Disturbance-mediated shifts in ecosystem function and implications for biogeochemical cycling in forested systems

By

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TABLE OF CONTENTS

ACKNOWLEDGMENTS ..... *iv*

INTRODUCTION *1*

MOTIVATION **2**

RESEARCH GOALS **3**

1. Chapter 1 3
2. Chapter 2 4
3. Chapter 3 4

REFERENCES **5**

CHAPTER 1: *8*

Effects of disturbance-mediated changes to intraspecific genetic diversity and litter chemistry in trembling aspen stands on litter decomposition *8*

ABSTRACT **9**

INTRODUCTION **11**

MATERIALS AND METHODS **14**

Study site history 14
Field Decomposition Experiments 16
Field sample processing and decomposition rate calculations 17
Litter addition laboratory incubation 18
Litter decomposition chemistry 20
Phospholipid fatty acid analysis 20
Stand-level characteristics 21
Statistical analyses 23

RESULTS **25**

Field experiment litter decomposition 25
Laboratory litter-addition soil incubation 27
Litter decomposition chemistry 28
Litter microbial PLFA biomass 29
Regression models 30

DISCUSSION **31**

Plant productivity, intraspecific diversity, and disturbance all affect litter decomposition 33
In-situ vs. common garden decomposition – insights into "Home-field Advantage" 35
Site-specific microenvironmental factor effects on litter turnover 36

CONCLUSIONS **38**

ACKNOWLEDGMENTS **39**

FIGURES AND TABLES **40**

SUPPLEMENTARY TABLES **52**

REFERENCES **55**

CHAPTER 2: *62*

Breaking it down: Soil aggregate disintegration and carbon dynamics in genetically diverse experimental aspen stands *62*

ABSTRACT **63**

INTRODUCTION **65**

METHODS **68**

Study site history 68
Aggregate and mineral-associated C 69
Aggregate stability and disintegration kinetics 70
Litter chemistry 72
Litter turnover estimates 72
Stand-level characteristics 73
Statistical analyses 74

RESULTS **75**

Soil C fractions 75
Aggregate disintegration kinetics and stability 76
Predictors of soil C fraction contributions 76
Predictors of soil aggregate disintegration and stability 77

DISCUSSION **78**

Plant genetic diversity and productivity are linked to soil C dynamics 79
Litter decomposition dynamics are connected to aggregate stability 81
Insights into the capacity for future C accrual in aspen systems 82

CONCLUSIONS **85**

ACKNOWLEDGMENTS **86**

FIGURES AND TABLES **87**

SUPPLEMENTARY FIGURES AND TABLES **97**

REFERENCES **103**

CHAPTER 3: *113*

Successional trajectory shapes litter decomposition and microbial dynamics in post-agricultural urban forests in St. Croix, U.S. Virgin Islands *113*

ABSTRACT **115**

INTRODUCTION **116**

MATERIALS AND METHODS **119**

Study Sites 119
Litter collection and decomposition experiments 121
Litter chemistry 122
Microbial community biomass and enzyme activity 125
Statistical Analyses 127

RESULTS **129**

Litterfall 129
Litter decomposition rates 129
Litter chemistry 130

Microbial community biomass and enzymatic activity 134

DISCUSSION **136**

Litter chemistry is tied to successional forest species composition 136

Controls of decomposition environment on litter chemical trajectory 138

Seasonal and interannual variations in microbial community composition and activity 140

Potential mechanisms driving root decomposition 141

CONCLUSION **143**

ACKNOWLEDGMENTS **144**

FIGURES AND TABLES **145**

SUPPLMENTARY FIGURES AND TABLES **159**

REFERENCES **165**

CONCLUSIONS AND FUTURE DIRECTIONS ***176***

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"Let it go, let me dance – and shake it 'til the pearls fall off"

-Jessie Ware

INTRODUCTION

Plant-soil-microbial feedbacks play a complex role in global biogeochemical cycling, but we still do not fully understand the magnitude and direction of these feedbacks, nor how they are influenced by biodiversity, forest succession, and disturbance. For my PhD research, I investigated how biodiversity and disturbance influences microbial activity and soil C dynamics in two forest ecosystems, including experimental aspen stands in southern Wisconsin and seasonally dry, post-agricultural subtropical forests in St. Croix, U.S. Virgin Islands.

The experimental aspen stands in Wisconsin each included multiple trembling aspen (*Populus tremuloides*) genotypes that differ in their growth strategies and phenotypic trait expression, influenced by disturbance-induced competitive interactions among genotypes (Cope et al. 2021). Disturbance was simulated through a reduction in tree density by 75% in half of the stands, which altered the competitive environment and led to differing genotype dominance among the stands, driven mostly through mortality of less competitive genotypes. *Disturbance-mediated changes to ecosystem function, including litter decomposition and soil C dynamics are rarely examined through the lens of intraspecific diversity, providing a unique opportunity to investigate how intraspecific competition and disturbance influence these processes.*

The seasonally dry, subtropical forests in St. Croix are both successional forests that followed different regeneration pathways after centuries of colonial sugarcane agriculture, including 40-year-old forests that were reforested with timber plantations before undergoing natural succession, and 10-year-old forests that were intensive pastures prior to forest regeneration. Differences in forest composition between the successional forests is primarily driven by the dominance of a nitrogen-fixing species *Leucaena leucocephala*, which became rapidly established in the 10-year-old forests, resulting in greater stem density, lower total

biomass, and lower species richness than 40-year-old forests (Atkinson and Marín-Spiotta 2015). Land use-driven disturbance history in these forests is a key determinant of forest composition, likely altering microbial composition and function. *Although subtropical, seasonally dry forests have some of the highest rates of agricultural conversion and habitat fragmentation, the effects of land use change and forest disturbance legacy on soil microbial activity are rarely examined there, providing an opportunity to better understand how forest succession influences important microbially-mediated ecosystem functions.*

MOTIVATION

There is large uncertainty in predictions of anthropogenic-accelerated climate change effects on terrestrial biogeochemical cycling and important soil-plant feedbacks mediated by soil microorganisms (Zabinski and Gannon 1997, Bezemer et al. 2006, Kardol et al. 2013, van der Putten et al. 2013). The sensitivity of soil as a carbon (C) sink or source in the face of future changes to climate and land use is particularly unknown. Soil organic carbon (SOC) is one of the largest terrestrial C reservoirs, encompassing a global mass of ≈2300 Pg C, which is ≈75% of the total land biosphere C on earth (Jobbágy and Jackson 2000). Increases in plant- and microbially-derived C inputs to the soil have been proposed to potentially offset increased CO_2 emissions (Eglin et al. 2010, Nowak et al. 2011, Schmidt et al. 2013) but the net retention of soil C is highly dependent on a combination of biotic and abiotic conditions (Schmidt et al. 2011). Soils can also switch from C sinks to sources of C loss through microbial decomposition of plant residues and soil organic matter (SOM), thus the vulnerability of long-term C storage and cycling is closely tied to environmental conditions, which are closely tied to disturbance.

Much of what we know about relationships between disturbance and C cycling comes from studies looking at severe events that can change the composition of an entire ecosystem, which likely have very different ramifications than more subtle events. Disturbances of intermediate intensity, frequency, or scale have shown to enhance forest biodiversity and productivity (Connell 1978, Belsky 1992, Molino 2001) and have been shown to influence ecosystem composition, structure, and function in a variety of geographic settings (e.g., Stueve et al. 2011, Halpin and Lorimer 2016). The effects of disturbance on ecosystem dynamics are not often examined through the lens of intraspecific biodiversity or subtropical forest succession. Some research suggests that that genetic variation within species could be an important and overlooked driver of ecosystem function, potentially even to the same extent as diversity between species (Des Roches et al. 2018). Widespread abandonment of previously cultivated lands and pastures has promoted forest regeneration across the tropics, leading to rapid changes in plant biodiversity (Poorter et al. 2021) and likely also ecosystem function. Disturbance-mediated changes to ecosystem functions, driven by competition-induced changes to stand-level genetic diversity and post-agricultural forest succession remain poorly understood.

RESEARCH GOALS

1. Chapter 1: Evaluate how disturbance history, decomposition environment, and litter chemistry influence decomposition dynamics of litter mixtures derived from multiple aspen genotypes by monitoring changes in litter mass loss, litter chemistry, and microbial community biomass over time.

2. Chapter 2: Establish the potential roler tree density, stand-level traits, and litter decomposition dynamics have on soil C and aggregate dynamics in genetically diverse trembling aspen stands and how these relationships may affect future soil C accrual.
3. Chapter 3: Examine how forest successional age and composition, decomposition environment, and abiotic conditions influence the chemical trajectory of litter during decomposition, and how litter decomposition dynamics are connected to microbial community biomass and activity.

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CHAPTER 1:

Effects of disturbance-mediated changes to intraspecific genetic diversity and litter chemistry in trembling aspen stands on litter decomposition

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ABSTRACT

Intraspecific genetic diversity is often an overlooked facet of ecological research, despite emerging evidence that it may play a key role in important ecosystem functions across spatial scales. Recent work has shown that plant trait expression among genetically-diverse trembling aspen (*Populus tremuloides*) stands is strongly connected to disturbance-mediated intraspecific competition among aspen genotypes. These strong connections between plant traits, genetic diversity, and disturbance history all likely have direct implications on ecosystem functions, such as litter decomposition. We conducted three field experiments over 477 days to test whether decomposition dynamics among 18 experimental trembling aspen stands, each consisting of 14 distinct genotypes, were driven by interactions between litter chemistry and decomposition environment, or either of these factors exclusively. Half of the experimental stands had tree density reduced by 75% in 2014, allowing us to examine potential influences of disturbance history on decomposition dynamics at the stand level. Here, we demonstrate that disturbance-mediated changes to stand-level genetic diversity, along with variations in litter chemistry and decomposition environment, influences decomposition of litter mixtures derived from multiple aspen genotypes. Specifically, litter mixtures derived from disturbed and undisturbed stands decompose similarly when placed within their respective stand of origin (*in-situ*), but litter derived from disturbed stands decomposed faster *in-situ* compared to in a common site. Litter turnover times for samples decomposing *in-situ* showed strong negative correlations with litter condensed tannin concentration and litter carbon-to-nitrogen ratios after 477 days, whereas turnover times in a common garden showed a strong positive correlation with source stand-level genetic diversity. Microbial biomass for many phospholipid fatty acid guilds showed negative correlations with changes in litter mass over time, with some significant interactions with

disturbance history. These findings indicate that disturbance-mediated changes in stand-level genetic diversity may drive litter decomposition dynamics by altering key microbial decomposer communities and consequently, how litter is processed in different decomposition environments.

INTRODUCTION

Biodiversity-ecosystem function (BEF) relationships generally focus on *interspecific* diversity or taxonomic assemblages consisting of multiple species, and rarely examine phenotypic, functional, and genetic diversity within a single species, or *intraspecific* diversity (Whitham et al. 2003, Crutsinger et al. 2006, Bolnick et al. 2011, Odling-Smee et al. 2013). Some researchers speculate that intraspecific variation could have strong implications for ecological dynamics and ecosystem function, potentially even to the same extent as or more than interspecific variation (Des Roches et al. 2018, Raffard et al. 2019). Furthermore, there is emerging evidence that intraspecific diversity plays a key role in ecosystem function across various spatial scales (e.g., Madritch et al. 2014), suggesting that intraspecific-BEF relationships are a potentially crucial facet of ecology and could be particularly important in the current state of rapid global environmental change.

Changes to plant diversity and the soil environment can influence C substrate and nutrient availability, with implications for microbial growth and ecological interactions between individual microbial taxa. Such changes can alter the prevalence and distribution of microbial functional groups, ultimately affecting plant-microbial interactions and ecosystem function (Daniel 2005, Fierer 2017). Intraspecific, or genetic diversity has been shown to have non-additive, synergistic effects on some ecosystem processes, such as litter decomposition and nutrient mineralization (Madritch et al. 2014, Crutsinger 2016), but these relationships remain largely unexplored to date. Plant intraspecific diversity has been shown to significantly affect soil microbial community composition in deciduous forest stands (Schweitzer et al. 2008), 2008), in bioenergy cropping systems (Ulbrich et al. 2020), and in annually tilled systems (Aira et al. 2010).

Plant litter decomposition rates are affected by abiotic factors, such as mean annual precipitation and temperature, as well as by litter chemistry and the functional capacity of the soil and forest floor microbiome (Balser and Firestone 2005, Dale et al. 2015, Bond-Lamberty et al. 2016, Fierer 2017). One of the most genetically variable plant species, trembling aspen (*Populus tremuloides*) (Mitton and Grant 1996) is a model system for investigating the effects of genetic diversity on microbially-mediated ecosystem processes, such as litter decomposition. Plant trait expression and C allocation among trembling aspen genotypes have been shown to vary in response to changes to competitive environments following disturbance (Kruger et al. 2020, Cope et al. 2021a, 2021b). Disturbance-mediated changes to competition environments could have implications on biogeochemical cycling and key ecosystem functions, as some research suggests that intraspecific variations in plant traits alone can influence litter decomposition (Wright et al. 2004, Cornwell et al. 2008). Stand-level characteristics, such as net primary productivity (NPP) and leaf area index (LAI) could also be important regulators of litter decomposition, because they affect environmental conditions at the forest floor by providing new substrates for microbes to decompose and affect rainfall inception, respectively (Nadelhoffer 2000, DeGryze et al. 2004, Fang et al. 2019).

In addition to plant trait expression and stand-level characteristics, litter chemistry should also influence litter decomposition dynamics among aspen genotypes. Previous work has demonstrated that variations in aspen genotype litter chemistry, such as carbon-to-nitrogen (C:N) ratios and lignin and tannin concentration, have been closely tied to litter decomposition rates (Schweitzer et al. 2004, Madritch et al. 2006). For example, Madritch and Lindroth (2011) conducted a litter decomposition study using single genotype litter mixtures and found both litter C:N ratio and condensed tannin (CT) concentration were strongly correlated with decomposition

rates. Litter with high CT concentrations could partially inhibit decomposition rates in litter mixtures at the stand level due to its inhibitory and toxic effects on microbes (Fierer et al. 2001, Hoorens et al. 2002), where a single stand of trembling aspen consists of litter derived from multiple genotypes.

Disturbance has been shown to lead to variations in leaf chemistry and microbial community composition and function in deciduous forests (Osburn et al. 2019, Cope et al. 2021a), supporting existing frameworks that environmental conditions can alter microbial functions (Roller and Schmidt 2015, Bond-Lamberty et al. 2016, Fierer 2017). Interactions between litter chemistry, disturbance history, and microbial community structure and function are expected to also influence litter decomposition dynamics when litter decomposes *in-situ* vs. in another environment. One commonly invoked decomposition framework states that microbes are most efficient at decomposing litter derived from their native environment compared to litter derived from elsewhere – a concept called "Home-Field Advantage Hypothesis" (Gholz et al. 2000, Ayres et al. 2009). While this decomposition framework has been supported by numerous studies (e.g., Vivanco and Austin 2008, Asplund et al. 2018, Lin et al. 2019) and in a study using single aspen genotype litters (Madritch and Lindroth 2011), it has not yet been evaluated for genetically diverse litter mixtures at the stand level.

In this study, we measured litter decomposition in the field over 477 days in experimental trembling aspen populations (stands) at the University of Wisconsin-Madison Arlington Agricultural Research station, using litter mixtures derived from multiple aspen genotypes. We were specifically interested in whether disturbance history and its effects on litter mixture chemistry influence litter decomposition dynamics at the stand level. Our three experiments included: (1) an *in-situ* experiment where leaf litter mixtures decomposed within their respective

aspen stand of origin (testing combined effects of decomposition environment and litter mixture chemistry), (2) a common garden experiment where litter mixtures decomposed in a common site (testing the effects of litter mixture chemistry alone), and (3) a common substrate experiment where wooden popsicle sticks decomposed in experimental aspen stands (testing the effects of decomposition environment alone). To better understand the potential inhibitory effects of CTs on litter decomposition dynamics and to measure the immediate response of microbes to litter additions, we conducted a short 11-day laboratory incubation where we added aspen litter sources that differed in their CT concentrations to soil mesocosms. Lastly, we asked whether stand-level genetic diversity and disturbance history were related to changes in litter mixture microbial community biomass, and if changes in microbial biomass were reflected in changes in litter mass lost over time. Because disturbance history can cause variations in litter chemistry and alter microbial community composition and function, we hypothesized that disturbance would affect stand-level litter decomposition dynamics in our system.

MATERIALS AND METHODS

Study site history

Our experimental site was established in 2010 and consisted of 18 experimental trembling aspen stands, located at the University of Wisconsin-Madison's Arlington Agricultural Research Station in Arlington, WI (43.3°N, 89°W). The 0.2-ha site was previously a hybrid poplar (*Populus nigra x P. Maximowiczii*) bioenergy crop research site before being plowed and disced prior to tree planting. Each experimental stand was originally comprised of 64 individual trees representing 16 genotypes collected from natural aspen stands throughout Wisconsin. However, microsatellite analysis later confirmed the existence of only 15 unique genotypes, 14

of which are found in all 18 stands (C. Cole, K. Mock, R. Lindroth, unpublished data). Genotypes were planted randomly and approximately equally distributed with 0.5 x 0.5 m spacing (40,000 trees ha^{-1} density) in all four 2 x 2-meter quadrants within each plot. Plots were bordered by additional, non-experimental aspen trees (hereafter border trees). Soils here are mapped within Huntsville series (Cumulic Hapludolls) formed in paleo floodplains capped with loess or wind-blown silt.

In spring 2014, half of the experimental stands were randomly selected for thinning to simulate an intermediate-level disturbance and decrease competitive inhibition amongst the trees by reducing tree density by 75% (10,000 trees ha^{-1}) (Figure 1A), leaving only one representative individual stem per genotype per disturbed stand. Since disturbance, there has been a divergence in genotypic composition over time among the stands, reflected in changes to genotype relative height growth rates and relative genotype frequency under different disturbance histories (Figure 1B). These changes corresponded to differential selection of fast vs. slow growing genotypes that thrive in high vs. low competitive environments, respectively (Cope et al. 2021b). Undisturbed stands, characterized by high intraspecific competition, are marginally less genetically diverse and have greater variability in stand-level genetic diversity than disturbed stands (Figure 1C), due to greater selection of fast-growing genotypes and mortality of slow-growing genotypes (Figure 1D). It is important to highlight that this divergence occurred over an ecologically relevant timescale (5 years) which is a very small fraction of the standard lifespan of an aspen tree, indicating that changes to competitive environments, mediated by disturbance, can shift the expression of stand-level plant traits and C allocation strategies (see Cope et al., 2021a, Kruger et al., 2020).

Field Decomposition Experiments

To determine whether decomposition dynamics are driven by litter mixture chemistry, decomposition environment, and their combined effect, we conducted three field decomposition experiments simultaneously over 477 days from December 2020 to March 2022. These experiments included (1) an *in-situ* experiment (chemistry x environment combination), where litter mixtures decomposed within their stand of origin, (2) a common garden experiment (litter mixture chemistry alone), where litter mixtures decomposed in an older aspen stand, not where litter in this experiment was derived from, and (3) a common substrate experiment (decomposition environment alone), where the same substrate was deployed within the original experimental stands.

In July 2020, we installed nylon mesh bird netting approximately 2 meters from the forest floor, secured to the border trees, around the perimeter of all 18 experimental aspen stands to minimize leaf litter cross-contamination during leaf senescence the following fall. We collected litter that fell within large trash bags secured to the forest floor within the interior each stand during fall 2020. Litter was composited and dried at 50°C then ≈5 grams of litter was weighed into 10 x 10 cm 12 decomposition bags made out of 1 mm fiberglass mesh window screening for each experiment, corresponding to 3 replicate bags for 4 retrieval dates. For the *in-situ* experiment, litter bags were randomly deployed in their respective stands of origin using randomly generated azimuths and directions from the center of all 18 stands, yielding a total of 216 bags for the *in-situ* experiment (4 retrieval dates x 3 replicate bags x 18 stands = 216 bags total).

The site used for the common garden experiment was a small aspen stand established in 2001 and located approximately 100 m from the main experimental aspen stands. This site was

chosen because its forest floor and soil microbiome has been shaped by 20 years of aspen inputs. 144 litter bags were deployed at this site, collected from a subset (12) of the original experimental aspen stands, selected based on accurate representation of the range of stand-level genetic diversity within disturbed and undisturbed stands (4 retrieval dates x 3 replicate bags x 12 stands = 144 bags total). For the common substrate experiment, 144 litter bags containing wooden popsicle sticks were deployed within 12 of the experimental aspen stands – the same subset of stands selected for common garden experiment (4 retrieval dates x 3 replicate bags x 12 stands = 144 bags total).

All bags were installed in direct contact with the soil surface and secured using anti-rust, 11-gauge zinc-galvanized steel landscape staples. A total of 504 decomposition bags were be deployed on December 7, 2020 (day 0) and collected on April 5, 2021 (day 120), July 7, 2021 (day 213), October 31, 2021 (day 328), and March 25, 2022 (day 477).

Field sample processing and decomposition rate calculations

Upon retrieval from the field, all litter samples were transported on ice back to the University of Wisconsin-Madison and weighed after removing any excess soil on the outside of decomposition bags. A subsample was stored at stored at -80°C for subsequent microbial analysis. A second subsample from each bag was oven-dried at 50°C for at least 48 hours to correct for field moisture. After being dried and ground, another litter subsample was combusted in a muffle furnace at 500°C for a minimum of 4 hours to estimate ash content. The final mass reported for all samples is expressed on an ash-free dry mass basis (Ostertag et al. 2008).

We calculated litter decomposition rates (k-values) using an exponential decay model based on the relationship between the log mass remaining and time in R-studio, using the *gsnls* package (Chau, 2023), calculated as:

(1) $M_t = M_0 e^{-kt}$

where M_t is the litter mass remaining at time t in days (t = 120, 213, 328, and 477 days) expressed as percent of its initial ash-free dry mass, M_0 is the initial litter mass at time 0 (expressed as 100%), and k is the first order decay constant. We calculated annual decay rates and their inverse to estimate mean litter turnover time in years. Estimated litter turnover times were calculated for all aspen stands individually and grouped by disturbance history for all three experiments.

Litter addition laboratory incubation

To better understand the influence variations in litter chemistry and disturbance history might have on the immediate response of soil microbes to litter additions, we performed a short 11-day laboratory incubation and measured CO_2 evolution over time. Potential differences in microbial activity on this short timescale would not have been captured in our longer field experiment. Originally, this experiment was planned to continue for 30+ days but was terminated after 11 days due to the COVID-19 campus shutdown.

Our experimental setup for this litter addition incubation included 3 laboratory replicates from each of the 18 experimental aspen stands, using 30 grams of field soil in a specimen cup and adding deionized water to reach 60% water filled pore space (Linn and Doran, 1984), which was maintained throughout the experiment. We added 0.5 grams of litter (ground to 1 mm on a Wiley Mill, size 20 mesh insert) derived from three replicate trees for two aspen genotypes (PI-3

and Sau-3) into replicate cups, yielding 6 cups in total per disturbance history (2 litter types x 3 replicates). We chose these two genotypes specifically because of their differences in litter condensed tannin (CT) concentration; PI-3 = 4.52% ± 0.36 and Sau-3 = 2.57% ± 0.21 ($p = 0.04$). Litter CTs have been shown to inhibit litter decomposition and be highly variable amongst aspen genotypes (Madritch et al. 2006, Rubert-Nason and Lindroth, 2021) and thus we wanted to isolate their effects on microbial activity in the laboratory to better understand their potential influence on litter decomposition in the field. We randomly selected three stands per disturbance history to use as control jars that did not receive litter additions to account for background soil respiration not associated with litter additions. CO_2 flux values from these jars were subtracted from the total CO_2 flux on each sampling day to provide an estimate of litter-derived respiration over background soil respiration.

All specimen cups were placed in 1-quart mason jars sealed with lids fitted with rubber septa and were lightly capped to allow for ventilation and placed in a dark cabinet between collection times to avoid measuring autotrophic respiration. After samples were initially brought to 60% water filled pore space, they were vented for 24 hours prior to the first CO_2 collection period. 10 mL of headspace was collected immediately after venting the chambers and tightly sealing the mason jars and then again 60 minutes later using a syringe; headspace samples were injected into exetainer glass vials prior to analysis. We collected gas samples on days 1, 3, 5, 8, and 11. All gas samples were analyzed on an infrared CO_2 analyzer at the University of Wisconsin-Madison Grassland Ecology Laboratory (LI-820 CO_2 Analyzer, Li-Cor, Lincoln, NE, USA).

Litter decomposition chemistry

To identify potential mechanisms underlying variations in litter decay rates, we monitored changes in litter chemistry during the *in-situ* and common garden experiments, including carbon-to-nitrogen (C:N) ratio, lignin:N ratio, lignin, fiber, cellulose, and condensed tannins (CT) concentration. Litter samples were analyzed for total C and total N to determine the C:N ratio using a Flash 2000 Elemental Analyzer (Thermo Scientific, Wilmington, DE, USA), run in duplicate with replicate error of < 10% using atropine as calibration and check standards. Acid-detergent fiber, lignin, and cellulose were estimated gravimetrically using an Ankom 2000 fiber analyzer (Ankom Technology, Macedon, NY, USA). CTs were quantified using an acid-butanol method (Porter et al., 1985) with analytical standards purified from *Populus tremuloides* foliage (Hagerman and Butler 1989). Prior to analysis, all litter samples were vacuum-dried for at least 48 hours prior to being ground to 1 mm on a Wiley Mill (size 20 mesh insert) and ground again using a ball-mill; fiber, lignin, cellulose estimations require coarser leaf material compared to C:N and CT analysis. CT data were only collected on initial litter samples and samples retrieved on day 120, because analysis of CT concentration from a subset of samples collected on day 213 were below the laboratory detection limit. All samples were stored at -20°C prior to analyses.

Phospholipid fatty acid analysis

Differences in estimated wooden stick turnover times from our common garden experiment suggested that there might be differences in microbial decomposer communities between disturbance histories at the experimental aspen stands (see Results section). To further explore potential differences in litter decomposer community biomass, we used phospholipid

fatty acid (PLFA) analysis to estimate changes in the microbial biomass on our litter samples decomposing within our *in-situ* experiment (Oates et al. 2017). Litter subsamples were composited from 3 litterbag replicates from each stand for each retrieval date (72 litter samples total) before being sent to Regen AgLab in Nebraska for analysis, following standard procedures and protocols for PLFA extraction and quantification (Balser et al. 2019, Bligh and Dyer, 1959). (Bligh and Dryer, 1959, Hamel et al. 2006, Peterson and Klug 1994). Identifiable lipids between 14-19 carbon chain length can be used as an index for the biomass of distinct microbial guilds, following methods from Smith et al. (2015) and Hamel et al. (2006).

Stand-level characteristics

Genetic diversity

We estimated stand-level genetic diversity based on the Shannon Diversity Index (Shannon and Weaver, 1949) and Simpson's Index (Simpson, 1949), both of which are the combined measurement of the number of species in a community and the relative frequency of each species. We used these diversity indices to estimate stand-level genetic diversity, based on the number of aspen genotypes and the relative frequency of each genotype. We calculated these diversity indices using the *vegan* package in R-Studio (Oksanen et al., 2012).

Leaf area index

We calculated leaf area index (LAI) for the 18 stands using UAV (DJI M600 Pro) tours equipped with a LiDAR imager (Velodyne Puck VL-16) in mid-August 2020. LAI is a quantitative expression of the leaf area per unit ground area and commonly used as an indicator of plant growth rates, as it is linked to plant photosynthesis and evapotranspiration (Breda 2003).

Experimental stands were isolated in LiDAR images by overlaying cylindrical buffer zones (3.5-meter diameter) in the center of each stand with ArcGIS (version 10.9) and then calculated LAI using the *canopyLazR* in R-Studio (Kamoske et al. 2021).

Net primary production estimates

As part of a larger study (Pomplun 2022), we were interested in how stand-level variations in annual net primary production (NPP) might affect important ecosystem processes within these experimental aspen stands, particularly litter decomposition soil carbon dynamics. NPP was calculated based on the summation of: (1) aboveground woody biomass growth, (2) foliage production, (3) coarse root production (> 2 mm), and (4) fine root production (< 2 mm) (Clark et al. 2001). Estimating aboveground wood growth required initial and final tree size measurements for use in allometric scaling. We collected size measurements (diameter at breast height [*D*] and total height [*H*]) for every experimental tree after leaf drop in mid-October in both 2019 and 2020. Then, for each tree we subtracted initial (Fall 2019) from final (Fall 2020) estimates of aboveground woody biomass, which were derived using an allometric equation generation with data from a destructive harvest of 25 trees in our experimental aspen stands. The resulting allometric model, which utilized both *D* and *H* as predictors, provided robust estimates (R^2 = 0.995, RMSE = 1.1) of aboveground woody mass (kg) across all 18 aspen populations:

$$(2)\ \ln(woody\ mass) = 2.17D^{0.50} + 0.17H - 0.01DH - 4.25$$

We estimated foliage production by subtracting the estimated initial from final total number of leaves of each tree within each population. The total number of leaves was nondestructively estimated in the crown of one randomly selected tree per genotype in 18 stands. Using a *D*-based allometric equation specific to *Populus tremuloides* growing in Wisconsin

forests (Ruark et al. 1987), we estimated coarse root production by subtracting initial from final coarse root biomass. We then estimated fine root production using a modified ingrowth root core method adapted from Person (1980). Following ground thaw in April 2020, we withdrew 10 randomly selected soil cores (wedge-shaped corer, ≈46 cm deep and covering 18.70 cm^2 of the ground surface) from each of the 18 experimental stands. Each resulting hole was lined with bags of thin plastic mesh to allow for root growth and bags were refilled with the extracted soil after removing debris and roots. Cores were retrieved in July 2020 and in December 2020 and processed by hand-extracting roots and rinsing them of debris. Isolated roots were pooled per retrieval date per population, oven dried at 60°C for 72 hours, then weighed.

Statistical analyses

All statistical analyses were performed in R-Studio (version 4.0.5; R Core Team 2021). We developed linear mixed effects models with restricted maximum likelihood using the *lme4* package (Bates et al. 2015), using disturbance history and time as fixed effects and stand as a random effect to determine potential differences in changes in variables of interest over time between disturbance histories for each of the three experiments: (1) changes in litter mass, litter chemistry, and PLFA biomass for the *in-situ* experiment, (2) litter mass and litter chemistry for the common garden experiment, and (3) wooden stick mass for the common substrate experiment. We also developed linear mixed effects models to evaluate potential differences in microbial CO_2 respiration over time for our litter addition laboratory incubation, using disturbance history, genotype litter source (chemistry), and time as fixed effects and stand as a random effect.

Due to differences in sample sizes between the three experiments, we used a Kruskal-Wallis test (nonparametric analysis of variance) followed by pairwise comparisons using the Bonferroni p-value adjustment method using the *PMCMRplus* package (Pohlert 2018) to evaluate potential differences in distributions and medians in final litter/stick masses remaining after 477 days in the field. We used the same nonparametric approach to compare changes in litter turnover times and litter chemistry between disturbance histories among the *in-situ* and common garden experiment. To more directly evaluate how litter processing, inferred by differences in litter chemistry, may differ between disturbance histories in our *in-situ* vs. common garden experiments, we compared differences in chemical variables within each retrieval time point, rather than between time points.

We developed simple linear regression models to understand which variables could potentially best explain the variability in estimated mean litter turnover times between *in-situ* vs. common garden experiments, to provide insight for how litter decomposition dynamics are driven by site-specific conditions. For this comparison specifically, we were interested in how variations in stand-level genetic diversity (Shannon and Simpson's), LAI, NPP, and initial and final litter chemistry were potentially correlated to litter turnover time within the experimental aspen stands and the common garden site. Due to differences in sample sizes between the *in-situ* vs. common garden datasets, resulting in non-normal data distributions, we developed these linear models separately for each experiment.

Exploratory regression analysis using total PLFA biomass averaged across timepoints as a potential predictor variable of litter turnover time did not show any significant relationships. This was likely due to significant effects of time on litter microbial biomass across PLFA guilds (see Results section), and thus, any potential relationship microbes may have with litter turnover

time may have been masked by averaging across timepoints. Knowing that microbial biomass is a potentially important mediator of litter decomposition dynamics, we developed nonlinear regression models to predict changes in the percent litter mass remaining over our 477-day *in-situ* experiment, using the biomass of individual PLFA microbial guilds. For this approach, we fit litter mass remaining data to the following equation:

(3) $M = a * e^{b*Biomass}$

where M is the litter mass remaining expressed as percent of its initial mass, *a* and *b* are fitting parameters, and *Biomass* indicates the PLFA biomass of either arbuscular mycorrhizal fungi (AMF), non-AMF, gram negative bacteria (Gm-), gram positive bacteria (Gm+), or Actinobacteria on litter samples over time. Fitting parameters were estimated based on coefficients from an exponential decay model using all litter mass remaining data, based on the exponential decay relationship used to estimate k-values. Because we submitted pooled litter samples for the PLFA analysis at the stand level, these nonlinear regression models were developed for the entire dataset and for disturbed and undisturbed stands (n = 9 per disturbance history).

RESULTS

Field experiment litter decomposition

Litter mass decreased over time for the *in-situ* and common garden experiments over the 477-day experiments. For the *in-situ* experiment, we found no significant effects of disturbance history on litter mass loss, unlike the results from the common garden experiment where we found significant interactions between disturbance history and time (Figure 2A). In the common garden experiment, litter derived from undisturbed stands lost a greater percentage of its original

mass over time compared to litter derived from disturbed stands (30.36 ± 7.09% vs. 45.72 ± 1.49%; $p < 0.0001$). Our common substrate experiment only showed significant effects of time and not decomposition environment on the changes in wooden stick masses (Figure 2B). Nonparametric analysis of variance revealed that distributions and medians of the final percent litter/stick masses remaining after 477 days differed significantly between the three experiments ($p < 0.0001$), with litter from the *in-situ* experiment having the lowest median values, although the highest interquartile range, of the three experiments (Table 1).

Non-linear exponential decay models best described litter decomposition rates for all three experiments in this study. Average R^2 values were 0.83 for the *in-situ* experiment, 0.89 for the common garden experiment, and 0.60 for the common substrate experiment, but R^2 values varied considerably among the stands across all three of the experiments (Table S2). We attribute the variability in model performance for the *in-situ* and common garden experiments to the lack of observed changes in litter masses between 0 and 120 days (96.44% and 94.16% on average, respectively); these values did were not significantly different from initial litter masses, suggesting no mass loss occurred over this period. The litter mixtures used in these experiments were also mostly under snow during this time. Although other experiments have found litter decomposition to occur under snowpacks (see Blok et al. 2016), snowpacks were likely not thick enough to provide sufficient insulation and promote wintertime decomposition. We attribute the lower model R^2 values from the common substrate experiment to increases in stick masses at different stages of decomposition, which makes it difficult to build predictive statistical models. The significant increase in stick mass could be explained by the incorporation of fungal hyphae while they were in contact with the soil in the field. Regardless of the variability in model

performance, we feel confident that these models adequately capture litter decay rates in our experiments, but likely underestimate actual litter decomposition rates and turnover times.

