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Wood decomposition in a warmer world

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To the Graduate Council:

I am submitting herewith a dissertation written by Emily Elizabeth Austin entitled "Wood decomposition in a warmer world." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Aimée T. Classen, Major Professor

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Wood decomposition in a warmer world

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Dedication

To Susan Elizabeth Morse

and Mary Elizabeth Stevenson

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Abstract

Climatic warming is altering species distributions and ecosystem functions across the globe. Wood is an important carbon pool and the fungal communities in wood are relatively simple compared to those in soil. These factors make decomposing wood an ideal system for exploring the influence of decomposer community on the response of decomposition to warming. My research has focused on the effects of warming wood decomposition rates and wood decomposing communities. Using field and lab- based manipulative experiments and field observations I explore the influence of tree species, wood decomposition stage, geography and warming on fungal community structure and activity. In chapter one I observe differences in the fungal community structure and potential activity between wood from *Pinus taeda* and *Liquidambar styraciflua* logs. In chapter two I observe changes in fungal and bacterial abundance, fungal community structure, and decomposition rate across logs in different stages of decomposition, but there is no interaction between decomposition stage and warming on respiration rates. In chapter three I observe differences in the decomposer community in *Acer rubrum* wood at two sites and differences in the response of wood decomposition to warming. My work shows that the effects of warming on decomposition rate and carbon flux from decomposing wood can vary by site and between wood types and suggest

more work is needed to understand the role of decomposer communities in altering the response of wood decomposition to warming.

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Introduction

"What structures community composition?" is a fundamental question that has been addressed in numerous macro-ecological studies, but remains less understood in microbial ecology. Microorganisms play a major role in decomposition and nutrient cycling, and the structure of microbial communities can influence important ecosystem functions such as decomposition. Changes in climate can have direct affects on ecosystem functions, and indirectly alter ecosystem functions through changes in microbial community structure and abundance. Climatic warming directly increases decomposition rate, and carbon dioxide, an important greenhouse gas, is a product of decomposition. Thus increasing decomposition rates with warming climate can lead to a positive feedback. However, we don't know how the effects of climate on microbial communities will indirectly affect ecosystem functions such as decomposition.

Microbial communities are structured by abiotic factors such as climate and substrate quality as well as by interactions with plants, soil organisms or other microbes. The factors structuring microbial communities are often assumed to be the same as those structuring plant and animal communities. However, recent advances in microbial ecology have had some surprising results. For example, soil bacterial communities are globally structured by pH

rather than by temperature, latitude or elevation as observed in plant communities.

As the global climate is changing there will be direct effects of climate on decomposition rates as well as indirect effects via changes in the wood environment or the decomposer communities (Figure 1)

Fungi play a major role in decomposition; fungi are the main drivers of soil organic matter decomposition (Yuste, 2011) and the only organisms with lignocellulasic enzymes needed to decompose wood.

Wood is a major carbon pool, comprising up to 20% of total carbon in forests (Harmon) and more than 90% of aboveground carbon (Cooke and Rayner). Wood maintains a large role in the carbon cycle in part because of its slow decomposition rate. Low chemical quality, recalcitrant compounds, and large volume all contribute to the slow decomposition rate of woody debris. The functional component of living woody material (and the nutrient rich portion) is in the cambium, a thin outer layer around the trunk and branches leading up to the leaves. In part because it is accessible, in part because of the high nutrient quality, the cambium is colonized first by "sugar fungi" which consume the nutrient rich portion leaving behind the inner wood. The inner material of woody trunks and branches is purely structural and made of low- nutrient compounds with strong chemical bonds such as lignocellulose. Bacteria and fungi require nitrogen to build enzymes for decomposition, however the high

C: N ratio in most of wood limits decomposition. Cord forming fungi must import nitrogen from the surrounding environment in order to decompose the structural components of wood such as lignocellulose.

