded that of all other treatments. Previous work found relative fungal abundance to increase with C content of soil, and decrease with high levels of N (Bardgett and McAlister 1999, Fierer et al. 2009). Given higher deposition of woody debris in ice storm treatments than control, we expected fungal abundance in soils to increase with ice storm treatments. It is unclear why the effect was only observed in the mid-level ice storm treatment. Predictably, fungal abundance was also higher in organic than mineral soil, as was DOC, in accordance with widespread observations (Oehl et al. 2005, Clemmensen et al. 2013).

The effect of ice storms on EEA varied by C, N, or P. Interestingly, C acquiring EEA varied only across soil horizons, P acquiring EEA varied according to ice storm treatment and soil horizon, independently, and ice storm treatment effects on N acquiring EEA varied across soil horizons (treatment × horizon interaction). This suggests that ice storms differentially affect P and N demand and cycling, with storm intensity altering P EEA expression and the effect on N EEA expression depending on soil characteristics. Furthermore, both P and N EEAs are inversely related to environmental concentrations of those elements (Olander and Vitousek 2000, Treseder and Vitousek 2001), suggesting that the effect of ice storms on the availability of limiting nutrients varies by nutrient. Our finding that N EEA responded to ice storm treatments and C EEA did not coincides with recent observations of greater N cycle sensitivity to climate variation compared to the C cycle (Durán et al. 2017), and lends further evidence for future decoupling of C and N cycles under climate change (Schimel and Bennett 2004, Li et al. 2007, Durán et al. 2017).

Additionally, the direction of response of N EEA to ice storms often depended on the soil horizon. For example, in the mid treatment, N EEA was reduced relative to control in organic soil, and increased relative to control in mineral soil. The N:P EEA also exhibited this pattern, with mid × 2 icing increasing N:P EEA compared to control in organic soil, and having the opposite effect in mineral soil. Previous research has similarly found the response of HBEF soils to climate variation to differ with depth, with deeper soils exhibiting greater climate sensitivity (Durán et al. 2017). Organic and mineral soils differ in C recalcitrance, water holding capacity, microbial biomass, and control exerted by plants and microbes, which decreases with depth, versus physical conditions, which increases with depth (Dungait et al. 2012, Morse et al. 2014). The differences we observed by soil horizon may therefore reflect the differing influence of ice storms on biological and physical processes relevant to biogeochemical cycling. Finally, in several instances the mid and mid × 2 treatments had opposite effects on enzyme expression. Specifically, in mineral soil the C:N EEA of mid icing was reduced relative to control, while that of mid × 2 increased relative to control. Also, N:P EEA in organic soil was increased by mid × 2 and decreased by mid relative to controls. This suggests that increasing ice storm frequency can alter the direction of the response of microbial activity to ice storms.

The effect of ice storms on DOC, TDN, and DOM biodegradability all varied across soil horizons. Soil DOC was elevated by low and mid icing compared to control, but only in mineral soil. This increased availability of DOC in the ice storm treatment could reflect changes in abiotic or biotic processes leading to DOC production. The direction of the effect of mid icing on soil TDN depended on soil horizon, with mid icing reducing TDN relative to control in organic soil, and having the opposite effect in mineral soil. Following the 1998 ice storm at HBEF, elevated soil nitrate levels were observed in organic and mineral horizons (Houlton et al. 2003), as opposed to our observation of elevated TDN in only mineral horizons. The inconsistent and low magnitude response we observed in soil solution TDN as compared to earlier findings is consistent with other observations of ecosystem N oligotrophication and lessened response of the N cycle to disturbance at HBEF (Durán et al. 2016, Groffman et al. 2018).

Ice storm treatments altered aromaticity and humification of DOM. As indicated by $SUVA_{254}$ (Weishaar et al. 2003), DOM aromaticity increased (indicating lower biodegradability) in low and high icing in organic soil, and mid × 2 icing in mineral soil relative to controls. This is perhaps attributable to the increase in woody litter in ice storm plots, which could alter leached compounds and microbial activity, and ultimately DOM chemical composition. For example, EE degradation of ligno-cellulose compounds can lead to greater aromaticity of DOM (Vujinovic et al. 2019). However, the $SUVA_{254}$ values we recorded are comparatively low (e.g., Vujinovic et al. 2019) and although statistically significant differences exist, the values are not distinct enough to represent significant ecological differences. Additionally, humification of DOM as measured by HIX (Zsolnay et al. 1999) decreased in mid-icing relative to control in organic soil only. Although this is opposite the response that we expected, similar to $SUVA_{254}$, the differences we observed are statistically but not ecologically significant. Nonetheless, this could represent a shift in the DOM pool under ice storm conditions.

3.6. Conclusion

Our work provides evidence that ice storms can result in complex ecosystem responses, as evidenced by altered patterns of soil fungal abundance, EEA, concentrations of soil DOC and TDN, and biodegradability of DOM. The increase in fungal abundance under mid icing conditions could have implications for soil C storage and N cycles. Ice storm treatments differentially affected C, N, and P EEA, providing evidence of altered microbial nutrient demand and potential decoupling of element cycles following extreme events. The direction of response of N EEA and TDN concentration to icing varied by soil horizon, demonstrating the importance of soil characteristics to both

N availability and microbial demand. DOC response to ice storms in only the mineral horizon provides further evidence of the importance of soil horizon to quantifying ice storm effects. Repeated mid-level ice storms also altered the direction of response of enzyme stoichiometry, indicating that ice storm frequency can dramatically alter microbial nutrient demand. Although ice storms altered the aromaticity and humification of DOM, this effect was small and ecologically insignificant. These patterns in fungal abundance, EEA, and C, N, and DOM biodegradability emerged from an otherwise variable response to ice storm treatments, perhaps reflecting the heterogenous nature of both soils and ice storm damage. Collectively, our results indicate that ice storms could alter soil processes and biogeochemistry, with the effects differing by element (C, N, or P), soil horizon, storm severity, and return interval.

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3.8. Figures

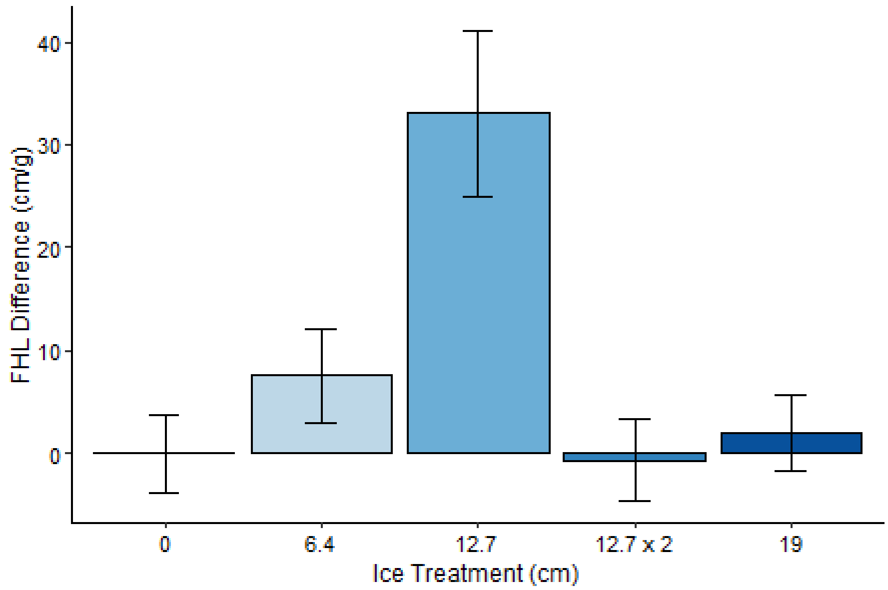


Fig. 3.1. Average difference in fungal hyphal length (FHL) between each ice storm treatment and the control plots. Error bars are the standard error of the mean of each treatment.

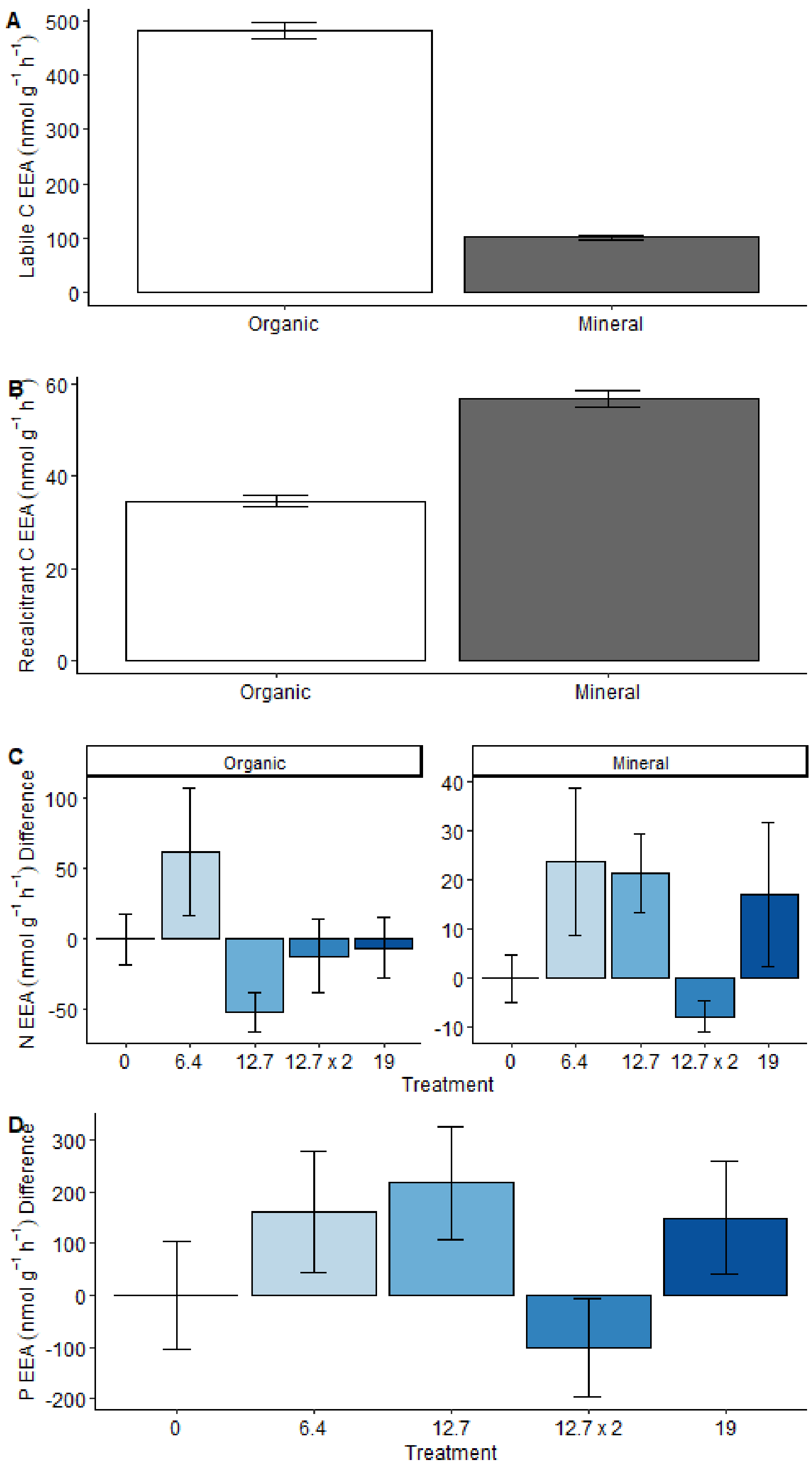


Fig. 3.2. Extracellular enzyme activity (EEA) of (A) labile C EEs (AG + BG + BX + CBH); (B) recalcitrant C EEs (PPO+PER); (C) difference between each treatment and control in N EEs (NAG + LAP) for each soil horizon; (D) difference between each treatment and control in P EE (AP). All error bars show the standard error of the mean. C EEA varied by soil horizon, N EEA varied by the interaction between ice storm treatment and soil horizon, and P EEA varied by ice storm treatment (mid > control).