Nonparametric analysis of variance between the *in-situ* and common garden experiments and disturbance histories revealed that litter turnover times did not differ between disturbance histories in either experiment, but litter from in the in-situ experiment (both disturbed and undisturbed stands) had a significantly lower turnover times compared to litter derived from disturbed stands decomposing in a common garden (both $p < 0.05$) (Figure 3A). Estimated turnover times of the wooden sticks from the common garden experiment were significantly lower when the sticks decomposed in undisturbed stands compared to disturbed stands ($p = 0.05$) (Figure 3B).

Laboratory litter-addition soil incubation

We conducted a short 11-day incubation experiment where we add litter derived from two different aspen genotypes, with significantly different CT concentration, to soil microcosms to measure the immediate response of microbes to litter additions and determine potential inhibitory effects of CTs on microbial activity. Soil microbes from disturbed stands respired more CO_2 on day 1 than microbes from undisturbed stands, regardless of CT concentration of amended litter (Figure 4A). Average CO_2 respiration on day one was 58.52 ± 3.78 μg CO_2 g soil^{-1} day^{-1} for disturbed stands and 44.27 ± 2.44 μg CO_2 g soil^{-1} day^{-1} for undisturbed stands. Over the 11-day experiment, we found marginally significant interactions between disturbance history and time on cumulative respiration ($p = 0.06$), with soils from undisturbed stands losing ≈25% less cumulative CO_2 than soils from disturbed stands (Figure 4B), again, without any significant effects of litter CT concentration. Total cumulative CO_2 fluxes were 511.22 ± 63.27 and 384.69

± 36.83 μg CO_2 g $soil^{-1}$ day^{-1} for disturbed and undisturbed stands, respectively. Results from our soil only control jars, which did not receive any litter additions, showed some interesting disturbance history effects on microbial activity, with soils from undisturbed stands respiring more than twice as much cumulative CO_2 than soils from disturbed stands over the 11-day experiment (216.65 ± 11.59 vs. 98.93 ± 12.25 μg CO_2 g $soil^{-1}$ day^{-1}) (Figure 4C).

Litter decomposition chemistry

Linear mixed effects models revealed significant effects of time on variations in litter CT, fiber, lignin, and cellulose concentration and lignin:N ratios and significant interactions between disturbance history and time on litter C:N ratios in the *in-situ* field decomposition experiment (Table 2). Initial litter C:N ratios were not significantly different, but litter derived from undisturbed stands was slightly higher than litter derived from disturbed stands (30.42 ± 0.81 vs. 28.73 ± 0.58). After 477 days, litter C:N ratios were not significantly different between disturbance histories but did decrease slightly more for litter decomposing in disturbed vs. undisturbed stands (20.88 ± 0.46 vs. 19.66 ± 0.45). For the common garden experiment, only time affected variations in litter chemistry (Table 3).

Non-parametric analysis of variance of litter chemistry revealed interesting changes in litter chemistry between experiments and disturbance histories (Figure 5). For example, litter lignin and fiber concentration were both lower for litter samples decomposing in the common garden vs. the *in-situ* experiment at 120 days, but common garden samples reached greater lignin and fiber values by 213 days than samples decomposing *in-situ* (Figure 5B and 5C). Litter decomposing in the common garden had lower cellulose concentration at 213 days than for the *in-situ* experiment, but only litter derived from disturbed stands *in-situ* had significantly more

cellulose concentration than litter derived from undisturbed stands decomposing in the common garden by 328 days (Figure 5D). Lignin:N ratios reflect the same differences on days 120 and 213 between experiments and disturbance histories as the lignin data, with samples decomposing *in-situ* having higher lignin:N ratios on day 120 and lower ratios on day 213 compared to samples decomposing in a common garden (Figure 4F). Litter C:N ratios and CT concentration did not vary between experiments and disturbance histories at any time points during the 477-day experiments (Figure 5A and 5E).

Litter microbial PLFA biomass

Total PLFA-estimated microbial biomass on litter samples from the *in-situ* experiment did not change throughout the 477-day experiment, although the biomass of individual guilds did (Figure 6). Microbial biomass for all PLFA guilds varied over time but most showed no significant interactions between disturbance history and time. For example, AMF biomass was greater on days 328 and 477 compared to days 120 and 213 (Figure 6A). Litter samples decomposing in disturbed stands compared to undisturbed stands had the same amount of AMF biomass at the end of the experiment (day 477), yet there was considerably less variation in the former. Non-AMF biomass remained relatively constant throughout the experiment but was significantly lower on day 213 than day 328 (Figure 6B). Gm+ bacteria biomass was significantly lower on day 120 compared to the rest of the days of the experiment, where it remained relatively constant (Figure 6D). Actinobacteria biomass showed a similar increase during the experiment, although values were lower on day 477 compared to day 328 (Figure 6E).

Gm- bacteria biomass and fungal-to-bacterial ratios were the only PLFA data that showed significant interactions between disturbance history and time (Figure 6C and 6F). Gm- bacteria

biomass was significantly lower for litter decomposing in disturbed stands than undisturbed stands on day 120. While Gm- biomass generally increased over the course of the experiment for litter decomposing in disturbed stands, it remained relatively constant for litter decomposing in undisturbed stands (Figure 6C). Fungal-to-bacterial ratios were greater for litter decomposing in disturbed stands compared to undisturbed stands on day 120 (Figure 6F). These relative differences between disturbance history at early stages of decomposition reveal differential responses of microorganisms to litter availability. Based on results from day 120, it appears that bacteria are found first on litter mixtures in undisturbed stands, whereas fungi appear in greater abundances in disturbed stands. Averaged across all timepoints, disturbed stands also had greater fungal-to-bacterial ratios than undisturbed stands (0.44 ± 0.04 vs. 0.35 ± 0.02, respectively) ($p = 0.05$), providing further evidence that forest floor microbiomes in disturbed stands might be fungally dominated.

Regression models

Only stand-level tree genetic diversity, final litter C:N ratio, and initial litter CT concentration emerged as significant predictor variables for estimated mean litter turnover time, although these relationships varied between the *in-situ* vs. common garden experiments (Table S3). Stand-level genetic diversity showed a significant positive correlation with litter turnover time for litter decomposing in a common garden ($R^2 = 0.44$) (Figure 7A). Litter turnover time had a strong negative correlation with final litter C:N ratio when it decomposed *in-situ* ($R^2 = 0.48$) (Figure 7B). Initial litter CT concentration also showed a strong negative relationship with litter turnover time for the *in-situ* experiment (Figure 7C), although it explained less variance than final litter C:N ratio (≈31% vs. ≈44%, respectively).

Nonlinear regression models showed that multiple microbial PLFA guilds had relatively strong negative relationships with changes in percent litter mass over time (Table 4). In undisturbed stands, both litter AMF and actinobacteria biomass showed stronger relationships with changes in the percent litter mass remaining compared to undisturbed stands (R^2 = 0.67 and 0.59 vs. 0.42 and 0.48, respectively). Gm- bacteria biomass showed a relatively weak correlation with litter mass remaining for disturbed stands (R^2 = 0.22), but no significant correlation for undisturbed stands. Litter Gm+ bacteria biomass explained ≈33% of the variation in the percent litter mass remaining, regardless of disturbance history. Surprisingly, non-AMF biomass did not emerge as a significant variable for predicting changes in the percent litter mass remaining (Table 4)

DISCUSSION

Aspen genotype trait expression and foliar chemistry have been shown to respond to changes to intraspecific plant competition, mediated by disturbance-related changes to stand-level genetic diversity in experimental stands (Cope et al. 2021a, 2021b) and decomposition dynamics of single-genotype aspen litter has been linked to variations in microbial community composition and environmental conditions (Madritch et al. 2006). Here, we demonstrate that such disturbance-mediated changes to stand-level genetic diversity influence stand-level decomposition dynamics of litter mixtures derived from multiple aspen genotypes, where the predictive capabilities of genetic diversity and litter chemistry on litter turnover time differed based on decomposition environment. Results from our 477-day decomposition experiment showed that litter decomposing *in-situ* had lower estimated turnover times compared to litter decomposing in a common garden. This finding suggests that litter mixture chemistry alone

might drive decomposition dynamics for litter mixtures consisting of litter derived from multiple aspen genotypes, given that the common garden experiment isolated the effects of litter chemistry on litter decomposition dynamics in this study.

However, only litter mixtures derived from disturbed stands had lower turnover times when they decomposed *in-situ* compared to in a common garden, while litter mixtures derived from undisturbed stands had similar turnover times in both experiments. Our short laboratory litter-amendment incubation experiment showed soil microbes in disturbed stands respired more CO_2 over time than microbes in undisturbed stands but our field experiment found no differences in litter turnover times between disturbance histories. Additionally, our incubation did not show any significant differences in cumulative CO_2 respiration between low and high tannin litter additions, while we found significant negative correlation between litter CT concentration and litter turnover time for our field experiment. Disturbance history showed significant interactions with time for some microbial guilds while litter decomposed in the field and also significantly influence common substrate decay rates – an experiment explicitly meant to isolate the effects of decomposition environment and microbial functional capacity. Mechanistically however, it appears that when litter sources are derived from multiple aspen genotypes, litter mixture chemical complexity overpowers differences in microbial functional capacity between disturbance histories, and thus, disturbance history alone is not enough to alter litter decomposition dynamics at the stand level. Based on these results taken together, we conclude that litter decomposition dynamics are influenced by a combination of litter mixture chemistry and decomposition environment.

Plant productivity, intraspecific diversity, and disturbance all affect litter decomposition

In theory, net primary productivity (NPP) and leaf area index (LAI) should influence litter decomposition by altering decomposition environments within the experimental stands. Annual NPP estimates and LAI values from this site vary among 18 stands (Kruger et al. *in prep*, Pomplun 2022) but we found no significant relationships between litter turnover time and these stand-level characteristics. However, NPP and LAI were both positively correlated with final litter C:N ratios (R^2 = 0.35 and 0.32, respectively), likely due to increased plant productivity increasing C allocation to leaf growth and subsequently litter C conentration. While including LAI did not improve model performance, using both final litter C:N ratios and NPP in multiple regression analysis improved model performance for predicting litter turnover times for the *in-situ* experiment (Figure 8). The positive slope of NPP in this multivariate model indicates that higher NPP may correspond to higher litter turnover times (slower decomposition). Stands with high NPP in our system also have greater fine root biomass (R^2 = 0.63), which could shift microbial nutrient acquisition strategies from leaf decomposition exclusively to both leaf and root litter or stimulate soil C decomposition (DeGryze et al. 2004, Fontaine et al. 2007).

Results from the incubation showed that soil microbes in disturbed stands respired more cumulative CO_2 than microbes from undisturbed stands, with no significant effects of litter chemistry (high vs. low CT concentration). The lab incubations did not replicate conditions in the field, and also occurred over a shorter time-span. In particular, significant interaction effects of NPP and litter C:N ratios on litter decomposition dynamics from our field study were not simulated in our laboratory incubation, along with other significant interactions between litter chemistry and stand-level genetic diversity. We used single litter mixtures in our incubation and litter mixtures derived from multiple genotypes in our field experiment. In our *in-situ*

experiment, litter turnover times did not differ between disturbance histories, but were negatively correlated with initial litter CT concentration. This discrepancy suggests that microbes in disturbed stands potentially have a greater capacity to decompose litter than those in undisturbed stands, but only when litter is derived from a single genotype. Additionally, final litter CT concentration was negatively correlated with stand-level genetic diversity in the field (R^2 = 0.29), suggesting litter mixtures from more genetically diverse stands correspond to greater litter CT loss. Other studies have found similar relationships between litter mixture diversity and changes in CT concentration when litter is derived from multiple plant species (e.g., Ristok et al. 2017). The use of single litter residues in our incubation evidently did not capture the important role genetic diversity has in decomposition of litter mixtures derived from multiple aspen genotypes and how variations in plant genetic diversity may be affecting changes in litter chemistry during decomposition.

Our linear regression results showed a strong positive relationship between stand-level genetic diversity and litter turnover time for litter decomposing in a common garden but not when decomposing *in-situ*. Disturbance-mediated changes to biodiversity can influence soil faunal community composition and alter biodiversity across several trophic levels (Hättenschwiler et al. 2005, Bastian et al. 2008, Gessner et al. 2010). Soil detritivores (e.g., arthropods, earthworms, nematodes etc.) play a critical role in litter decomposition by shredding coarse litter into finer fragments and detritivore community diversity has been shown to increase with plant biodiversity (Tonin et al. 2018). Visual inspection of the litterbags upon retrieval indicates there were potential differences in detritivore communities between the experimental stands and the common garden site – ants were found in many of the bags from the common garden site and worms were found in the bags from the experimental stands. These observational

differences in detritivore communities could also be the result of inherent differences in stand age, characteristics, and land use history between the common garden site and the experimental stands. Although tree genetic diversity showed no relationship with litter turnover times for the *in-situ* experiment, it evidently had a lasting effect on litter decomposition dynamics in the common garden experiment, potentially by facilitating a different (and perhaps more diverse) detritivore community.

In-situ vs. common garden decomposition – insights into "Home-field Advantage"

Our study found significant differences in many litter chemistry variables at different timepoints between *in-situ* and common garden experiments, suggesting that microbial litter processing during decomposition is at least partially mediated by decomposition environment. However, litter turnover times only showed strong negative correlations with litter chemistry for the *in-situ* experiment, although the trend for litter turnover vs. CT concentration was still negative for the common garden experiment. Other studies have shown CTs limit microbial decomposition by acting as a microbial toxin or binding to proteins (Madritch et al. 2007, Ushio et al. 2013, Adamczyk et al. 2013). If differences in chemical characteristics at different times during decomposition are mediated by site specific differences, then it is likely that forest floor microbiome structure differs between the experimental aspen stands and the common garden, with implication for variations in turnover times.

While it is likely that microbial communities do become adapted to specific litter sources ("Home-field Advantage" (HFA) hypothesis), few studies monitor changes in community composition during the decomposition process, and thus, the timing of microbial adaptation is unclear. Schroeter et al. (2022) recently showed that while decomposer communities were

specialized toward specific litter sources, decomposer community composition changed at different stages of decomposition, as seen in other research (e.g., Strickland et al. 2009). Additionally, their work showed that litter metabolome profiles from forest and grassland ecosystems converged over only 22 days, suggesting that litter microbiomes may only differ between environments at early stages of decomposition. Although the experimental design of our study differs from most that test HFA (i.e., reciprocal transplants of litter derived from multiple environments), our results still provide insight into how microbes decompose litter derived from their native community vs. litter derived elsewhere.

We observed significant changes in microbial biomass across most PLFA guilds for the *in-situ* experiment and in litter mixture chemistry between the *in-situ* and common garden experiments at early stages of decomposition. Given results from our nonlinear regression analysis, we can infer those changes in litter microbial PLFA biomass were related to changes in litter mass in some capacity and partially mediated by disturbance history. Litter turnover times also only differed between *in-situ* vs. common garden decomposition for litter derived from disturbed stands, suggesting interactions between disturbance history, litter chemistry, and microbial composition also play a role in HFA. Future work using DNA sequencing will reveal whether changes in litter bacterial and fungal composition correspond to changes in litter chemistry during decomposition and whether disturbance history influences those relationships.

Site-specific microenvironmental factor effects on litter turnover

Litter turnover times were significantly lower when litter decomposed *in-situ* vs. in a common garden, particularly for litter derived from disturbed stands. Variations in stand-level characteristics and microenvironmental variables between decomposition environments could

have played an important role in litter decomposition dynamics in our study. Interactions between tree density and LAI can alter environmental conditions at the forest floor that influence decomposition, because LAI directly effects precipitation interception (Jarlan et al. 2008) and moisture strongly regulates microbial decomposition (Lee et al. 2014). In the experimental stands, tree density showed a stronger positive correlation with LAI for undisturbed and disturbed stands ($R^2 = 0.58$ vs. 0.35, respectively), indicating increase tree density corresponds with increased LAI and potentially increased canopy interception.

Averaged across all retrieval dates, litter moisture concentrations did not differ between disturbed and undisturbed stands but were significantly lower for samples decomposing *in-situ* compared to in the common garden (25.47 ± 0.84 % vs. 32.70 ± 1.55%; $p < 0.0001$), providing evidence that precipitation interception is also lower at in the common garden, although we did not quantify LAI at this site. Litter moisture also had a strong and negative correlation with litter turnover time in the common garden experiment ($R^2 = 0.51$) but no correlation for the *in-situ* experiment. In theory, lower tree density and LAI should correspond with lower litter decomposition rates due to more rapid drying of litter on the forest floor (Zhang and Zak 1995, Tucker and Reed 2016, Sierra et al. 2017), but differences in correlations between litter turnover times and litter moisture concentrations between environments suggest moisture controls on litter decomposition were site-specific in our study. Additional monitoring of other microenvironmental variables, such as temperature, would provide further evidence for how stand-level characteristics may mediate litter decomposition dynamics in different environments, particularly at the ecosystem scale.

CONCLUSIONS

Disturbance-mediated changes to stand-level genetic diversity and competitive environments have been shown to strongly influence plant-trait expression of aspen genotypes in experimental stands (Cope et al., 2021a, 2021b) and decomposition dynamics of single-genotype aspen litter has been linked to microbial communities and site-specific conditions (Madritch and Lindroth, 2011). Here, we demonstrate that such disturbance-mediated changes to stand-level genetic diversity influence stand-level decomposition dynamics of litter mixtures derived from multiple aspen genotypes. We conclude that litter decomposition dynamics among the 18 experimental trembling aspen stands in our study were driven by interactions between litter chemistry and decomposition environment. Disturbance history appears to influence litter decomposition in *in-situ* vs. in common garden settings and litter microbial biomass and community composition. Variations in litter chemistry between disturbance histories within our *in-situ* and common garden experiments indicate that both disturbance history and decomposition environment strongly regulate litter processing, mediated by microbial decomposers and potentially other detritivores. This discovery significantly improves our understanding of intraspecific biodiversity-ecosystem function relationships and leaves room for future research on how disturbance-mediated changes to genetically diverse forest stands may influence microbial decomposer and detritivore community composition. Our research also has implications for understanding how future disturbance events or management practices may affect litter decomposition dynamics in natural aspen stands that also consist of multiple genotypes.

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FIGURES AND TABLES

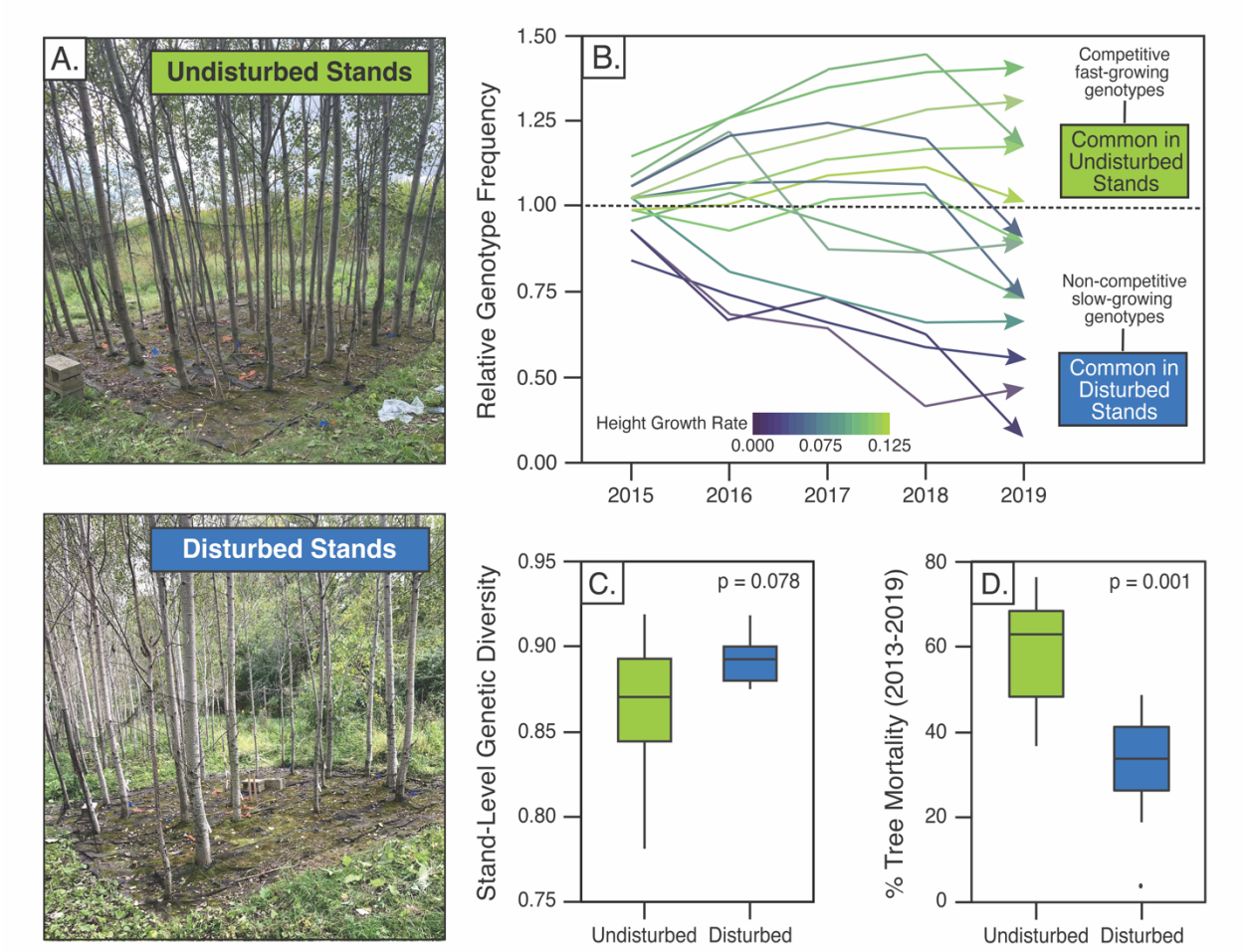

Figure 1: Experimental aspen site history and effects of disturbance treatment on A.) changes in relative growth rates and frequency aspen genotypes over time (adapted from Cope et al. (2021), PNAS.2103162118); relative genotype frequency values below 1 indicate higher frequency in disturbed stands, B.) stand-level genetic diversity (adapted Simpson's Index) between disturbance histories since the thinning treatment, and C.) percent tree mortality over a 6-year period between disturbance histories. P-values in upper right corners of panels B and C correspond to results from one-way ANOVA between disturbance histories.

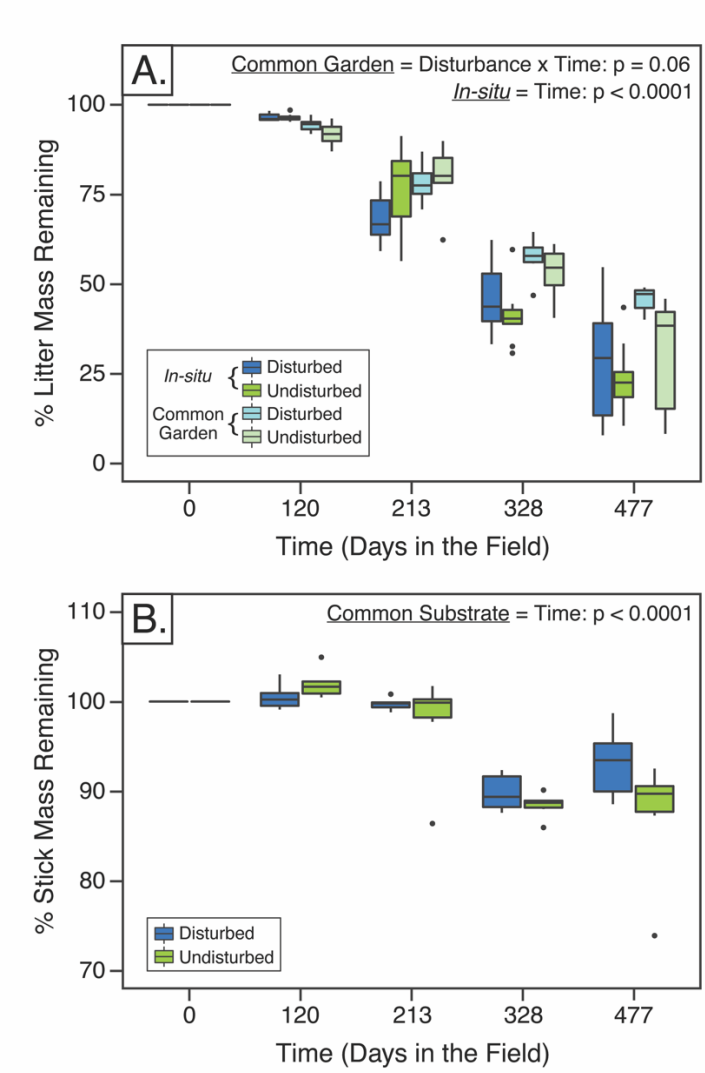

Figure 2: Boxplots showing medians and distributions in the percent mass remaining over time for the three decomposition experiments conducted in this research. A.) Mass remaining over time for litter samples from the *in-situ* and common garden experiments. B.) Mass remaining over time for wood stick samples from the common substrate experiment. Significant effects and p-values shown in the upper right corners correspond with results with linear mixed effects models (see Table S1 for full model results). Note the difference in the y-axis scales between panels.

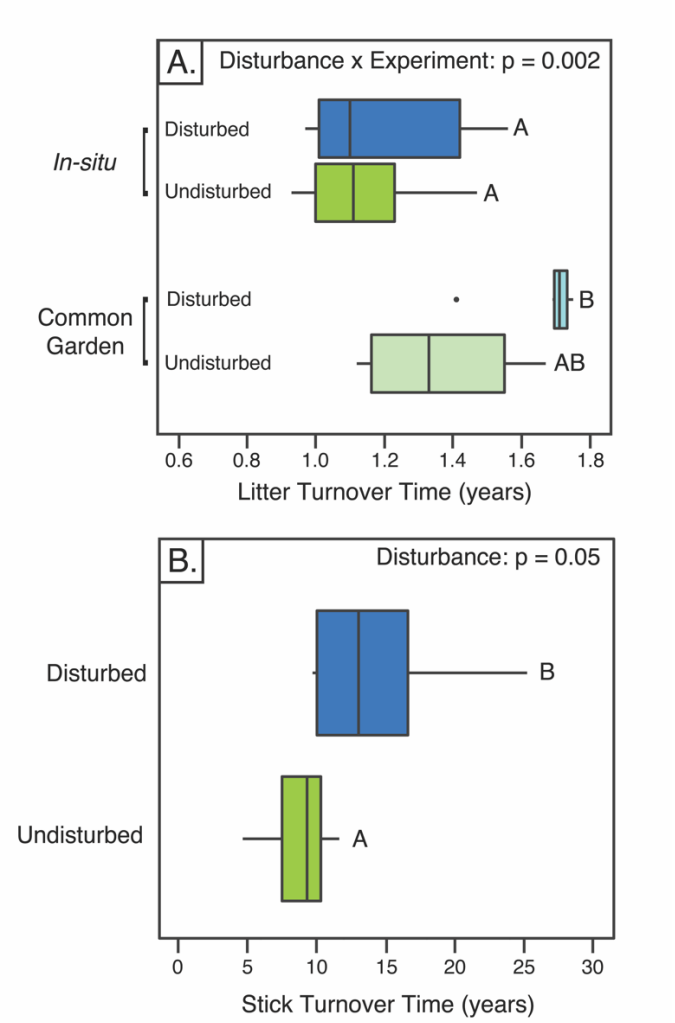

Figure 3: Boxplots showing medians and distributions of estimated turnover times based on k-value calculations for A.) litter samples derived from disturbed and undisturbed stands for the *in-situ* and common garden experiments, and B.) wooden popsicle stick samples from the common substrate experiment. Uppercase letters in panel A indicate significant differences in distributions between groups, based on results from non-parametric analysis of variance and pairwise comparisons based on Bonferroni p-value adjustments ($p \leq 0.05$). Uppercase letters in panel B indicate significant differences between disturbance histories, based on results from one-way ANOVA ($p \leq 0.05$). Note the difference in x-axis scales between panels.

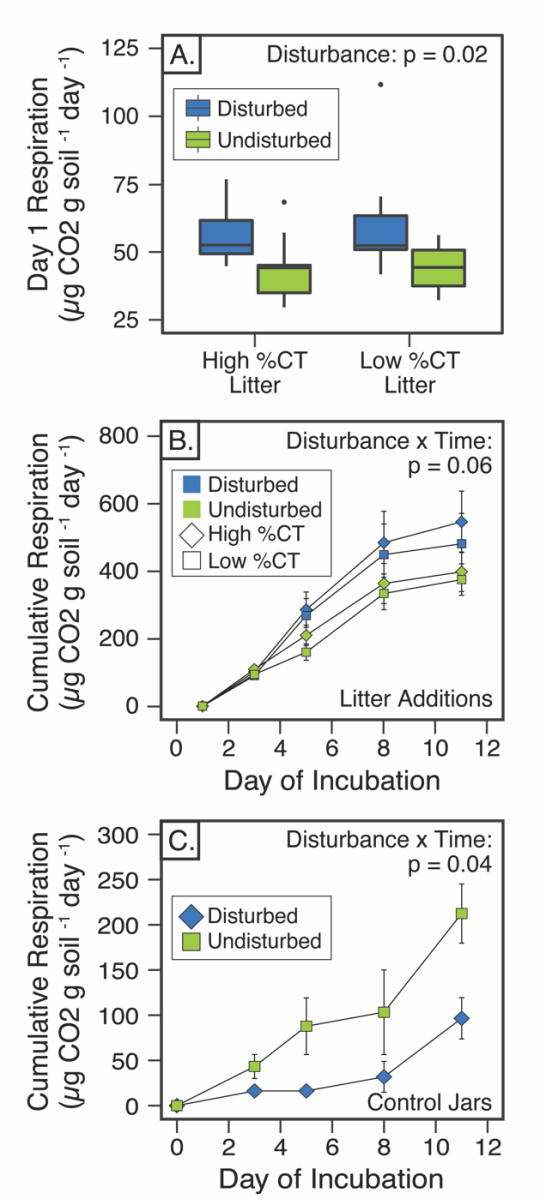

Figure 4: Results from 11-day litter addition incubation experiment. A.) Boxplots showing medians and distributions of CO_2 respiration on day 1 of the experiment between litter chemistry types (i.e., aspen genotypes with high vs. low condensed tannin (CT) concentration) and soils derived from disturbed and undisturbed stands, B.) cumulative respiration following additions of high CT vs. low CT litter between disturbance histories, and C.) cumulative respiration of control jars (i.e., jars containing only soil that did not receive any litter additions) between disturbance histories. Note the difference in y-axis scales between panels.

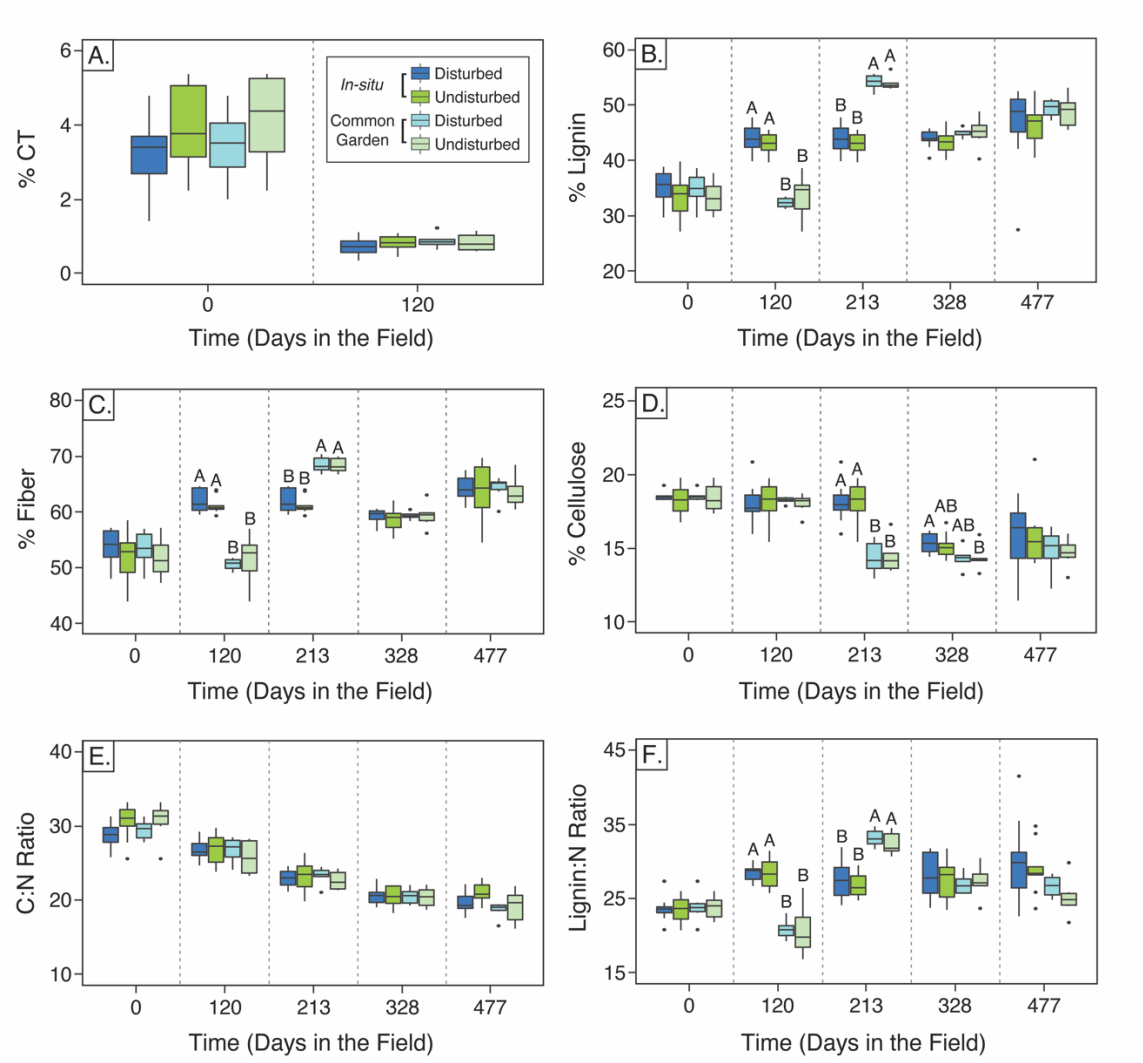

Figure 5: Boxplots showing medians and distribution for changes in litter chemistry data over time for the *in-situ* and common garden litter decomposition experiments, including changes in A.) condensed tannin (CT) concentration, B.) lignin concentration, C.) fiber concentration, D.) cellulose concentration, E.) carbon-to-nitrogen (C:N) ratios, and F.) lignin-to-nitrogen (lignin:N) ratios. Capital letters indicate significant differences between decomposition experiments and disturbance histories for chemical characteristics at a given time point, based on results from non-parametric analysis of variance and pairwise comparisons of means based on Bonferroni p-value adjustments ($p \leq 0.05$); the absence of uppercase letters indicates no significant differences in distributions at a given time point. Note differences in y-axis scales between panels.