Fungal community interactions can influence the composition of wood decomposing fungi. Once established, the fungi have competitive and combative interactions that influence the community structure in a given piece of wood (L Boddy, 2000). The species of fungi that arrives first can influence the fungal community structure and later the activity rate of that community Research indicates that the composition of fungal communities responsible for wood decomposition is a function of substrate quality, climate and species interactions. In addition to altering the climate, climatic changes may cause shifts in plant species composition (Breshears et al. 2005), which will cause changes in substrate quality (C:N ratio, water holding capacity, lignin content) and microclimate. Basidiomycetes, a major group of wood decomposing fungi, have an optimal growth rate between 25-30 degrees C and optimal moisture content of 30-250% dry mass.

These changes will likely result in altered fungal community composition and thus shifts in the rate of fungal activity (Waldrop et al., 1999) (e.g. wood decomposition), a key component of the global C cycle. Wood is often thought of as a C sink because it has a high concentration of recalcitrant lignin; however, white-rot fungi exude enzymes that are able to break down lignin and

cellulose, making wood a C source as well. *Understanding wood degrading fungal community response to changes in substrate and climate will enable scientists to better predict how climate change will alter the global C cycle.*

My dissertation will address: How do changes in climate and substrate quality alter fungal community composition and what are the impacts of those shifts on decomposition rates? Specifically, my work will test the following hypotheses: (i) Changes in wood quality will alter fungal activity; (ii) Fungal abundance, diversity, and activity will increase with temperature but be constrained by moisture; and (iii) Warming and substrate quality will interact in their effect on fungal activity with greater responses to temperature in higher quality substrates.

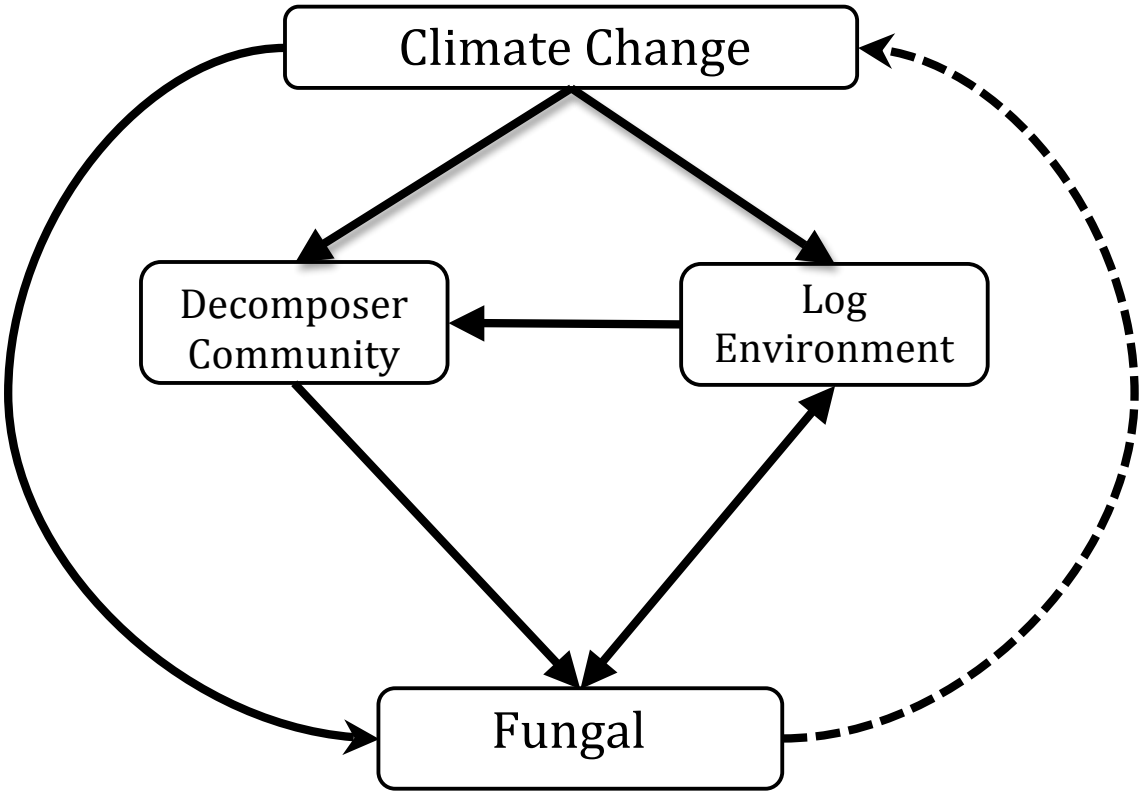

Figure 1: A schematic diagram representing the research areas addressed in my dissertation. The effect of log environment on fungi and fungal activity are addressed in chapters one, where the log species is manipulated and in chapter two where the log age is manipulated. The effects of climate on fungal activity directly and indirectly via changes in fungi is addressed in chapters two and three and the indirect effects of climate on fungal activity via changes in the log environment is addressed in chapter three.