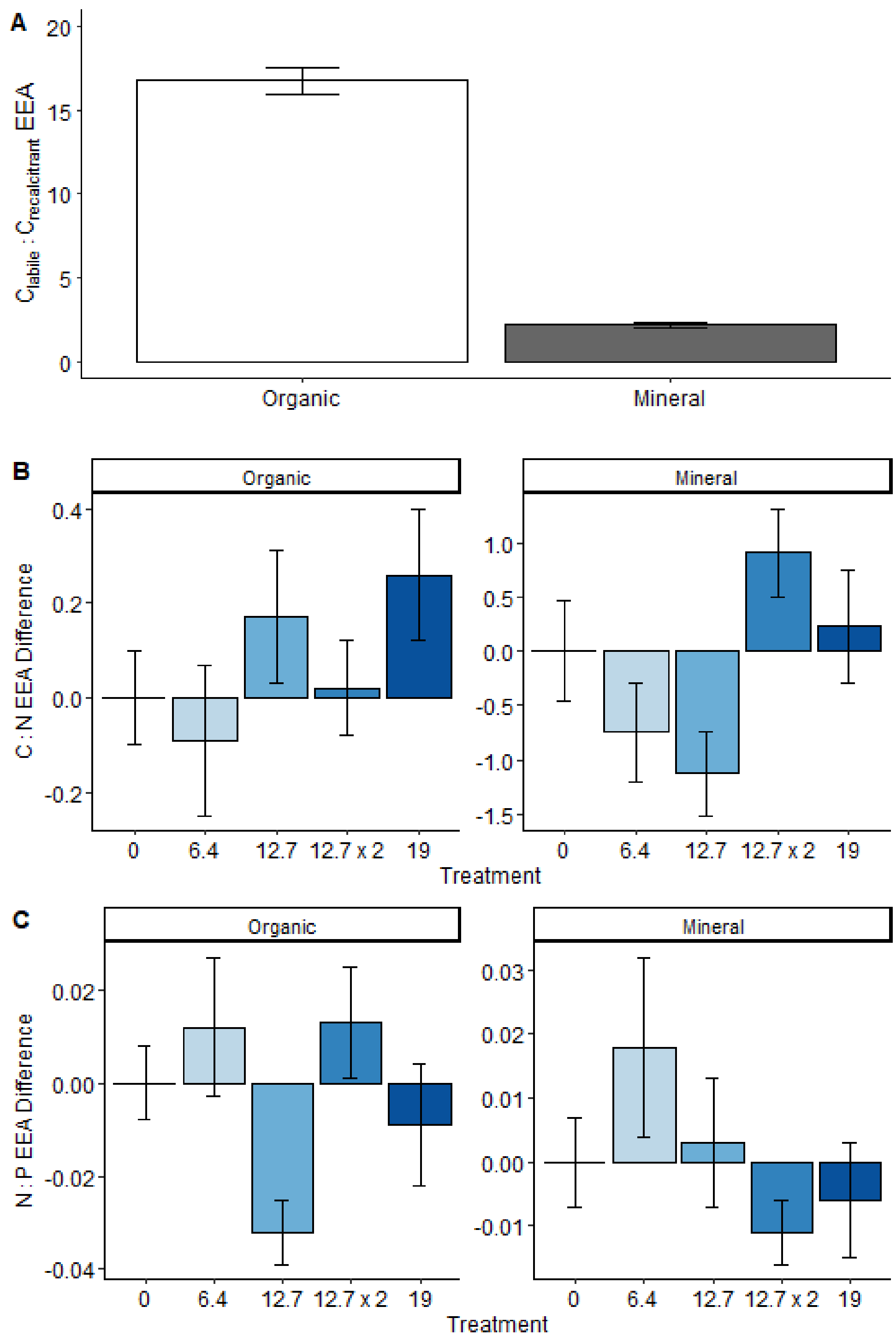
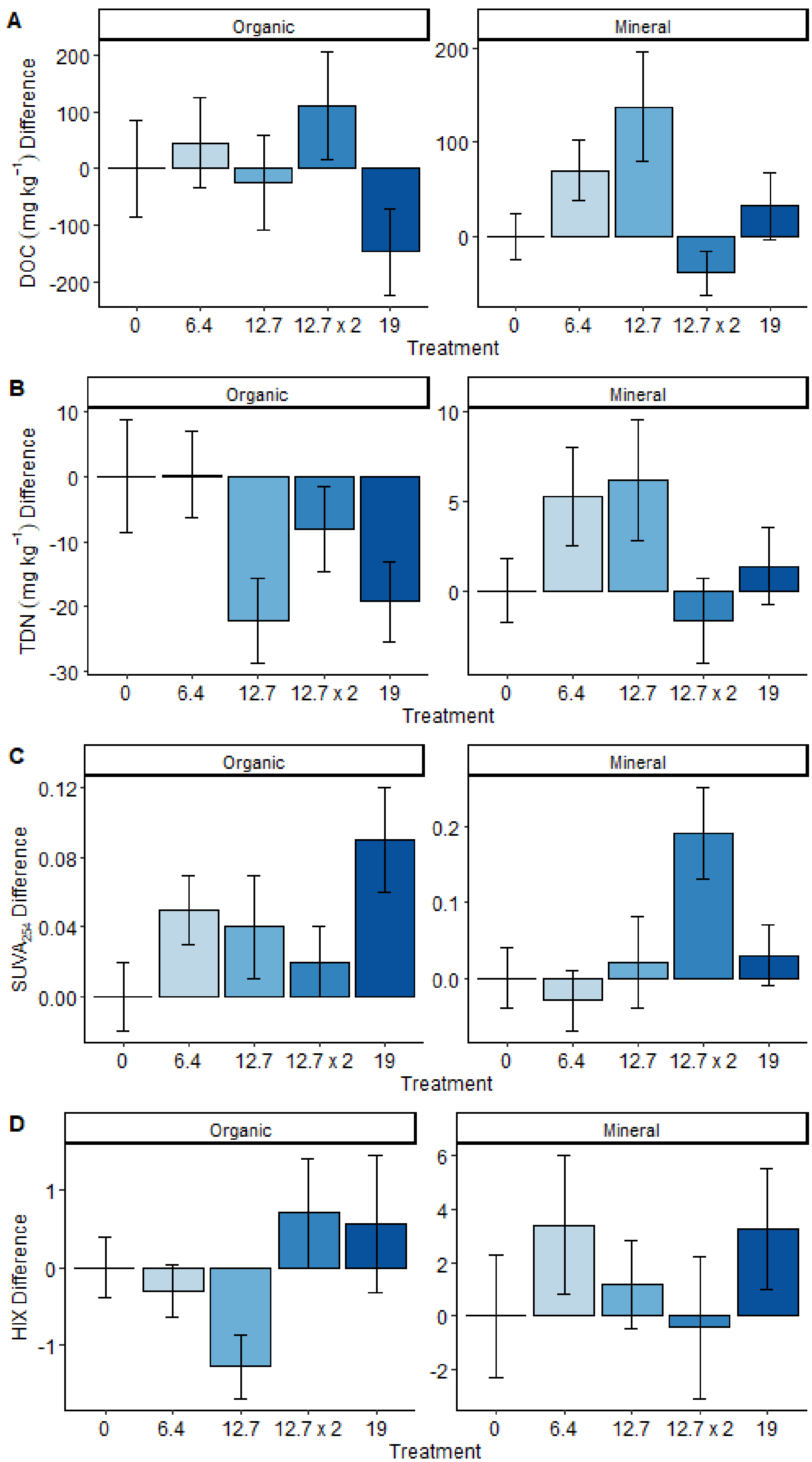


Fig. 3.3. Stoichiometric ratios of EEA graphed according to significant differences: C_{labile}:$C_{recalcitrant}$ was significantly different by soil horizon , C:N EEA varied significantly by treatment × horizon, and N:P EEA varied significantly by treatment × horizon. (A) C_{labile}:$C_{recalcitrant}$; (B) difference in C:N EEA for each soil horizon between each treatment and control; (C) difference in N:P EEA for each soil horizon between each treatment and control. All error bars show the standard error of the mean. Note the different y axis limits for the organic and mineral horizons.



A
Organic
Mineral
DOC (mg kg-1) Difference
Treatment
B
Organic
Mineral
TDN (mg kg-1) Difference
Treatment
C
Organic
Mineral
SUVA254 Difference
Treatment
D
Organic
Mineral
HIX Difference
0
6.4
12.7
12.7 x 2
19
Treatment

Fig. 3.4. (A) Difference in DOC availability in each soil horizon between treatments and control; (B) difference in TDN availability in each soil horizon between treatments and control; (C) difference in $SUVA_{254}$ in each soil horizon between treatments and control; (D) difference in HIX in each soil horizon between treatments and control. All error bars show the standard error of the mean. Note the different y axis limits for the organic and mineral horizons.

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CHAPTER 4: MICROBIAL PARAMETERS IMPROVE A GLOBAL MODEL OF MASS LOSS BUT NOT NITROGEN RELEASE DURING DECOMPOSITION

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4.1. Abstract

Traditionally, decomposition models have only implicitly included microbial activity through its well-known relationship to climate and litter quality. Decomposition, however, is a complex, microbially mediated process that varies with microbial activity and community characteristics. Microbial decomposition partitions organic carbon (C) between the atmosphere and soil C, the largest terrestrial C reserve which is of great importance to controlling the rate of climate change. In an attempt to improve C model projections, and due to the central role of microbes in regulating biogeochemical cycles, researchers are increasingly calling for explicit inclusion of microbial characteristics and processes in Earth system models. However, the benefit of adding microbial parameters has been largely unexplored and the computational costs could be large. Our objective was to add microbial parameters to a decomposition model that previously included only climate and litter quality in order to compare the microbial models to the first order (microbially implicit) model. We formulated two basic mass loss models: one which included microbial transformations of litter, and the other which incorporated a microbial biomass pool. Within each of these model structures we varied estimation of microbial carbon use efficiency (CUE) and used model selection techniques to identify the best model. We then formulated nitrogen (N) release models to accompany the base mass loss model and the best of each type of microbial model (flow vs. pool). Inclusion of microbial parameters improved mass loss models as compared to the base model which only implicitly included microbes. The best model included transfers from the labile and slow mass pools to the recalcitrant pool to represent microbial transformations of litter, and it estimated microbial CUE as a constant value for each litter quality pool. Conversely, the base model was selected as best for N release, suggesting that further work is necessary to model the microbial influence on N dynamics during decomposition. Our results demonstrate that inclusion of microbial parameters can improve mass loss decomposition models that rely on traditionally available datasets (i.e., mass loss, N loss, climate, litter quality).