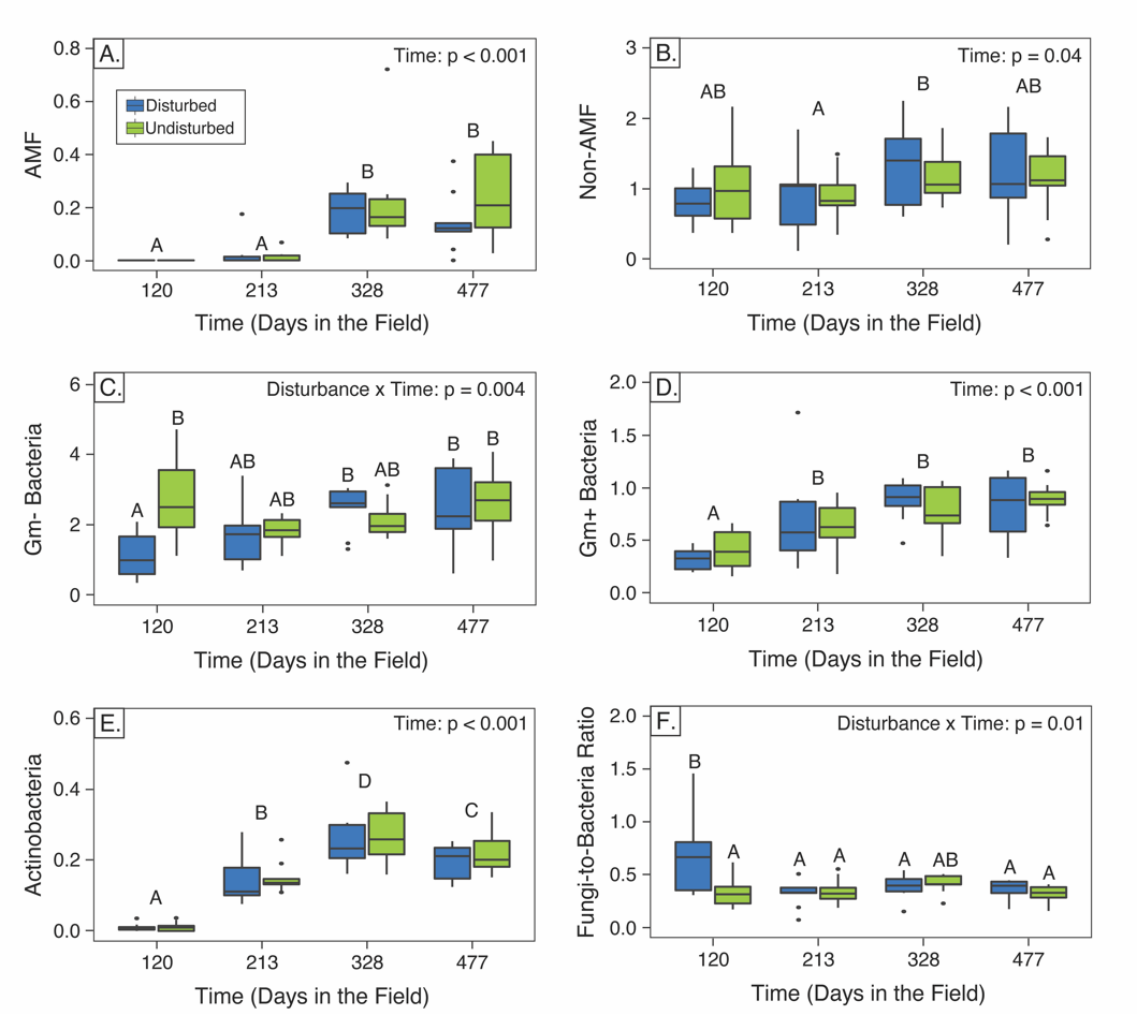

Figure 6: Boxplots showing medians and distributions of litter biomass for various phospholipid fatty acid (PLFA) guilds between disturbance histories during the *in-situ* decomposition experiment. Guild assignments shown include A.) arbuscular mycorrhizal fungi (AMF), B.) non-AMF, C.) gram negative (Gm-) bacteria, D.) gram positive (Gm+) bacteria, E.) actinobacteria, and F.) ratios of fungi-to-bacteria. P-values in the upper right corners of each panel also correspond to linear mixed effects model results. Capital letters correspond with significant differences between decomposition experiment time points (panels A, B, D, and E) or time x disturbance history interactions (panels C and F) based on results from linear mixed effects models ($p \leq 0.05$). PLFA biomass units for panels A-E are expressed in µmol g litter $^{-1}$. Note the difference in y-axis scales between panels.

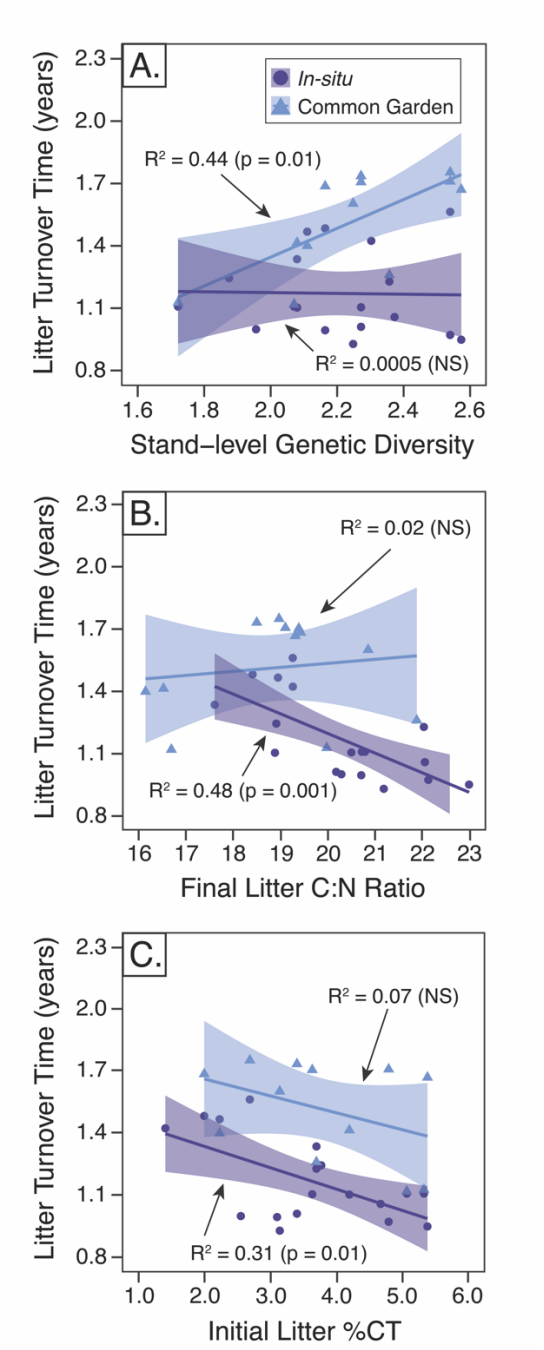

Figure 7: Best-fit simple linear regression results for predicting litter turnover times between *in-situ* vs. common garden experiments. A.) Stand-level genetic diversity (shannon diversity), B.) final litter C:N ratios after 477-days of decomposition, and C.) initial litter condensed tannin (CT) concentration. Shaded areas around regression lines represent 95% confidence intervals.

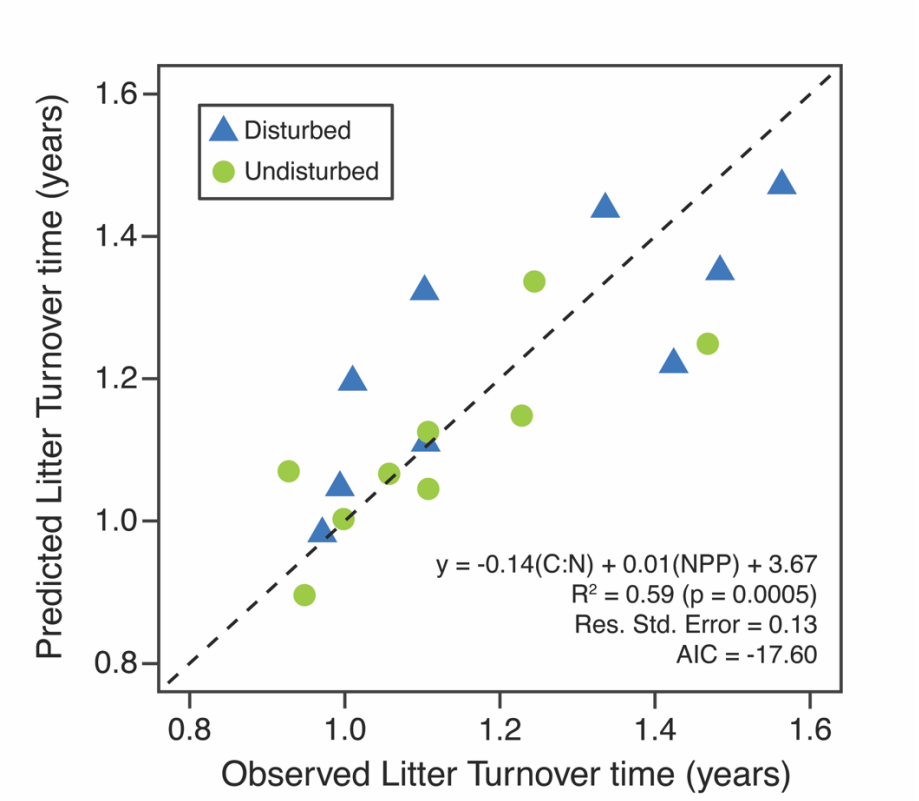

Figure 8: Results from multiple linear regression analysis for predicting litter turnover time based on final litter C:N ratio and net primary production (NPP). The dashed line represents a 1:1 line. Comparisons of this model vs. simple linear regression models for final litter C:N ratio and NPP as single predictive variables can be found in Table S3.

Experiment	Minimum	1st Quartile	Median	3rd Quartile	Max	Bonferroni Group
In-situ	7.89	14.98	23.28	34.57	54.72	C
Common Garden	8.28	39.08	42.55	46.78	49.78	B
Common Substrate	73.88	88.86	90.53	93.14	89.70	A

Table 1: Summary statistics of final litter/stick percent mass remaining after 477 days in the field for the three experiments conducted in this research. Capital letters in the final column indicate significant differences between data medians and distributions for decomposition experiments, based on results from non-parametric analysis of variance and pairwise comparisons of means based on Bonferroni p-value adjustments ($p \leq 0.05$).

Chemical Characteristic	Days in the field	Disturbed	Undisturbed	Disturbance History	Time	Disturbance History x Time
% CT	0	3.21 ± 0.35	3.98 ± 0.40	$F_{1,16}$ = 2.56 P = 0.13	**$F_{1,16}$ = 109.53 P < 0.0001**	$F_{1,16}$ = 1.51 P = 0.24
	120	0.71 ± 0.08	0.81 ± 0.08			
% Lignin	0	35.38 ± 1.01	33.41 ± 1.33	$F_{1,16}$ = 0.46 P = 0.51	**$F_{1,64}$ = 40.79 P < 0.0001**	$F_{1,64}$ = 0.32 P = 0.86
	120	43.78 ± 0.87	42.96 ± 0.64			
	213	43.75± 0.88	42.96 ± 0.64			
	328	43.83 ± 0.56	43.37 ± 0.75			
	477	46.14 ± 2.58	46.38 ± 1.26			
% Fiber	0	53.81 ± 1.04	51.67 ± 1.57	$F_{1,16}$ = 1.25 P = 0.28	**$F_{1,64}$ = 48.79 P < 0.0001**	$F_{1,64}$ = 0.29 P = 0.88
	120	61.77 ± 0.68	61.16 ± 0.52			
	213	61.80 ± 0.67	61.16 ± 0.52			
	328	59.09 ± 0.47	58.43 ± 0.80			
	477	64.16 ± 0.77	63.32 ± 1.08			
% Cellulose	0	18.43 ± 0.10	18.26 ± 0.33	$F_{1,16}$ = 0.002 P = 0.97	**$F_{1,62}$ = 26.10 P < 0.0001**	$F_{1,62}$ = 0.11 P = 0.98
	120	18.00 ± 0.46	18.20 ± 0.45			
	213	18.05 ± 0.46	18.20 ± 0.45			
	328	15.26 ± 0.22	15.06 ± 0.28			
	477	18.02 ± 2.38	16.93 ± 1.31			
Lignin:N Ratio	0	23.63 ± 0.58	23.64 ± 0.59	$F_{1,16}$ = 0.25 P = 0.62	**$F_{1,64}$ = 14.40 P < 0.001**	$F_{1,64}$ = 0.08 P = 0.99
	120	28.57 ± 0.41	28.53 ± 0.67			
	213	27.66 ± 0.85	27.12 ± 0.85			
	328	28.34 ± 1.05	28.33 ± 1.20			
	477	29.84 ± 1.98	29.00 ± 1.15			
C:N Ratio	0	28.73 ± 0.58	30.42 ± 0.81	$F_{1,16}$ = 1.01 P = 0.33	**$F_{1,64}$ = 308.48 P < 0.001**	**$F_{1,64}$ = 2.50 P = 0.05**
	120	26.81 ± 0.51	27.03 ± 0.70			
	213	22.96 ± 0.43	23.20 ± 0.67			
	328	20.60 ± 0.40	20.62 ± 0.44			
	477	19.66 ± 0.45	20.88 ± 0.46			

Table 2: Summary statistics and linear mixed model results for changes in litter chemistry over time between disturbance histories for the *in-situ* experiment. Note that condensed tannin (CT) concentration data were not collected after 120 days of the experiment, so models were developed only based on CT concentration on day 0 and day 120. Summary statistic values represent means ± one standard error. Bolded model results represent significant effects of disturbance history, time, disturbance history x time interactions.

Chemical Characteristic	Days in the field	Disturbed	Undisturbed	Disturbance History	Time	Disturbance History x Time
% CT	0	3.45 ± 0.41	4.14 ± 0.54	$F_{1,10} = 0.71$ $P = 0.42$	**$F_{1,10} = 97.07$** **$P < 0.0001$**	$F_{1,10} = 1.49$ $P = 0.25$
	120	0.88 ± 0.08	0.83 ± 0.10			
% Lignin	0	34.78 ± 1.29	33.31 ± 1.26	$F_{1,10} = 0.11$ $P = 0.75$	**$F_{1,40} = 167.66$** **$P < 0.0001$**	$F_{1,40} = 0.44$ $P = 0.78$
	120	32.34 ± 0.37	33.49 ± 1.69			
	213	54.09 ± 0.58	53.92 ± 0.52			
	328	44.99 ± 0.33	45.00 ± 1.17			
	477	49.39 ± 0.66	63.46 ± 1.18			
% Fiber	0	53.25 ± 1.38	51.66 ± 1.52	$F_{1,10} = 0.11$ $P = 0.75$	**$F_{1,40} = 167.66$** **$P < 0.0001$**	$F_{1,40} = 0.44$ $P = 0.78$
	120	50.52 ± 0.43	51.42 ± 1.90			
	213	68.43 ± 0.60	68.31 ± 10.56			
	328	59.28 ± 0.28	59.29 ± 0.92			
	477	64.19 ± 0.90	63.46 ± 1.18			
% Cellulose	0	18.47 ± 0.15	18.35 ± 0.40	$F_{1,10} = 0.20$ $P = 0.67$	**$F_{1,40} = 92.69$** **$P < 0.0001$**	$F_{1,40} = 0.37$ $P = 0.83$
	120	18.17 ± 0.09	17.93 ± 0.28			
	213	14.31 ± 0.46	14.39 ± 0.48			
	328	14.28 ± 0.30	14.30 ± 0.35			
	477	14.80 ± 0.62	14.62 ± 0.41			
Lignin:N Ratio	0	23.86 ± 0.86	23.80 ± 0.66	$F_{1,10} = 0.43$ $P = 0.53$	**$F_{1,40} = 64.00$** **$P < 0.0001$**	$F_{1,40} = 0.41$ $P = 0.80$
	120	20.91 ± 0.53	20.75 ± 1.46			
	213	33.27 ± 0.53	32.54 ± 0.69			
	328	27.15 ± 0.65	27.41 ± 0.96			
	477	26.65 ± 0.60	25.18 ± 1.09			
C:N Ratio	0	29.48 ± 0.55	30.61 ± 1.10	$F_{1,10} = 0.002$ $P = 0.96$	**$F_{1,40} = 162.78$** **$P < 0.0001$**	$F_{1,40} = 1.49$ $P = 0.22$
	120	26.77 ± 0.96	25.76 ± 0.99			
	213	22.63 ± 0.49	22.63 ± 0.53			
	328	20.51 ± 0.44	20.37 ± 0.55			
	477	18.65 ± 0.45	19.14 ± 0.93			

Table 3: Summary statistics and linear mixed model results for changes in litter chemistry over time between disturbance histories for the common garden experiment. Note that condensed tannin (CT) concentration data were not collected after 120 days of the experiment, so models were developed only based on CT concentration on day 0 and day 120. Summary statistic values represent means ± one standard error. Bolded model results significant effects of disturbance history, time, or disturbance history x time interactions.

PLFA Guild	Disturbance History	a	b	Residual standard error	p-value	R^2
AMF	All Data	81.67	-4.36	20.21	< 0.0001	0.55
	Disturbed	79.74	-4.09	22.78	< 0.0001	0.42
	Undisturbed	83.68	-4.62	17.82	< 0.0001	0.67
Non-AMF	All Data	77.33	-0.25	28.79	0.03	0.06
	Disturbed	78.10	-0.24	28.59	0.09	0.08
	Undisturbed	76.48	-0.25	29.80	0.21	0.05
Gm- bacteria	All Data	83.50	-0.16	28.46	0.01	0.08
	Disturbed	93.19	-0.23	26.26	0.004	0.22
	Undisturbed	64.25	-0.04	30.45	0.74	0.003
Gm+ bacteria	All Data	111.47	-0.99	24.47	< 0.0001	0.32
	Disturbed	110.86	-0.98	24.16	0.0002	0.35
	Undisturbed	112.28	-0.99	25.47	0.0005	0.30
Actinobacteria	All Data	97.37	-3.58	20.32	< 0.0001	0.54
	Disturbed	95.36	-3.43	21.61	< 0.0001	0.48
	Undisturbed	99.45	-3.71	19.53	< 0.0001	0.59

Table 4: Non-linear regression results for predicting the percent litter mass remaining as a function of the biomass of different litter PLFA guilds for the whole dataset and between disturbance histories. Parameters a and b correspond to equation 3.

SUPPLEMENTARY TABLES

Experiment	Disturbance History	Time	Disturbance History x Time
In-situ	$F_{1,16} = 0.46$ $P = 0.51$	$\mathbf{F_{1,64} = 189.24}$ $\mathbf{P < 0.0001}$	$F_{1,64} = 0.38$ $P = 0.82$
Common Garden	$F_{1,10} = 3.63$ $P = 0.09$	$\mathbf{F_{1,40} = 143.55}$ $\mathbf{P < 0.0001}$	$F_{1,40} = 2.42$ $P = 0.06$
Common Substrate	$F_{1,10} = 3.56$ $P = 0.09$	$\mathbf{F_{1,40} = 37.52}$ $\mathbf{P < 0.0001}$	$F_{1,40} = 2.17$ $P = 0.09$

Table S1: Linear mixed model results for changes in litter percent mass remaining over time between disturbance histories for all three decomposition experiments. Bolded model results indicate significant effects of disturbance history, time, or disturbance history x time interactions.

Experiment	Stand	Intercept	k-value estimate	R^2
In-situ	BL01	105.30	0.00175	0.797
	BL02	112.70	0.00259	0.7979
	BL03	110.00	0.00295	0.8872
	BL04	106.30	0.00248	0.8535
	BL05	109.20	0.00289	0.8943
	BL06	107.90	0.00248	0.8965
	BL07	112.10	0.00276	0.841
	BL08	111.00	0.00247	0.8532
	BL09	113.20	0.00282	0.8177
	BL10	109.80	0.00220	0.8862
	BL11	105.30	0.00175	0.8433
	BL12	116.80	0.00205	0.5823
	BL13	108.0	0.00223	0.8717
	BL14	111.20	0.00271	0.8639
	BL15	103.70	0.00185	0.7695
	BL16	114.00	0.00274	0.7377
	BL17	108.00	0.00187	0.8373
	BL18	106.00	0.00192	0.9011
Common Garden	BL01	108.30	0.00156	0.8793
	BL03	107.50	0.00171	0.8182
	BL04	105.20	0.00160	0.9488
	BL05	105.00	0.00164	0.9425
	BL06	107.90	0.00194	0.8795
	BL08	109.30	0.00243	0.7907
	BL09	104.80	0.00160	0.9398
	BL10	116.50	0.00245	0.7472
	BL13	107.50	0.00217	0.8826
	BL14	103.90	0.00158	0.966
	BL15	103.80	0.00162	0.9444
	BL17	105.20	0.00196	0.9102
Common Substrate	BL01	100.40	0.00018	0.4265
	BL03	102.10	0.00060	0.7397
	BL04	101.90	0.00026	0.5538
	BL05	102.00	0.00026	0.727
	BL06	100.70	0.00016	0.4425
	BL08	102.70	0.00029	0.6344
	BL09	99.36	0.00011	0.1501
	BL10	101.50	0.00040	0.5684
	BL13	101.70	0.00024	0.6046
	BL14	103.20	0.00028	0.7600
	BL15	102.60	0.00028	0.8171
	BL17	102.00	0.00030	0.8038

Table S2: Nonlinear regression results for estimating k-values based on changes in litter mass remaining for the three field experiments in this research.

Variable	Experiment	Intercept	Slope	Res. Std. Error	R^2	p-value	AIC
Shannon Diversity	In-situ	1.21	-0.02	0.21	0.0005	0.93	-1.21
	Common Garden	-0.04	0.69	0.18	0.49	0.01	-3.27
Simpson's Diversity	In-situ	0.85	0.36	0.21	0.003	0.82	-1.27
	Common Garden	-2.68	4.76	0.17	0.54	0.007	-4.35
NPP	In-situ	1.21	-0.001	0.21	0.006	0.76	-1.31
	Common Garden	1.44	0.003	0.25	0.01	0.74	4.71
LAI	In-situ	1.37	-0.06	0.20	0.06	0.31	-2.41
	Common Garden	1.28	0.07	0.25	0.05	0.51	4.29
Initial C:N	In-situ	2.35	-0.04	0.19	0.19	0.07	-4.98
	Common Garden	1.71	-0.01	0.25	0.003	0.86	4.81
Final C:N	In-situ	3.11	-0.10	0.15	0.48	0.002	-12.86
	Common Garden	1.15	0.02	0.25	0.02	0.66	4.61
Initial %Lignin	In-situ	1.33	0.01	0.25	0.001	0.91	-4.09
	Common Garden	0.89	0.02	0.25	0.06	0.46	4.17
Final %Lignin	In-situ	1.56	-0.01	0.20	0.06	0.33	-2.29
	Common Garden	1.32	0.004	0.25	0.001	0.91	4.83
Initial %Fiber	In-situ	0.08	0.02	0.19	0.17	0.09	-4.47
	Common Garden	0.60	0.02	0.24	0.06	0.43	4.06
Final %Fiber	In-situ	1.99	-0.01	0.20	0.07	0.31	-2.42
	Common Garden	1.18	0.01	0.25	0.003	0.87	4.81
Initial %Cellulose	In-situ	-0.73	0.10	0.20	0.13	0.13	-3.77
	Common Garden	0.14	0.08	0.25	0.05	0.49	4.25
Final %Cellulose	In-situ	0.71	0.03	0.21	0.09	0.26	-0.23
	Common Garden	1.40	0.01	0.25	0.002	0.90	4.83
Initial %CT	In-situ	1.54	-0.10	0.17	0.35	0.01	-8.91
	Common Garden	1.83	-0.08	0.23	0.16	0.20	2.76
Final %CT	In-situ	1.26	-0.12	0.21	0.02	0.59	-1.55
	Common Garden	1.75	-0.28	0.25	0.06	0.45	4.14

Table S3: Simple linear regression model results for predicting litter turnover time from diversity and litter chemistry variables.

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CHAPTER 2:

Breaking it down: Soil aggregate disintegration and carbon dynamics in genetically diverse experimental aspen stands

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ABSTRACT

Genetic and competition-induced variations in physiological traits among tree genotypes can complicate forest management strategies that seek to increase plant productivity and soil carbon (C) accrual, having large implications for accurate projections of soil C-climate feedbacks simulated by models under future warming scenarios and increased atmospheric CO_2. Trembling aspen (*Populus tremuloides*) is a genetically diverse, widespread tree species with strong competitive responses in plant trait expression, making it a model system to study relationships between intraspecific plant competition and soil C dynamics and evaluate how those relationships potentially affect future soil C accrual. In this study, we examined how stand-level characteristics, litter decomposition dynamics, and variations in intraspecific plant competition (mediated by changes in tree density) affect soil C storage and aggregate stability among genetically diverse experimental trembling aspen stands in southern Wisconsin. We quantified relative C contributions and aggregate disintegration rates and stability using a new laser diffraction technique for three soil C fractions, including < 53 μm (mineral-associated), 53-250 μm (microaggregates), and 250-2000 μm (macroaggregates). Our results showed that stand-level genetic diversity, net primary productivity, and fine root biomass were strongly correlated with relative soil C fraction proportions, with some interactive effects of tree density. Aggregate disintegration rates for aggregates 250-2000 μm were negatively correlated with genetic diversity, demonstrating for the first time to our knowledge that increased plant genetic diversity corresponds with greater aggregate stability. Disintegration rates for aggregates < 250 μm decreased with increasing litter condensed tannin – a secondary plant metabolite with no previously known connection to soil aggregate stability. We determined that most of the bulk soil C stock in these soils is retained within the > 53 μm fraction (consisting of microaggregates

and macroaggregates), likely due to aggregates accounting for the greatest total soil C mass, but all soil C fractions evaluated in this research have the potential to accrue additional C. While these conditions, along with soil mineralogy, are conducive for future C accrual in our system, projected increased climate warming and atmospheric CO_2 have shown to accelerate C cycling, limit soil C stabilization, alter litter chemistry, and increase intraspecific competition in other experimental aspen stands. Our work demonstrates that intraspecific plant competition and genetic diversity influence soil C dynamics and has implications for both future forest management strategies and soil C-climate feedback modeling efforts.

INTRODUCTION

Forest soils contain > 40% of global C and store up to ≈860 Pg of C (McGarvey et al. 2015, Nave et al. 2019, IPCC-AR6, 2022) and soil C sequestration accounts for ≈30% of total net ecosystem C sequestration in temperate forests (Pan et al. 2011) but the response of the forest soil C sink to future climate change remains uncertain. While these systems are currently increasing their total C and soil C storage, due to advances in our understanding of how land management practices increase ecosystem C sequestration, future climate change may switch these systems from C sinks to C sources. Field and experimental data and global $\Delta^{14}C$ mean age estimates show lower retention of fresh plant-derived C inputs within stable soil fractions compared to modeled projections (Richter et al. 1999, Hofmockel et al. 2011, Todd-Brown et al. 2013, He et al. 2016, Groenigen et al. 2017, Shi et al. 2020), demonstrating high uncertainty for how increased warming and plant productivity might affect future forest soil C accumulation. Interactions between forest productivity and future C accrual could be also strongly affected by variations in physiological traits, growth dynamics, and C allocation strategies among tree genotypes. Trembling aspen (*Populus tremuloides*) is a widespread deciduous tree species and one of the most genetically diverse species known to science (Mitton and Grant 1996, Callahan et al. 2013). Variations in tree density can alter intraspecific plant competition and has been shown to affect plant trait expression and growth rates within experimental aspen stands (Cope et al. 2021a, 2021b). Establishing interactions between genetic diversity, plant productivity, intraspecific plant competition, and soil C dynamics can better our understanding of the capacity for forest soils to accrue future C, using trembling aspen as a model system.

Reductions in tree density, mimicking selective harvesting forest management practices, can decrease competitive inhibition among aspen genotypes and alter C allocation strategies

(Kruger et al. 2020). Tree foliage and litter production have been shown to rapidly recover following selective harvesting in aspen stands – a practice that also has been shown to increase forest soil C storage relative to clear cutting (LaganiãRe et al. 2010, Strukelj et al. 2015, Mayer et al. 2020). However, selective harvesting has also been shown to decrease C allocation to roots without changing forest productivity estimates (Noormets and Nouvellon 2015) and its effects on links between litter decomposition rates and soil C accrual are unclear. Because fine root turnover represents a large proportion of total of C cycling in forests (Kong and Six 2010) and litter decomposition is a primary pathway in the terrestrial C cycle (Aerts 1997, Krishna and Mohan 2017), potential variations in above and belowground productivity mediated by reductions in tree density could have significant implications for soil C dynamics.

Variations in litter chemistry play a strong role in mediating litter decomposition rates at the ecosystem level (Meentemeyer 1978, Vitousek et al. 1994, Aerts 1997, Madritch et al. 2006). Litter carbon-to-nitrogen (C:N) ratios are the most widely used metric in litter decomposition studies for monitoring changes in litter chemistry, with higher decomposition rates corresponding to lower C:N ratios. In addition to C:N ratios, many secondary plant compounds can affect litter decomposition rates, such as polyaromatics like condensed tannins (CT), which are resistant to microbial decay and have been shown to accumulate in soil C (Kögel-Knabner 2000, Tfaily et al. 2015), although their role in soil C stabilization and accrual is unknown. Aspen foliage CT concentrations are highly variable among aspen genotypes and has been shown to be negatively correlated with litter decomposition rates in field studies and inhibit microbial respiration in the presence of other C compounds in laboratory experiments (Madritch et al. 2006, 2007, Rubert-Nason and Lindroth 2021). Foliar CT concentration can be higher in low density aspen stands with less intraspecific competition among genotypes compared to in

high density stands (Cope et al. 2021a). Such controls of intraspecific competition on variations in foliar chemistry could have subsequent consequences on litter decomposition dynamics and the capacity for soils to accrue C.

The fate of plant-derived material in soil is directly connected to microbial decomposer activity and transfers into soil C fractions that are more physically isolated from microorganisms. This material can become occluded within aggregates or bound on mineral surfaces through production of microbial products (e.g., amino acids, polysaccharides) and formation of organo-mineral complexes (Six et al. 2002, 2004, Jastrow et al. 2007, Sollins et al. 2007, Tiemann and Grandy 2015, Buckeridge et al. 2020). Given that aggregate formation and organo-mineral complexation are closely tied to microbial processes and soil microbiome and rhizosphere characteristics vary among in *Populus* genotypes (Schweitzer et al. 2004, 2008, Gottel et al. 2011, Madritch and Lindroth 2011, Shakya et al. 2013, Mueller et al. 2020), mechanisms of soil C protection and long-term storage should also be mediated by genetic diversity. Research from bioenergy cropping systems demonstrates the capacity for *Populus* communities to accrue slowly cycling C within protected soil C fractions, comparable to natural systems (DeGryze et al. 2004, Kahle et al. 2013, Sprunger and Robertson 2018, Szymanski et al. 2019, von Haden et al. 2019) but the potential effects of intraspecific competition are unknown.

Our research goal was to determine how soil C dynamics are influenced by stand-level characteristics among genetically diverse experimental trembling aspen stands that vary in their competitive pressures, due to differences in tree density. We compared relative C contributions to total soil C and aggregate stability and disintegration rates among three soil fractions, including < 53 μm (mineral-associated), 53-250 μm (microaggregates), and 250-2000 μm (macroaggregates), between low and high density stands. We were also interested in how

intraspecific plant competition, stand-level genetic diversity, plant productivity, and litter decomposition dynamics relate to relative fraction C contributions and aggregate stability dynamics. We hypothesized that variations in competitive pressures among aspen genotypes in low vs. high density stands would alter relationships between soil C dynamics and stand-level characteristics and litter decomposition dynamics, affecting the capacity for future soil C accrual in these systems.

METHODS

Study site history

Our 0.2 ha site consisted of 18 experimental stands of trembling aspen, established in 2010 at the University of Wisconsin-Madison's Arlington Agricultural Research Station in Arlington, WI (43.3°N, 89°W). This site was previously a grassy meadow before being plowed and disked. Each experimental stand originally consisted of 64 individual trees representing 16 unique genotypes collected from natural aspen stands throughout Wisconsin. Microsatellite analysis later confirmed only 15 unique aspen genotypes, 14 of which are found in all 18 stands (C. Cole, K. Mock, R. Lindroth, unpublished data). Trees were propagated from root stock and planted within each of the 4 x 4 m plots, which were randomly and approximately equally distributed with 0.5 x 0.5 m spacing (40,000 trees ha^{-1} density). Soils at this site are mapped within Huntsville series (Cumulic Hapludolls), which are silt loam soils formed in paleo floodplains capped with wind-blown silt sediment.

In spring 2014, half of the experimental stands were randomly selected for thinning to simulate an intermediate-level disturbance and decrease competitive inhibition by reducing tree density by 75% (10,000 trees ha^{-1}) (Figure 1A), leaving only one representative individual stem

per genotype per low density stand. Since the thinning treatment, variations in relative growth rates and genotype frequency led to a divergence in genotypic composition between high and low density stands (Figure 1B) (Cope et al. 2021b). This divergence in genotype composition over only a 5-year period, where slow-growing genotypes were outcompeted by fast-growing genotypes under higher competitive pressures, reflects how intraspecific competition can quickly shape ecological selection through increasing tree mortality of less competitive genotypes (Figure 1C). The greater dominance of fast-growing, competitive genotypes in high density stands also has led to more variable and marginally lower stand-level genetic diversity compared to low density stands, where other environmental factors have likely shaped their genotypic composition. (Figure 1D).

Aggregate and mineral-associated C

We analyzed surface soils (0-10 cm) from of each of the 18 stands. Samples were collected from two subplots, each representing a composite of 5 soil cores, sampled based on randomly generated azimuths and distances from the center of each 4 x 4 m plot. Soil C fraction data presented in this paper represents the average of the 2 subplot samples per stand. Soil C fractions were isolated from 25 g of field-moist soil into three water-stable fractions using wet-sieving, including macroaggregates (250-2000 μm), microaggregates (53-250 μm), and mineral-associated organic matter (MAOM) (< 53 μm), following methods described by Cates and Ruark (2017) for similar soils. We recognized that the MAOM fraction consists of C bound to primary mineral particles and microaggregates small enough to pass through a 53 μm sieve. Each fraction was analyzed for organic C using a Flash 2000 Elemental Analyzer (Thermo Scientific, Wilmington, DE, USA). Samples were run in duplicate with replicate errors < 10%, using

aspartic acid and soil reference material as check standards. We then calculated the absolute mass of C for each fraction, based on the respective % C and the total mass of each fraction obtained from wet sieving. The relative proportion of C within each fraction is based on the total C recovered within the C fractions, expressed as g C fraction g C^{-1}.

Aggregate stability and disintegration kinetics

We quantified aggregate stability and aggregate disintegration kinetics using air-dried, intact soil samples from all 18 experimental stands using a Malvern Mastersizer 2000 MU laser particle analyzer. Briefly, samples are circulated in DI water and constantly stirred for >180 minutes, while constant mechanical stress is applied from the stirring propeller, the pump, and effects of circulation under pressure, following methods of Kasmerchak et al. (2019) and based off past work using laser diffraction to monitor aggregate stability (e.g., Field and Minasny 1999, Fristensky and Grismer 2008, Bieganowski et al. 2010). Repeated measurements of the particle size distribution were taken every ≈2 minutes for the first 2 minutes of circulation, and then within ≈10-minute intervals until the total circulation time exceeded 180 minutes. After >180 minutes, the circulating sample was then dispersed chemically by adding 10 mL of 50 g L^{-1} Na-MP to the beaker and physically by being exposed to ultrasound, yielding a final fully dispersed measurement (Kasmerchak et al. 2019).