Chapter I: Contrasting patterns of functional and phylogenetic fungal diversity in decomposing logs of Loblolly Pine (*Pinus taeda)* and Sweetgum (*Liquidambar styraciflua)*

Abstract

Emerging molecular community ecology techniques present opportunities to investigate how microbial community composition is related to function. Here, we measured loblolly pine and sweetgum fungal communities in decomposing logs to see if changes in community structure were related to changes in function. We analyzed the diversity of LSU fungal ribosomal RNA (rRNA) genes and a functional gene (CBH-1) coding cellobiohydrolase, an enzyme important to wood decomposition and carbon cycling, using clone library sequencing and quantitative PCR. We found differences in fungal community characteristics between the tree species. However, these differences were not driven by changes in fungal richness but by shifts in individual fungi occurring in the two tree species and their functional gene repertoires. Overall, there was greater richness of the CBH-1 functional genes than of rRNA genes across all the logs evaluated. This pattern is consistent with the dominant species of wood decomposing fungi present containing multiple orthologous cellobiohydrolase functional gene compliments. Sweetgum logs had a much higher fungal abundance (rRNA gene copy number) than pine logs, and a higher richness of CBH-1 gene types. Our molecular ecology study supports the findings of previous fungal fruiting body surveys that cataloged contrasting communities in these two host tree species. Together, these results illustrate

that the simple fungal communities in decomposing wood with low phylogenetic richness and highly variable community composition may make excellent model systems for addressing fundamental structure/function relationships in natural systems.

Key words: fungi; community structure; wood; decomposition; cellobiohydrolase

Introduction

Understanding what structures communities has been a fundamental question in ecology; however, until the last decade it has been less explored in microbial communities due to limitations in our ability to accurately identify and quantify species occurrence patterns. These limitations arose primarily because fungal fruiting structures, which are easy to see, do not reflect the naturally occurring diversity in fungal communities (Horton & Bruns, 2001), and our ability to culture and identify microorganisms (bacteria, archaea and fungi) in many habitats was limited by inadequate methods (Schadt *et al.*, 2003; Dawson & Pace, 2002). New developments in molecular microbial ecology show that the distribution of microbial communities, such as bacteria, are best described by soil pH and not factors such as temperature and precipitation that best describe the distributions of plants and animals (Rousk *et al.*, 2010; Fierer & Jackson, 2006). It is possible that wood degrading fungi, which are important regulators of the carbon cycle, might also be structured by wood properties and not by overall climate (Ovaskainen *et al.*, 2010). Even in the low richness fungal communities like those of wood degrading fungi, questions such as "What determines the most dominant fungal species?" and "Do wood species or physical and chemical characteristics predict fungal species composition?" are

still mostly unknown (but see Fukami *et al.*, 2010 and Boddy, 1983 as notable exceptions).

What fungi contribute to wood-degrading fungal community structure has been debated, with some studies suggesting ascomycetes are the most dominant community member (Kulhánková & Béguiristain, 2006), while others argue basidiomycetes are the most dominant community member (Nordén *et al.*, 2004). The variation in these results might arise, in part, because there is variation in the methods used to describe fungal communities. The ability to culture some but not all fungal species as well as things like seasonal differences in fructification patterns have contributed to some of the confusion of what species are responsible for wood degradation in forested ecosystems. For example, fruiting body surveys may be biased towards basidiomycotous fungi, as most large mushrooms are basidiomycetes. While most research agrees that basidiomycetes are more host-specific than ascomycetes in decomposing wood, recent studies suggest that geography may also play strong roles in determining fungal community composition in wood (Jönsson *et al.*, 2008; Kulhánková & Béguiristain, 2006; Ferrer & Gilbert, 2003).