Keywords: decomposition, mass loss, nitrogen loss, microbial model, microbial parameters

4.2. Introduction

Ecosystem productivity relies on the availability of nutrients and energy sources that are released through decomposition, a complex, microbially-mediated ecosystem process (Schimel and Weintraub 2003). Carried out largely by microbial extracellular enzymes, decomposition rates vary with factors that affect microbial populations and their activity, including climate variables and litter quality (Meentemeyer 1978, Aber et al. 1990), soil structure (Allison and Jastrow 2006), and microbial physiology (Allison et al. 2010, Allison 2012, Wieder et al. 2013). Despite their central importance, microbes are only implicitly included in large scale Earth system models (Schimel 2001) through their relationship to litter quality and climate (e.g., Parton et al. 1994, Gholz et al. 2000, Adair et al. 2008). It is increasingly hypothesized that explicit inclusion of microbial activity or community dynamics would improve Earth system models (Lawrence et al. 2009, Allison 2012, Treseder et al. 2012, Wieder et al. 2013). For example, analysis of 82 datasets on carbon (C) and nitrogen (N) cycling found that environmental variables left 44% of the variation in the data unexplained, suggesting that adding microbial data could potentially increase model accuracy (Graham et al. 2016). Furthermore, environmental perturbations that have resulted in unexpected litter decay responses call into question current models and understanding of decomposition (Moorhead and Sinsabaugh 2006). However, possible improvement to decomposition models through inclusion of microbial factors has been largely unexplored.

Microbial decomposition partitions organic C between the atmosphere and longer-term soil C storage, with old soil C comprised nearly entirely of microbial products (Allison 2006). The efficiency of this conversion is described as carbon use efficiency (CUE), the ratio of microbial biomass accumulation to respiration during decomposition of organic material (Sinsabaugh et al. 2013). The fundamental importance of microbial metabolism is represented in ecological models as CUE because it determines the energetic and biogeochemical flows from the soil detrital food web (Miltner et al. 2012). Environmental, stoichiometric, microbial, and substrate characteristics all interact to determine CUE of a microbial community (Manzoni and Porporato 2009). For example, CUE can decline due to nutrient limitation (Larsson et al. 1995) and be relatively high when C availability is low (Sinsabaugh et al. 2013). However, variation in CUE due to differing environmental and stoichiometric factors is not typically included in Earth system models (Sinsabaugh et al. 2013).

The microbial role in nutrient cycling and climate regulation in particular makes microbes and their inclusion in Earth system models crucially important to projecting the impacts of climate change (Cavicchioli et al. 2019). As the largest terrestrial C pool, soils contain two to three times the amount of C in the atmosphere (Jobbágy and Jackson 2000), such that perturbations to decomposition rates and related SOM creation could alter global C dynamics. Due to the importance of projecting soil C dynamics under changing global climate scenarios, the microbial role in soil organic matter (SOM) formation (Schmidt et al. 2011, Miltner et al. 2012, Cotrufo et al. 2015), and the wide variation in soil C projections (Todd-Brown et al. 2013), models of soil C dynamics in particular have been developed to include microbial parameters. These have included microbial biomass pools (Sulman et al. 2014, Wieder et al. 2014, Campbell et al. 2016), microbial product pools (Campbell et al. 2016), turnover of microbial biomass (Wieder et al. 2014), microbial CUE (Allison et al. 2010, Wieder et al. 2013, Wieder et al. 2014, Campbell et al. 2016), extracellular enzyme activity (EEA) (Allison et al. 2010), and microbial growth strategy (i.e., r vs. k) (Wieder et al. 2014). Several microbial models of litter decomposition have also been developed which included microbial traits (Allison 2012) and microbial biomass and products pools (Campbell et al. 2016). However, the improvement in microbial model projections as compared to microbially implicit first order models has been largely varied or unexamined (Treseder et al. 2012), and they often incorporate microbial parameters developed in the lab at small spatial and temporal scales (Wieder et al. 2015). Given the additional effort and complexity required in microbial as compared to first order models (Treseder et al. 2012), further exploration of the benefits of adding microbial parameters to large scale models is necessary.

Here, we examined the improvement made to a microbially implicit decomposition model following addition of microbial parameters. Microbes were explicitly included through incorporating either (i) microbial flows between litter quality pools; or (ii) a microbial biomass and products pool with its own decomposition rate. Within each of these microbial model structures, we investigated different representations of microbial CUE. To do this, we compiled a global dataset of decomposition studies which we used to parameterize models of mass loss and N release. We ran a model comparison for mass loss models, and selected the best of each model group (i.e., microbial flow vs. microbial pool) to develop an accompanying N model. We expected both types of microbial models (microbial flow and microbial pool) to better predict mass loss and N release during decomposition than the microbially implicit model (H1). We further hypothesized that regulation of microbial CUE according to litter N would improve modeled mass loss (H2). Evidence of improvement to large-scale decomposition models using widely available data (i.e., mass loss, N loss, initial litter chemistry, climate) by the addition of microbial parameters would suggest that incorporation of microbes can benefit global change projections without excessive and added complexity.

4.3. Methods

4.3.1. Data sources

We compiled 5 decomposition datasets to use in this project (Table 1), with extensive details of each project in their original publications (noted here). Briefly, the Assembly of Research on Traits and DECOmposition (ARTDeco) dataset includes species-specific decomposition data from 66 experiments which included 818 plant species, and spanned 6 continents (Cornwell et al. 2008). We subset the ARTDeco dataset to exclude irrigated studies, as well as those that did not have initial litter lignin and cellulose contents, resulting in inclusion of data from 14 sites and 101 litter types. The Canadian Intersite Decomposition Experiment (CIDET) examined relationships between decay rates, substrate quality, and climate through a litterbag study using 10 litter types across 19 Canadian sites (Trofymow and CIDET Working Group 1998). The European Decomposition Data (EuroDeco) was collected from 47 sites ranging from subarctic, to subtropical and Mediterranean with the goal of determining the influence of climate versus substrate quality on decomposition rates (Berg et al. 1993). The Global Tropical (GT Dec) dataset measured litter decomposition in 23 tropical forests, spanning 14 countries (Powers et al. 2009). Finally, the Long-term Intersite Decomposition Experiment Team (LIDET) was a reciprocal litterbag study which transplanted leaf and root litter from 26 species and 27 sites in North and Central America over a 10 year period (LIDET 1995). Our compiled dataset includes leaf and root decomposition data from 144 plant species in 130 sites across the globe (Fig. 1), which spanned from a period of months to a maximum of ten years.

In addition to site characteristics, our compiled dataset included litter mass and N remaining at each collection time, as well as initial litter cellulose, lignin, C, and N. We calculated the initial lignin fraction as the portion of cellulose + lignin comprised of lignin. We used monthly temperature and precipitation data from the University of East Anglia Climatic Research Unit (CRU 2019) that was collected during each experimental period to calculate site-specific Lloyd and Taylor climatic decomposition indices (CDI_{LT}), which combine temperature and moisture effects on decomposition with a variable Q_{10} (Quotient 10) temperature function (Lloyd and Taylor 1994b). Using the CDI_{LT} produced the best model fit in a previous model comparison study using the LIDET dataset (Adair et al. 2008). The dataset used to parameterize the mass loss model contained over 6,000 observations. Due to limited data on N remaining at each collection time, the dataset used to model N contained 3,600 observations.

4.3.2. Mass Loss Models

We used a three-pool mass loss model developed using LIDET data as the base model for this study (Adair et al. 2008). It is a microbially implicit (Schimel 2001), negative exponential model (Olson 1963) that divides litter mass into three pools (labile, slow (cellulose), and recalcitrant), each with its own decay rate (Fig. 2a). The size of the labile and slow pools is defined by the initial litter lignin/N ratio, and lignin content determines the size of the recalcitrant pool. The site and year-specific CDI_{LT} modifies the decomposition rate of all three pools. Additionally, the relative amount of cellulose and lignin (lignin fraction, L_s = lignin/[lignin+cellulose]) modifies the decomposition rate of the slow pool (Adair et al. 2008).

To explore the effect of including microbial parameters on the mass loss model's predictive ability, we developed two basic microbial models: a microbial flow model that incorporated transfers between pools to represent microbial transformations of litter to a more recalcitrant state (Fig. 2b), and a microbial pool model that included a fourth pool with its own decay rate to account for microbial biomass and products (Fig. 2c). We then varied each of these models to explore the best estimation of microbial CUE (Table 2), as follows:

1. Microbial flow model: microbial transformations of labile and slow C moved to the recalcitrant pool, representing the formation of microbial products (Fig. 2b).
    a. Microbial CUE was set at 0.3, the average value for litter decomposition (Sinsabaugh et al. 2013).
    b. Microbial CUE varied from 0.3-0.6 for the labile pool and 0.2-0.5 for the slow pool (Wieder et al. 2014, Campbell et al. 2016) depending on initial litter N content.
    c. The slope and intercept of microbial CUE as it varies with initial litter N was estimated from the data set and used to calculated CUE.
    d. The slope of microbial CUE as it varies with initial litter N was estimated from the data set and used to calculated CUE.
    e. Microbial CUE for the labile and slow pools was estimated from the dataset as a constant value (i.e., not varying with initial litter N).

2. Microbial pool model: A microbial pool with its own decay rate was added, to account for immobilization and include microbial products (Fig. 2c). Initial microbial biomass was set at 1% of initial litter mass, based on the estimate that microbial biomass C comprises 1-3% of soil C (Smith and Paul 1990).
   a. Microbial transformations of labile, slow, and very slow C moved to a separate microbial biomass and products pool with its own decay rate that was not modified by CDI_{LT}. Microbial CUE was set at 0.3 (Sinsabaugh et al. 2013).
   b. Same model as 2a, except that the decay rate of the microbial biomass and products pool was modified by $CDI_{LT.}$
   c. Microbial transformations of the labile and slow pools moved to the microbial biomass pool, and the slope and intercept of microbial CUE as it varies with initial litter N was estimated from the data set. In this model, lignin did not transfer into the microbial biomass and products pool based on the hypothesis that lignin is not energetically favorable for microbes to decompose (Moorhead et al. 2013, Campbell et al. 2016).
   d. Same as model 2c, except only the slope of microbial CUE (not the intercept) as it varies with initial litter N was estimated from the data set.
   e. Same model as 2c, except that microbial CUE was estimated from the dataset as a constant value (i.e., not varying with initial litter N).