Although this method provides nearly infinite possibilities for examining aggregate disintegration across size classes (Figure S1), we only quantified aggregate disintegration kinetics for the same fractions we isolated using wet sieving (250-2000 μm, 53-250 μm, and < 53 μm), following modified equations from Kasmerchak et al. (2019). Aggregate size fraction dynamics show that percent volume of particles < 53 μm increase exponentially as particles > 53

μm exponentially decrease over time, representing the progressive breakdown of larger aggregates and the progressive increase of smaller aggregates (Figure 2). Because the instrument used in this analysis determines particle size based on the cross-sectional area of all particles in the circulation chamber, the < 53 μm represents both aggregates and individual mineral particles of this size. All samples analyzed by Kasmerchak et al. (2019) assumed two populations of aggregates, those that disintegrate quickly and those that disintegrate slowly over time. In our samples, only modeled parameter estimates for < 53 μm fraction had significant p-values using this two-population approach, and were modeled using the following equation:

$$(1)\ \%_{<53\ \mu m} = A_{0,1}(1 - e^{-k_1 t}) + A_{0,2}(1 - e^{-k_1 t})$$

where $A_{0,1}$ is the total percentage of aggregates < 53 μm that disintegrate rapidly, $A_{0,2}$ is the percentage that disintegrate slowly, k_1 is the decay rate of aggregates that breakdown quickly, k_2 is the decay rate of aggregates that breakdown more slowly, and t is circulation time in minutes. For aggregates 53-250 μm and 250-2000 μm, we assumed only one population of aggregates that disintegrate over time and modeled their disintegration kinetics using the following equation:

$$(2)\ \%_{>53\ \mu m} = A(e^{-kt}) + C$$

where A represents the total percentage of aggregates that disintegrate during circulation, k is the decay rate, t is circulation time in minutes, and C is the percent volume of particles > 53 μm in the final particle size distribution prior to being chemically and physically dispersed. No *C* term was necessary for equation 1 (t = 0) due to minimal fine fraction content at the start of the experiment. Models were developed using nonlinear least-squares fitting with the *gslnls* package in R-studio (Chau, 2023).

The ratio of the fully dispersed measurement (FD) relative to the final measurement prior to dispersion (180 mins) is indicative of the relative abundance of especially stable aggregates

that persist throughout > 180 minutes of circulation, which we have defined as the stability index ratio, where higher ratios correspond with greater stability. The proportion of aggregates in the < 53 μm fraction increase in percent volume following dispersion and were calculated as FD/180 mins. Aggregates > 53 μm decrease in percent volume following chemical and physical dispersion and were calculated as 1-FD/180 mins.

Litter chemistry

To understand the potential connections between litter chemistry and soil C dynamics, we collected stand-level data on initial litter carbon-to-nitrogen (C:N) ratio and condensed tannin (CT) concentration. Litter samples were collected in fall 2020 from each experimental stand and analyzed for total C and total N to determine C:N ratio using a Flash 2000 Elemental Analyzer (Thermo Scientific, Wilmington, DE, USA). All samples were run in duplicate with replicate error of < 10% using atropine as calibration and check standards. CT were quantified using an acid-butanol method (Porter et al., 1985) with analytical standards purified from *Populus tremuloides* foliage (Hagerman and Butler 1989). All samples were stored at -20°C prior to analysis, after being vacuum-dried for at least 48 hours and then ground to 1 mm on a Wiley Mill (size 20 mesh insert) and then ground again using a ball-mill.

Litter turnover estimates

Given the role litter decomposition plays in terrestrial nutrient cycling, we wanted to evaluate potential connections between litter decomposition and soil C and aggregate dynamics. Litter samples were collected over a 477-day decomposition experiment using litter mixtures derived from the 18 experimental stands. Samples were corrected for field moisture connect and

potential soil material contributions to litter mass at each retrieval time point. We calculated litter decomposition rates (k-values) using an exponential decay model based on the relationship between the log mass remaining and time, calculated as:

$$(3)\ M_t = M_0 e^{-kt}$$

where M_t is the litter mass remaining at time t in days (t = 120, 213, 328, and 477 days) expressed as percent of its initial mass, M_0 is the initial litter mass at time 0 (expressed as 100%), k is the first order decay constant, which can then be multiplied by 365.24 to approximate the litter decay rate over the course of one year. We then used the inverse of this annual decay rate to estimate mean litter turnover time in years.

Stand-level characteristics

We estimated stand-level diversity of aspen genotypes based on the Shannon diversity index (Shannon and Weaver, 1949) and Simpson's index (Simpson, 1949) using the *Vegan* package in R-Studio (Oksanen et al., 2012).

To directly relate variations in plant productivity to soil C and aggregate dynamics among the experimental aspen stands, we estimated annual net primary production (NPP) based on the summation of: (1) aboveground woody biomass growth, (2) foliage production, (3) coarse production (> 2 mm), and (4) fine root production (< 2 mm) (Clark et al. 2001). Estimating aboveground wood growth required initial and final tree size measurements for use in allometric scaling. We collected size measurements (diameter at breast height [*D*] and total height [*H*]) for every experimental tree after leaf drop in mid-October in both 2019 and 2020. Then, for each tree we subtracted initial (Fall 2019) from final (Fall 2020) estimates of aboveground woody biomass, which were derived using an allometric equation generation with data from a

destructive harvest of 25 trees in our experimental aspen stands. The resulting allometric model, which utilized both *D* and *H* as predictors, provided robust estimates (R^2 = 0.995, RMSE = 1.1) of aboveground woody mass (kg) across all 18 aspen populations:

$$(4)\ \ln(woody\ mass) = 2.17D^{0.50} + 0.17H - 0.01DH - 4.25$$

We estimated foliage production by subtracting the estimating the initial from final total number of leaves of each tree within each population. The total number of leaves was nondestructively estimated in the crown of one randomly selected tree per genotype in all 18 stands. Using a *D*-based allometric equation specific to *Populus tremuloides* growing in Wisconsin forests (Ruark and Bockheim 1987), we estimated coarse root production by subtracting initial from final coarse root biomass. We then estimated fine root production using a modified ingrowth root core method adapted from Perrson (1980). Following ground thaw in April 2020, we withdrew 10 randomly selected soil cores (wedge-shaped corer, ≈45.70 cm deep and covering 18.70 cm^2 of the ground surface) from each of the 18 experimental stands. Each resulting hole was lined with bags of thin plastic mesh to allow for root growth and refilled the bags with the extracted soil after removing debris and roots. Cores were retrieved in July 2020 and in December 2020 and processed by hand-extracting roots and rinsing them of debris. Isolated roots were pooled and from each core per retrieval date per population, oven dried at 60°C for 72 hours, then weighed.

Statistical analyses

All statistical analyses were performed in R-Studio (version 4.0.5; R Core Team 2021). We used two-way ANOVA to determine potential significant interactions of tree density on soil C fraction contributions to total C recovered from wet sieving, aggregate disintegration kinetic

parameters, and stability index ratios. We also used one-way ANOVA to compare fraction C contributions within high and low density stands, and to compare decay constants and stability index ratio among aggregate size fractions obtained from laser diffraction. We developed simple linear regression models to understand which individual variables could best explain the fraction C contributions, aggregate disintegration kinetics, and stability index ratios. For this analysis, we were interested how variations in stand-level genetic diversity (Shannon and Simpson's diversity indices), NPP, litter turnover time, litter C:N ratio, and litter CT concentration were potentially correlated with soil C and aggregate stability dynamics. We also included individual stand-level components of our NPP estimates in this analysis, including fine root biomass and tree foliage, to provide insight which potential contributions of NPP to have stronger relationships with soil C and aggregate dynamics in our system. Lastly, we used linear regression to explore how soil fraction C contributions might potentially influence aggregate disintegration and aggregate stability.

RESULTS

Soil C fractions

Soil macroaggregates (250-2000 μm) had significantly more fraction C than microaggregates (53-250 μm) and MAOM (< 53 μm), regardless of overlying tree density (Figure 3). Within low density stands, the MAOM and microaggregate fractions had significantly lower C contributions compared to the macroaggregate fraction. C contributions among high density stands were significantly different between the three fractions: microaggregates > MAOM > macroaggregates. Tree density did not affect C contributions within each fraction, which were highly variable among the 18 stands (Figure S2). It is important to note that all soil C

fractions also include some primary soil particles although the relative contributions to total fraction mass vary, particularly for the macroaggregate fraction where mineral soil particle contributions are minimal.

Aggregate disintegration kinetics and stability

Across all experimental stands, decay constants varied by soil fraction, with < 53 μm fraction pool 2 (k_2 values) and 53-250 μm fraction k values being an order of magnitude lower than 250-2000 μm fraction k values and < 53 μm fraction pool 1 (k_1 values) (Figure 4A). Aggregate stability index ratios differed among the three fractions, decreasing in stability with increasing fraction size (Figure 4B).

Decay constants (k_1) for the < 53 μm fraction were marginally greater in the low density stands than in the high density stands ($p = 0.10$). High density stands had greater decay constants for microaggregates ($p = 0.02$), and marginally lower stability index ratios ($p = 0.08$). Tree density had no significant effect on < 53 μm fraction k_2 values, 53-250 μm fraction k values, or stability index ratios for the < 53 μm and 53-250 μm fraction (Table 1).

Predictors of soil C fraction contributions

Of the variables used in linear regression models, only stand-level characteristics emerged as significant predictors of fraction C contributions; these included genetic diversity, NPP, and fine root biomass (Table S1). Tree density showed significant interactions with genetic diversity, NPP, and fine root biomass on predicting macroaggregate fraction C (Figure 5A-C). For low density stands, macroaggregate C showed a very strong negative correlation with genetic diversity ($R^2 = 0.69$) and a marginally significant negative correlation with NPP ($R^2 = 0.34$, p =

0.08). Tree density only had a significant interaction with genetic diversity for microaggregate fraction C, with low density stands showing a strong positive correlation (Figure 5D). Microaggregate fraction C showed a positive correlation with NPP and fine root biomass, regardless of tree density (Figure 5E, F). MAOM fraction C showed no significant relationships with genetic diversity or NPP (Figure 5G and 5H), but high density stands showed a strong negative correlation with fine root biomass ($R^2 = 0.45$) (Figure 5I).

Predictors of soil aggregate disintegration and stability

Decay (k_1) rates for the MAOM fraction (< 53 μm) were positively correlated with MAOM soil C fraction in low density stands ($R^2 = 0.51$, $p = 0.07$), but not high density stands. Across all 18 stands, average MAOM stability index ratios were positively correlated with MAOM fraction C ($R^2 = 0.32$). There were no significant relationships between < 53 μm k_2 values and k values for the 53-250 μm and 250-2000 μm fractions with fraction C (Table 2).

Litter C:N ratios, litter CT concentration, litter turnover time, and stand-level genetic diversity, emerged as significant predictors of aggregate decay constants (Table S2-S4). There were no significant interaction effects of tree density. K_1 values for the < 53 μm fraction were significantly and negatively correlated with litter C:N ratios and litter CT concentration, although CT concentration explained considerably greater variation ($R^2 = 0.25$ vs. 0.47, respectively) (Figures 6A, 6B). Litter turnover time and stand-level genetic diversity showed no significant relationships with k_1 values (Figures 6C and D). While < 53 μm fraction k_2 values showed no significant relationship with litter C:N ratio (Figure 6E), they a had strong negative correlation with litter CT concentration ($R^2 = 0.27$) (Figure 6F) and a strong positive correlation with litter turnover time ($R^2 = 0.44$) (Figure 6G). K_2 values showed no significant correlations with genetic

diversity (Figure 6H). K values for the 53-250 µm fraction also showed no significant correlation with litter C:N ratios but were negatively correlated with litter CT concentration and a positively correlated with litter turnover time (Figures 6J and 6K) – explaining similar variations as for < 53 µm fraction k_2 values. Stand-level genetic diversity also showed no significant relationship with k values for this fraction (Figure 6L.). 250-2000 µm fraction k values did not show any significant relationships with litter chemistry (Figures 6M and 6N) or litter turnover time (Figure 6O) but a marginally significant negative relationship with stand-level genetic diversity (R^2 = 0.21, p = 0.06) (Figure 6P).

Litter C:N ratios, litter CT concentration, litter turnover time, and NPP emerged as significant predictors of aggregate stability index ratios (Table S2-S4) with some significant interactions of tree density. Stability index ratios for 250-2000 µm fractions alone in high density stands were weakly positively correlated with litter C:N ratio (R^2 = 0.40, p = 0.07) (Figure 7A-C). In low density stands, stability index ratios for both < 53 µm and 53-20 µm fractions (R^2 = 0.40 and 0.68, respectively) were negatively correlated with litter CT concentration (Figure 7D-F). Among the three fractions, only stability index ratios for the 53-250 µm showed a strong positive correlation with litter turnover time (R^2 = 0.52), regardless of tree density (Figure 7G-I). Only the 250-2000 µm fraction had significant relationships with NPP, with high density stands showing a very strong positive correlation (R^2 = 0.61) (Figure 7J-L).

DISCUSSION

Our results showed the macroaggregate fraction stored a greater proportion of soil C than the microaggregate and MAOM fraction regardless of tree density, although differences in relative fraction C contributions varied among stands within the same tree density treatment.

Aggregate disintegration rates were an order of magnitude lower for < 53 μm k_2 values and the 53-250 fraction compared to < 53 k_1 values and the 250-2000 μm fraction, suggesting that 53-250 μm sized aggregates and the population of slowly decaying aggregates within the < 53 μm fraction disintegrate much more slowly. Aggregate stability index ratios decreased with increasing aggregate fraction size, demonstrating that smaller sized aggregates are ultimately more stable and persist longer than larger ones under mechanical stress. Our linear regression results showed that relative C contributions among soil fractions were strongly correlated with interactions between disturbance-mediated changes to tree density and stand level characteristics, but aggregate stability variables were strongly correlated to litter decomposition dynamics, litter chemistry, and stand-level characteristics, without any significant interactions with overlying tree density.

Plant genetic diversity and productivity are linked to soil C dynamics

Past work found positive effects of increased plant species diversity and C storage among soil fractions (Blanco-Canqui and Lal 2004, Tiemann et al. 2015, de Moraes Sá et al. 2018) and aggregate stability (Gyssels and Poesen 2003, Reich et al. 2012, Pérès et al. 2013). Our findings suggest that stand-level genetic diversity may affect the relative proportion of C stored within the macroaggregates vs. microaggregates within low density stands, based on opposing trends with these two soil C fractions. In other words, increased genetic diversity corresponds with increased C storage within microaggregates, which have longer turnover times and are more stable than macroaggregates (Six et al. 2000, 2001). Past work has shown increased microaggregate C storage under diverse perennial grass in similar soils in Wisconsin (Tiemann and Grandy 2015), but our study is the first to our knowledge that provides direct evidence that increased plant

intraspecific, genetic diversity corresponds with increased soil C occlusion within microaggregates. Our results further expand on biodiversity-ecosystem function relationships by showing increased plant genetic diversity corresponds to lower aggregate disintegration rates for the microaggregate fraction. Variations in plant diversity can increase microbial diversity and the production and chemistry of aggregate binding agents that affect soil aggregate stability, and hence, C dynamics (e.g., Wilson et al. 2009, Cai et al. 2022).

Soil C in aggregate fractions also showed variable responses to net primary productivity (NPP), which is influenced by intraspecific genetic diversity and plant competition at the stand level (Pomplun, 2022). Microaggregate C increased with increasing NPP (regardless of tree density) whereas macroaggregate C decreased with increasing NPP in low density stands. We found strong correlations between fine root biomass but not tree foliage with all three soil C fractions, supporting an important role for belowground NPP in soil C dynamics in our system. Root inputs can stimulate microbial decomposition (Fontaine et al. 2007, de Graaff et al. 2014), yet root-derived C is a dominant component of soil organic matter (Mendez-Millan et al. 2010, Schmidt et al. 2011). Within our system, fine root biomass was positively correlated with macroaggregate C in high density stands, negatively correlated with macroaggregate C in low density stands, positively correlated with microaggregate C for all stands, and negatively correlated with MAOM C in high density stands. These relationships indicate that increases in belowground productivity increase C storage within aggregates but may limit C storage within the MAOM fraction. These findings are consistent with the role of root structures and root-derived C in the binding of macro and microaggregates (Tisdall and Oades 1982, Six et al., 2002; Jastrow et al. 2007), whereas C from other sources, such as microbial necromass, are stored within the MAOM fraction (Mueller et al. 2012, See et al. 2022, Sokol et al. 2022).

Litter decomposition dynamics are connected to aggregate stability

Few studies have connected aggregate stability to litter decomposition and litter chemistry in the field, but some experiments have linked variations in plant residue chemistry to aggregate stability in the laboratory (Al-Maliki et al. 2017, Sarker et al. 2018). Our results demonstrate that variations in litter chemistry and litter turnover time are linked to aggregate disintegration kinetics. The strong negative correlation between litter CT concentration and k values for the < 53 μm and 53-250 μm fractions demonstrates how connections between chemical controls on microbial litter processing and decomposition rates might affect aggregate stability. Some studies have found litter CT concentration to inhibit litter decomposition rates (Schweitzer et al. 2004, Madritch et al. 2006) and others have found the molecular composition of soil organic matter molecular composition to include contributions from tannins (Tfaily et al. 2015), but we know of no studies to date that directly connect CTs to aggregate stability.

Based on opposing trends for aggregate k values and litter turnover times, it appears that microbial activity corresponds with lower litter CT concentration in our study, and thus increased microbial activity is linked to higher aggregate disintegration rates. CTs and other polyphenols have been shown to inhibit litter decomposition and nitrogen mineralization in forest systems (Schimel et al. 1998, Schweitzer et al. 2004, Hättenschwiler and Vitousek 2000). Alternatively, CTs could play a similar role as root exudates with respect to aggregate binding properties, which play a large role in forming stable organo-mineral complexes that promote microaggregate stability (Jastrow et al. 2007, Kögel-Knabner et al. 2008, Bradford et al. 2008). Tree density interactions on the relationships between aggregate stability index ratios and litter chemistry

suggest that competition-mediated ecological trajectories of genotype dominance among the stands also may affect soil aggregate stability dynamics.

Insights into the capacity for future C accrual in aspen systems

Past work has highlighted trembling aspen forests to have the greatest soil C accumulation potential compared to other forests, with minimum negative effects from tree harvesting (Seely et al. 2002). The relative contributions of > 53 μm vs. < 53 μm C fraction stocks to bulk soil C stocks (see Cotrufo et al. 2019) and soil mineralogy can describe the capacity of soils in our study to accrue additional C. Using relative fraction C contributions to total C and bulk soil C stocks from these sites, we found only that > 53 μm fraction C stock (combined microaggregates and macroaggregates) to have a strong positive correlation with bulk soil C stocks ($R^2 = 0.72$, $p < 0.0001$) (Figure 8). C storage capacity within this fraction is not limited by mineral exchange site saturation, and thus, it can accrue additional C through future aggregate formation. MAOM fraction C content averaged 2.18 ± 0.07% C across the 18 stands, which is well below the point of C saturation reported for forest soils in Europe (Cotrufo et al. 2019), indicating this fraction also has the potential to accrue additional C. Soil clay mineralogy in our study area is dominated by smectite (Jacobs et al. 2011), which has large cation exchange capacity and a large specific surface area, both of which enhance C stabilization (Kaiser and Guggenberger 2003, Georgiou et al. 2022). Together, this information suggests that (1) the majority of bulk soil C within these aspen stands is stored within aggregates, (2) the MAOM fraction is below the point of C saturation, and (3) soil mineralogy should favor C stabilization, all of which indicate these soils could accrue C in the future.

Although soils at our sites theoretically have the capacity to accrue additional C, interactions between tree density and root biomass may influence C stability mechanisms in our system. Our regression results showed that fine root biomass was negatively correlated with MAOM fraction C and positively correlated with macroaggregate fraction C in high density stands. Higher root biomass corresponds with increased macroaggregate formation (Tisdall and Oades 1982, Oades and Waters, 1991; Six et al. 2004) but root growth and turnover can also stimulate microbial activity and soil organic matter decomposition (Hoosbeek et al. 2004, Fontaine et al. 2007, de Graaff et al. 2014, Perveen et al. 2014), potentially limiting C accrual within the MAOM fraction by accelerating cycling of non-occluded, dead plant material. Although we did not quantify live vs. dead root biomass in our study, previous work from aspen forests in Canada found that live root biomass is proportional to tree density and basal area (DesRochers and Lieffers 2001). High density stands have significantly higher total basal area than low density stands ($p = 0.01$) and thus, likely also have higher live root biomass, which could be promoting macroaggregate formation compared to in low density stands. Root traits and morphologies are highly variable among aspen genotypes (Hajek et al. 2013, Laganière et al. 2017) and additional variation in root trait expression induced by intraspecific plant competition could further limit MAOM fraction C accumulation between high vs. low density stands.

Projected future increased climate warming, atmospheric CO_2 concentrations, and plant productivity has implications for future soil C dynamics in trembling aspen systems. Simulated elevated atmospheric CO_2 experiments in trembling aspen stands have shown to increase fine root production, soil respiration, and root-specific fungal biomass and significantly alter soil metagenomes (King et al. 2001, Janus et al. 2005, Lesaulnier et al. 2008, Lukac et al. 2009). 10 years of elevated CO_2 stimulated increased aspen root and leaf litter production and accelerated

decomposition of old C stored in the MAOM fraction, while only accruing new C within unprotected soil fractions (Hofmockel et al. 2011). Variations in growth responses and litter chemistry among aspen genotypes have also been observed under elevated CO_2 conditions (Lindroth et al. 2001) as well as increased mortality of less competitive aspen genotypes due to intraspecific competition (Kubiske et al. 2007, Zak et al. 2007). Collectively, these findings strongly indicate that future climate warming and elevated atmospheric CO_2 concentrations may alter plant productivity, genetic diversity, and soil C and microbial dynamics of natural aspen stands.

Within the context of our study, future climate change may promote increased fine root biomass and limit C accrual within the MAOM fraction under high intraspecific competition, limiting plant-derived C occlusion within mineral-bound soil fractions. While increased fine root biomass could promote aggregate formation, it could also promote decomposition of older soil C reserves faster than they can accumulate new C. Increased plant productivity may stimulate microbial activity, accelerate litter decomposition, and alter litter chemistry, all of which would decrease aggregate stability and accelerate C cycling. Continued intraspecific competition will favor the growth of fast-growing genotypes and the mortality of slow growing genotypes, potentially even in environments with low tree density due to differences in C allocation strategies. Despite favorable conditions for C accrual in our system, future climate change may limit long-term soil C stabilization and accrual within protected soil fractions. Additional soil fraction C turnover estimates, constrained by $\Delta^{14}C$ data, among our experimental aspen stands would add insight into current long-term soil C reservoirs in our system and could be used to parameterize soil-C climate feedback model projections under warming climate scenarios.

Results from our study also have direct implications for future forest management practices that promote C sequestration in the face of rapid climate change.

CONCLUSIONS

Plant genetic diversity, plant productivity, litter decomposition dynamics, and intraspecific competition among plant genotypes may have important implications for soil C dynamics and the soil C accrual in temperate forests. Here, we demonstrate these ecosystem characteristics and functions among genetically diverse trembling aspen stands have strong links and complex interactions on relative soil fraction C contributions and aggregate stability dynamics. Using a novel technique to quantify the rates at which aggregates disintegrate under constant mechanical stress, we also show that increased intraspecific genetic diversity also corresponds to lower rates of disintegration of aggregates >250 μm. Condensed tannins, a secondary plant metabolite, and litter turnover time were inversely related to aggregate disintegration rates and stability for aggregates < 250 μm. The greatest proportion of current bulk soil C stocks in our system is stored within soil aggregate fractions, which have the potential to accrue future C through continued aggregate formation. While soils within these stands are below the mineral-associated fraction C saturation point, we determined that the potential capacity to accrue additional C within this fraction might be limited in high density stands by increased in fine root biomass. Although soils in this study have the capacity to accumulate C in the future, projected climate change and increase atmospheric CO_2 may accelerate C cycling and increased intraspecific competition among aspen genotypes, limiting future C accumulation within stable soil fractions. Results from this study highlight the need to incorporate intraspecific competition-induced and variations in tree physiology into soil C-climate feedback models in

order to accurately predict how future climate change may alter forest soil C dynamics. This work also has direct implications for future forest management strategies that aim to increase soil C sequestration in systems characterized by high genetic diversity and intraspecific competition, such as many natural aspen stands found throughout North America.

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FIGURES AND TABLES

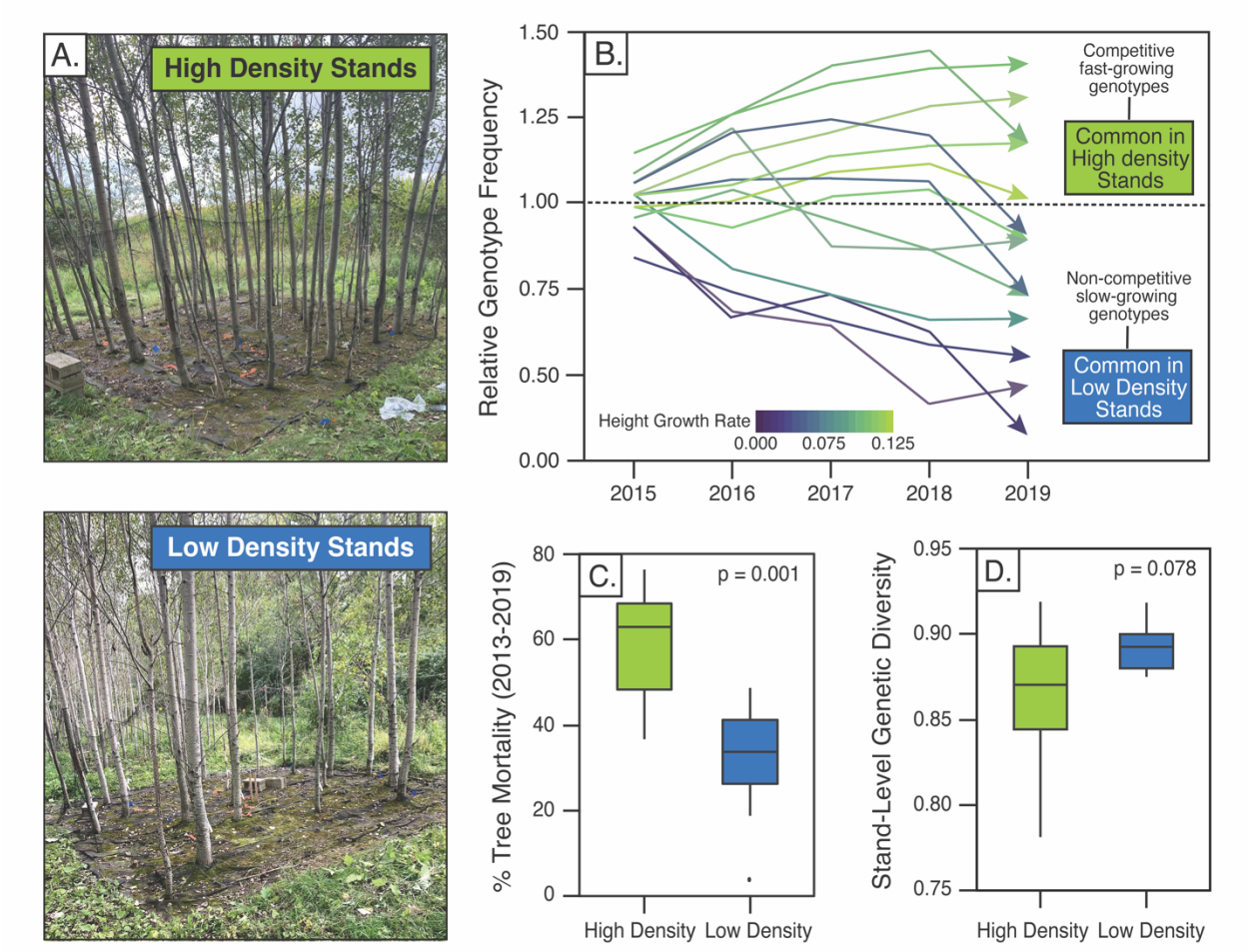

Figure 1: A.) Images showing differences in tree density between high vs. low density stands, B.) changes in relative growth rates and frequency aspen genotypes over time since thinning treatment (adapted from Cope et al. (2021), PNAS.2103162118); relative genotype frequency values below 1 indicate higher frequency in disturbed stands, C.) percent tree mortality over a 6-year period between disturbance histories, and D.) stand-level genetic diversity (simpson's diversity index) between high vs. low density stands, P-values in upper right corners of panels B and C correspond to results from one-way ANOVA between disturbance histories.

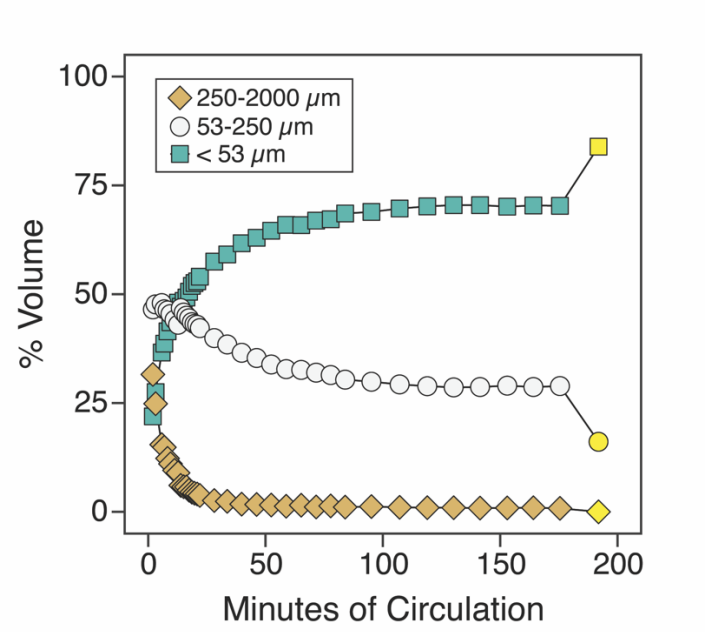

Figure 2: Examples of aggregate stability dynamics over time for the three fractions examined in this research, using the laser diffraction procedure. Fully dispersed measurements are shown with the same symbol for each fraction but in gold. Aggregate data shown here are from plot BL17.

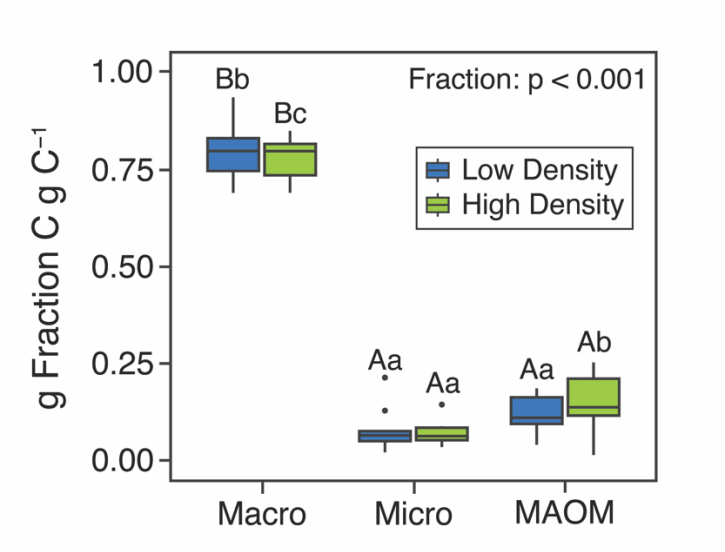

Figure 3: Boxplots showing medians and distributions among fraction C contributions to total C recovered from wet sieving. Uppercase letters indicate significant differences between fractions among all 18 stands and lowercase letters indicate significant differences between fractions within tree density treatments, based on results from one-way ANOVA ($p < 0.05$).

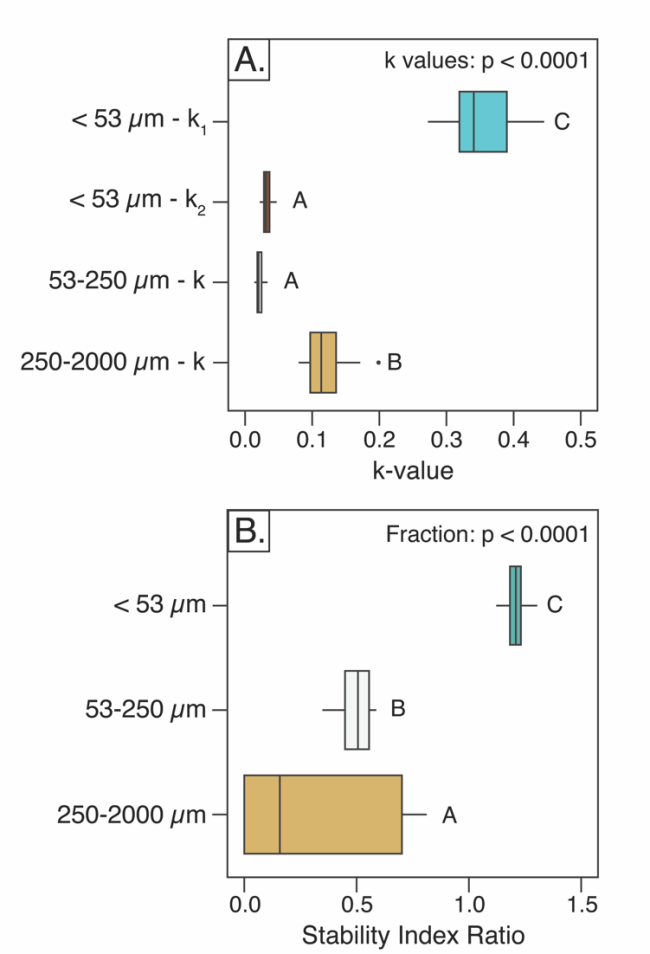

Figure 4: Boxplots showing medians and distributions for three aggregate fractions of A.) k-value estimates for aggregate disintegration kinetics and B.) stability index ratios. Note the < 53 µm fraction includes two k value estimates that correspond to aggregate populations that disintegrate rapidly and more slowly over time, whereas the 53-250 µm and 250-2000 µm fractions each only have one k value. Uppercase letters indicate significant differences between k-value estimates and size fractions, based on results from one-way ANOVA ($p \leq 0.05$); p-values are shown in the upper right corner of each panel. Note differences in the x-axis between panels.