Many wood decomposing fungi colonize new logs by forming hyphal cords that grow through the soil in search of new substrate (Lamour *et al.*, 2007). After dispersal, strong competitive interactions work to determine the fungal community in a given log. This may lead to more similar fungal communities in

logs that are closer together in space. As an established fungal species grows to dominate a log, the extra resources gained may give the more dominant fungus an advantage with an ability to grow longer cords and discover resources first. Strong competition for space and resources in wood gives an advantage to the earliest establishing organisms. Priority effects are thus though to be paramount, because what fungus arrives first on a log may dominate and structure the simple community of that log for long periods (Weslien *et al.*, 2011; Boddy, 2000). This may have important subsequent consequences for the function of the community, namely the rate and mode of decomposition process.

Here, we present an initial study to explore how decomposing communities vary in composition and function in logs of different tree species with distinct wood traits. Loblolly pine (*Pinus teada*) and Sweetgum (*Liquidambar styraciflua*) logs were selected in monoculture sites as well as in a site where both species co-occur in the Ridge and Valley Provence of the Tennessee Valley. The southeastern U.S. hosts diverse tree species and a diverse mycota of lignocolous fungi because it is the northern range limit for many tropical and subtropical species and is the southern range limit for many temperate species. Additionally, the wood degrading fungal communities of these tree species have been extensively cataloged through fruiting body surveys and culture based efforts (Nakasone, 1996). Using clone library sequencing and quantitative PCR

of the large subunit and small subunit of the ribosomal RNA (rRNA) gene, we assess fungal community structure and abundance in each log. To assess function of the communities, we used clone library sequencing of the CBH-I gene family (coding for cellobiohydrolases). Specifically, we ask: (1) does the richness, community structure, or abundance of fungi differ between wood substrate and among sites? (2) How does our molecular survey compare with previous culture and fruiting body efforts in these tree species? (3) Does the variation in sequences of the functional CBH-I gene family reflect the observed variation in fungal community structure represented by universal fungal phylogenetic markers such as the large subunit section of the rRNA gene?

Methods

Site descriptions and experimental design

In 2009, we selected three sites located at the National Environmental Research Park at Oak Ridge National Laboratory in Oak Ridge, TN (35°58'N, 84°18'W), no permits or special permission is required for researchers at Oak Ridge National Lab. One site was a unmanaged monoculture stand of mature loblolly pine *(Pinus taeda)* that was highly affected by bark beetle outbreaks in the late 1990s and early 2000s, one site was a sweetgum *(Liquidambar styraciflua)* stand planted in 1988, and one site was a stand of loblolly pine and sweetgum growing intermixed in a plantation dating from ~1980 (here after "common

site"). Each site occurred within a 5-mile radius and has not been actively managed for weed control or thinned in the previous decade. These former plantation/monoculture sites were selected so we could easily identify the species of wood chosen for the experiment. We selected several logs of similar size and decomposition stage from each site, and collected wood samples of ~2 to 5 grams using an increment borer that was wiped with 70% EtOH between sampling events to prevent cross-contamination. DNA was successfully extracted from one pine log at the pine only site, one sweetgum log at the sweetgum only site, and from two pine logs and two sweetgum logs at the common site (for a total of 3 pine and 3 sweetgum logs across three sites).

Molecular Methods and Analyses

To extract DNA from the logs, we used the Powersoil 96-well DNA isolation kit MoBio Labaratories (Carlesbad,CA) with the addition of freezing samples in liquid N and grinding in a mortor and pestle prior to carrying out the bead beating step using a Retch Mixer Mill Model MM400 for 20 min on the highest speed setting (20). To assess DNA concentration and quality and confirm our successful extraction from the logs we used a ND-1000 spectrophotometer at 230, 260, and 280 nm (Nanodrop Technologies, Wilmington, DE). Initially, the study included several logs from each site that were unfortunately consumed in unsuccessful attempts to extract DNA using MoBio protocols without the liquid

N additions and bead beating modifications. Thus, we were only able to extract DNA from three loblolly and three sweegum logs limiting the inferences achievable here.