4.3.3. Nitrogen release models

Nitrogen release models (Table 3) were formulated to accompany the base model and the best version of mass loss models 1 (model 1e) and 2 (model 2e) as determined by the model comparison (see "Parameter Estimation and Model Comparison" below). The N model calculated the initial percentage of N in the recalcitrant pool based on the initial litter N:C. Because the slow pool was assumed to be cellulose, it contained no N and all remaining N was placed in the labile pool. Nitrogen loss was then calculated as mass loss (predicted by the mass loss model) multiplied by the N:C of each litter quality pool.

4.3.4. Parameter Estimation and Model Comparison

For both mass loss and N dynamics, we solved the model systems of differential equations using the dsolve function in Matlab (R2019a Update 1, The Mathworks, Inc., Natick, MA, USA). We estimated model parameters using maximum likelihood estimation (mle2) in the R package bblme (Bolker and R Development Core Team 2017). We used Akaike's Information Criterion (AIC) modified for small sample sizes (AICc) to rank the models according to which is closest to the unknown truth as represented by the data (Burnham and Anderson 2002). The model with the lowest AICc score is supported by the data as being closest to the unknown truth. We calculated the difference between the AICc score of the model most supported by the data and all other models (Δr = AICc of each model - AICc of best model). Models within 1-2 AICc points of the best model are supported by the data, while models with $\Delta r > 7$ have no support in the data (Burnham and Anderson 2002). We also calculated the Akaike weight (w_r) for each model, which indicates the probability that the best model would be identified as the best again given new data and the same set of comparison models (Burnham and Anderson 2002).

4.4. Results

4.4.1. (H1) Microbial models will better predict mass loss and N dynamics during decomposition than the microbially implicit base model.

Modeled mass loss during decomposition was improved by the addition of microbial parameters (Table 5). All the microbial models we tested performed better than the base model as assessed by AICc ranking. The best model (Flow model 1e) incorporated microbial transformations from the labile and slow pools to the recalcitrant pool, and estimated CUE for each pool as a constant value. This model explained over half the variability in the dataset ($R^2 = 0.57$), and had a high probability of being chosen again as the best model given the same set of comparison models and new data (w_r = 0.8647). The decay rates for the three pools indicated fast decomposition of both the labile (k_1 = 12.987) and slow (k_2 = 10.290) pools, and slow decomposition of the lignin pool over time (k_3 = 0.137). Only two other models had any support in the data ($\Delta r < 7$), and neither was a close competitor to the best model ($\Delta r > 2$) nor had high probability of being chosen as the best model from the same set of models tested with new data (w_r = 0.0793, 0.0514 for second and third best model, respectively). The second-best model (Flow model 1c, $\Delta r = 4.8$, $R^2 = 0.57$) incorporated microbial transformations from the labile and slow pools to the recalcitrant pool and estimated microbial CUE as the relationship between initial litter N with an intercept and slope for both the labile and slow pools. The third best model (Pool model 2e, $\Delta r = 5.6$, $R^2 = 0.57$) included a microbial biomass pool, and estimated microbial CUE as a constant value. Both the second and third best models estimated decay rates with very similar values to the best model (Table 5).

As opposed to the mass loss models, N dynamics during decomposition were best represented by the base (microbially implicit) model (Table 6). This model estimated that the N:C of the recalcitrant pool was 4.7% of the litter N:C (a = 4.708). It had 100% probability of being selected as the best model given the same set of models and new data (w_r = 1). Although model selection identified it as the best model, it was only able to explain 6% of the variability in the N loss data during decomposition. The two microbial models had no support in the data (Δr = 5916 and 6075 for the pool and flow model, respectively). However, both the microbial flow model (R^2 = 0.0966) and the microbial pool model (R^2 = 0.1426) explained a greater portion of the variability in the data than the base model.

4.4.2. (H2) Regulation of microbial CUE according to litter quality will improve modeled mass loss.

The best mass loss model estimated CUE as a constant value for each litter quality pool, rather than through its relationship to initial litter N content. Although the second-best model did estimate CUE based on the relationship between an intercept and slope with litter N, the slope for both the labile and slow pools was estimated to be zero, effectively making the intercept a constant estimate of microbial CUE for each litter pool. Estimates of microbial CUE for the labile and slow pools were consistent across the three best models, with labile CUE values falling in the range of 0.249 - 0.254 and slow CUE in the range of 0.303 - 0.311.

4.5. Discussion

Addition of microbial parameters improved a traditional litter mass loss model. However, accompanying N loss models were not improved by addition of microbial parameters. The best mass loss model estimated microbial CUE for each litter quality pool, rather than through its relationship to initial litter N. Our results illustrate that inclusion of microbial parameters can improve first order mass loss models based on traditionally available decomposition data, but that further work is needed to develop a mechanistic microbial model of N release during decomposition.

4.5.1. (H1) Microbial parameters improved mass loss, but not N release, decomposition models

All the microbial mass loss models we tested performed better than the base, microbially implicit model, and the best model simply included CUE-regulated flows between litter quality pools to represent microbial transformations. Although many studies that incorporate microbial parameters do not compare their models to traditional, microbially implicit models, there are several exceptions. These include incorporating microbes to explain soil respiration pulses following rewetting events (Lawrence et al. 2009), improvements to global soil C projections by including temperature and C sensitive CUE (Wieder et al. 2013), and improved SOM pool response to warming after inclusion of enzyme kinetics and microbial functional types (Wieder et al. 2014). Collectively, these results suggest that models that include microbial parameters are better able to capture the response of ecosystem processes to global change phenomena. Given the multiple climatic and biogeochemical alterations that Earth system models are faced with in their projections, including microbial parameters may be particularly important to generating better model predictions. Our model was not confronted with global change scenarios, but performed better on long-term, large-scale decomposition data, demonstrating the overall value of adding microbial processes.

Our global decomposition dataset included only typically available data such as mass loss, N loss, climate, and litter quality. This demonstrates that microbial representations in models can be achieved in the most commonly collected decomposition datasets, namely litterbag decomposition studies, which remain the most widespread method for measuring decomposition (Kurz-Besson et al. 2005). This contrasts with many microbially explicit models that rely on parameter values estimated in laboratory studies (Wieder et al. 2015), which were estimated at small spatial and temporal scales. Conversely, rate constants in first-order (i.e., microbially implicit) models (e.g., Adair et al. 2008, Zhang et al. 2008) have been estimated using broad temporal and spatial data (Wieder et al. 2015), much like we achieved with the current dataset.

As opposed to mass loss, modeled N release during decomposition did not improve following inclusion of microbial parameters. Although the microbial model was able to explain over twice the variability in the dataset as compared to the base model (14% vs. 6%), the model selection clearly identified the base model as the best for predicting a new dataset. In contrast to our low R^2 values, previous models of N dynamics during decomposition found high predictive ability based solely on initial litter N concentration and mass remaining during decomposition (Parton et al. 2007), information which similarly formed the basis of our N model. That non-mechanistic model was able to explain 77% of the variability in N release patterns in the LIDET dataset (Parton et al. 2007). When applied to our compiled global dataset, it explained 20% of the variability in N loss during decomposition, demonstrating much possibility for improvement in predictions yet also performing better than the mechanistic models we developed here.

Basic stoichiometric theory indicates that substrate stoichiometry and microbial demand drive decomposition (Melillo et al. 1982, Hessen et al. 2004). Conversely, increased nutrient availability may lead to decreased decomposition rates (Moorhead and Sinsabaugh 2006), as greater N availability can decrease microbial use of recalcitrant C (i.e., reduce microbial N mining; Wang et al. 2004). To include the role of microbial stoichiometry in N release during decomposition, we modified our N loss model to allow N use to be determined by mass loss and the microbial ratio of N:C (data not shown). In this model, microbes could access enough N to decompose all available C, eliminating nutrient limitations in accordance with basic stoichiometric theory. This should allow microbes to demonstrate the pattern of N immobilization followed by release that is characteristic of litter decomposition (Parton et al. 2007). However, this model performed worse than our base model which incorporated mass loss and the litter N:C ratio. Previous research found that arid and humid ecosystems display different N release patterns (Parton et al. 2007), suggesting that the variety of ecosystems included in our dataset may require more in-depth individual analysis to identify common drivers of N dynamics during decomposition.

4.5.2. (H2) The best models estimated CUE as a constant value for each litter pool, not based on initial litter N.

Our best mass loss model estimated microbial CUE as a constant value for each litter quality pool (labile and slow) that had transfers into the recalcitrant pool. The fast and slow pool CUEs were estimated as 0.254 and 0.311, respectively. These values approximate the recommended value of 0.3 for broad scale models (Sinsabaugh et al. 2013), but are low compared to values previously used in microbial models (0.3-0.6; Wieder et al. 2014, Campbell et al. 2016). Because nutrient limitation can reduce CUE (Larsson et al. 1995), and decomposers can lower their CUE to utilize low N litters (Manzoni et al. 2008), we expected the best model to vary CUE with litter N content. Our results suggest that for broad-scale data, overall litter quality pools are a better determinant of CUE than N content specifically. CUE results from the interplay of environmental conditions, substrate quality, stoichiometry, and the activity and composition of the microbial community (Manzoni and Porporato 2009). It is therefore likely that across broad temporal and spatial scales, the variability in CUE observed with nutrient availability lessens and the best estimate is the process average (Sinsabaugh et al. 2013).

4.6. Conclusion

Our work provides evidence that the predictive capability of first order mass loss models using widely available datasets can be improved by including representations of microbial activity. However, we also found our microbial N models to perform worse than conventional microbially implicit models, indicating a need for further work to incorporate microbial mechanisms related to the N cycle into large scale models.

Microbial CUE was best estimated for each litter quality pool, as opposed to initial litter N content, and approximated large-scale average CUE for litter decomposition. These results suggest that addition of microbial processes can improve large scale mass loss model accuracy, potentially improving projections of future environmental conditions.

4.7. Tables and Figures

4.7.1. Tables

Table 4.1. Datasets included in model development and comparison. L/N = lignin to nitrogen ratio. In all cases, leaf litter was decomposed aboveground and roots were decomposed belowground.

Data Set	Time (years)	# Sites	# Litter types	L/N range	Site location, biomes	Citation
ART DECO Assembly of Research on Traits and DECOmposition	0.6-4.8	14	101 leaf	0.1-51% (lignin) 0.2-5% (N)	Tropical, subtropical, temperate	Cornwell et al 2008
CIDET Canadian Intersite Decomposition Experiment	6	19	10 leaf	15.59-55.26	Canadian boreal, subarctic, temperate, cordilleran	Trofymow and CIDET Working Group 1998
EuroDeco European Decomposition Data	3-8	47	12 leaf 5 root	8.6-98.7	Temperate and boreal conifer sites across 11 European countries	Berg et al 1993
GT Dec Global Tropical	1-1.2	23	2 leaf	9.9, 20	Global, wet and dry tropical forests	Powers et al 2009
LIDET Long-term Intersite Decomposition Experiment Team	10	27	6 leaf 3 fine root 18 “wildcard” leaf	0.94-59.49	N. & S. America, arctic through tropical	LIDET 1995

Table 4.2. Differential equations for mass loss models. The base model is a three pool, negative exponential model with a climatic decomposition index based on the Lloyd and Taylor (1994) temperature function (CDI_{LT}) modifying all pools and a litter quality modifier (e^{-bL_s}) for pool 2. See table 4 for definitions of terms.