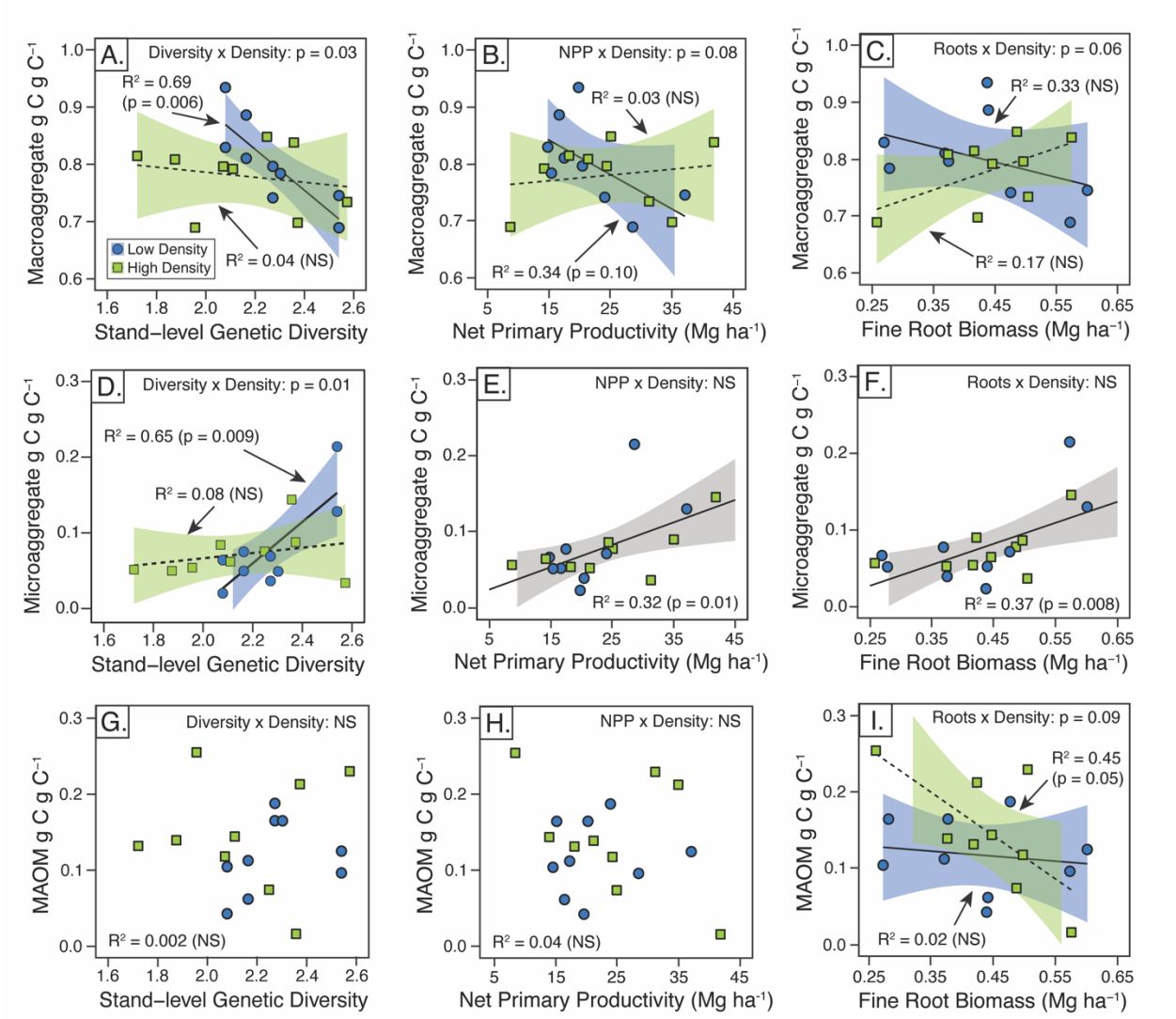

Figure 5: Linear regressions showing relative soil C fraction contributions to total C recovered from wet sieving for macroaggregates and A.) stand-level genetic diversity (Shannon index), B.) net primary productivity (NPP), C.) fine root biomass, microaggregates and D.) stand-level genetic diversity, E.) NPP, F.) fine root biomass, and mineral associated organic matter (MAOM) and G.) stand-level genetic diversity, H.) NPP, I.) fine root biomass. For each column, panels read from top to bottom: macroaggregates, microaggregates, and MAOM. Panels A, B, C, D, and I display regressions for low vs. high density stands separately based on significant and marginally significant interactions between stand-level characteristics and tree density, whereas panels E, F, G, and H show regression results using data from all 18 stands (no significant interaction with tree density). Significant interactions between variables and tree density p-values are shown in the upper right corner (NS = not significant). Panels without regression lines or ribbons (95% confidence intervals) show no significant relationships. Note y-axis scales differ between panels corresponding to macroaggregates compared to microaggregates and MAOM and x-axis scales are the same within each column.

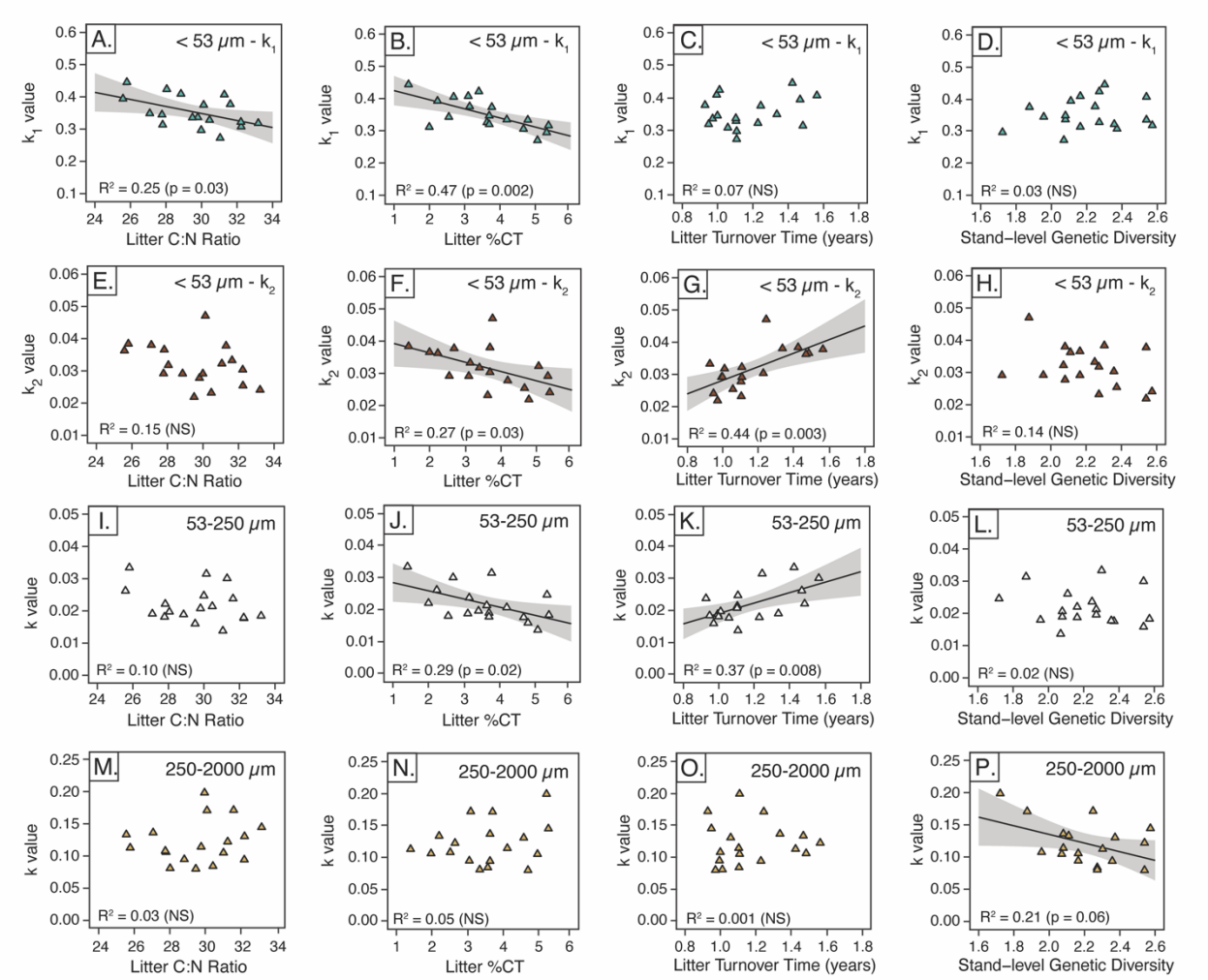

Figure 6: Best-fit linear regression models for predicting aggregate decay rates from stand-level characteristics, including < 53 μm k_1 values and A.) litter carbon-to-nitrogen (C:N) ratios, B.) litter condensed tannin (CT) concentration, C.) litter turnover time, D.) stand-level genetic diversity (shannon index), < 53 μm k_2 values and E.) litter C:N ratios, F.) litter CT concentration, G.) litter turnover time, H.) stand-level genetic diversity, 53-250 μm k values and I.) litter C:N ratio, J.) litter CT concentration, K.) litter turnover time, L.) stand-level genetic diversity, and 250-2000 μm k values and M.) litter C:N ratios, N.) litter CT concentration, O.) litter turnover time, and P.) stand-level genetic diversity. For each column, panels read from top to bottom: k_1 values for the < 53 μm fraction, k_2 values for the < 53 μm fraction, k values for the 53-250 μm fraction, and k values for the 250-2000 μm fraction. Panels without regression lines or ribbons (95% confidence intervals) show no significant relationships. Note y-axis scales differ for panels between decay rates and aggregate size fractions, and x-axis scales are the same within each column.

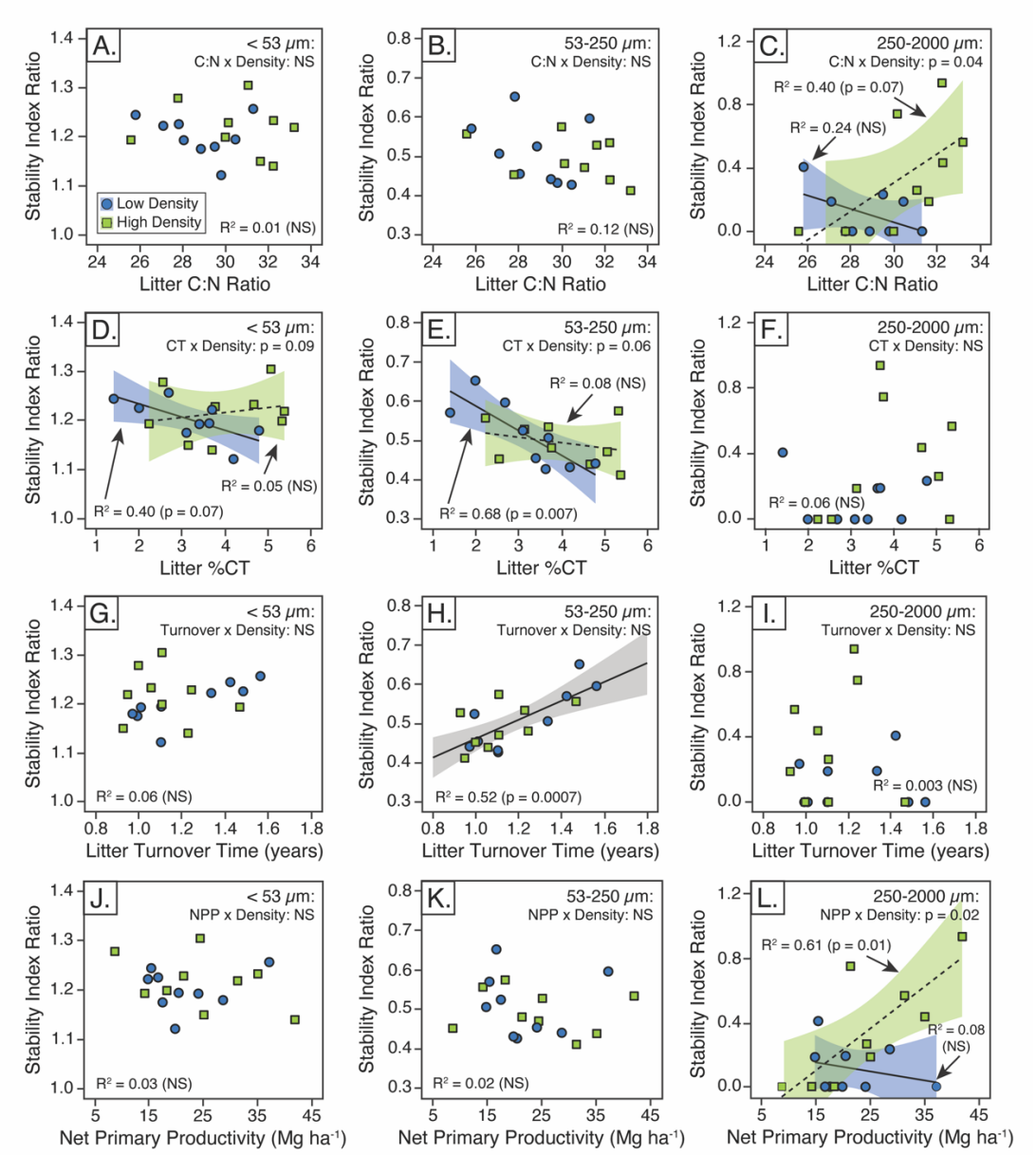

Figure 7: Best-fit linear regression models for predicting aggregate stability index ratios from stand-level characteristics, including A-C) litter carbon-to-nitrogen (C:N) ratios, D-F) litter condensed tannin (CT) concentration, G-I.) litter turnover time, and J-L.) annual net primary productivity (NPP) estimates. For each row, panels read from left to right: the < 53 µm fraction, the 53-250 µm fraction, and the 250-2000 µm fraction. Panels C, D, E, and L display regressions for low vs. high density stands separately based on significant and marginally significant interactions between stand-level characteristics and tree density, whereas panel A, B, F, G, H, I, J, and K, show linear regression results using data from all 18 stands (no significant interaction with tree density). Significant interactions between variables and tree density p-values are shown in the upper right corner (NS = not significant). Panels without regression lines or ribbons (95% confidence intervals) show no significant relationships. Note y-axis scales differ for panels of aggregate size fractions, and x-axis scales are the same within each row.

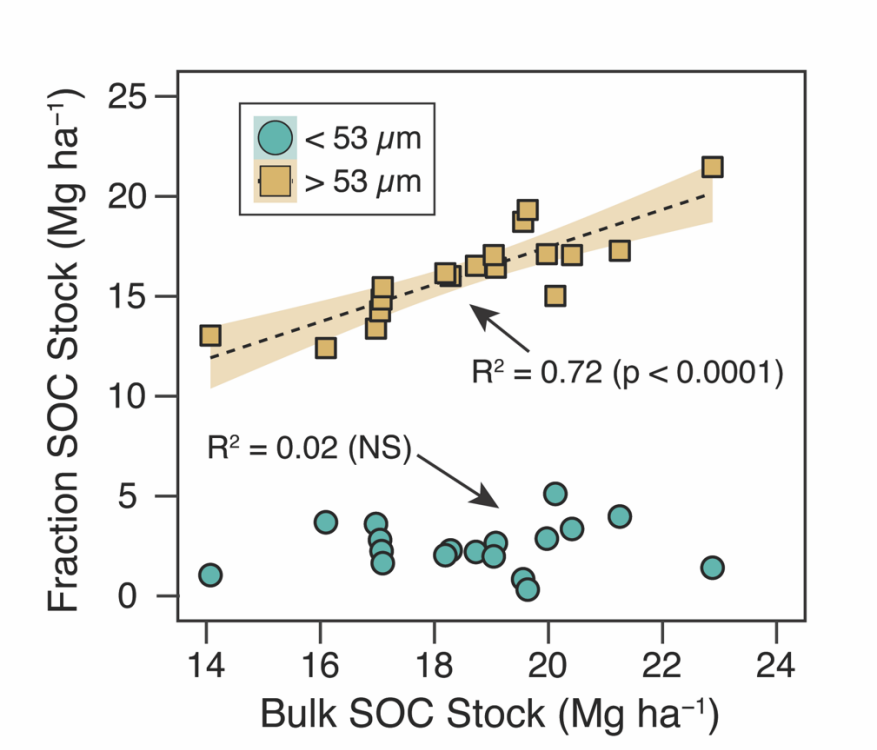

Figure 8: Comparison of linear regressions for > 53 µm (combined microaggregates and macroaggregates) and < 53 µm (mineral-associated organic matter) soil organic C (SOC) stocks vs. bulk SOC stocks. NS = not significant.

Stability Parameter	Aggregate Fraction	Tree Density	Mean ± SE	Tree Density Effect
k values	< 53 µm – k_1	Low	0.37 ± 0.02	**$F_{1,16}$ = 3.13**
		High	0.33 ± 0.01	**P = 0.10**
	< 53 µm – k_2	Low	0.03 ± 0.002	$F_{1,16}$ = 0.008
		High	0.03 ± 0.002	P = 0.93
	53-250 µm – k	Low	0.02 ± 0.002	$F_{1,16}$ = 0.16
		High	0.02 ± 0.002	P = 0.70
	250-2000 µm – k	Low	0.10 ± 0.01	**$F_{1,16}$ = 7.21**
		High	0.14 ± 0.01	**P = 0.02**
Stability Index Ratio	< 53 µm	Low	1.20 ± 0.01	$F_{1,16}$ = 0.43
		High	1.22 ± 0.02	P = 0.52
	53-250 µm	Low	0.51 ± 0.03	$F_{1,16}$ = 0.27
		High	0.50 ± 0.02	P = 0.61
	250-2000 µm	Low	0.11 ± 0.05	**$F_{1,16}$ = 3.51**
		High	0.35 ± 0.12	**P = 0.08**

Table 1: Summary statistics and one-way ANOVA results testing significant effects of tree density on aggregate stability parameters and stability index ratios for three aggregate fractions. Note only the < 53 µm fraction models include two decay rates (k-values) representing two populations of aggregates, whereas the 53-250 µm and 250-2000 µm fractions only have one decay rate. Significant and marginally significant effects of tree density are bolded in the final column.

Aggregate Size Fraction	g C Fraction g C^{-1}	Stability Parameter	Tree Density	Intercept	Slope	Res. Std. Error	R2	p-value	Tree Density Effect
< 53 µm	MAOM (< 53 µm)	k_1 value	**Low**	**0.30**	**0.65**	**0.04**	**0.51**	**0.07**	**$F_{1,14}$ = 3.44 P = 0.08**
			High	0.34	-0.03	0.04	0.004	0.87	
			All	0.34	0.08	0.05	0.01	0.67	
		k2 value	Low	0.03	0.007	0.007	0.002	0.90	$F_{1,14}$ = 0.36 P = 0.56
			High	0.04	-0.03	0.007	0.11	0.39	
			All	0.03	-0.02	0.007	0.03	0.50	
		Stability Index Ratio	Low	0.16	0.35	0.04	0.16	0.28	$F_{1,14}$ = 0.07 P = 0.80
			High	**1.15**	**0.44**	**0.05**	**0.39**	**0.07**	
			All	**1.15**	**0.42**	**0.04**	**0.32**	**0.01**	
53-250 µm	Micro-aggregates (53-250 µm)	k value	Low	0.02	-0.02	0.006	0.05	0.58	$F_{1,14}$ = 0.43 P = 0.52
			High	0.03	-0.07	0.005	0.15	0.30	
			All	0.02	-0.03	0.006	0.07	0.30	
		Stability Index Ratio	Low	0.52	-0.08	0.09	0.004	0.88	$F_{1,14}$ = 0.34 P = 0.57
			High	0.46	0.45	0.06	0.07	0.50	
			All	0.50	0.05	0.07	0.001	0.89	
250-2000 µm	Macro-aggregates (250-2000 µm)	k value	Low	0.008	0.12	0.02	0.20	0.23	$F_{1,14}$ = 0.11 P = 0.75
			High	-0.009	0.19	0.04	0.10	0.41	
			All	0.05	0.09	0.03	0.04	0.46	
		Stability Index Ratio	Low	0.63	-0.65	0.15	0.11	0.39	$F_{1,14}$ = 0.71 P = 0.41
			High	-0.54	1.14	0.36	0.04	0.62	
			All	0.46	-0.29	0.29	0.004	0.79	

Table 2: Simple linear regression and tree density interactions results for predicting aggregate stability parameters from soil C fraction contribution to total C recovered from wet sieving (g C Fraction g C^{-1}). Significant and marginally significant model results are shown in bold.

SUPPLEMENTARY FIGURES AND TABLES

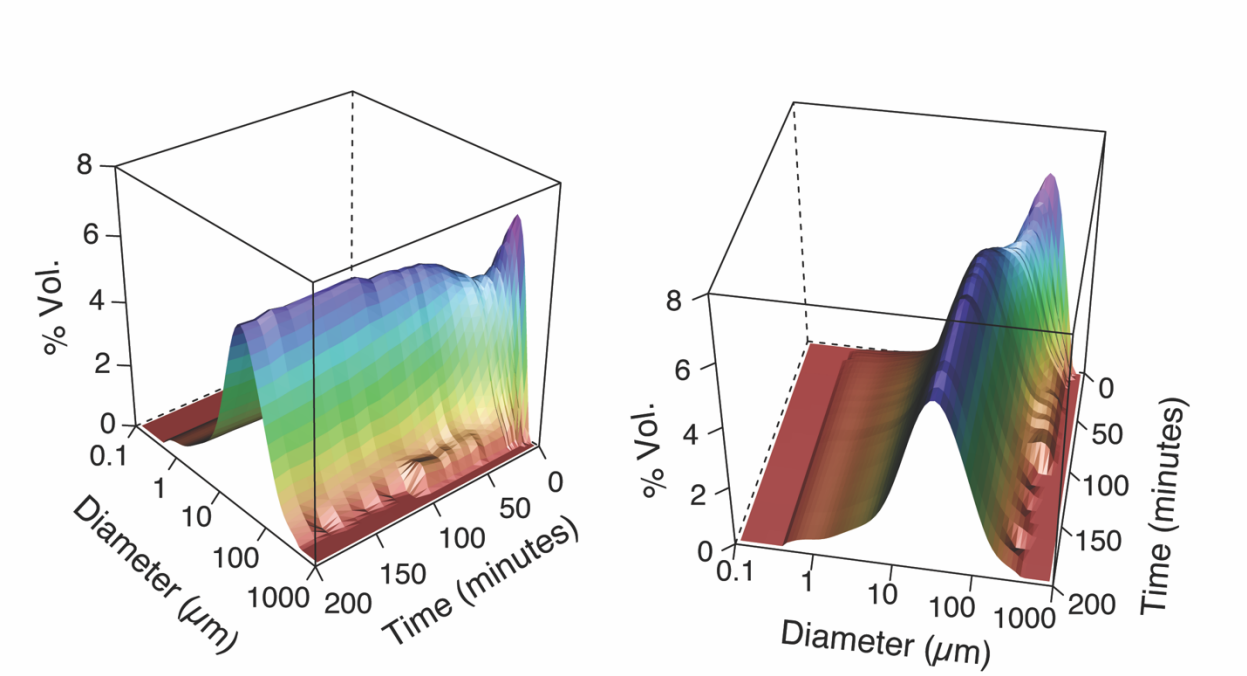

Figure S1: Two views of the same continuous surface plot showing the percent volume for each size bin within the full particle size distributions over time, using the laser diffraction method to monitor aggregate disintegration. Height of surface represents relative abundance of each particle size class as it changes over time from 0 to > 180 minutes of circulation. Data are shown here from plot BL04.

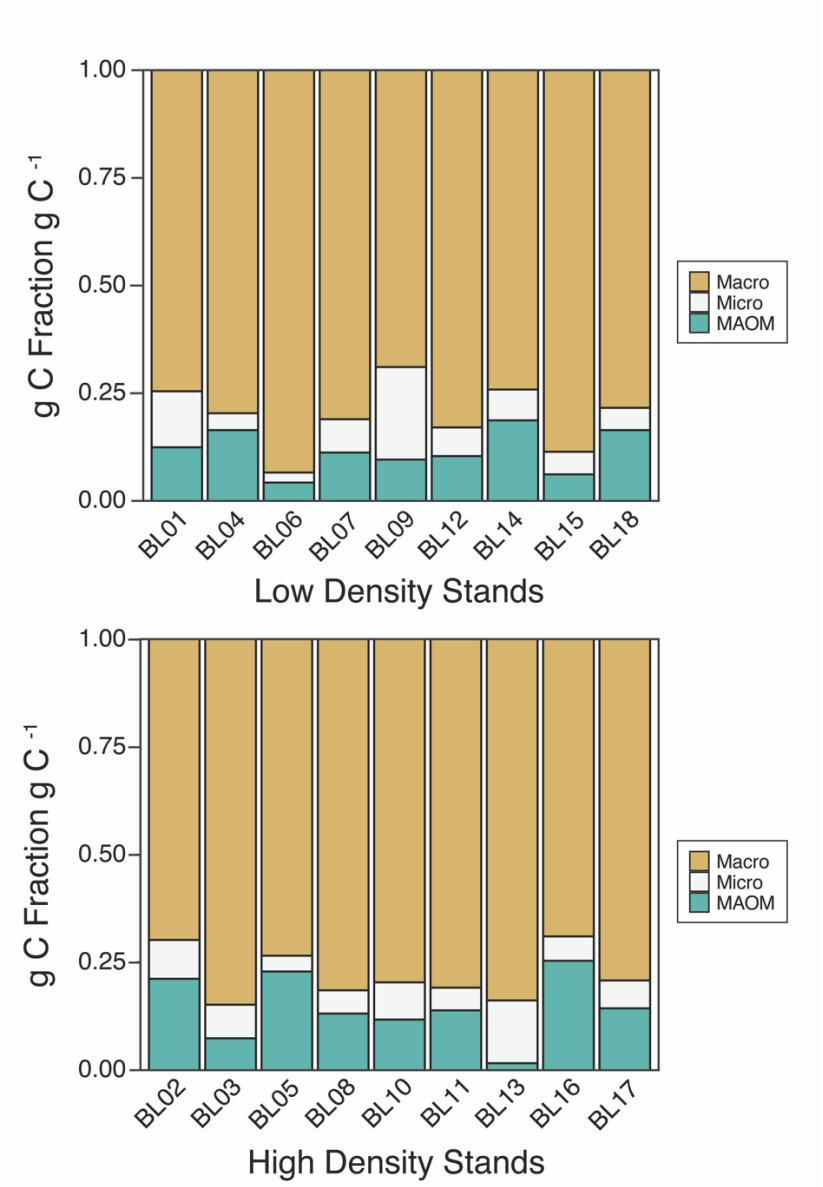

Figure S2: Fraction contributions to total carbon recovered from fractionation via wet sieving for within individual plots for high and low density and high density stands.

Soil C Fraction	Stand-level Characteristic	Intercept	Slope	Res. Std. Error	R^2	p-value	Density Interaction
MAOM (< 53 µm)	Shannon Diversity	0.11	0.01	0.07	0.002	0.87	0.64
	Simpson's Diversity	0.14	-0.01	0.07	1.90e-05	0.99	0.56
	NPP (Mg ha^{-1})	0.17	-0.001	0.06	0.04	0.42	0.34
	Fine Root Biomass (Mg ha^{-1})	0.24	-0.24	0.06	0.15	0.12	0.09
	Foliage (Mg ha^{-1})	0.19	-0.02	0.06	0.05	0.38	0.96
	Leaf Area Index	0.18	-0.02	0.06	0.04	0.41	0.96
	Litter Turnover Time (years)	0.23	-0.08	0.06	0.06	0.32	0.49
	Litter C:N Ratio	0.18	-0.002	0.06	0.004	0.81	0.96
	Litter %CT	0.12	0.003	0.06	0.002	0.85	0.59
Micro-aggregates (53-250 µm)	Shannon Diversity	-0.14	0.10	0.04	0.25	0.03	0.01
	Simpson's Diversity	-0.39	0.54	0.04	0.15	0.11	0.01
	NPP (Mg ha^{-1})	0.01	0.003	0.04	0.32	0.01	0.16
	Fine Root Biomass (Mg ha^{-1})	-0.04	0.27	0.04	0.37	0.008	0.49
	Foliage (Mg ha^{-1})	0.004	0.03	0.04	0.17	0.09	0.22
	Leaf Area Index	0.009	0.02	0.04	0.16	0.10	0.26
	Litter Turnover Time (years)	0.09	-0.008	0.04	0.001	0.89	0.55
	Litter C:N Ratio	-0.05	0.004	0.05	0.04	0.41	0.62
	Litter %CT	0.06	0.006	0.05	0.02	0.56	0.27
Macro-aggregates (250-2000 µm)	Shannon Diversity	1.04	-0.11	0.06	0.15	0.11	0.03
	Simpson's Diversity	1.24	-0.53	0.07	0.07	0.29	0.02
	NPP (Mg ha^{-1})	0.83	-0.002	0.07	0.04	0.42	0.08
	Fine Root Biomass (Mg ha^{-1})	0.81	-0.04	0.07	0.003	0.83	0.06
	Foliage (Mg ha^{-1})	0.81	-0.01	0.07	0.0006	0.77	0.47
	Leaf Area Index	0.81	-0.01	0.07	0.0006	0.76	0.45
	Litter Turnover Time (years)	0.69	0.09	0.07	0.07	0.29	0.82
	Litter C:N Ratio	0.87	-0.01	0.07	0.007	0.74	0.71
	Litter %CT	0.82	-0.01	0.07	0.02	0.56	0.82

Table S1: Simple linear regression results and density interactions for predicting soil C fraction contribution to total C recovered from wet sieving (g C Fraction g C^{-1}) from stand-level characteristics and litter decomposition dynamics. NPP = net primary productivity

Aggregate Stability parameter	Stand-level Characteristic	Intercept	Slope	Res. Std. Error	R^2	p-value	Density Interaction
k_1 value	Shannon Diversity	0.28	0.03	0.05	0.03	0.51	0.53
	Simpson's Diversity	0.007	0.39	0.05	0.08	0.27	0.52
	NPP (Mg ha^{-1})	0.38	-0.001	0.05	0.04	0.45	0.42
	Fine Root Biomass (Mg ha^{-1})	0.38	-0.001	0.05	0.04	0.45	0.80
	Foliage (Mg ha^{-1})	0.39	-0.09	0.05	0.03	0.47	0.72
	Leaf Area Index	0.40	-0.02	0.05	0.05	0.37	0.73
	Litter Turnover Time (years)	0.40	-0.01	0.05	0.07	0.31	0.57
	Litter C:N Ratio	0.68	-0.01	0.04	0.25	0.03	0.99
	Litter %CT	0.045	-0.03	0.04	0.47	0.002	0.72
k_2 value	Shannon Diversity	0.06	-0.01	0.006	0.14	0.13	0.68
	Simpson's Diversity	0.07	-0.05	0.007	0.06	0.32	0.97
	NPP (Mg ha^{-1})	0.04	-0.0002	0.006	0.06	0.34	0.85
	Fine Root Biomass (Mg ha^{-1})	0.04	-0.02	0.007	0.06	0.33	0.95
	Foliage (Mg ha^{-1})	0.04	-0.005	0.006	0.20	0.06	0.55
	Leaf Area Index	0.04	-0.003	0.006	0.21	0.06	0.54
	Litter Turnover Time (years)	0.007	0.02	0.005	0.44	0.003	0.91
	Litter C:N Ratio	0.07	-0.001	0.006	0.15	0.11	0.62
	Litter %CT	0.04	-0.003	0.006	0.27	0.03	0.40
Stability Index Ratio	Shannon Diversity	1.25	-0.02	0.05	0.01	0.70	0.24
	Simpson's Diversity	1.33	-0.14	0.05	0.01	0.70	0.26
	NPP (Mg ha^{-1})	1.23	-0.001	0.05	0.03	0.48	0.29
	Fine Root Biomass (Mg ha^{-1})	1.26	-0.12	0.05	0.07	0.29	0.24
	Foliage (Mg ha^{-1})	1.27	-0.03	0.05	0.13	0.14	0.55
	Leaf Area Index	1.27	-0.02	0.05	0.11	0.19	0.55
	Litter Turnover Time (years)	1.14	0.06	0.05	0.06	0.31	0.12
	Litter C:N Ratio	1.28	-0.003	0.05	0.01	0.64	0.84
	Litter %CT	1.22	-0.003	0.05	0.007	0.75	0.09

Table S2: Linear regressions for predicting < 53 μm fraction aggregate stability parameters from stand-level characteristics and litter decomposition dynamics and significant effects of tree density. NPP = net primary productivity.

Aggregate Stability parameter	Stand-level Characteristic	Intercept	Slope	Res. Std. Error	R^2	p-value	Density Interaction
k value	Shannon Diversity	0.03	-0.004	0.006	0.02	0.57	0.19
	Simpson's Diversity	0.04	-0.02	0.006	0.02	0.61	0.20
	NPP (Mg ha^{-1})	0.02	-0.0001	0.006	0.03	0.50	0.41
	Fine Root Biomass (Mg ha^{-1})	0.03	-0.01	0.0006	0.03	0.46	0.74
	Foliage (Mg ha^{-1})	0.02	-0.002	0.006	0.07	0.30	0.79
	Leaf Area Index	0.03	-0.002	0.006	0.06	0.33	0.66
	Litter Turnover Time (years)	0.003	0.02	0.005	0.37	0.008	0.75
	Litter C:N Ratio	0.005	-0.0008	0.005	0.10	0.21	0.83
	Litter %CT	0.03	-0.003	0.005	0.29	0.02	0.23
Stability Index Ratio	Shannon Diversity	0.61	-0.05	0.07	0.03	0.51	0.49
	Simpson's Diversity	0.84	-0.38	0.07	0.03	0.46	0.59
	NPP (Mg ha^{-1})	0.53	-0.0009	0.07	0.02	0.63	0.89
	Fine Root Biomass (Mg ha^{-1})	0.49	0.03	0.07	0.002	0.86	0.71
	Foliage (Mg ha^{-1})	0.55	-0.02	0.07	0.03	0.47	0.84
	Leaf Area Index	0.55	-0.02	0.07	0.03	0.47	0.82
	Litter Turnover Time (years)	0.22	0.24	0.05	0.52	0.0007	0.38
	Litter C:N Ratio	0.82	-0.01	0.07	0.12	0.15	0.84
	Litter %CT	0.62	-0.03	0.06	0.33	0.01	0.06

Table S3: Linear regressions for predicting 53-250 μm fraction aggregate stability parameters from stand-level characteristics and litter decomposition dynamics and significant effects of tree density. NPP = net primary productivity.

Aggregate Stability parameter	Stand-level Characteristic	Intercept	Slope	Res. Std. Error	R^2	p-value	Density Interaction
k value	Shannon Diversity	0.27	-0.07	0.03	0.21	0.06	0.81
	Simpson's Diversity	0.64	-0.59	0.03	0.34	0.01	0.84
	NPP (Mg ha^{-1})	0.13	-0.0003	0.03	0.008	0.73	0.82
	Fine Root Biomass (Mg ha^{-1})	0.13	-0.04	0.03	0.02	0.62	1.00
	Foliage (Mg ha^{-1})	0.11	0.04	0.03	0.006	0.76	0.77
	Leaf Area Index	0.11	0.003	0.03	0.007	0.75	0.80
	Litter Turnover Time (years)	0.11	0.006	0.04	0.001	0.89	0.25
	Litter C:N Ratio	0.04	0.003	0.03	0.03	0.48	0.72
	Litter %CT	0.10	0.007	0.04	0.05	0.36	0.35
Stability Index Ratio	Shannon Diversity	-0.40	0.29	0.29	0.05	0.36	0.51
	Simpson's Diversity	-1.12	1.54	0.29	0.03	0.78	0.81
	NPP (Mg ha^{-1})	-0.17	0.02	0.25	0.29	0.02	0.02
	Fine Root Biomass (Mg ha^{-1})	0.04	0.45	0.29	0.03	0.53	0.07
	Foliage (Mg ha^{-1})	0.07	0.07	0.29	0.02	0.56	0.12
	Leaf Area Index	0.07	0.05	0.29	0.02	0.56	0.16
	Litter Turnover Time (years)	0.32	-0.07	0.29	0.003	0.83	0.93
	Litter C:N Ratio	-1.54	0.06	0.26	0.22	0.05	0.04
	Litter %CT	0.009	0.06	0.29	0.06	0.32	0.47

Table S4: Linear regressions for predicting 250-2000 μm fraction aggregate stability parameters from stand-level characteristics and litter decomposition dynamics and significant effects of tree density. NPP = net primary productivity.