To examine fungal phylogenetic and functional community structure we created clone sequence libraries of the D1/D2 section of the 28S (LSU) rRNA gene and the CBH-I gene respectively. We used the following reaction mixtures to amplify the 28S rRNA gene: 2 µl 10x PCR buffer, 2.2 µl 25 mM $MgCl_2$, 2.5 µl 10 mg ml^{-1} BSA, 1.6 µl 25mM dNTPs, 1 µl 10pmol μl^{-1} each primer LROR (5'-AGA GTT TGA TCC TGG CTC AG -3') and LR5 (5'-GGT TAC CTT GTT ACG ACT T-3') (Hopple & Vilgalys, 1994), 1 µl of undiluted wood DNA solution, 0.6 µl Taq-polymerase and H_2O to a final volume of 20 µl (10.1µl) and amplified under the following conditions: 95°C for 2 min followed by 35 cycles of 94°C for 45 seconds, 57.5°C for 50 seconds and 72°C for 105 seconds (increasing 1 second with each cycle), and final extension step at 72°C for 5 min in GeneAmp PCR system 9700 thermocyclers (PE Applied Biosystems). The reaction mixture for amplification of the CBH-I gene included 4 µl 10x PCR buffer, 1.2 µl 25mM $MgCl_2$, 1 µl 10 mg ml^{-1} BSA, 1 µl 10pmol μl^{-1} each primer, fungcbh1F (5' ACC AA[C,T] TGC TA[C,T] ACI [A,G]G[C,T] AA 3') and fungcbh1R (5' GC[C,T] TCC CAI AT[A,G] TCC ATC 3') (Edwards *et al.*, 2008), 1.6 µl 25 mM dNTPs, 0.6 µl Taq-polymerase, 2 µl wood DNA solution, and water to a final volume of 20 µl then amplified under the following

conditions: 94°C for three minutes followed by 35 cycles of 94°C for 30 seconds, 42.5°C for 45 seconds, 72°C for 60 seconds, and a final extension step at 72°C for 15 minutes. Positive control (DNA extracted from *Phanerocheate chrysosporium*) and negative controls (sterile filtered water) were used in all PCR reactions.

To confirm successful amplification we used electrophoresis in 1.5% agarose in Tris-acetate EDTA buffer. To continue with cloning and sequencing, we extracted PCR products from the agarose gel using a Qiagen Gel purification kit (Qiagen). We cloned the PCR product (1 µl, and 4 µl reactions) using the TOPO-TA PCR 2.1 cloning kit (Invitrogen Corporation). Salt solution was omitted from the reaction and ligation at room temperature was extended to 30 min. White *E. coli* colonies were picked from agar plates and amplified using the Illustra Templiphi DNA amplification kit according to the kit protocol except using ½ the volume of dilution buffer and premix in 11µl reactions.

To sequence and identify the sample DNA we performed cycle sequencing reactions using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA), the LROF primer and the following master mix: 1µl 5x sequencing buffer, 1.7µl H_2O, 1µl BigDye, 0.3 µl 20 pmol μl^{-1} primer, and 1 µl DNA template. We ethanol precipitated and re-suspended the product in 10 µl of Hi-Di Formamide (Applied Biosystems) to run on an ABI PRISM 3730 DNA analyzer (Applied Biosystems). We edited sequences in Sequencher program version 4.7

(Gene Codes Corporation) where vector remnants and low quality ends of the sequences were removed.

Sequences were assembled, manually edited, then aligned and trimmed to a common homologous region of approx. 417-574 bp in GENEIOUS program (Biomatters v5.0.4). Short, poor quality or ambiguous sequences were removed and the sequences then de novo clustered within QIIME at 95% identity (Caporaso *et al.*, 2010). We identified fungi in the logs by comparing the representative fungal sequence OTUs clustered above using a Naïve Bayesian classifier (Wang *et al.*, 2007) implemented against the SILVA LSU database (Pruesse *et al.*, 2007) within QIIME and by using a BLAST analysis of top identified hits against the NCBI database by bit score (Altschul *et al.*, 1990). Only classification results at greater than 80% confidence level cutoff are shown. For the additional analyses below, phylogenetic trees were generated using neighbor-joining method in GENEIOUS and bootstrap analysis was performed with 100 replicates. Rarefaction analyses was performed using dotur.exe (Schloss & Handelsman, 2005). Logarithmic rarefaction curves fit to 3% sequence similarity and the equations were extended to 3000 samples where most curves had leveled off. Edited sequences were submitted to GenBank (ACC numbers xxx-xxx, numbers will be added prior to publication). Distance matrices, which we used to conduct P-tests (Martin, 2002) were calculated

between treatments using the Unifrac web application available at http://bmf2.colorado.edu/unifrac (Lozupone & Knight, 2005).