Model	CUE	Equation
Base Model	NA	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT}$
Flow 1a	0.3	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + (-k_3M_{3t}CDI_{LT} + ck_1M_{1t}CDI_{LT} + ck_2M_{2t}CDI_{LT}e^{-bL_s})$
Flow 1b	Varies with initial litter N	*Same as 1a*
Flow 1c	$\beta_0 + \beta_1$N	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + (-k_3M_{3t}CDI_{LT} + (\beta_3 + \beta_4N)k_1M_{1t}CDI_{LT} + (\beta_5 + \beta_6N)k_2M_{2t}CDI_{LT}e^{-bL_s})$
Flow 1d	β_1N	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + (-k_3M_{3t}CDI_{LT} + (\beta_4N)k_1M_{1t}CDI_{LT} + (\beta_6N)k_2M_{2t}CDI_{LT}e^{-bL_s})$
Flow 1e	Estimated constant	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + (-k_3M_{3t}CDI_{LT} + fk_1M_{1t}CDI_{LT} + sk_2M_{2t}CDI_{LT}e^{-bL_s})$
Pool 2a	0.3	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT} + (k_4M_{4t} + ck_1M_{1t}CDI_{LT} + ck_2M_{2t}CDI_{LT}e^{-bL_s} + ck_3M_{3t}CDI_{LT})$
Pool 2b	0.3	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT} + (k_4M_{4t}CDI_{LT} + ck_1M_{1t}CDI_{LT} + ck_2M_{2t}CDI_{LT}e^{-bL_s} + ck_3M_{3t}CDI_{LT})$
Pool 2c	$\beta_0 + \beta_1$N	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT} + (k_4M_{4t}CDI_{LT} + (\beta_3 + \beta_4N)k_1M_{1t}CDI_{LT} + (\beta_5 + \beta_6N)k_2M_{2t}CDI_{LT}e^{-bL_s})$
Pool 2d	β_1N	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT} + (k_4M_{4t}CDI_{LT} + (\beta_4N)k_1M_{1t}CDI_{LT} + (\beta_6N)k_2M_{2t}CDI_{LT}e^{-bL_s})$
Pool 2e	Estimated constant	$\frac{dM}{dt} = -k_1M_{1t}CDI_{LT} + -k_2M_{2t}CDI_{LT}e^{-bL_s} + -k_3M_{3t}CDI_{LT} + (k_4M_{4t}CDI_{LT} + fk_1M_{1t}CDI_{LT} + sk_2M_{2t}CDI_{LT}e^{-bL_s})$

Table 4.3. Differential equations for N loss models, based on the base mass loss model and the best of the microbial flow and pool models (see Table 2). See table 4 for definitions of terms.

Model	**CUE**	**Equation**
Base Model	NA	$\frac{dN}{dt} = -k_1 M_{1t} CDI_{LT} \frac{N_{1t}}{C_i} + -k_3 M_{3t} CDI_{LT} \frac{N_{3t}}{C_i}$
Flow 1e	Estimated constant	$\frac{dN}{dt} = -k_1 M_{1t} CDI_{LT} \frac{N_{1t}}{C_i} + (k_3 M_{3t} CDI_{LT} \frac{N_{3t}}{C_i} + f k_1 M_{1t} CDI_{LT} \frac{N_{1t}}{C_i})$
Pool 2e	Estimated constant	$\frac{dN}{dt} = -k_1 M_{1t} CDI_{LT} \frac{N_{1t}}{C_i} + -k_3 M_{3t} CDI_{LT} \frac{N_{3t}}{C_i} + (-k_4 M_{4t} CDI_{LT} \frac{N_{4t}}{C_i} + f k_1 M_{1t} CDI_{LT} \frac{N_{1t}}{C_i} + s k_3 M_{3t} CDI_{LT} \frac{N_{3t}}{C_i})$

Table 4.4. List of model parameters and definitions for models in tables 2 and 3.

Parameter	Definition
M_t	Mass remaining at time, t (%)
t	Time (years)
M_1	Initial mass of fast pool (%)
M_2	Initial mass of slow pool (%)
M_3	Initial mass of recalcitrant pool (%)
M_4	Initial mass of microbial biomass pool (%)
N_{1t}	Initial N content of fast pool (%)
N_{3t}	Initial N content of recalcitrant pool (%)
N_{4t}	Initial N content of microbial biomass pool (%)
C_i	Initial C content of litter (fraction)
k_1	Decay rate of fast pool
k_2	Decay rate of slow pool
k_3	Decay rate of recalcitrant pool
k_4	Decay rate of microbial biomass pool
CDI_{LT}	CDI with Lloyd and Taylor temperature function (Lloyd and Taylor 1994)
N	Initial litter N content
b	Parameter for lignin fraction (L_s) in pool 2
β_1	Parameter 1 for estimating initial size of labile pool
β_2	Parameter 2 for estimating initial size of labile pool
β_3	Labile pool intercept for estimating CUE using initial N
β_4	Labile pool slope for estimating CUE using initial N
β_5	Slow pool intercept for estimating CUE using initial N
β_6	Slow pool slope for estimating CUE using initial N
L_S	Lignin/(Lignin + Cellulose)
c	Microbial CUE, 0.3 (Sinsabaugh et al. 2013)
f	Estimated CUE of fast pool
s	Estimated CUE of slow pool

Table 4.5. Mass loss model diagnostics and parameter values. Models are in order of best to worst models, from left to right, based on Akaike's Information Criterion (AICc). Δ*r*, difference in AICc of each model and the best model; wr, Akaike weight; na, not applicable. All other terms are defined in table 4.

Model	1e	1c	2e	2c	2b	1a	1b	2a	1d	2d	Base
Type	Flow	Flow	Pool	Pool	Pool	Flow	Flow	Pool	Flow	Pool	Implicit
CUE	Constant	$\beta_0+\beta_1 N$	Constant	$\beta_0+\beta_1 N$	0.3	0.3	0.2-0.6	0.3	$\beta_1 N$	$\beta_1 N$	na
R^2	0.5732	0.5732	0.5730	0.5730	0.5705	0.5703	0.5675	0.5562	0.5439	0.5487	0.5401
AICc	51988	51993	51994	51998	52032	52033	52143	52238	52428	52434	52470
Δr	0	4.8	5.6	10.5	44.1	44.6	154.9	250.1	440.4	445.9	482.1
w_r	0.8647	0.0793	0.0514	0.0046	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
k_1	12.987	12.394	12.890	11.567	8.488	8.433	6.982	10.917	5.599	7.116	5.411
k_2	10.290	10.151	10.333	10.582	12.155	12.113	7.215	13.474	1.765	3.572	1.283
k_3	0.137	0.136	0.137	0.142	0.118	0.131	0.063	0.464	0.025	0.024	0.011
k_4			0.138	0.137	0.186			0.018		0.0	
b	4.404	4.374	4.407	4.454	4.509	4.625	3.770	5.176	3.138	4.301	2.680
β_1	50.183	50.880	50.309	50.081	50.400	50.424	50.124	50.479	50.058	25.030	50.082
β_2	0.070	0.070	0.071	0.071	1.980	1.251	1.517	0.994	0.013	0.003	0.012
β_3		0.253		0.240							
β_4		0.0		0.0					0.148	0.0	
β_5		0.310		0.306							
β_6		0.0		0.0					0.188	0.226	
f	0.254		0.249								
s	0.311		0.303								

Table 4.6. Nitrogen loss model diagnostics and parameter values. Models are in order of best to worst models, from left to right, based on Akaike's Information Criterion (AICc). Δr, difference in AICc of each model and the best model; wr, Akaike weight; a, proportion of N in lignin pool; na, not applicable. All other terms are defined in table 4.

Model	Base	2e	1e
Type	Implicit	Pool	Flow
CUE	na	Constant	Constant
R^2	0.0648	0.1426	0.0966
AICc	6929	12845	13004
Δr	0	5916	6075
w_r	1	< 0.001	< 0.001
a	4.708	3.969	1.137

4.7.2. Figures

Fig. 4.1. Map of sites from which decomposition data were collected.

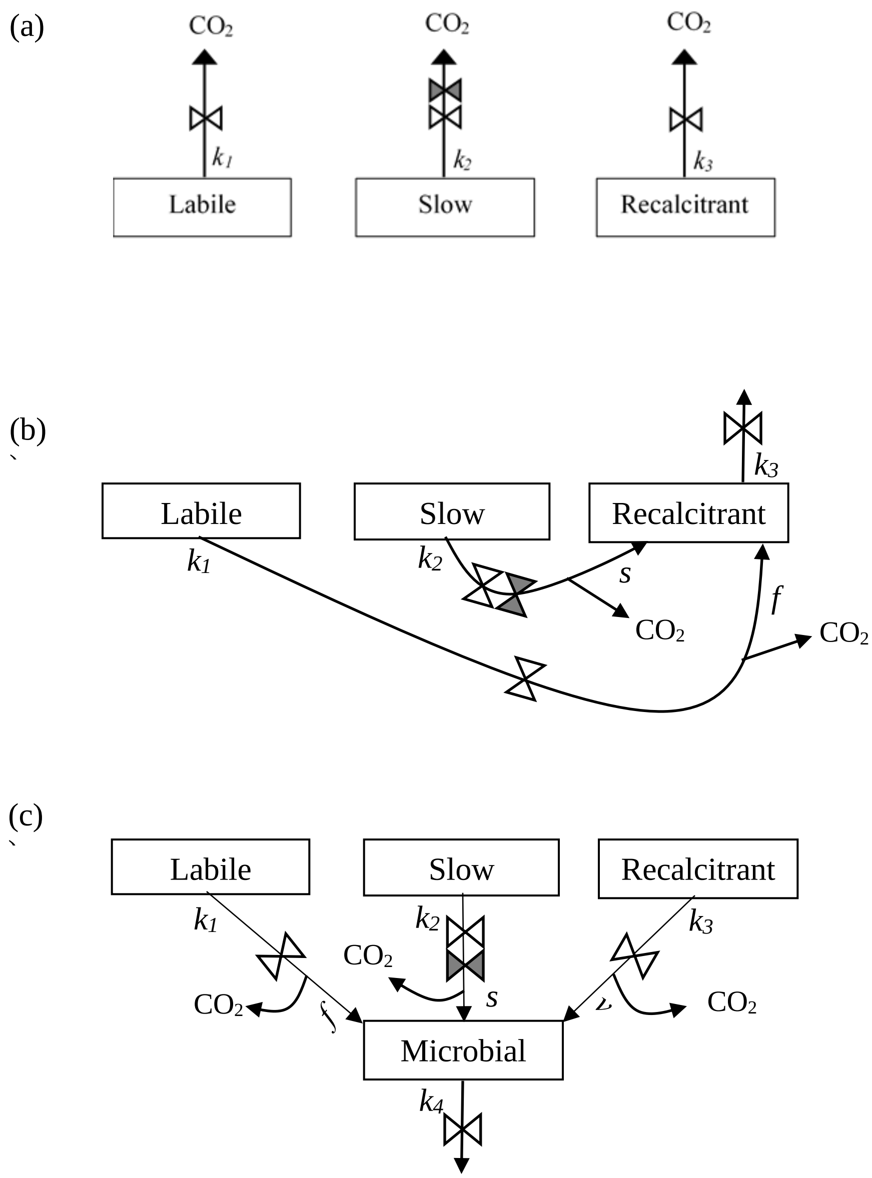

Fig. 4.2. The LIDET model depicts mass loss of leaf and fine root litter. For all panels, decomposition rate of each pool is denoted as k_x, white bowties denote the CDI_{LT} climate modifier (all pools), and the gray bowtie represents the cellulose/lignin modifier (slow pool only). The terms *f*, *s*, and *v* refer to microbial CUE for the fast, slow, and very slow pools, respectively. (a) The base model is a three pool, negative exponential model that does not incorporate any microbial parameters. (b) The microbial flow model incorporated transfers from the labile and slow pools to the recalcitrant pool to represent microbial transformations of litter. (c) The microbial pool model included a microbial biomass and products pool with its own decay rate, which received inputs from the litter pools.