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CHAPTER 3:

Successional trajectory shapes litter decomposition and microbial dynamics in post-agricultural urban forests in St. Croix, U.S. Virgin Islands

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ABSTRACT

Dry tropical and subtropical forests have undergone significant fragmentation worldwide. Differences in post-agricultural land use trajectories and the timing of secondary forest succession have altered tropical forest community composition, with important consequences on ecosystem functions mediated by microorganisms, such as biogeochemical cycling. Our research tests the effects of forest composition and successional age, litter chemistry, and decomposition environment (*in situ* vs. transplant experiments) on leaf and root litter decay rates in a 688-day experiment in subtropical dry forests in St. Croix, U.S. Virgin Islands. Early successional 10-year-old forests were dominated by a nitrogen fixing tree species whereas older 40-year-old forests consisted of multiple tree species. We monitored changes in leaf litter chemistry, microbial community biomass, and the potential activity of a suite of hydrolytic extracellular enzymes over time. Our results showed that leaf litter derived from 10-year-old forests decomposed faster than litter derived from 40-year-old forests, regardless of decomposition environment, likely due to differences in initial litter C:N ratios between successional forests. Diffuse-reflectance infrared Fourier-transformed (DRIFT) spectral data suggested that both decomposition environment and forest age could affect the chemical trajectory of litter during decomposition, particularly for mixed species litter. Despite similar initial C:N ratios, roots from 10-year-old forests decomposed more quickly, regardless of decomposition environment. Time alone significantly affected forest floor and soil microbial community biomass and soil enzyme activities, whereas forest age or forest age x time interactions affected forest floor enzyme activities. Together, our results indicate that successional age and forest composition influence litter decomposition dynamics in subtropical dry forests, mediated by initial litter C:N ratios, litter mixture diversity, and decomposition environment.

INTRODUCTION

Decomposition of plant detritus in soil is an important step in nutrient cycling and soil organic matter formation in terrestrial ecosystems. At latitudinal to global scales, climate is the strongest predictor of litter decomposition rates, followed by litter chemistry and other plant traits (Ayres et al. 2009, Bradford et al. 2016, Cornwell et al. 2008, Gholz et al. 2000, Meentemeyer 1978, Ostertag et al. 2022, Vitousek et al. 1994, Waring 2013). At local to regional scales, decomposer community, soil nutrient availability and other environmental factors become more important in explaining variability in litter decomposition rates (Bradford et al. 2017, Gartner & Cardon 2004, Gonzalez and Seastedt 2022, Powers et al. 2009, Vivanco and Austin 2008). Changes in plant composition and soil properties due to land-use legacies hence are expected to affect litter decay and other ecosystem processes.

Seasonally dry forests globally have an extensive history of deforestation and remain some of the most perturbed and least conserved forests today (Miles et al. 2006, Quesada et al. 2009). In many areas, intensive agricultural land use has been replaced by urbanization and expansion of residential and commercial development. Today, many lowland and coastal areas in the tropics contain a mosaic of successional or secondary forest patches interspersed with urban and suburban land uses (Atkinson and Marín-Spiotta 2015, Coomes et al. 2016). Tropical urban secondary forests may show effects of historical land use and influences of surrounding human activities on ecosystem processes, such as productivity and nutrient cycling (Borges et al. 2021, Cusack & McCleery 2014).

Few studies have examined litter decomposition in subtropical, seasonally dry forests with well-documented long-term histories of human alteration. At regional scales, litter decomposition rates are most regulated by climate and litter chemistry and less so by microbial

community composition or biomass, particularly in tropical dry forests (Cornwell et al. 2008, Gonzalez and Seastedt 2022, Meentemeyer 1978). In a large pantropical study, Powers et al. (2009) confirmed the importance of abiotic controls, showing that litter decay rates decreased linearly with annual precipitation, with >95% mass loss occurring within one year at most sites. Other evidence from tropical systems strongly suggests that biotic controls may be more important in humid tropical systems than in dry forests (e.g., Gonzalez and Seastedt 2022). Other research has shown differences between above vs. belowground litter decomposition dynamics. For example, Gholz et al. (2000) showed that leaf litter decomposed faster than roots in wet forests and roots decomposed faster than leaves in dry forests.

Litter chemistry influences decomposition rates more than climate at the ecosystem scale. Litter with low C:N and/or lignin:N ratios is generally more easily decomposed by microbes, but high concentrations of non-structural C, high concentrations of phenolics, and low concentrations of polyphenols have also been linked to high litter decomposition rates (Paudel et al. 2015, Ostertag et al. 2008). It is thought that microbes are most efficient at decomposing litter from their native communities, regardless of litter chemistry – a hypothesis referred to as "home-field advantage" (Ayres et al. 2009, Gholz et al. 2000). However, Strickland et al. (2009) showed that relationships between microbial communities and litter chemistry may not exclusively explain litter decomposition rates. In their study, forest communities decomposed grass and forest litter similarly, whereas grassland communities were most efficient at decomposing grasses only. Based on these results, it appears that litter chemistry, specifically chemical complexity, may exert a stronger control on litter decomposition than home-field advantage alone (see Freschet et al. 2012 and Gießelmann et al. 2011)

Land use history can also affect litter decomposition in tropical forests by altering successional legacies and patterns in plant and microbial composition (Lohbeck et al. 2015, Ostertag et al. 2008, Paudel et al. 2015, Schilling et al. 2016, Xuluc-Tolosa 2003). For example, emerging post-agricultural forests can hold novel species assemblages that are strongly influenced by the past land use type, as well as surrounding landscapes (Hobbs et al. 2014, Lugo and Helmer 2004, Mascaro et al. 2012). Shifts in plant species or functional groups can subsequently change the chemistry and quantity of plant-derived inputs to the soil. For example, nitrogen (N)-fixing tree species, with lower litter C:N ratios, which require less energy for microorganisms to decompose, are especially abundant in successional dry tropical forests (Atkinson and Marín-Spiotta 2015, Gei et al. 2018). Land use legacy and forest successional trajectory also influence soil physical and chemical properties (Batterman et al. 2013, Bautista-Cruz and del Castillo 2005), the spatial distribution of soil resources (Ashagrie et al. 2005), and soil microbial community composition (Díaz-Vallejo et al. 2021, Smith et al. 2014).

In this study, we measured leaf and root litter decomposition in subtropical dry forests in St. Croix to determine influences of forest composition, forest age, and decomposition environment on litter decomposition dynamics. We performed a 688-day *in-situ* and reciprocal litter transplant field decomposition experiment in two types of successional forests: (1) 10-year-old mono-species forests dominated by early successional N-fixing tree species and (2) 40-year-old currently unmanaged mixed-species forests that were former experimental timber plantations (Atkinson and Marín-Spiotta 2015). Both successional forests emerged on landscapes with a history of almost 200 years of intensive sugarcane agriculture. We hypothesized that differences in litter chemistry (C:N ratio) between the 10-year- and 40-year-old secondary forests, driven by compositional differences in tree communities, would explain decomposition rates. We also

expected that native litter would decompose faster in its site origin compared to in the opposite forest site. We used two methods, diffuse reflectance infrared Fourier transform spectroscopy and nuclear magnetic resonance spectroscopy, to examine how litter C functional groups and the molecular composition would vary during the decomposition process, both when litter decomposed *in-situ* and in the opposite forest type. Lastly, we monitored changes in microbial community composition and microbial function as an attempt to infer how microbial communities potentially regulate leaf vs. root litter decomposition in these forests.

MATERIALS AND METHODS

Study Sites

Our field sites were located in the subtropical dry forest life zone (Ewel and Whitmore 1973) in St. Croix, U.S. Virgin Islands in the Caribbean (17°44'N, 64°43'W). Mean annual temperature at these sites is 26.6 °C and mean annual precipitation is 1,052 mm (1970-2014) (NOAA NCDC), with a wet season from September to November and a dry season from January through April (Figure 1). All sites were located between 10-120 m above sea level on Mollisols (Typic Haplustolls), consisting of shallow, well-drained, slowly permeable soils formed in material weathered from soft limestone bedrock.

Island-wide decline and subsequent abandonment of sugarcane production in St. Croix as recently as the middle of the 20th century has resulted in forests at different stages of succession (Daley 2010). Our study included three replicate field sites of two successional forest types, 10-year- and 40-year-old forests that emerged after different land-use trajectories post-sugarcane cultivation. The 10-year-old secondary forests experienced about 30 years of grazed pasture use following sugarcane before recent forest regeneration. At the time of sampling, these forests

were almost entirely dominated by small stems (< 10 cm DBH) of an early successional N-fixing tree species*, Leucaena leucocephala* (Atkinson and Marin-Spiotta 2015). The 40-year-old secondary forests experienced low intensity pasture use after sugarcane, followed by establishment of experimental timber plantations. Two of the plantations were part of the U.S. Department of Agriculture Estate Thomas Experimental Forest, where planting of mahogany (*Swietenia mahagoni*) and teak (*Tectona grandis*) occurred during 1954–1972 (Weaver 2006). Harvesting began in 1963 and active management ceased after Hurricane Hugo in 1989 (Weaver 2006). The third plantation site was planted with mastic (*Sideroxylon foetidissimum*) on privately owned property adjacent to the University of the Virgin Islands. While management practices at the three plantation sites differed, planting, harvesting, and abandonment of active management occurred at comparable times and the sites have since been overtaken by forest successional processes. At the time of sampling, the former plantations harbored diverse communities including native dry forest species *Bourreria succulenta, Trifasia trifolia*, *Myris elemifera, Krugiodendron ferreum, and Randia aculeate*, although species composition remained distinct from nearby successional forests of the same age with a common history of pasture use following sugarcane but that had not been planted (Atkinson and Marín-Spiotta 2015). Soil characteristics differed between the two forest ages (Table 1), with 40-year-old secondary forests having greater soil pH values, soil organic carbon (SOC) content, and nitrate content, and soils under 10-year-old secondary forests having greater ammonium content, phosphorus, and calcium content.

Litter collection and decomposition experiments

We collected aboveground litterfall from two of the three replicate field sites per successional forest type biweekly for 12 months beginning in August 2011 for the field decomposition study. We collected litter from five litter baskets per site, randomly stratified within each fifth of a 491-m^2 circular plot. Litter collectors were made of rectangular plastic laundry baskets with inner dimensions of 22 cm × 16.5 cm, lined with 1-mm fiberglass mesh window screening suspended from the rim of the basket, to avoid pooling of water on the mesh. Collector bases were perforated for drainage. Any large woody stems and branches that fell into or across the litter baskets were discarded. We did not separate leaves from other fine debris, fruits, or flowers, hence aboveground material collected from the baskets is a composite of litter. Live fine roots (< 1 cm diameter) were handpicked and washed from soil collected from the top 10 cm at each site. All plant material was oven dried to a constant weight at 50°C and weighed.

The litter and root decomposition field experiments began in August 2012. Dried litter was homogenized by site, and 5 g litter was placed in bags (10 x 10 mm) made of 1 mm fiberglass mesh window screening. Roots (0.5 g) were placed in mesh bags (10 x 10 mm) lined with 0.3 mm nylon bridal tulle to prevent the loss of material.

Replicate litterbags of each type (5 for litter and 4 for roots) per collection time point were sewn together with nylon thread and closed with plastic zip ties with an attached aluminum identification tag. In the field, the lines of litterbags were placed radiating from a randomly chosen point marked by a PVC pipe. A third replicate field site for the 40-year-old and the 10-year-old successional forests was used for the decomposition experiments. In the *in-situ* litter experiment, litter produced at the two 10-year-old and 40-year-old secondary forest source sites

was homogenized by site and placed back into the replicate sites of origin (where litterfall was collected) and into the third replicate site for each forest type. We set out 80 bags per site in six sites for a total of 480 bags for aboveground litter and 64 bags per site for a total of 384 root bags. The root decomposition experiment followed the same procedure except that the root bags were each placed in a vertical slit in the top 10 cm to allow for contact with the soil.

We collected litter bags at 71, 153, 312, and 688 days and root bags at 153, 312, and 688 days (August 18, 2012, October 28, 2012, January 18, 2013, June 25, 2013, and July 7, 2014) (Figure 1) and immediately shipped on ice overnight to the University of Wisconsin-Madison. All samples were processed by removing them from nylon mesh bags, wiping off visible soil, and drying at 50°C for at least 48 hours. We calculated mass loss rates for each bag.

Litter chemistry

Initial litter and root chemistry was determined on a sub-sample of composited litter and roots collected at the source sites and used for the decomposition experiments. Oven-dried litter and fine root samples were ground in a Wiley mill using a 40-mesh insert. Total C and N concentrations were determined on dried, ground samples using a Flash 2000 CN Analyzer (Thermo Scientific, Wilmington, DE, USA) at the University of Wisconsin-Madison. All samples were run in duplicate with replicate error < 10%, using aspartic acid as calibration and check standards. We monitored changes in carbon functional groups of initial aboveground leaf litter for the samples collected at throughout the experiment using two complementary approaches.

DRIFT spectroscopy

Visual inspection of diffuse reflectance Fourier transformed infrared (DRIFT) spectroscopy combined with multivariate statistics is a robust and low-cost way of measuring changes in organic C functional groups over time. We collected 375 DRIFT spectral signatures that correspond to various collection periods from our 688-day aboveground litter decomposition experiment, including litter derived from 10-year- and 40-year-old successional forests and *in-situ* vs. transplanted decomposition environments (n = 3-5 per time point per forest type per decomposition environment). DRIFT spectra for these samples were acquired using a Bruker Vertex 70 (Bruker Opticsk, Billerica, MA, USA) equipped with a wide range Si beam splitter and mid infrared detector with Csl windows and a Pike Autodiff (Pike Technologies, Madison, WI, USA) diffuse reflectance accessory at the Woodwell Climate Research Center. Spectra were acquired on finely ground material over 6,000-180 per cm with a resolution of 4 per cm. For each sample, 60 scans were collected and averaged using the OPUS software Package (Bruker Optics) and then corrected for background signal (average of 60 scans) and transformed into absorbance spectra. The acquired DRIFT spectra were then normalized using the standard normal variation transformation. Based on prominent peaks and shoulders observed in DRIFT spectra, we identified 13 bands that represent organic functional groups (Table 2).

Decomposition indices based on subsets of DRIFT spectral signatures (Margenot et al. 2015) could provide more information on how forest age and decomposition environment might affect the chemical trajectory of litter during the decomposition process. To related differences in functional group composition to the chemical trajectory during decomposition, we calculated two index ratios from spectral bands previously used to determine the relative degree of decomposition for soil organic matter (see Margenot et al. 2015 and Veum et al. 2014):

(1) $Index\ I = \frac{B4+B12+B13}{B2+B3+B6+B7}$

$$(2)\ Index\ II = \frac{B2+B3+B4+B6+B7+B12+B13}{B1+B9+B10+B11}$$

Index I represents decomposition as a ratio of aromatic to aliphatic functional groups. Ratios of bands representing these functional groups have been shown to increase with increasing decomposition (Hsu and Lo 1999). Index II is a ratio of C-H to C-O bonds of organic functional groups and increases with increasing chemical recalcitrance (Veum et al. 2014). Index I uses the inverse association between the relative enrichment of aromatic functional groups and decrease in aliphatic functional groups during decomposition (equation 1). However, all 7 DRIFT spectral band intensities used in Index I are found in the numerator of Index II (equation 2), because although they represent aromatic and aliphatic functional groups, they also represent C-H bonds of organic functional groups. The denominator of Index II includes C-O bonds of functional groups – which are not associated with the Index I calculation.

^{13}C-NMR Spectroscopy

A subset of leaf litter samples (n = 44) was analyzed by solid-state cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy (CPMAS ^{13}C-NMR) on a Bruker DSX 200 spectrometer (Bruker BioSpin) at the Lehrstuhl für Bodenkunde at the Technical University of Munich, Germany. Samples were filled into zirconium dioxide rotors and spun in a MAS probe at a rotation speed of 6.8 kHz to minimize chemical anisotropy. A ramped 1H pulse was used during a contact time of 1 ms to prevent Hartmann–Hahn mismatches. The delay time was set to 400 ms. Chemical shifts were referenced to tetramethylsilane (TMS = 0 ppm). Spectra were integrated within the following chemical shift (ppm) regions: 0–45 (alkyl C), 45–60 (n-alkyl C), 60–95 (O-alkyl C), 95–110 (di-O-alkyl C), 110–145 (aromatic C), 145–165 (O-aryl C) and 165–185 ppm (carboxyl and carbonyl C). The

integrated spectral areas were normalized to the total signal intensity for each spectrum. We also calculated alkyl-to-O-alkyl ratios, defined as the alkyl-C area (-10 to 45 ppm) divided by the summed areas of n-alkyl + O-alkyl + di-O-alkyl (45-110 ppm). All peak areas were used to calculate the proportion contribution of six different C compounds to total litter C using a molecular mixing model, including carbohydrate, protein, lignin, lipid, carbonyl, and char (Nelson and Baldock 2005, Plante et al. 2023). Measured litter C:N ratios were used to constrain the results.

Microbial community biomass and enzyme activity

Microbial community biomass and potential enzyme activity were measured from forest floor and surface mineral soil samples collected in July 2011, January 2012, August 2012, and January 2013 (Figure 1). At each site, forest floor samples were collected from five random locations, and below that five individual soil cores (2.5 cm diameter) from the surface (0-10 cm) were collected and composited. Consecutive sampling occurred in close proximity to the original samples. Samples were transported on ice to Madison, WI, where subsamples for community analysis were immediately freeze-dried and subsamples for enzyme assays were maintained in a freezer prior to analysis (Weintraub et al. 2007).

Microbial biomass and community structure was measured using a hybrid version of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) analysis (Kao-Kniffin and Balser 2008) as described in Smith et al. (2014). Briefly, lipids were extracted from approximately 3 g of ground soil and from 0.5 g of litter, using a chloroform and methanol extraction with a phosphate buffer in a 1:2:0.9 ratio and prepared for analysis on an Agilent 6890 gas chromatograph equipped with a flame ionization detector and an Ultra 2 capillary column

(Agilent Technologies, Wilmington, DE, USA). Peaks were identified with a mixed fatty acid methyl ester (FAME) standard (EUKARY) formulated to work with Sherlock microbial identification software (MIDI, Inc., Newark, DE, USA). Lipids were quantified by comparing peak areas from the sample to those of two FAME standards, methyl non-anoate and methyl non-adecanoate (9:0 and 19:0; Sigma, St. Louis, MO). Only fatty acids of < 19 carbon chain length that were identifiable and present at >0.5 mol %, were used in analyses.

Total microbial PLFA biomass was calculated as the sum of all peaks (as μmol PLFA g soil^{-1}) (Balser and Firestone 2005). Specific known and previously identified lipids were designated as belonging to specific guilds (see Smith et al. 2015), including: (1) the sum of 18:1 ω9c and 18:2 ω6,9c lipids as general fungal indicators, (2) the sum of monosaturated (excluding 16:1 ω5c) and cyclopropyl lipids at Gram-negative bacterial indicators, (3) anteiso-and iso-branched lipids as Gram-positive bacterial indicators, (4) the sum of 15:0, 15:0, 16:0, and 18:0 lipids as non-specific microbial indicators, and (5) the ratio of fungal biomarkers to bacterial biomarkers (excluding 16:1 ω5c) as an index for fungal:bacterial lipid ratio. The biomass for different lipid biomarkers is expressed as μmol g soil^{-1} or litter^{-1}.

We measured potential activity of six hydrolytic soil enzymes in forest floor and surface soil (0-10 cm) samples: β-glucosidase (BG), α -glucosidase (AG), cellobiohydrolase (CBH), and xylosidase (XYL) (involved in the breakdown of cellulose and hemi-cellulose compounds), N-acetylglucosaminidase (NAG) (catalyzes the breakdown of chitin and N polymers stored in SOM) and acid phosphatase (PHOS) (used for microbial phosphorous acquisition) (Wallenstein et al. 2009).

Extracellular enzyme assays were conducted on homogenized samples of 1-2 g of fresh soil or 0.5 g fresh litter using a modified fluorescent-linked substrate (4-methylumbelliferone,

MUB) microplate protocol (German et al. 2011, Sinsabaugh et al. 2008), as described in Smith et al. (2015). Samples were read on a Beckman-Coulter DTX880 fluorescent microplate reader (Beckman-Coulter, Fullerton, CA, USA) at the University of Wisconsin-Madison. Potential enzyme activity, reported here as μmol g $soil^{-1}$ hr^{-1}, is based on the fluorescence of each assay after providing samples with each of the different enzyme substrates (Allison and Vitousek, 2005, Grandy et al. 2007, Wallenstein et al. 2009). Potential enzyme activity was calculated based on equations modified from German et al. (2011).

Statistical Analyses

Litterfall rates were calculated based on the amount collected in biweekly intervals. Litter decomposition rates were determined using an exponential decay model based on the relationship between log mass remaining and time:

(3) $Mt = M_0e^{-kt}$

where M_t is the mass remaining at time t (t = 71, 153, 312, 688 days for litter and t = 153, 312, 688 days for roots) expressed as % of the initial mass, M_0 is the initial litter mass at time 0, expressed as 100%, k is the first order litter decay constants. Litter decay constants were calculated for all sites, and then grouped by forest type (10-year- and 40-year-old forests) and then by decomposition environment (*in-situ* and transplanted). We used two-way ANOVA and a post-hoc Tukey HSD test to examine differences for our calculated k-values, total percent mass loss, and final C:N ratios between forest types and decomposition environments, after checking for normality and equal variances. All statistical analyses were conducted in R-Studio (version 4.0.5; R-Core Team, 2022).

To examine potential differences in DRIFT spectra we first used exploratory principal component analysis (PCA) using the *vegan* package in R-Studio (Oksanen et al. 2012), using time (days in the field) and the 13 DRIFT bands identified as variables. PCA is a commonly used ordination tool that highlights dominant linear trends by reducing large datasets into two or three dimensions (i.e., principal components) based on best predictor variables. This approached allow us to first determine which organic C functional groups had large loading scores for the data set, which we could then inspect more closely for spectral changes in DRIFT signal intensities during the decomposition process between forest types and decomposition environments. DRIFT spectra for 18 samples from day 688 had adsorption bands indicative of a carbonate signature (between 2389 and 2268 cm^{-1}). Given that soils in our study are Mollisols that formed in limestone, we determined this carbonate signature was due to mineral soil contamination, and those 18 samples were removed from the dataset prior to PCA.

We also performed exploratory PCA on using just time, Index I (equation 1), and Index II (equation 2) to evaluate how these DRIFT spectra derived indices might identify potential changes in litter chemistry between decomposition environments for both forest types. We then used linear mixed effects models with restricted maximum likelihood, using forest successional trajectory and time as fixed effects and site (nested within treatment) as a random effect to determine how our DRIFT derived decomposition indices changes over time between forest types and decomposition environments, using the *lme4* package in R-studio (Bates et al. 2015). Due to low sample replication, we were not able to perform statistical analysis on the NMR spectra areas or proportional contributions to our molecular mixing model over time between forest types and decomposition environments. Instead, we used these data to support our interpretation of changes in organic functional groups derived from DRIFT spectra.

We also performed exploratory PCA on PLFA and enzyme data separately for forest floor and surface soil samples. This analysis included soil characteristics from the 10-year- and 40-year-old forests and collection within the PLFA and enzyme data sets, to explore which environmental factors might influence the variability in microbial community biomass vs. enzyme activity. To test for differences between forest ages, time, and their interactions, we analyzed microbial community biomass and enzyme activity using a linear mixed effects model with restricted maximum likelihood. Forest age and time were treated as fixed effects and site (nested within treatment) was treated as a random effect, using the *lme4* package in R-studio.

RESULTS

Litterfall

Annual total litterfall mass from August 2011 to July 2012 did not differ between the 10-year-old (8.3 ± 0.8 Mg^{-1} ha^{-1} yr^{-1}) and 40-year-old secondary forests (7.9 ± 0.7 Mg^{-1} ha^{-1} yr^{-1}). Instead, the timing of inputs to the forest floor differed. Monthly litterfall rates were greater in the 40-year-old secondary forests during the end of the dry season (April-June, maximum = 815.71 kg ha^{-1}) and greater in the 10-year-old secondary forests during the wet season (September-December, maximum = 844.54 kg ha^{-1}) (Figure 2).

Litter decomposition rates

Litter derived from 10-year-old forests decomposed faster over the 688-day experiment compared to litter derived from 40-year-old forests, regardless of decomposition environment (Table 3). Litter decomposition rates from the 10-year-old forests were similar for *in-situ* (k= 2.08 ± 0.18) and transplant experiments (k=2.28 ± 0.27). Litter derived from 40-year-old forests

also did not differ between *in-situ* vs. transplanted experiments (k= 1.15 ± 0.15 and 1.13 ± 0.13, respectively). At 71 days, litter from the 10-year-old forests had lost ≈48% of its original mass, whereas litter from the 40-year-old forests had lost only ≈29% (Figure 3A). Although decomposition rates differed between forest types ($p < 0.001$), the total mass lost at the end of the experiment did not (Table 2), with 10-year- and 40-year-old forests losing ≈91% and ≈84% of their original mass by the end of the experiment, respectively.

Changes in litter root mass over the 688-day decomposition experiment were also best predicted by exponential decay models, despite initial increases in mass measured between time day 0 and day 153, likely from growth of fungal hyphae (Figure 3B). In contrast to leaf litter, the origin of the root material did not affect decomposition rates, although it did affect the final percent mass remaining at the end of the experiment. Roots from the 10-year-old forests decomposed at similar rates in the *in situ* (k = 0.70 ± 0.10) and transplanted (k = 0.44 ± 0.06) experiments, as did roots from the 40-year-old forests (*in situ* k = 0.53 ± 0.11; transplanted k = 0.65 ± 0.09). Roots from 10-year-old forests lost a greater percentage of their original mass than roots from 40-year-old forests, losing ≈77% and ≈63% by day 688, respectively (Table 2). There were also significant interactions between forest type and decomposition environment on the total % mass lost ($p < 0.01$) and k-values ($p < 0.03$).

Litter chemistry

Carbon-to-nitrogen ratios

Litter C:N ratios decreased over the course of the decomposition experiment for both forest types and decomposition environments. Litter from the 10-year-old forests showed the greatest rates of change in C:N ratios from day 0 to day 71, after which point it was held mostly

constant over the remainder of the experiment (Figure 3C). Final litter C:N ratios were significantly lower for the 10-year-old forests compared to 40-year-old forests, regardless of decomposition environment (Table 1). However, there were significant interactions between forest age and decomposition environment on the final litter C:N ratios ($p < 0.05$), suggesting that the trajectory of litter chemistry might be at least partially influenced by forest-specific conditions related to how microbes process litter. Final root C:N ratios were similar between 10-year- and 40-year-old forests (Table 2) and varied much less than aboveground litter during the decomposition experiment (Figure 3D).

DRIFT Spectra

Exploratory PCA analysis using the 13 DRIFT spectral bands and time (days in the field) showed clear changes in spectral signatures, irrespective of forest age or decomposition environment (Figure 4). All variables had similar loading scores on PC1, but B2 and B3 (representing aliphatic compounds) both had the largest loading scores on PC2 and explained 48.3% of the variation along this component alone (Table S1). Time and B9 (representing phenols and carboxylic acids) also had relatively high loading scores on PC2, although they were lower in magnitude. These same two DRIFT bands have large loading scores on PC3 and explained 35.9% of the variation of this component. Although B10 and B11 (representing polysaccharides) had relatively low loading scores on PC3, they explained 38.6% of the variation, suggesting other chemical constituents of litter may also be important.

Given the large variation explained along multiple principal components, we examined changes in relative signal intensities over time for two aliphatic spectral bands (B2 and B3) over time between forests and decomposition environments. For *in-situ* decomposition, DRIFT

spectral intensities quickly decreased from day 0 to day 71 for litter derived from 10-year-old forests (Figure 5A) and decreased more slowly over time for litter derived from 40-year-old forests (Figure 5B). When litter derived from 10-year-old secondary forests decomposed in 40-year-old forests (i.e., transplanted), the DRIFT spectral intensities also decreased quickly early in decomposition (Figure 5C) but changed modestly when 40-year-old litter decomposes in a 10-year-old forest (Figure 5D).

Exploratory principal component analysis (only using time, Index I, and Index II as variables) showed very little difference between decomposition environments for litter derived from 10-year-old forests, but showed clear separation between collection time points (Figure 6A). In contrast, litter derived from 40-year-old forests showed separation between decomposition environments, with more overlap at earlier stages of decomposition (Figure 6B).

Changes in litter Index I values over time showed significant effects of time for litter derived from 40-year-old (Figure 7A) and 10-year-old forests (Figure 7B), but no effect of decomposition environment. Changes in Index II values over time showed significant interactions between time and decomposition environment for litter derived from 40-year-old forests (Figure 7C) but only significant effects of time for litter derived from 10-year-old forests (Figure 7D). These results indicate that chemical complexity, particularly the relative proportion of C-H to C-O bond organic functional groups, likely influences changes in litter chemistry during decomposition process.

NMR Spectra

Changes in forest litter NMR spectra over time revealed different patterns in the timing and order of chemical transformations during the decomposition experiments. The greatest

changes in peak intensities occurred in the O-alkyl-C and Alkyl-C spectral regions for both forest ages and decomposition environments (Figure 8). Initial litter O-alkyl-C peak areas are higher for litter derived from 10-year-old forests compared to 40-year-old forests; this result is supported by the molecular mixing model, which shows greater abundance of carbohydrates in the 10-year-old forest litter (Table S2). The abundance of carbohydrates decreased over time for both forests and decomposition environments. Alkyl-C and O-alkyl-C both decreased and carboxyl-C peak areas increased over the decomposition process for both 10-year- and 40-year-old secondary forests, regardless of decomposition environment. Peak areas representative of aromatic compounds increased in both decomposition environments for litter derived from 10-year forests, but interestingly, decreased when litter derived from 40-year-old decomposed *in-situ* and increased when it decomposed in a 10-year-old forest (Table S2).

Alkyl-to-O-alkyl ratios varied considerably over time for litter derived from 10-year-old forests in both decomposition environments, ranging from 0.25-0.48 (Figure 9A). The largest decrease occurred at 153 days for the transplant experiment, although this time point is represented by only one sample. For 10-year-old forest litter decomposing *in-situ*, the largest decrease in Alkyl-to-O-alkyl ratios occurred at 312 days. Ratios for both decomposition environments reached similar values by the end of the experiment. Alkyl-to-O-alkyl ratios varied far less over time for litter derived from 40-year-old forests, only ranging from 0.43-0.55 (Figure 9B). Ratios for 40-year-old forest litter decomposing *in-situ* steadily increased over time, whereas litter decomposing in a 10-year-old forest sharply increased at 153 days and then decreased steadily until the end of the experiment.

Microbial community biomass and enzymatic activity

Forest Floor

Exploratory principal component analysis looking at variation in forest floor PLFA microbial community biomass and soil characteristics showed distinct clustering between 10-year- and 40-year-old forest and explained 49.0% of the variation in the dataset (Figure 10A). Soil characteristics had largest loadings on PC1, including ammonium, nitrate, calcium, phosphorus, pH, and SOC, and explained 86.6% of the variation, while fungal and bacterial biomass, moisture content, and time had the largest loadings along PC2 and explained 96.56% of the variation (Table S3). The lack of significant loadings for soil characteristics along PC2 indicate that differences in forest age might not be reflected in forest floor PLFA data. Results from our linear mixed effects model results support this interpretation, where we found consistently significant effects of time on forest floor PLFA community biomass but effects of forest age or interactions between forest age and time were rarely significant (Table 5). It is important to highlight that 40-year-old forests had greater forest floor bacterial biomass than the 10-year-old forests (0.20 ± 0.02 μmol g soil^{-1} vs. 0.12 ± 0.02 μmol g soil^{-1}) averaged across all sampling dates, indicating that older successional forests might have bacteria-dominated forest floor microbiomes.

Our PCA analysis also showed distinct clustering between forest types for the dataset that included forest floor enzyme activity and soil characteristics and explained 50.4% of the variation in the data along PC1 and PC2 (Figure 10B). Soil characteristics had the largest loadings along PC1 and explained 60.8% of the variation and had relatively large loadings on PC2 (Table S4). Enzyme activities (other than PHOS) had higher magnitude loadings and explained greater variation along PC2 than soil characteristics (22.4% vs. 67.5%). Large loading

scores of both soil characteristics and enzyme activity along PC2 for this dataset (unlike for the forest floor PLFA dataset) are reflected in our mixed model results, which showed significant effects of by time, forest age, and interactions between the two on enzyme activity (Table 5). The 10-year-old forests had greater CBH, total C EEA (BG + AG + XYL + CBH), and total EEA activities compared to 40-year-old forests; BG values were marginally greater for 10-year- vs. 40-year-old forests ($p = 0.07$). Forest floor AG, XYL, and CBH (all enzymes involved in C acquisition) activities showed significant interactions between forest age and time, suggesting successional age might play an important role in microbial litter processing and cycling of C in these forests.

Soil

PCA analysis looking at variation in soil PLFA and soil characteristics showed distinct clustering, with PC1 and PC2 explaining 63.8% of the variation in this dataset (Figure 10C). Unlike for the forest floor PLFA data, bacterial and fungal biomass guilds had the largest loadings along PC1 and explained 72.5% of its variation, while soil characteristics and litterfall had largest loadings on PC2 and explained 80.47% of the variation along this component (Table S5). Interestingly, time and moisture content together explained 82.5% of the variation along PC3 (data not shown; Table S5), indicating that while litter inputs, soil characteristics, and microbial biomass had the largest loadings on the first two components, variations in moisture over time might have high explanatory power in this dataset. Our linear mixed model results showed that microbial biomass for almost all guilds varied significantly by time (all $p < 0.001$) and only some had significant forest age x time interactions (Table 5). Soil fungal-to-bacterial ratios were marginally different between forest ages ($p = 0.07$), with 10-year-old forests having

greater ratios than 40-year-old forests averaged across sampling dates (0.76 ± 0.05 vs. 0.60 ± 0.05 μmol g soil^{-1}, respectively). These results suggest that soil microbiomes in younger successional forests could be dominated by fungi, which we did not see in the forest floor PLFA data, suggesting that microbial communities between forest floor and soil could be distinctly different.

PCA analysis for soil enzyme activities and soil characteristics showed distinct clustering between forest types, with PC1 and PC2 explaining 54.91% of the variation in this dataset (Figure 10D). Interestingly, almost all variables in this dataset had high magnitude loading scores on PC1 and enzyme activities, soil nutrients, and time had the largest loadings on PC2 (66.07% of the variation; Table S6). The high loading magnitude time alone has on PC1 and PC2 suggest that time could be a very important variable for this dataset (Table S6). Indeed, our soil enzyme activity data showed only significant effects of time and no significant effects of forest age. These results also suggest a decoupling between forest floor and soil microbial activity, given that forest age had significant effects on many of the enzymes involved in C-acquisition in the forest floor samples but explains no variability in the soil enzyme activity data.