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APPENDIX A: CHAPTER 2 SUPPLEMENTARY MATERIAL

Supplementary Results

Growing season magnesium and aluminum losses

In 2015, Mg losses were 60% higher from coarse soils (7.4 g ± 0.2) than fine soils (4.7 g ± 0.3) and soil and treatment interacted to alter Mg loss dynamics. Mg loss from coarse soils in 2015 was higher from controls (8.4 g ± 0.4) than from snow exclusion (7.1 g ± 0.1) or warmed (6.7 g ± 0.2) mesocosms. In the same year on fine soils, warmed (5.4 g ± 0.3) mesocosms had the highest Mg losses, followed by controls (4.9 g ± 0.2) and finally snow exclusion mesocosms (3.7 g ± 0.4). Aluminum loss in 2014 was 30% greater from coarse (541 mg ± 26) than fine (409 mg ± 26) soils, and had no relationship to climate treatment. In 2015, Al loss varied on the different soil-treatment groups (soil × treatment interaction), such that its loss from coarse soils was consistent across climate treatments (control: 518 mg ± 43; warmed: 510 mg ± 13; snow exclusion: 541 mg ± 45), but fine soil Al loss was highest from control (510 mg ± 61) and warmed (512 mg ± 30) mesocosms as compared to snow exclusion (390 mg ± 20).

Asynchrony carbon and nutrient losses

In 2015, DOC losses were 25% greater from coarse (588 mg ± 64) than fine soils (442 mg ± 48). Nitrogen (TDN, NO_3^-, NH_4^+) losses during asynchronies were also significantly altered by soil type and climate treatment, with more effects in the second spring. While TDN was not significantly related to soil or treatment in 2014, soil and the interaction between soil and treatment significantly altered TDN losses in 2015. That year, TDN losses from coarse soils (333 mg ± 91) were ten times greater than from fine soils (33 mg ± 7). The response to treatment varied by soil, such that coarse soil warmed losses (437 mg ± 164) were more than twice that from snow exclusion (190 mg ± 59), with control losses intermediate (373 mg ± 221). Conversely, on fine soils, snow exclusion (59 mg ± 8) experienced the highest TDN losses, followed by warmed (27 mg ± 5), and finally control (13 mg ± 7) treatments. Unlike TDN, NO_3^- was significantly related to treatments both years of the study (Figs. 4c and 4d). In 2014 (Fig. 4C), the response of NO_3^- loss to treatment varied by soil type. On coarse soils, NO_3^- losses were comparable across treatments (control: 273 mg ± 85; warmed: 418 mg ± 65; snow exclusion: 313 mg ± 78). However, on fine soils, snow exclusion (719 mg ± 80) experienced the highest NO_3^- losses, followed by warmed mesocosms (444 mg ± 110) and finally controls (167 mg ± 56). In 2015 (Fig 4d), NO_3^- loss from coarse soils was nearly twenty times higher (335 mg ± 80) than from fine soils (17 mg ± 6) and the effect of treatment varied by soil type. On coarse soils, warming (433 mg ± 133) resulted in significantly more NO_3^- leaching than snow exclusion (231 mg ± 46), and the control (340 mg ± 208) was intermediate to both. On fine soils, losses were significantly elevated from snow exclusion (39 mg ± 11) as compared to control (6 mg ± 2) and warmed mesocosms (6 mg ± 3). Ammonium losses did not vary significantly by either soil or treatment either year of the study. Finally, PO_4^{3-} losses were significantly higher from fine (2014: 18 mg ± 1; 2015: 20 mg ± 2) than coarse soils (2014: 13 mg ± 1; 2015: 12 mg ± 1) both springs, with no significant effect of climate treatment.

Loss of cations related to soil acidification during asynchronies varied significantly by both soil and climate treatment, with the effects of climate treatment tending to emerge in the second year of the study (Table 5). Both springs, Ca^{2+} loss was higher from coarse (2014: 35 g ± 3; 2015: 23 g ± 1) than fine soils (2014: 17 g ± 1; 2015: 13 g ± 1). In 2015, the control (21 g ± 2) experienced higher Ca^{2+} leaching than snow exclusion (16 g ± 2), with warmed (18 g ± 2) Ca^{2+} losses being intermediate. The effect of treatment on Mg loss varied across soil types both years of the experiment. In 2014, coarse soil losses were comparable across treatments (control: 4.6 g ± 0.5, warmed: 4.1 g ± 0.3, snow exclusion: 4.2 g ± 0.9). Conversely, warming (4.8 g ± 1.2) and snow exclusion (4.7 g ± 0.4) on fine soils resulted in 80% more Mg loss than from the control (2.7 g ± 0.4). In 2015, Mg losses from coarse soils were higher from the control (3.4 g ± 0.2) than from warmed (2.6 g ± 0.1) or snow exclusion (2.5 g ± 0.04) treatments. On fine soils, the warmed treatment (2.5 g ± 0.3) experienced higher Mg losses than controls (2.0 g ± 0.1), and snow exclusion experienced the lowest losses (1.4 g ± 0.1). Finally, Al loss in 2014 was 15% higher from coarse (311 mg ± 30) than fine (263 mg ± 21) soils. In 2015, the effect of treatments on Al loss varied by soil type. On coarse soils, snow exclusion (141 mg ± 8) Al losses were elevated over control (117 mg ± 14) and warmed (130 mg ± 10) treatments. On fine soils, warmed Al losses (163 mg ± 5) were significantly higher than from snow exclusion (110 mg ± 21), with control losses (139 mg ± 28) being intermediate.

In 2014, Mg losses during asynchrony did not vary by soil type, but decreased with asynchrony length. In 2015, Mg losses were higher from coarse than fine soils, and longer asynchronies were associated with higher Mg losses on fine soils, while on coarse soils longer asynchronies were associated with slightly lower Mg losses.

Supplementary Tables

Table S1. Physical and chemical properties of the two soils used in the mesocosms. CEC = cation exchange capacity. Data marked with * are from Beard et al. (2005), and were measured when the site was established in 1995. pH measurements were made in 2015, and all other data were measured in 2013 before initiation of climate treatments. Data are means with standard errors in parentheses where available.

Soil Property	Coarse	Fine
Bulk density (g cm^{-3})	1.724	1.498
CEC (meq 100 g^{-1})*	18.2	0.9
Clay (%)*	1.15	0.95
Silt (%)*	0.56	0.66
Sand (%)*	63.92	81.17
Fine gravel >2 mm (%)*	34.26	17.30
pH	7.639 (0.173)	6.156 (0.193)
Ca (mg kg^{-1})	1773.75 (68.74)	70.00 (4.68)
P (mg kg^{-1})	4.50 (0.80)	1.55 (0.22)
K (mg kg^{-1})	42.83 (4.36)	48.79 (3.89)
Mg (mg kg^{-1})	35.36 (1.22)	10.69 (0.90)
Na (mg kg^{-1})	5.83 (0.31)	4.93 (0.33)
Al (mg kg^{-1})	7.74 (0.15)	11.16 (0.44)
Fe (mg kg^{-1})	9.07 (0.57)	4.27 (0.45)
Mn (mg kg^{-1})	20.26 (0.73)	12.91 (0.94)
S (mg kg^{-1})	32.16 (1.31)	6.91 (1.07)
% C	0.698 (0.039)	0.325 (0.033)
% N	0.045 (0.004)	0.031 (0.003)
C:N	15.891 (0.851)	10.520 (0.350)

Table S2. Rooting depth characteristics and relative location of the South Burlington, VT planting site in relation to tree species ranges for saplings planted in the mesocosms.

Location	Shallow rooted	Deep rooted
At south of range	paper birch (*Betula papyrifera* Marshall)	quaking aspen (*Populus tremuloides* Michx.)
At north of range	black cherry (*Prunus seronita* Ehrh)	American chestnut (*Castanea dentate* (Marshall) Borkh.)

Table S3. Climate treatments significantly altered mean soil temperatures (X_2^2 = 39.8, $p <$ 0.0001, R^2 = 0.73), snow depth AUC (2014: X_2^2 = 399.1, $p < 0.0001$, R^2 = 0.94; 2015: X_2^2 =294.7, $p < 0.0001$, R^2 = 0.94), and soil freezing AUC (2014: X_2^2 =187.6, $p < 0.0001$, R^2 = 0.89; 2015: X_2^2 =9.2, p = 0.01, R^2 =0.36) throughout the replicated climate change mesocosm experiment. Values are means with standard errors of the mean in parentheses.

	2014			2015		
	Control	Warmed	Snow exclusion	Control	Warmed	Snow exclusion
Mean soil temp, 5 cm depth (°C)	8.7 (0.12)	9.6 (0.19)	8.6 (0.19)	8.2 (0.16)	8.8 (0.14)	7.9 (0.12)
Snow AUC (cm days)	1529.8 (49.8)	568.1 (24.9)	1226.7 (39.3)	1157.4 (11.5)	720.0 (23.9)	968.6 (11.4)
Frost AUC (cm days)	2338.6 (134.6)	2766.2 (243.4)	5433.2 (136.1)	4264.1 (213.4)	4090.3 (129.3)	4653.8 (110.5)

Table S4. Bud and leaf development descriptions for assessments of sapling spring phenology (West and Wein 1971).

Stage	Defining Characteristics
0	Buds dormant with scales closed
1	Buds display silver/green tip, greenness between scales
2	Buds green and tight or scales slightly separated
3	Buds expanding, leaves unfolding
4	Internodes/petioles visible, leaves hanging but not enlarged
5	Internodes/petioles visible, leaves enlarged

Supplementary Figures

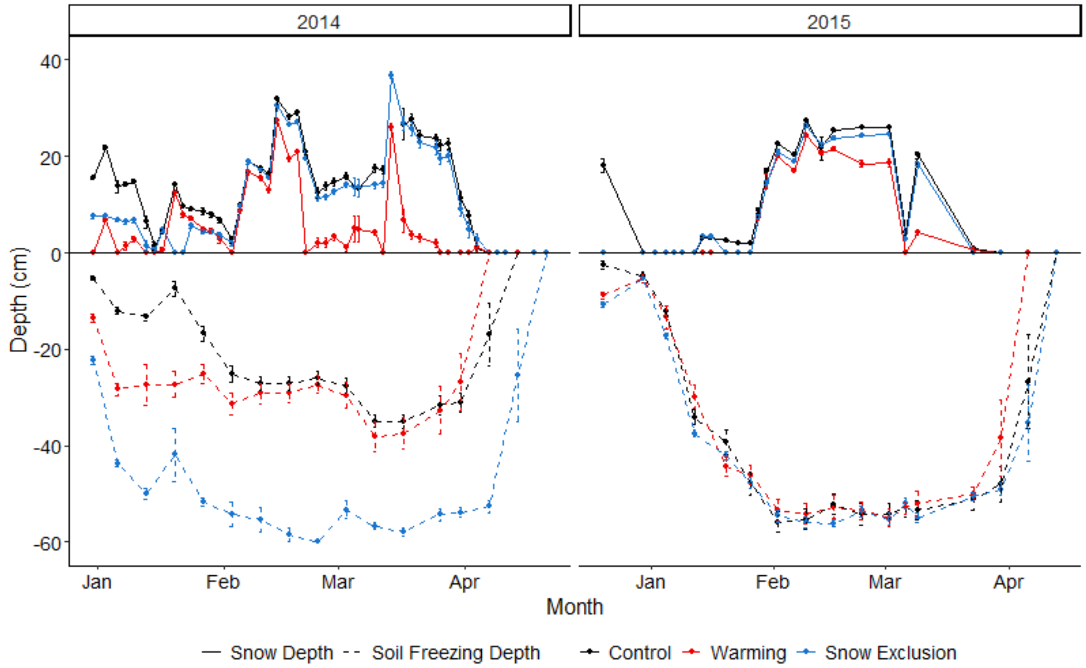


Fig. S1. Mean snow and soil freezing depth by climate treatment in 2014 and 2015. Error bars are ±1 standard error. Values above zero (solid lines) represent snow depth, and values below zero (dashed lines) represent soil freezing depth.

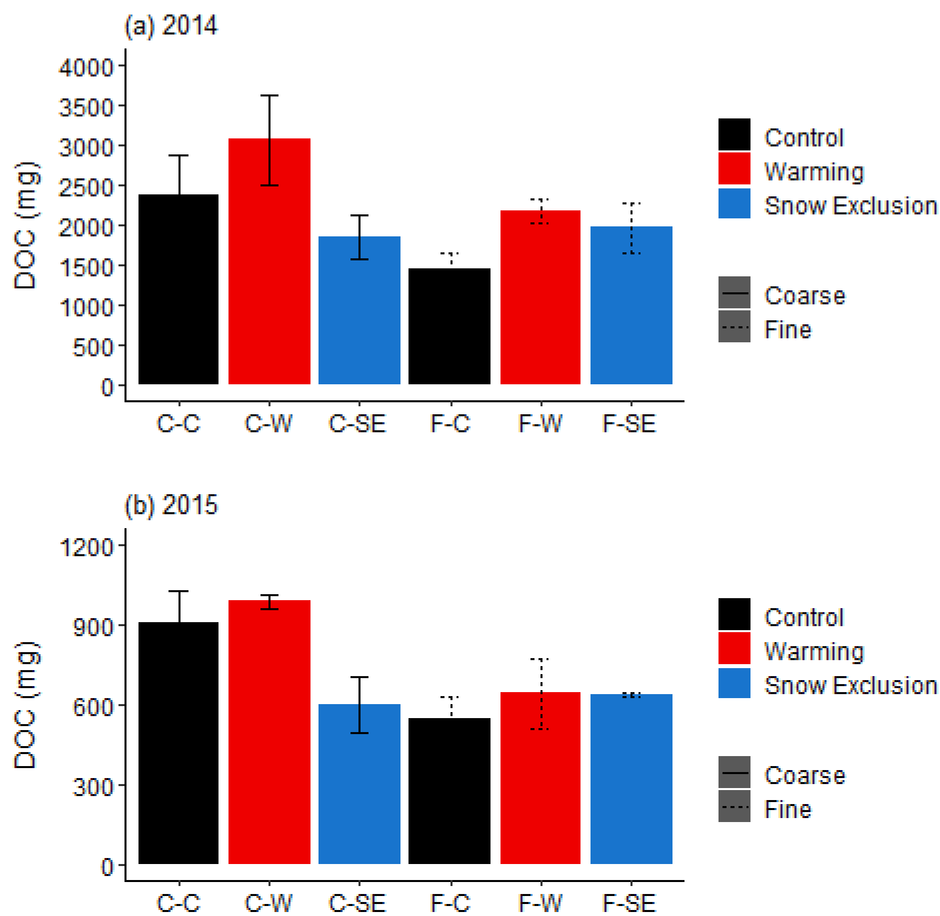


Fig. S2. Total DOC leachate loss from forest mesocosms in (a) 2014 and (b) 2015. Note different y axis limits. X axis codes are soil (C = coarse soil, solid lines or F = fine soil, dashed lines) followed by treatment (C= control (gray), W = warming (red), SE = snow exclusion (blue)).

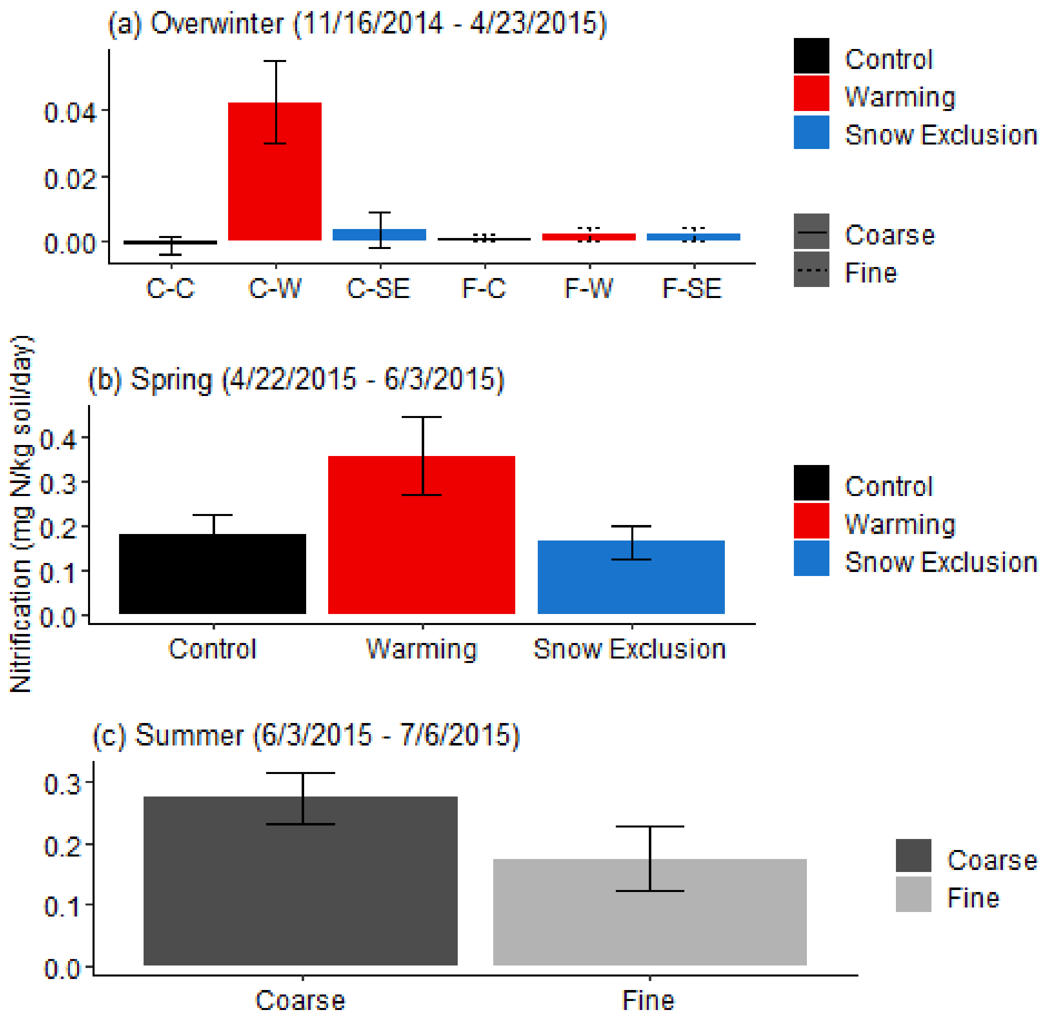


Fig. S3. In situ nitrification measured in forest mesocosm soils by season: (a) winter; (b) spring; and (c) summer. Note the varying y axis limits in each panel.