DISCUSSION

Litter chemistry is tied to successional forest species composition

The litter decomposition experiments showed that litter from 10-year-old forests decomposed faster than litter from 40-year-old forests, regardless of decomposition environment. Litter derived from 10-year-old forests also had significantly lower initial C:N ratios than litter derived from 40-year-old forests. Given these results, it appears that the strong differences in

litter chemistry between our 10-year- and 40-year-old forests, due to differences in plant composition, could strongly regulate differences in leaf litter decomposition rates.

The effects of forest type on decomposition rates by litter origin masked unexpected similarities between one of the 10-year-old sites and one of the 40-year-old sites. Despite differences in initial C:N ratios (≈18 and 29, respectively), litter from these two sites had similar decomposition rates, with k-values of 1.42 for their respective *in-situ* decomposition experiments. The k-value for this 40-year-old forest site is relatively high compared to the other 40-year secondary forest site, which had a k-value of 0.94, despite having similar initial litter C:N ratios. Conversely, the k-values for one of 10-year-old secondary forest site were 1.6 times greater than another 10-year-old forest site. Variability in litter decay rates between sites of the same successional age, despite little variability in plant species composition and initial litter chemistry (C:N ratio), suggests that other environmental factors might influence decomposition dynamics.

Differences in species composition are not always reflected in ecosystem processes, such as decomposition. Even if species composition and aboveground biomass changes significantly along a successional gradient, litter chemistry and litter decay rates could still be highly variable (see Marín-Spiotta et al. 2007 and Ostertag et al. 2008). However, in our study, litter chemistry differed significantly between secondary forests, due to higher prevalence of the nitrogen fixing tree species (*Leucaena leucocephala*) in our 10-year-old forests, which tend to have low litter C:N ratios. In addition to litter C:N ratio data, our NMR data showed that litter derived from 10-year-old secondary forest had greater initial carbohydrate content, which is more easily decomposable than other compounds found in leaf litter (Cepáková and Frouz 2015), particularly

at earlier stages of decomposition (Osono and Takeda 2005). Given that our NMR data was not robust enough for statistical analysis, we can only interpret these data qualitatively.

The DRIFT data reveal complex interactions between changes in litter chemistry that are mediated by differences in microbial biomass between secondary forests in our study. The 10-year-old forest lost aliphatic H-C functional groups rapidly early in decomposition and compared to the 40-year-old forests that that lost these compounds more slowly, when litter decomposed *in-situ*. Although aliphatic compounds are hydrophobic and accumulate during litter decomposition, they also can leach from litter low with cellulose concentration and low abundances of polyphenols (Bonanomi et al. 2021, Hensgens et al. 2021, Osono and Takeda 2005). Although we did not quantify litter cellulose or polyphenol concentrations in this study, lower C:N ratios and higher decomposition rates for litter derived from 10-year-old forests suggest it is more chemically labile and less resistant to microbial decay, which could explain its rapid decrease in aliphatic DRIFT spectral intensities. Additionally, our PLFA data showed that 40-year-old forests had greater forest floor bacterial biomass than 10-year-old forests, which may explain why litter from 40-year-old forests lost aliphatic compounds more slowly, since aliphatic compounds can also accumulate in litter via bacterial production (Kögel-Knabner 2002). These complex interactions between aliphatic compound loss/accumulation and litter chemistry appear to be tied not only to forest composition but also important differences in forest floor bacterial biomass – all of which are tied to forest succession.

Controls of decomposition environment on litter chemical trajectory

Our linear mixed effects models and PCA analysis of DRIFT data showed that the chemical trajectory of litter derived from 40-year-old forests is influenced by its decomposition

environment, even if litter decay rates are not. These results suggest that litter mixture complexity (i.e., litter derived from multiple tree species) more than C:N ratio alone might influence changes in litter chemistry during decomposition between decomposition environments. These results are at odds with the "home-field advantage" (HFA) hypothesis (Ayres et al. 2009, Gholz et al. 2000), which states that microbes are most efficient at decomposing litter from their native communities. Strickland et al. (2009) found similar results when comparing *in-situ* vs. transplanted decomposition of litters with very different initial chemistries, using grassland litter with low C:N ratios and forest litter with high C:N ratios. Their findings suggested that microbial communities sourced from both habitats could decompose the grass litter similarly, but not the forest litter. Some other recent studies have found the HFA hypothesis to be more valid for chemically complex litter sources (e.g., Palozzi and Lindo 2018, Veen et al. 2015), likely due to greater abundances of that soil biota at higher tropic levels than bacteria (e.g., nematodes, protists, fungal endophytes, etc.) (Fanin et al. 2021, Lindow and Brandl 2003, Wolfe and Ballhorn 2020).

Results from our DRIFT-based litter decomposition indices provide evidence for the role litter chemical complexity may play in HFA. Recall that we used two decomposition indices to monitor changes in litter chemistry between forest types and decomposition environments (Margenot et al. 2015). Changes in Index I values for litter derived for 40-year-old forests over time were not significantly different between decomposition environments but Index II showed significant time x decomposition environment interactions. Exploratory PCA analysis using these indices and time in the field showed separation between decomposition environments for litter derived from 40-year-old forests but not 10-year-old forests. While both Index I and II are sensitive to changes in chemistry and stoichiometry (Margenot et al. 2015), they describe

different aspects of decomposition and thus, should be supported by other chemistry data for correct interpretation. Results from our study illustrate the potential of DRIFT spectroscopy to provide direct measures of changes in C functional group composition during litter decomposition, but also demonstrates limitations of conclusions drawn exclusively from such approaches.

Seasonal and interannual variations in microbial community composition and activity

Observed temporal fluctuations in microbial community composition and activity and interactions between forest type and sampling time likely correspond to high variability in rainfall during the study. Our microbial sample collection times corresponded to two samples per season, however total monthly precipitation values varied considerably between dry and wet seasons over the collection period. Rainfall values for the wet season in July 2011 were ≈100 mm greater than dry season values in January 2013, but dry season precipitation values for January 2013 were comparable to wet season values in August 2013. This large variability in precipitation between wet and dry seasons between years in our study likely explains the strong significant effects of time on microbial community composition and activity.

Our results highlight the potential importance of moisture variability on microbial dynamics, particularly on soil microbial community composition. Exploratory PCA analysis using soil characteristics and soil PLFA data revealed time and soil moisture together explained ≈82.50% of the variation along PC3. Other studies have highlighted the strong effects that seasonality and variations in precipitation can have on soil microbial biomass, community composition, and activity in tropical forests (Bargali et al. 2018, Buscardo et al. 2018, Pajares et al. 2018, Saynes et al. 2005, Smith et al. 2015, Wieder et al. 2014). In ecosystems characterized

with high spatiotemporal variability in rainfall, seasonal water stress has been shown to promote the reactivation and growth of previously dormant, drought-tolerant microbes (Lebre et al. 2017, Lennon and Jones 2011, Rittershaus et al. 2013). In tropical dry forests specifically, microbial composition and expression of genes associated with C and N acquisition have been shown to differ drastically between wet and dry seasons (Lacerda-Júnior et al. 2019). Future work needs to expand on these findings to better understand how rainfall patterns in tropical dry forests may influence microbial cellular processes that influence nutrient cycling.

Potential mechanisms driving root decomposition

Unexpectedly, root decomposition rates did not differ between decomposition environments, despite some differences in soil microbial community biomass between successional forests, suggesting that microbial community composition may not constrain microbial function in our study sites. Results from our linear mixed models using soil PLFA data showed that fungal-to-bacterial ratios were significantly higher in 10-year-old forests compared to 40-year-old forests, but roots from 10-year-old forest had greater percent mass loss over time compared to 40-year-old forests, regardless of decomposition environment. This suggests that fungal biomass alone is not responsible for greater mass loss of roots derived from 10-year-old forests. Additionally, initial root C:N ratios did not differ between successional forests, indicating that litter chemistry also cannot explain differences in the total percent mass lost during the experiment.

In dry tropical forests, root litter often decomposes slower than leaf litter and decomposition rates vary between wet and dry seasons (Anaya et al. 2012, Gholz et al. 2000). Differences in litter decomposition rates are generally preserved across biomes (Makkonen et al.

2012) suggesting that plant traits and litter chemistry are tied to decomposition dynamics. In theory, plant diversity and species richness can affect litter decomposition rates by influencing litter chemistry, which in turn, affects the activity and composition of microbial decomposer communities (Gessner et al. 2010, Hättenschwiler et al. 2005). Fungi can more efficiently decompose chemically complex molecules found in roots (e.g., lignin) than bacteria (Hiscox et al. 2015, Schlatter et al. 2018). While links between soil pH and litter decomposition are poorly understood, soil pH has been shown to affect microbial community composition and soil fertility (Neina 2019, Rousk et al. 2010, Wardle et al. 2004). While 40-year-old forests had higher soil pH values, 10-year-old forests had higher soil phosphorus, ammonium, and calcium contents, which have been shown to increase decomposition rates in tropical fertilization experiments (e.g., Ostertag and Hobbie 1999). However, given the lack of significant effects of decomposition environment on root mass loss, soil fertility also is not likely the sole driver of root decomposition dynamics in our study.

Although we did not examine differences in morphological and chemical root traits between our secondary forests, they could potentially be important for explaining our observed differences in root mass loss over time, as shown in previous studies (Aulen et al. 2012, Silver and Miya 2001). Previous work has shown some specific root traits are particularly favorable for decomposition, and root traits and soil fertility can explain greater variations in root decay rates than species diversity (Guerrero-Ramírez et al. 2016, Liang et al. 2016). Morphological changes associated with forest succession in dry tropical forests involve trade-offs between enhanced growth and increased survival in early vs. late successional forests, respectively (King et al. 2006, Subedi et al. 2019). Likely, interactions between differences in fungal biomass, soil nutrients, and root morphology are responsible for greater percent mass loss of roots derived

from 10-year-old vs. 40-year-old forests in our study. We suggest future studies looking at root decomposition include characterization of morphological and chemical root traits, particularly in tropical and dry tropical forests.

CONCLUSION

This study provides insight on how decomposition dynamics in post-agricultural successional forests are influenced by not only changes in species composition and diversity, but also litter mixture complexity and site-specific conditions in soil properties and microbial biomass. Traditionally, differences in litter carbon-to-nitrogen ratios are thought to explain differences in litter decay rates, and microbes are generally thought to be most efficient at decomposing litter derived from their native environments. While we did find that litter derived from 10-year-old forests decomposed faster that litter from 40-year-old forests, regardless of decomposition environment, other spectral data from our study suggests that the chemical trajectory of chemically complex litter from 40-year-old forests is influenced by decomposition environment. Results from our study align with previous work that suggests home-field advantage hypothesis might be most appropriately applied to litter mixtures derived from multiple species but not litter derived from monoculture forests with low C:N ratios. While we found some differences in litter and soil microbial community biomass and enzyme activity, our results overwhelmingly showed that time in the field explained the greatest variability in microbial dynamics, suggesting that seasonal variability in precipitation might be an important driver of microbial composition and function in dry tropical forests. We suggest future decomposition studies in tropical ecosystems collect high resolution data on microbial

community composition and incorporate a combination of spectral and organic C chemistry to monitor changes in litter and root chemistry during decomposition.

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FIGURES AND TABLES

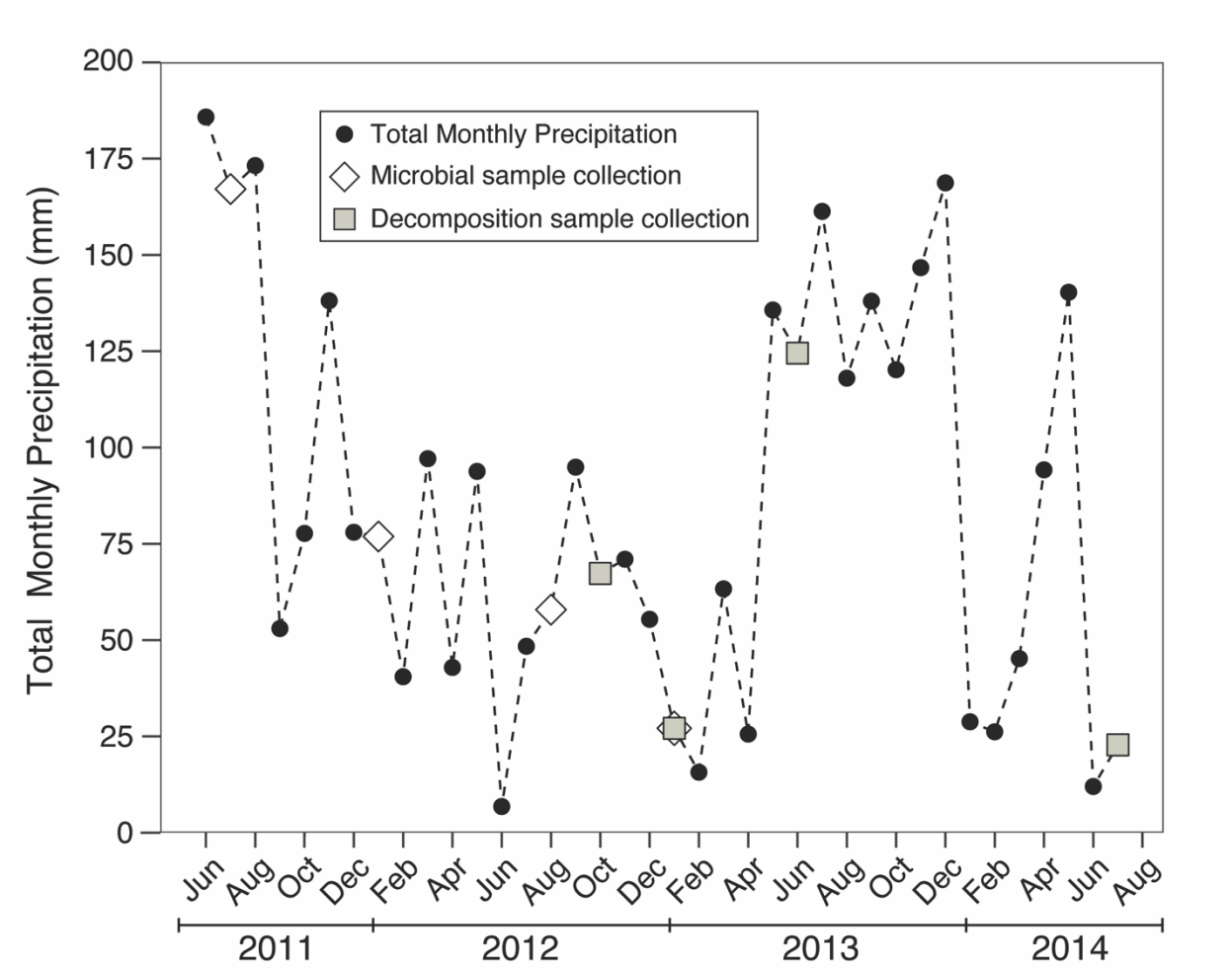

Figure 1: Total monthly precipitation during the study period from June 2011-July 2014. Points showing litter decomposition and surface soil (0-10 cm) sample collection correspond to periods of wet vs. dry seasons at the study sites. Note the overlapping collection period for decomposition and soil samples in February 2013. Precipitation data are derived from the NOAA repository for St. Croix.

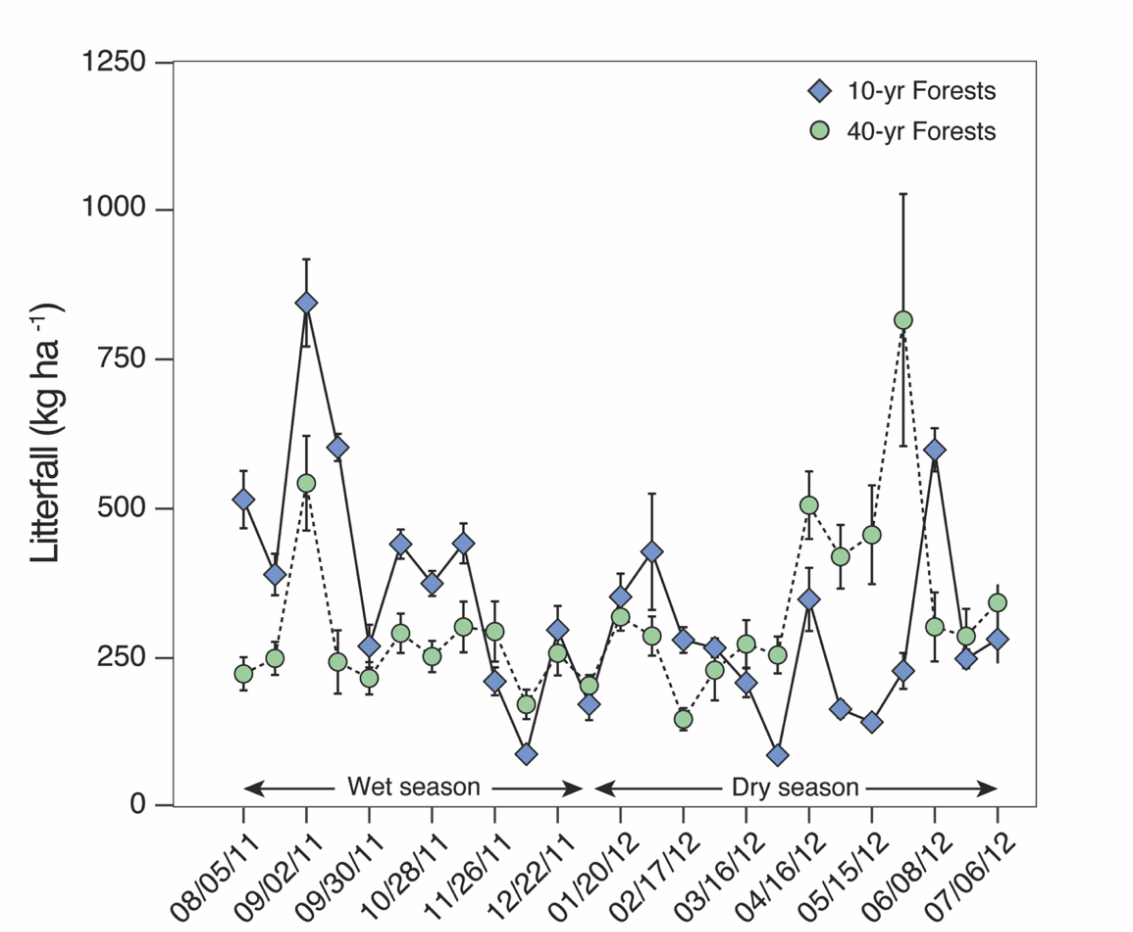

Figure 2: Average bi-weekly litterfall rates over the course of the litter collection period. Bars around the average correspond to standard error (n = 2 sites per forest type).

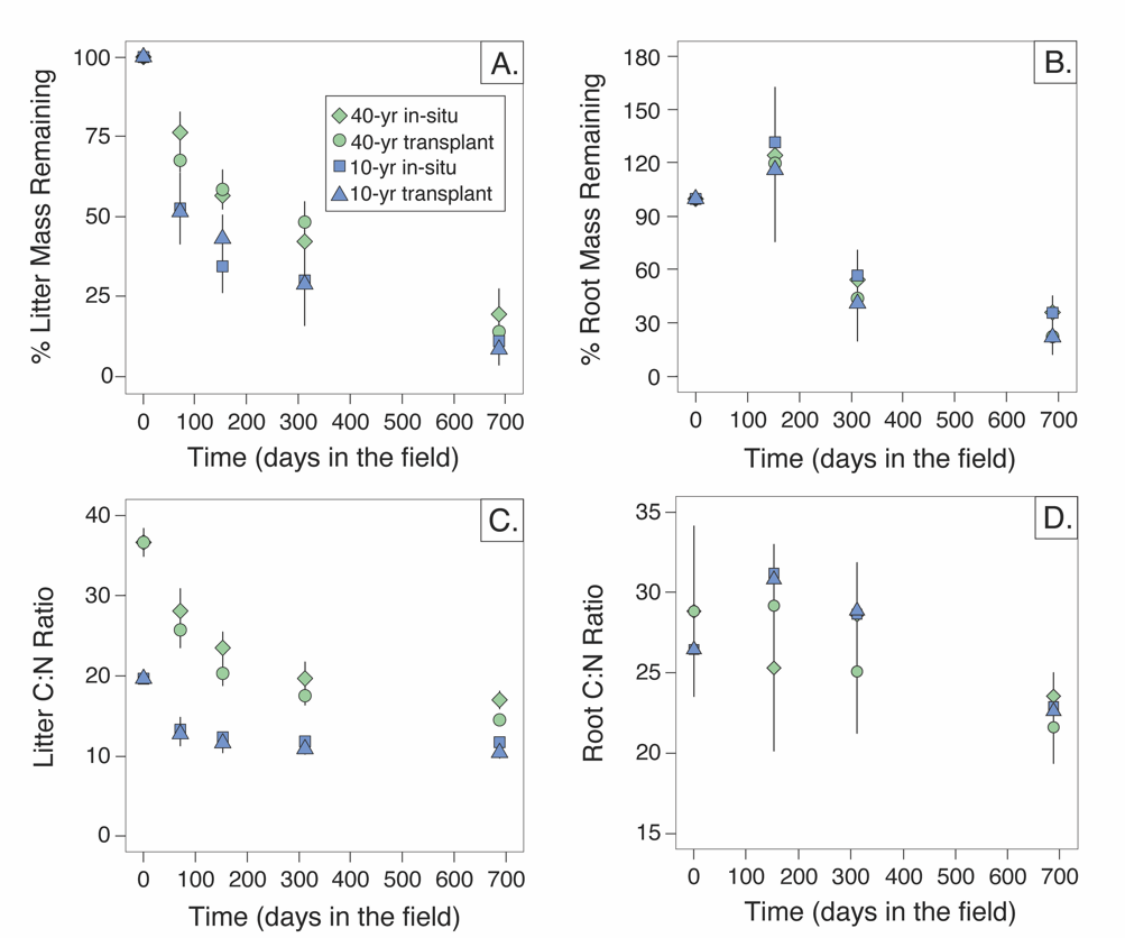

Figure 3: Comparison between 10-year- vs. 40-year-old successional forests and decomposition environments for A.) leaf litter mass loss, B.) roots litter mass loss, C.) leaf litter carbon-to-nitrogen (C:N) ratios, and D.) root litter C:N ratios over time. Points represent averages and error bars represent propagated errors based on site-level standard errors (n = 3-5) per forest type and decomposition environment per time point (n = 6). Note differences in y-axis scales between panels.

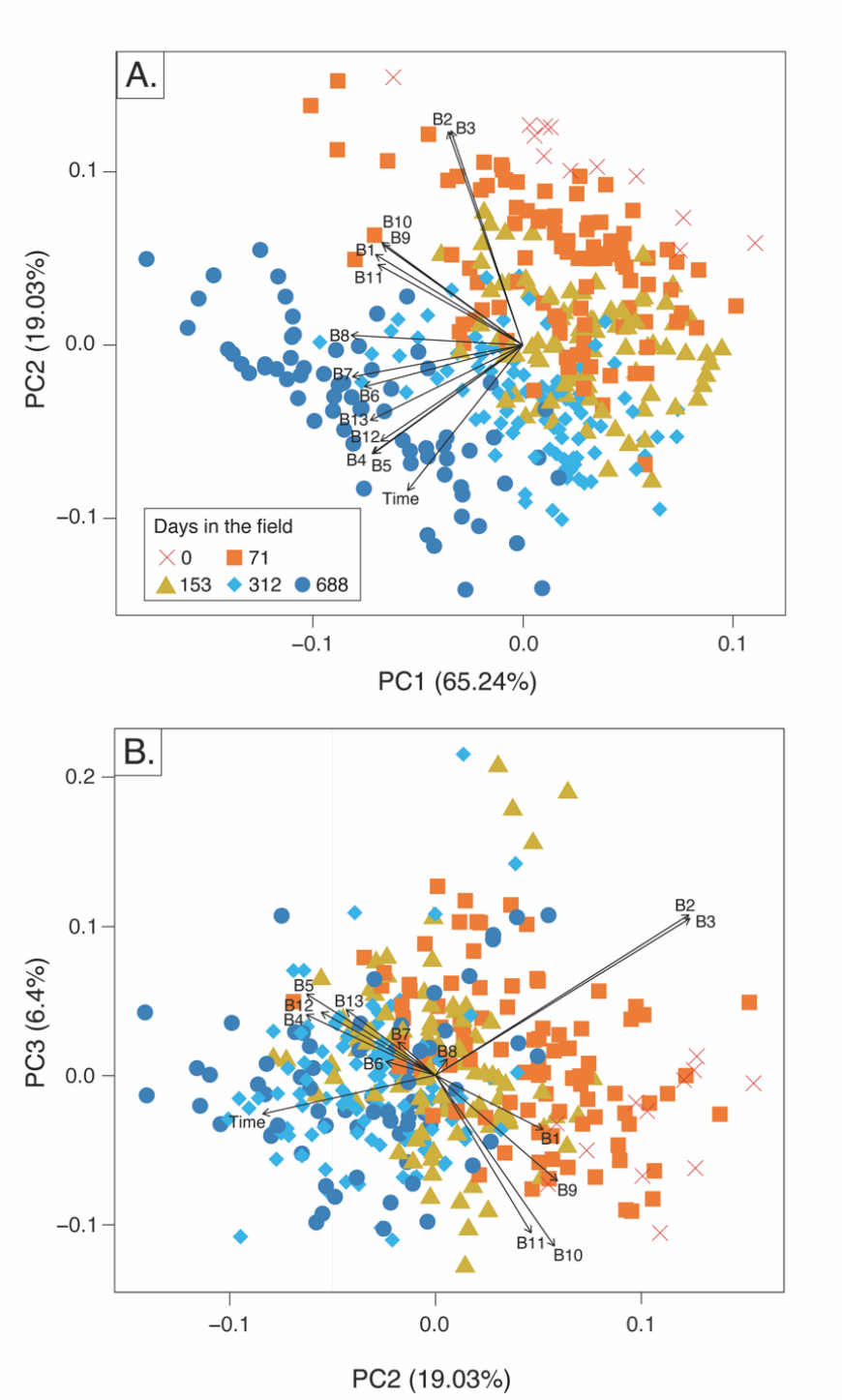

Figure 4: Principal component biplots of DRIFT spectral bands from our 688-day leaf litter decomposition experiment. Points represent spectral readings corresponding with number of days in the field for A.) principal component 1 and 2 and B.) principal component 2 and 3. Vectors represent time (days in the field) and the 13 DRIFT bands used in this research.

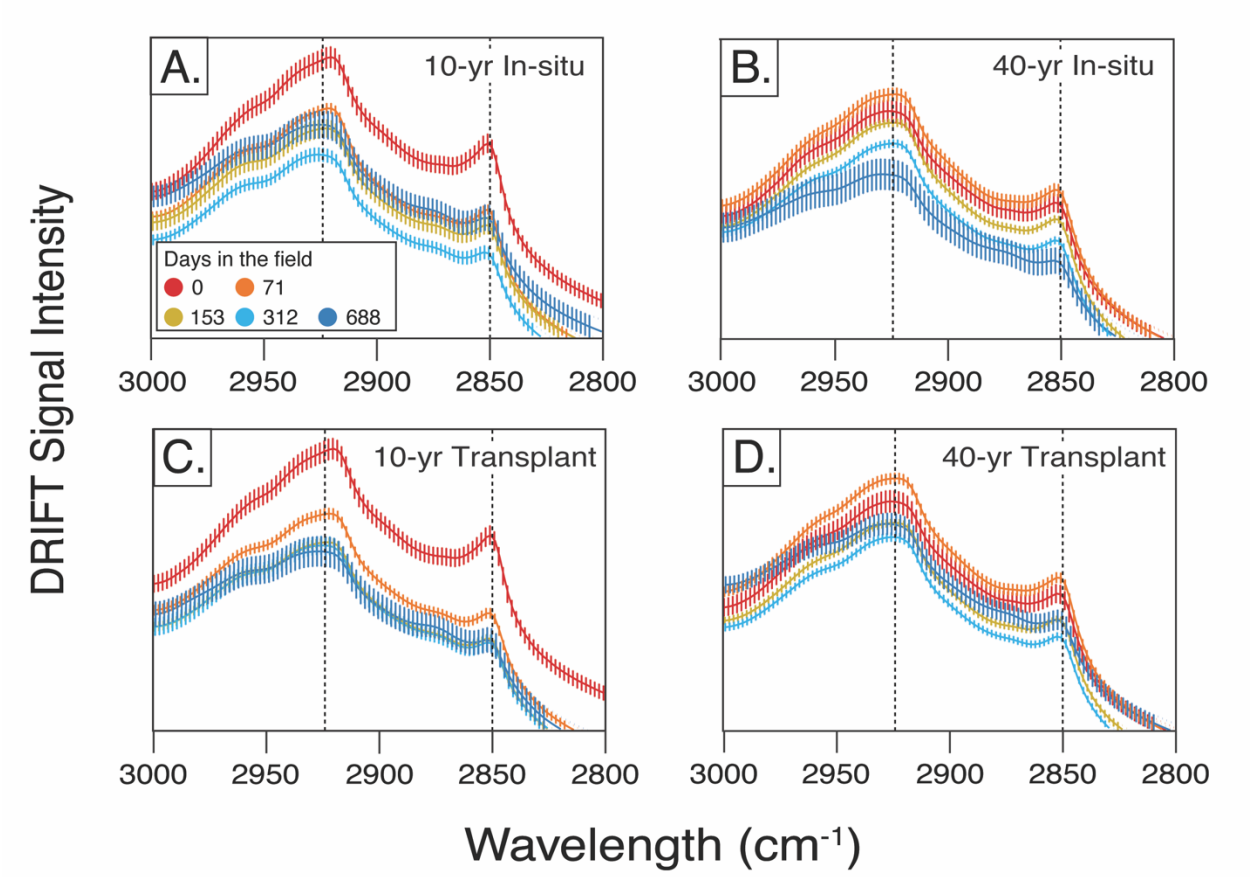

Figure 5: Changes in DRIFT spectra signal intensities over the 688-day litter decomposition experiment for B2 (2924 cm^{-1}) and B3 (2850 cm^{-1}) that correspond to aliphatic carbon functional groups for A.) 10-year-old forest litter decomposing in-situ, B.) 40-year-old forest litter decomposing in-situ, C.) 10-year-old litter decomposing in 40-year-old forests (transplant), and D.) 40-year-old litter decomposing in 10-year-old forests (transplant). Continuous lines represent mean signal intensities and error bars represent one standard error (n = 3-6). The vertical dashed lines on each panel represent B2 and B3 peaks.

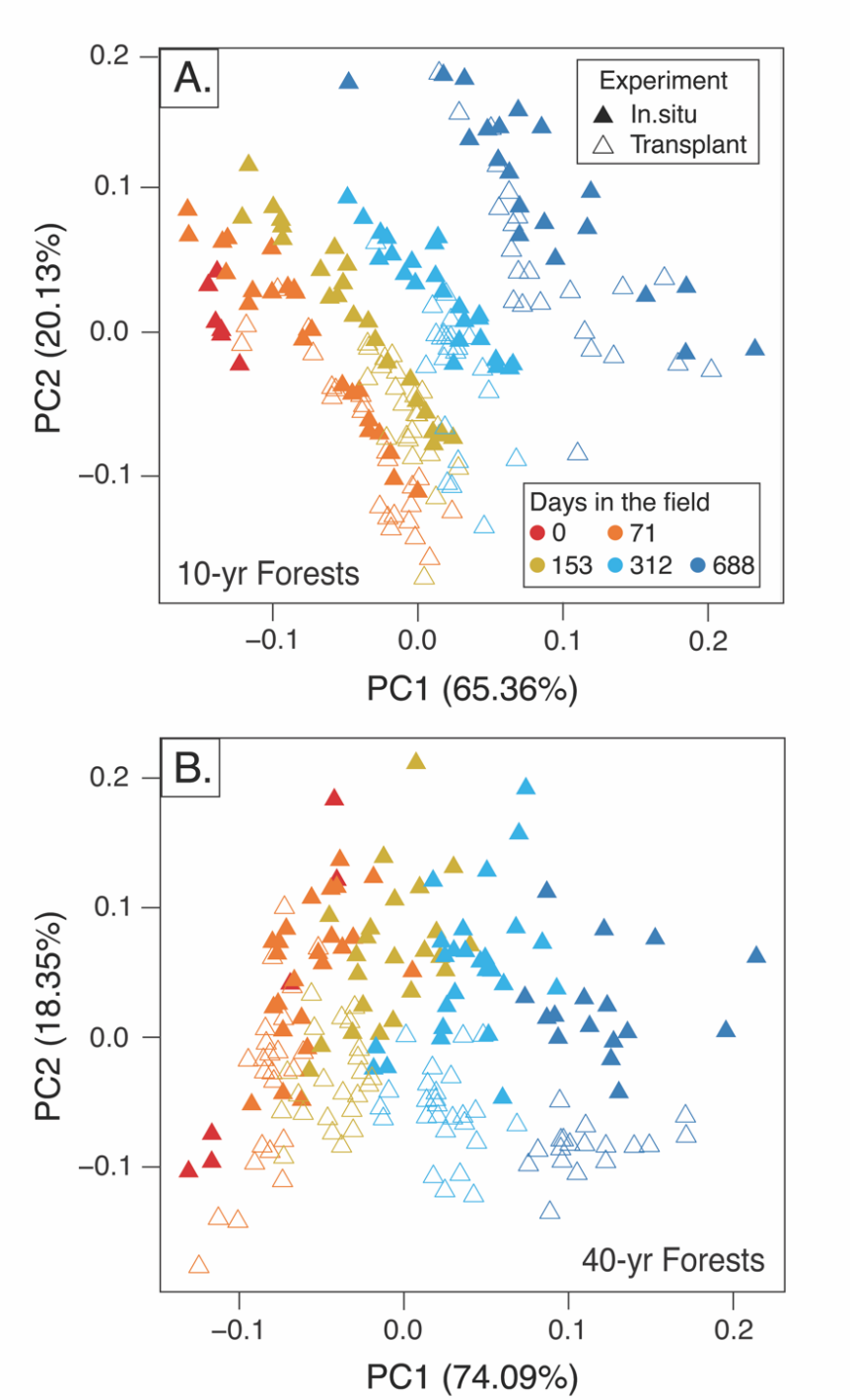

Figure 6: Principal component biplots of DRIFT indices from our 688-day leaf litter decomposition experiment. Points represent spectral readings corresponding with number of days in the field for and litter decomposition in-situ vs. a transplanted environment for A.) 10-year-old secondary forests and B.) 40-year-old secondary forests.

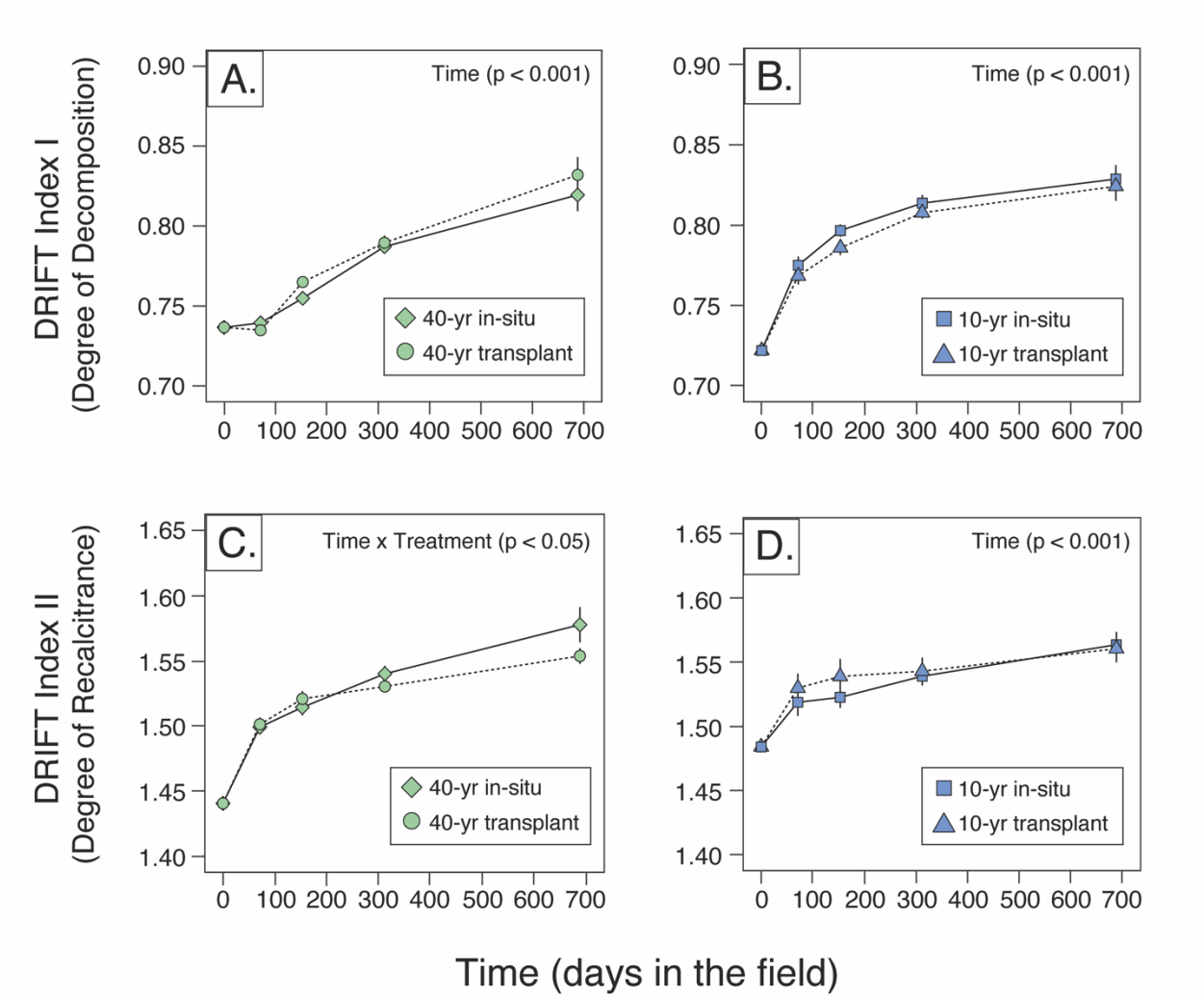

Figure 7: Changes in DRIFT spectral indices over the 688-day litter decomposition experiment. A.) Index I values for litter originated from 40-year-old secondary forests decomposing in-situ vs. in 10-year-old forests, B.) Index I values for litter originated from 10-year-old secondary forests decomposing in-situ vs. 40-year-old forests, C.) Index II values for litter originated from 40-year-old secondary forests decomposing in-situ vs. 10-year-old forests, D.) Index II values for litter originated from 10-year-old secondary forests decomposing in-situ vs. 40-year-old forests. Error bars represent standard errors from DRIFT index calculations (n = 3-5).

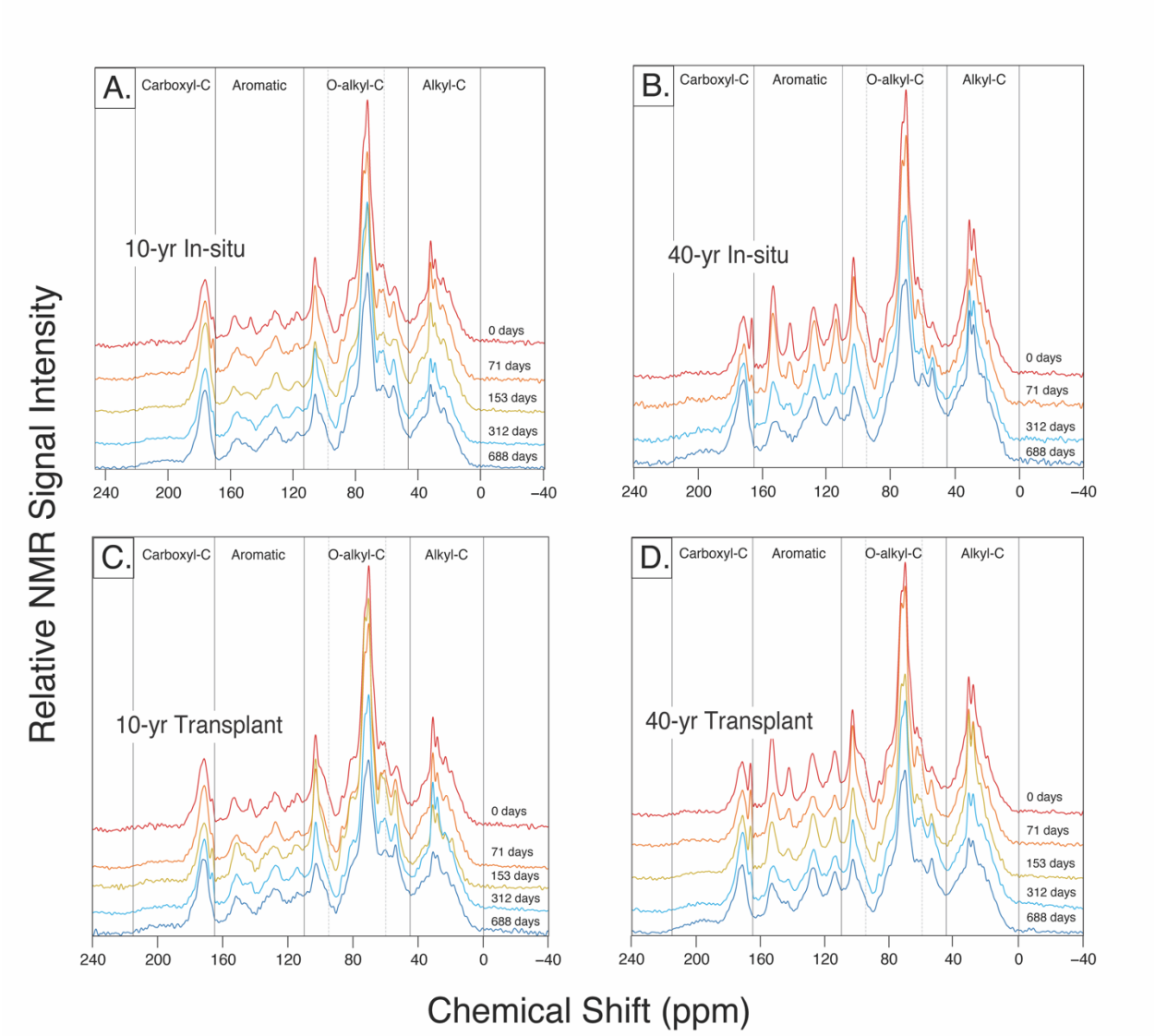

Figure 8: Changes in NMR spectra over time from our 688-day leaf litter decomposition experiment for A.) litter from 10-year-old secondary forests decomposing in-situ, B.) litter from 40-year-old secondary forests decomposition in-situ, C.) litter from 10-year-forests decomposing in 40-year-old forests, and D.) litter from 40-year-old forests decomposing in 10-year-old forests. Lines represent averages (n=2-6) or single readings, depending on forest origin, decomposition environment, and time in the field. Note that panel B does not have spectral values for litter decomposing for 153 days.

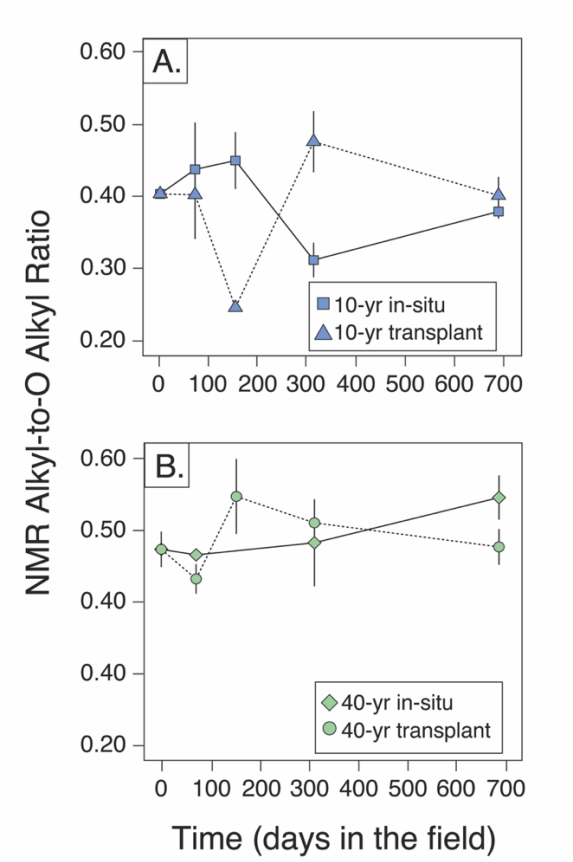

Figure 9: Changes in leaf litter Alkyl-to-O Alkyl ratios over our 688-day decomposition experiment, calculated as the NMR-derived alkyl-C area (-10 to 45 ppm) divided by the summed integral of n-alkyl + O-alkyl + di-O-alkyl (45 to 110 ppm) for A.) litter from 10-year-old secondary forests and B.) litter from 40-year-old secondary forests. Bars represent standard error per forest type and decomposition environment per time point (n = 2-6). Note that some points do not have error bars due to low replication (n = 1).

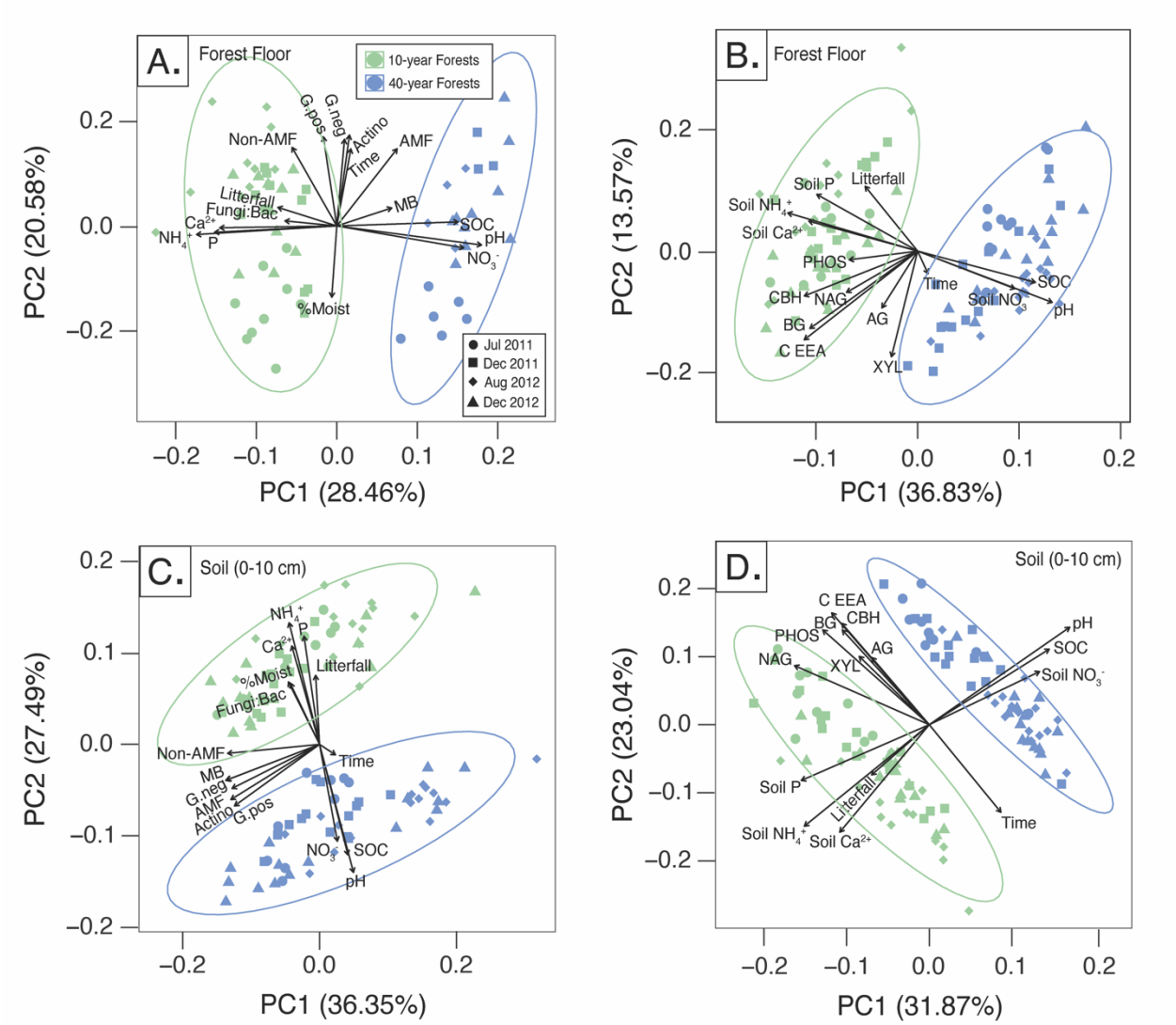

Figure 10: Principal component analysis biplots showing clustering between 10-year- and 40-year-old forests and soil characteristics for A.) forest floor phospholipid fatty acid (PLFA) guilds B.) forest floor extracellular enzyme activities (EEA), C.) soil PLFA, and D.) soil EEA. Colors correspond to successional forest ages and shapes correspond to collection dates (time in the field).

Soil Characteristic	10-year Successional Forests	40-year Successional Forests
Bulk density (g cm^{-3})	1.08 ± 0.04	1.11 ± 0.01
Soil moisture (%)	36.19 ± 1.80	33.61 ± 3.15
pH*	6.77 ± 0.02	7.03 ± 0.01
Organic C (%)*	3.12 ± 0.08	4.27 ± 0.19
NH_4^+ (mg Kg^{-1})*	17.16 ± 0.65	9.23 ± 0.36
NO_3^- (mg Kg^{-1})*	15.02 ± 1.85	21.09 ± 1.06
Available P (mg Kg^{-1})*	3.31 ± 0.38	1.68 ± 0.06
Ca content (mg Kg^{-1})*	11,126.29 ± 742.19	6,781.78 ± 160.25

Table 1: Summary of soil physicochemical characteristics between 10-year vs. 40-year successional forests. Data are presented as averages ± indicates one standard error (n = 3 per forest type). Asterisks represent variables that differ significantly between forest ages ($p \leq 0.05$).

Band Assignment	Drift Wavelength (cm^{-1})	Index Assignment	Carbon Functional Group
B1	3400	Index II	υ(N-H), υ(O-H)
B2	2924	Index I and II	aliphatic υ_{as}(C-H)
B3	2850	Index I and II	aliphatic υ_{as}(C-H)
B4	1650	Index I and II	aromatic υ(C = C)
B5	1575	-	amide δ(N-H) and υ(C-N)
B6	1470	Index I and II	aliphatic δ(C-H)
B7	1405	Index I and II	aliphatic δ(C-H)
B8	1390	-	aliphatic δ(C-H), potential contributions from carboxylate υ_s(C-O)
B9	1270	Index II	phenol υ_{as}(C-O), carboxylic acid υ(C-O)
B10	1110	Index II	polysaccharide υ_s(C-O)
B11	1080	Index II	polysaccharide υ_s(C-O)
B12	920	Index I and II	aromatic δ(C-H)
B13	840	Index I and II	aromatic δ(C-H), less substituted

Table 2: Functional group assignments for 13 bands used to evaluate changes in DRIFT spectra of leaf litter over time between forest types and decomposition environments. Based on Margenot et al. (2015). υ, stretching vibration; υ_{as}, asymmetric stretching vibration; υ_s, symmetric stretching vibration; δ, bending vibration

Decomposition Material	Forest Age	Decomposition Experiment	k-value (yr^{-1})	Total Mass Lost (%)	Final C:N Ratio
Litter	10-year	In-situ	2.08 ± 0.18 b	92.46 ± 1.61	10.38 ± 0.50 a
		Transplanted	2.28 ± 0.27 b	89.68 ± 2.64	11.70 ± 0.47 a
	40-year	In-situ	1.15 ± 0.15 a	82.03 ± 5.01	16.97 ± 0.96 b
		Transplanted	1.13 ± 0.13 a	86.11 ± 3.93	14.48 ± 0.96 b
Roots	10-year	In-situ	0.71 ± 0.10	77.93 ± 3.30 b	22.67 ± 0.71
		Transplanted	0.66 ± 0.09	77.60 ± 3.29 b	21.72 ± 1.95
	40-year	In-situ	0.53 ± 0.11	63.95 ± 2.75 a	23.55 ± 0.88
		Transplanted	0.44 ± 0.06	64.15 ± 1.14 a	22.89 ± 0.98

Table 3: Summary of litter decomposition decay rates, mass lost, mean residence time, and final C:N ratios between secondary forest types and decomposition experiments. Values represent means ± one standard error (n = 6 per each decomposition experiment per forest type). Lower case letters represent Tukey's HSD post-hoc test, indicating significant differences between decomposition environments and successional forest age, but not between decomposition materials ($p \leq 0.05$).

Response Variables		Forest Floor			Soil (0-10 cm)		
		Time	Forest Age	Time x Forest Age	Time	Forest Age	Time x Forest Age
PLFA Biomass	Total Microbial PLFA	**0.0006**	0.6890	0.8331	**< 0.0001**	0.7358	0.1353
	Fungal PLFA	0.1850	0.2239	0.6847	**0.0004**	0.3276	0.5299
	Bacterial PLFA	**0.0002**	**0.0425**	**0.0015**	**< 0.0001**	0.8013	**0.0049**
	Fungi-to-Bacteria	0.7021	0.3474	0.2706	0.6249	0.0708*	0.7452
PLFA Community	Actinobacterira	**0.0004**	0.3738	0.6517	**< 0.0001**	0.1347	**0.0012**
	G.neg	**0.0053**	0.0775*	**0.0076**	**< 0.0001**	0.2830	0.2410
	G.pos	**0.0004**	0.6360	0.1028	**< 0.0001**	0.3709	**0.0003**
	AMF	0.1650	0.0737*	0.3254	**0.0004**	0.6556	0.1997
	Non-AMF	0.2764	0.2646	0.7566	**0.0005**	0.1481	0.5905
Extracellular Enzyme Activities	BG	0.2420	0.0661*	0.8178	**0.0032**	0.8001	0.9232
	AG	**0.0115**	0.667	**0.0369**	**< 0.0001**	0.9997	0.6019
	XYL	0.2482	0.9717	**0.0118**	**< 0.0001**	0.4121	0.1018
	CBH	0.2207	**0.0257**	**0.0563**	**< 0.0001**	0.6393	0.1081
	Total C EEA	0.2494	**0.0426**	0.5491	**0.0056**	0.7560	0.9649
	NAG	**0.0396**	0.0911	0.1882	**0.0250**	0.1451	0.4836
	PHOS	**0.0336**	0.1999	0.4400	**0.0012**	0.6348	0.2516
	Total EEA	0.3076	**0.0395**	0.3261	**0.0007**	0.3378	0.6318

Table 4: Linear mixed model results for microbial phospholipid fatty acid guilds and extracellular enzyme activities testing significant effects of time, forest age, and time x forest age. Significant p-values are bolded ($p < 0.05$) and marginal p-values include asterisks ($p = 0.06$-0.10).

SUPPLMENTARY FIGURES AND TABLES

DRIFT Band	Loading Score			Contribution to %Variance		
	PC1	PC2	PC3	PC1	PC2	PC3
Time	-0.22	-0.33	-0.10	4.77	11.18	1.07
B1	-0.28	0.21	-0.15	7.80	4.34	2.11
B2	-0.14	0.49	0.43	1.96	24.06	18.37
B3	-0.14	0.49	0.42	1.85	24.27	17.56
B4	-0.29	-0.25	-0.25	8.17	6.25	2.62
B5	-0.28	-0.25	-0.25	8.01	6.22	4.71
B6	-0.30	-0.10	-0.10	9.01	0.92	0.14
B7	-0.32	-0.07	-0.07	10.44	0.53	0.80
B8	-0.33	0.02	0.02	10.59	0.05	0.19
B9	-0.27	0.34	0.34	7.15	5.58	7.98
B10	-0.27	0.12	0.23	7.04	5.30	20.83
B11	-0.27	0.19	0.19	7.55	3.43	17.72
B12	-0.27	-0.22	-0.22	7.25	4.89	2.85
B13	-0.29	-0.17	-0.17	8.34	2.99	3.08

Table S1: Principal component analysis loadings and contributions to variance for all DRIFT spectral bands for PC1-PC3.

Forest Age	Treatment	Time	NMR Region Areas				Molecular Mixing Model Proportion Contributions					
			Alkyl-C	O-alkyl-C	Aromatic	Carboxyl-C	Carbohydrate	Protein	Lignin	Lipid	Carbonyl	Char
10-yr	Initial	0	22.58	55.84	13.65	8.04	32.07	14.53	13.25	21.16	9.26	9.60
	In-situ	71	21.51 ± 1.36	49.68 ± 4.22	16.34 ± 0.92	12.26 ± 1.85	28.63 ± 3.21	15.39 ± 1.11	13.38 ± 0.04	21.47 ± 1.09	10.75 ± 1.74	10.43 ± 0.37
		153	22.35 ± 1.21	49.93 ± 2.32	14.46 ± 1.19	13.03 ± 0.92	28.46 ± 1.70	15.99 ± 0.34	13.09 ± 0.14	22.07 ± 0.54	11.66 ± 0.42	9.63 ± 0.59
		312	16.14 ± 1.46	54.22 ± 1.48	16.14 ± 1.86	11.10 ± 0.28	31.87 ± 1.15	13.96 ± 0.51	13.46 ± 0.26	18.67 ± 0.79	10.09 ± 0.28	10.30 ± 0.81
		688	19.68 ± 0.46	51.86 ± 1.16	16.29 ± 0.94	12.02 ± 0.85	28.91 ± 0.85	14.95 ± 0.24	13.50 ± 0.17	19.72 ± 0.44	11.18 ± 0.54	10.73 ± 0.49
	Transplant	71	20.34 ± 2.11	51.90 ± 3.36	16.12 ± 0.53	11.46 ± 1.21	29.61 ± 2.65	14.98 ± 1.02	13.41 ± 0.09	20.67 ± 1.49	10.31 ± 0.92	10.31 ± 0.33
		153	14.00	56.72	18.32	10.73	33.83	12.61	13.73	16.52	9.00	10.88
		312	24.11 ± 3.00	50.40 ± 1.82	16.70 ± 1.37	8.74 ± 3.23	27.45 ± 0.65	15.53 ± 0.24	13.74 ± 0.04	22.23 ± 1.84	9.09 ± 1.93	11.13 ± 0.53
		688	19.63 ± 0.96	48.91 ± 0.88	18.88 ± 0.45	12.34 ± 0.22	27.02 ± 0.70	15.17 ± 0.39	13.79 ± 0.10	20.05 ± 0.78	10.88 ± 0.26	11.60 ± 0.23
40-yr	Initial	0	23.35 ± 0.31	49.51 ± 1.82	20.10 ± 2.63	7.04 ± 0.97	29.36 ± 1.81	14.36 ± 0.05	14.11 ± 0.38	22.34 ± 0.06	7.02 ± 0.65	12.02 ± 1.04
	In-situ	71	22.31	48.00	22.38	7.49	28.15	14.05	14.45	21.40	6.95	13.08
		312	22.43 ± 2.60	46.62 ± 0.42	20.51 ± 2.44	10.41 ± 0.26	26.39 ± 0.52	15.21 ± 0.98	14.12 ± 0.31	21.65 ± 1.78	9.71 ± 0.29	12.64 ± 0.92
		688	23.97 ± 0.99	44.09 ± 0.66	19.52 ± 0.28	12.41 ± 0.17	24.05 ± 0.36	16.42 ± 0.10	13.89 ± 0.03	22.90 ± 0.19	11.05 ± 0.00	12.22 ± 0.07
	Transplant	71	21.96 ± 1.08	50.87 ± 0.11	18.25 ± 0.47	8.85 ± 1.60	30.68 ± 0.39	14.19 ± 0.09	13.81 ± 0.17	21.53 ± 0.25	7.44 ± 0.79	11.08 ± 0.38
		153	24.91 ± 1.67	45.86 ± 1.19	19.88 ± 1.88	9.23 ± 1.38	26.29 ± 0.66	15.67 ± 0.98	13.99 ± 0.42	23.76 ± 1.63	8.38 ± 1.01	11.97 ± 0.98
		312	24.68 ± 1.70	48.48 ± 2.61	18.24 ± 2.98	8.62 ± 1.07	25.86 ± 0.98	15.77 ± 0.53	13.95 ± 0.38	22.70 ± 1.06	8.97 ± 0.31	11.84 ± 1.36
		688	20.94 ± 0.42	44.16 ± 3.17	22.43 ± 2.26	12.44 ± 1.30	24.51 ± 1.87	15.25 ± 0.11	14.36 ± 0.22	20.53 ± 0.27	10.92 ± 1.09	13.64 ± 1.07

Table S2: Mean NMR region area values for components used in molecular mixing model and proportion contributions of different C compounds to overall litter composition between forest types and decomposition environments. Contributions were determined using a 6-component molecular mixing model. Values represent means ± one standard error (n = 2-6). Note some samples show no standard error due to limited replication (n = 1).

Variable	Loading score			Contribution to %Variance		
	PC1	PC2	PC3	PC1	PC2	PC3
Time	0.04	0.35	-0.24	0.17	12.25	5.99
Litterfall	-0.17	0.08	0.02	3.01	0.71	0.05
SOC	0.36	0.02	-0.03	12.73	0.03	0.11
Ammonium	-0.41	-0.04	0.17	17.02	0.15	2.75
Nitrate	0.37	-0.10	0.13	13.96	0.99	1.80
Phosphorus	-0.36	-0.03	0.01	12.93	0.08	0.00
Calcium	-0.34	-0.01	0.27	11.85	0.01	7.25
pH	0.43	-0.09	-0.06	18.14	0.75	0.39
%Moist	-0.02	-0.32	0.25	0.02	10.46	6.46
AMF	0.18	0.35	0.21	3.15	12.27	4.27
Non-AMF	-0.13	0.36	-0.40	1.73	12.70	15.71
Fungi:Bact	-0.15	0.02	-0.60	2.33	0.05	35.40
G.neg	0.02	0.39	0.23	0.05	15.46	5.18
G.pos	-0.04	0.41	0.38	0.16	16.38	14.25
Actino	0.04	0.41	0.06	0.14	17.03	0.37
PLFA Biomass	0.16	0.08	-0.02	2.62	0.67	0.02

Table S3: Principal component analysis loadings and contributions to variance for forest floor phospholipid fatty acid biomass and soil characteristics for PC1-PC3.

Variable	Loading score			Contribution to %Variance		
	PC1	PC2	PC3	PC1	PC2	PC3
Time	0.03	-0.10	-0.66	0.08	1.08	43.54
Litterfall	-0.15	0.30	0.10	2.09	8.87	0.91
SOC	0.32	-0.14	0.01	10.49	2.07	0.02
Ammonium	-0.36	0.18	-0.20	12.76	3.08	4.11
Nitrate	0.27	-0.18	-0.10	7.15	2.98	1.02
Phosphorus	-0.28	0.26	0.01	7.70	6.79	0.02
Calcium	-0.30	0.14	-0.34	8.97	1.90	11.31
pH	0.37	-0.34	0.13	13.70	5.56	1.71
NAG	-0.20	-0.19	0.16	3.82	3.61	2.52
PHOS	-0.19	-0.03	0.55	3.54	0.16	30.51
BG	-0.30	-0.36	0.04	8.79	12.66	0.19
AG	-0.10	-0.26	-0.14	0.97	6.81	1.82
CBH	-0.31	-0.21	0.08	9.72	4.25	0.68
XYL	-0.07	-0.49	-0.12	0.52	23.58	1.54
Total C EEA	-0.31	-0.41	0.03	9.73	16.62	0.12

Table S4: Principal component analysis loadings and contributions to variance for forest floor extracellular enzyme activity and soil characteristics for PC1-PC3.

Variable	Loading score			Contribution to %Variance		
	PC1	PC2	PC3	PC1	PC2	PC3
Time	0.07	-0.03	-0.72	0.46	0.11	52.33
Litterfall	-0.02	0.22	0.22	0..03	4.86	4.60
SOC	0.12	-0.35	0.03	1.47	12.45	0.10
Ammonium	-0.13	0.39	-0.05	1.68	15.09	0.26
Nitrate	0.08	-0.31	0.07	0.58	9.56	0.48
Phosphorus	-0.07	0.35	-0.09	0.44	11.93	0.75
Calcium	-0.12	0.31	-0.06	1.40	9.87	0.30
pH	0.15	-0.41	0.08	2.10	16.71	0.71
%Moist	-0.13	0.20	0.55	1.78	3.98	30.17
AMF	-0.37	-0.14	-0.11	13.94	1.98	1.17
Non-AMF	-0.39	-0.03	-0.08	15.37	0.08	0.69
Fungi:Bact	-0.15	0.20	-0.29	1.56	3.80	8.11
G.neg	0.40	-0.11	0.05	15.72	1.29	0.24
G.pos	0.38	-0.18	0.1	14.31	3.10	0.01
Actino	-0.36	-0.19	0.02	13.17	3.86	0.04
PLFA Biomass	-0.40	-0.12	-0.18	16.01	1.33	0.03

Table S5: Principal component analysis loadings and contributions to variance for soil phospholipid fatty acid biomass and soil characteristics for PC1-PC3.

Variable	Loading score			Contribution to %Variance		
	PC1	PC2	PC3	PC1	PC2	PC3
Time	0.18	-0.27	-0.02	3.27	7.20	0.02
Litterfall	-0.15	-0.15	-0.10	2.11	2.37	1.05
SOC	0.30	0.23	0.05	9.16	5.36	0.26
Ammonium	-0.31	-0.31	-0.02	9.94	9.68	0.05
Nitrate	0.28	0.16	-0.03	7.74	2.65	0.01
Phosphorus	-0.32	-0.17	0.01	10.41	2.91	0.01
Calcium	-0.23	-0.33	0.00	5.10	10.85	0.00
pH	0.36	0.30	0.01	12.56	8.91	0.02
NAG	-0.34	0.18	0.29	11.55	3.27	8.32
PHOS	-0.27	0.29	-0.04	7.22	8.43	0.18
BG	-0.22	0.29	0.43	4.80	8.40	0.18
AG	-0.15	0.21	-0.51	2.10	4.23	0.26
CBH	-0.22	0.31	-0.30	4.87	9.63	8.76
XYL	-0.18	0.21	-0.52	3.12	4.38	0.27
Total C EEA	-0.25	-0.25	0.31	6.05	11.73	9.70

Table S6: Principal component analysis loadings and contributions to variance for soil extracellular enzyme activity and soil characteristics for PC1-PC3.

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CONCLUSIONS AND FUTURE DIRECTIONS

Our study investigated how disturbance history affects ecosystem processes and biogeochemical cycling in forest systems, including genetically diverse experimental aspen stands in southern Wisconsin and successional forests of different ages in St. Croix in the U.S. Virgin Islands. This research has contributed to an emerging body of literature assessing the potential importance of intraspecific competition and genetic diversity on litter decomposition and soil C dynamics, and how subtropical land use change and forest succession affect microbial activity and litter decomposition dynamics. This dissertation research highlights the importance of disturbance history exerts on ecological interactions among and within tree species and the implications those interactions have on biogeochemical cycling.

Past work showed high variability in genotypic composition among the experimental aspen stands studied in this research, where slow growing genotypes were out competed in undisturbed stands by more competitive genotypes. Disturbed stands exhibited less variable and marginally greater stand-level genetic diversity than undisturbed stands, due to lower competitive pressures and lower tree density. Given that litter derived from disturbed stands had significantly lower litter turnover times when it decomposed *in-situ* vs. in a common garden, disturbance history, genetic diversity appears to impart a legacy on litter decomposition dynamics when decomposing in a foreign environment. We found no differences in litter turnover between disturbed and undisturbed stands for the *in-situ* experiment, but collectively, litter turnover times were negatively correlated with litter chemistry. Based on lower litter turnover times for litter samples decomposing *in-situ* vs. in a common garden, we conclude that site-specific variations in microenvironmental conditions and microbial and detritivore

communities also influence decomposition dynamics for aspen litter mixtures derived from multiple genotypes.

Disturbance-induced changes to tree density among the experimental aspen stands had significant interactions with stand-level characteristics on predicting soil fraction C contributions, particularly for macroaggregate and mineral-associated fractions. Using a new technique for quantifying aggregate disintegration rates using laser diffraction, we found that litter decomposition, litter chemistry, and stand-level genetic diversity have significant relationships with aggregate decay rates. This is the first study to our knowledge that demonstrates higher plant genetic diversity and litter condensed tannin concentration both correlate with lower rates of aggregate disintegration. Although relative fraction C stock contributions to bulk soil C, mineral-associated C content, and clay mineralogy at our sites are conducive for future soil C accrual, future climate warming and increased atmospheric CO_2 concentrations will likely accelerate soil C cycling and limit soil C accumulation, which has implications for natural aspen forests characterized by high genetic diversity and intraspecific.

Forest composition and species diversity different significantly between successional forests in our St. Croix study. 10-year-old successional forests were dominated exclusively by an early successional nitrogen-fixing tree species with low litter C:N ratios, whereas the 40-year-old successional forests consisted of numerous tree species. Although litter derived from 10-year-old forests decomposed faster than litter from 40-year-old forests regardless of decomposition environment, the chemical trajectory of litter was varied between decomposition environments for 40-year-old litter, suggesting that litter mixture diversity affects microbial litter processing. Despite no differences in root chemistry between successional forests, roots from 10-year-old

forest lost a greater percentage of their original mass than roots from 40-year-old forests, indicating that root traits and morphology might influence root decomposition in these systems.

Incorporating DNA sequencing and monitoring how changes in bacterial and fungal communities correspond to changes in litter chemistry during decomposition would provide insight into how variations in stand-level genetic diversity and litter chemistry affect microbial decomposer communities. This approach would better inform how disturbance history and intraspecific plant competition affect the ecological selection of microbes and microbial adaptation to litter sources that are not traditionally favorable for microbes to decompose. Radiocarbon modeling of soil C fractions would help constrain C turnover time and identify current long-term reservoirs in our experimental aspen stands. These estimated ages between soil C fractions could be used to parameterize future soil C-climate models and help inform future management strategies of natural forests that display high genetic diversity. Lastly, there needs to be more biogeochemical research conducted in tropical and subtropical successional forests with long and complex histories of land use change, particularly in regions that are currently undergoing rapid development and habitat fragmentation.