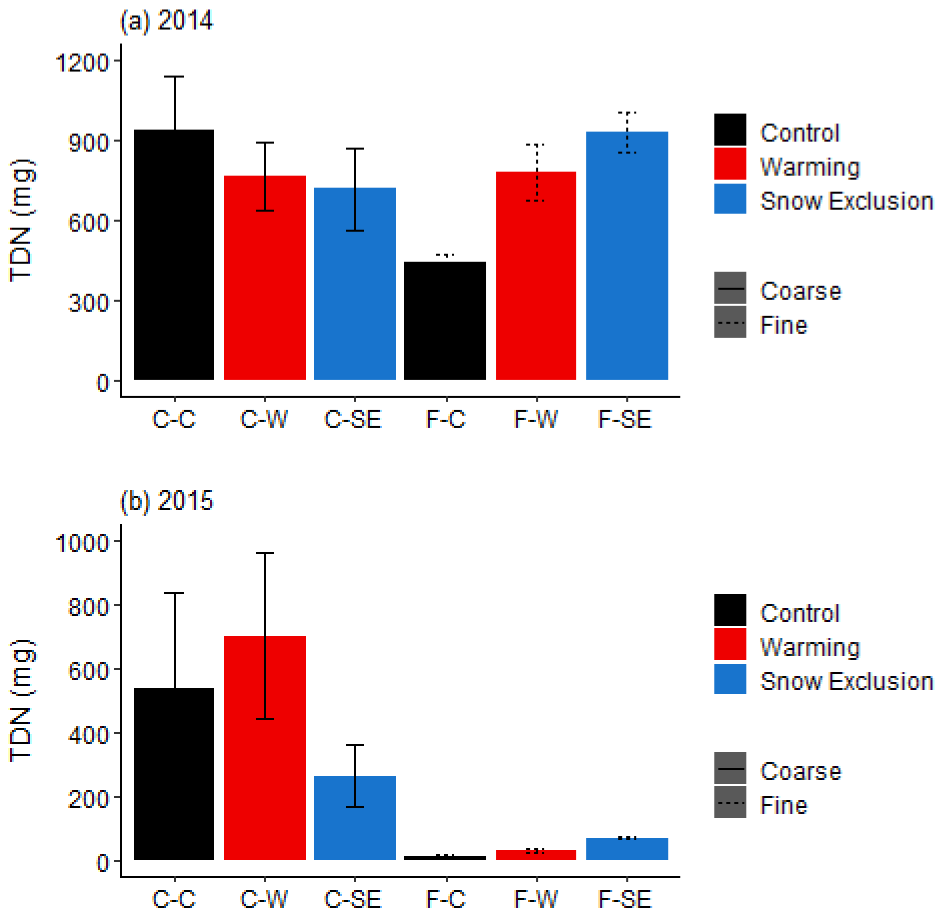
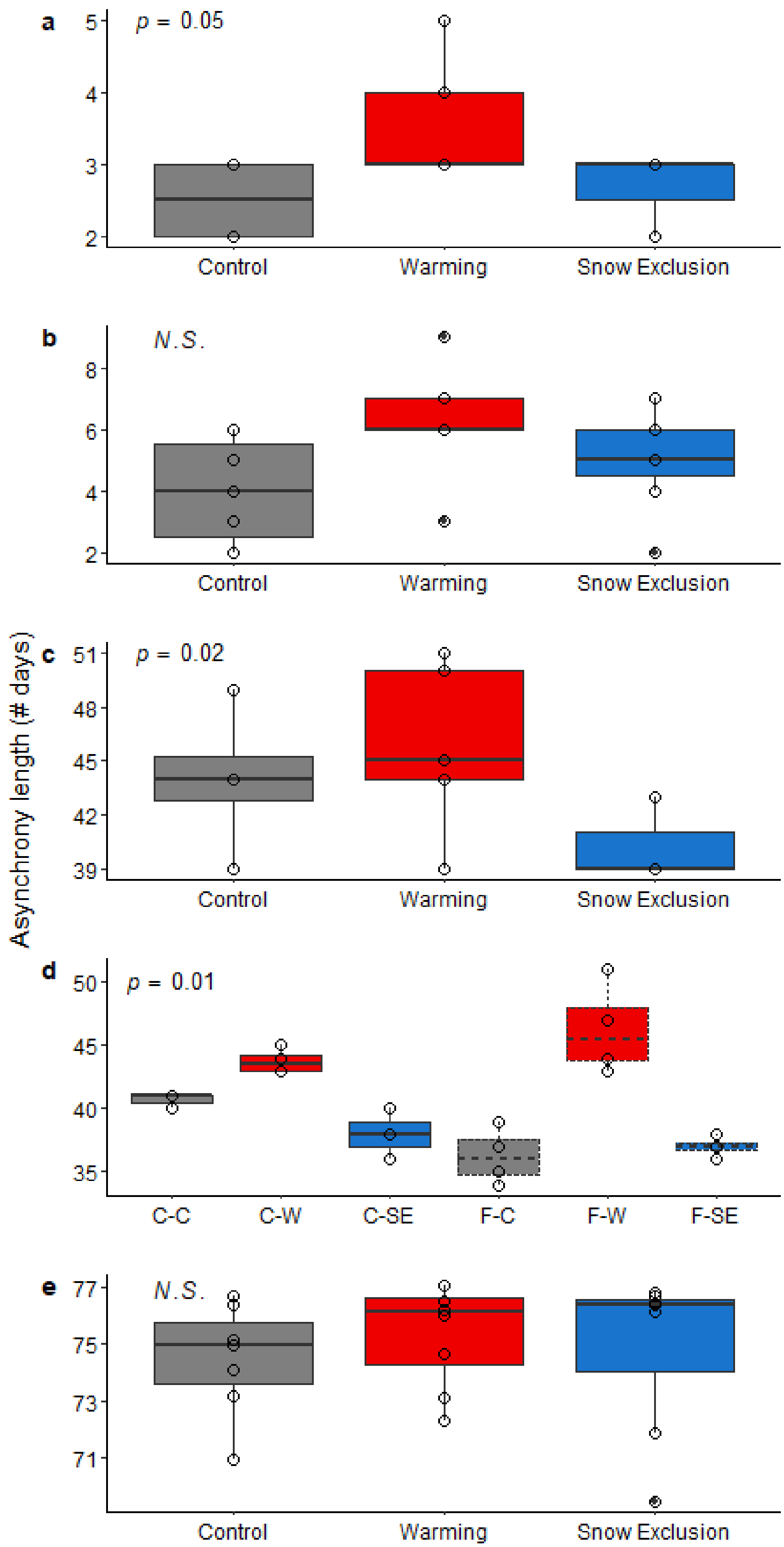


Fig. S4. Total dissolved N leachate losses from mesocosms in (a) 2014 and (b) 2015. Note different y axis limits. X axis codes are soil (C = coarse soil, solid lines or F = fine soil, dashed lines) followed by treatment (C= control (gray), W = warming (red), SE = snow exclusion (blue)).



a
p = 0.05
b
N.S.
c
p = 0.02
d
p = 0.01
e
N.S.
Asynchrony length (# days)
Control
Warming
Snow Exclusion
C-C
C-W
C-SE
F-C
F-W
F-SE

Fig. S5. Plant-microbe asynchrony length as measured in (a) winter 2014; (b) winter 2015; (c) spring 2014; (d) spring 2015; (e) fall 2014. Asynchrony length was calculated as the number of days with daytime soil temperatures > 4 °C at 5 cm depth during each season. Springtime asynchrony length in 2015 varied by soil × treatment, codes are C (coarse soil, solid lines) or F (fine soil, dashed lines) followed by treatment (C= control (gray), W = warming (red), SE = snow exclusion (blue)). Note the varying y axis limits in each panel. Open circles represent data points and filled circles represent outliers.

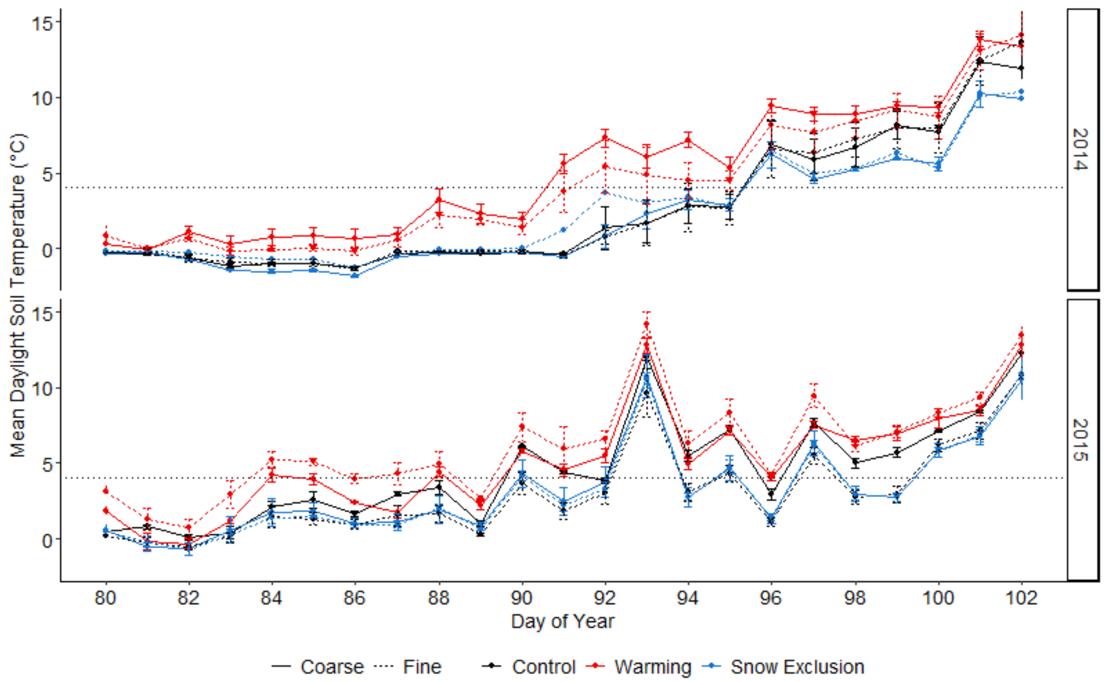


Fig. S6. Springtime soil temperatures (°C) by soil and climate treatment at 5 cm depth in forest mesocosms measured in 2014 and 2015. The dotted horizontal line marks 4 °C, the soil temperature at which rapid biological activity is thought to begin.

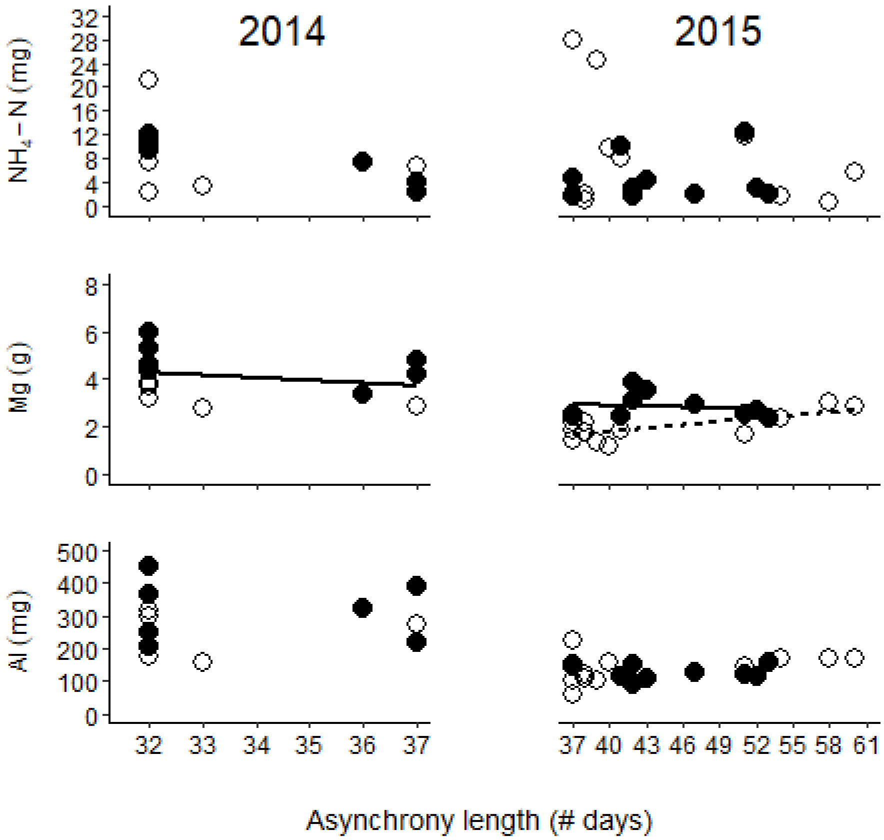


Fig. S7. Relationships between leachate water loss NH_4^+, Mg, and Al during plant-microbe activity asynchronies, soil type, and asynchrony length in 2014 (left column) and 2015 (right column). Asynchrony length was calculated as the number of days with soil temperatures over 4 °C at 5 cm depth while plants were dormant. Regression lines are shown for significant relationships. One regression line indicates losses varied significantly with asynchrony length, and two regression lines indicate that the relationship of analyte loss to asynchrony length varied by soil type. Solid circles and lines represent the coarse soil, open circles and dashed lines represent the fine soil.