To assess fungal abundance, we used quantitative PCR and rRNA gene copy number as a proxy as described previously (Castro *et al.*, 2010). Briefly we performed qPCR on the SSU rRNA gene using the primers nu-SSU-1196 (3' TCT GGA CCT GGT GAG TTT CC 5'), and nu-SSU-1536 (3' ATT GCA ATG CYC TAT CCC CA 5'). The qPCR reaction included 2 µl BSA, 0.5µl 10 pmol ml^{-1} each primer, 10 µl sybr green supermix (Bio-Rad, Hercules, CA), 1 µl wood DNA template and 6 µl dH_2O. Duplicate technical replicates were included for each sample in the qPCR. We included positive controls and standard curves from a dilution series of *Saccharomyces cerevisiae*, and negative controls of sterile water on each 96 well plate.

We tested fungal and CBH-I richness against a poisson distribution and all other data sets against a normal distribution. Abundance data were log transformed to fit the assumptions of the statistical tests and to standardize variance. We used two way ANOVAs to test for interactions between log type and site in structuring fungal richness, abundance and CBH-I richness. We used one-way ANOVA to test the effects of wood type on fungal abundance and χ^2 to test the effects of wood type on fungal richness and CBH-I gene richness. To determine the effects of wood type and site on fungal community composition, we used PRIMER (Plymouth Marine Laboratory, UK) to conduct

permutational multivariate analysis of variance (PERMANOVA). We performed PERMANOVA tests on Bray Curtis dissimilarity triangular matrices (Bray & Curtis, 1957) generated from relative abundance of OTUs. In order to illustrate the multivariate patterns of species composition, non-metric multidimentional scaling (nMDS) ordinations were performed using the Bray-Curtis dissimilarity triangular matrix.

Results

The fungal communities were unique in each log analyzed in this study, meaning no two clones closely matched the same fungal species among logs. Although within a log as many as 80% of the clones matched a single fungal species. The overall richness in logs varied, with clones matching 2 24 genera and rarefaction curves predicting between 3 and 43 operational taxonomic units (OTUs) with 97% sequence similarity. We did not detect differences in fungal richness between wood types (Figure 2; $\chi^2 = 2.19$, $p = 0.14$) although functional gene richness was higher in sweetgum logs (Figure 2; $\chi^2 = 5.28$, $p = 0.02$). Basidiomycotous rather than ascomoycotous fungi dominated the logs in this study. Basidiomycotous clones represented between 50 100 % of clone libraries for each log, whereas Ascomycota represented less than 50% of clones for each log. Pine logs were largely dominated by *Botryobasidium subconatum* at the pine only site, *Trechispora confinis*, and *Hyphodontia arguta* at the common

site; whereas, sweetgum logs were dominated by *Postia alni* and *Sistotrema biggsiae* at the common site, and *Basidiodendron caesiocinereum* and *Basidiodendron sp* GEL 4674 at the sweetgum only site (Figure 3). Although the logs shared similar richness and were all dominated by basidiomycetes as opposed to ascomycetes, the communities differed between each log.

Fungal community structure differed between sweetgum and loblolly pine wood (UNIFRAC $p = 0.01$, PERMANOVA pseudo-$F = 1.13$, $p < 0.05$) and among all three sites (UNIFRAC $p = 0.03$, PERMANOVA pseudo-$F = 1.11$, $p = 0.05$). This result is not surprising considering the low overlap between blast matches in each log. However, no differences in phylogenetic community structure were observed among the individual logs (unifrac $p > 0.15$) despite the lack of species overlap between any two logs.

As predicted, fungal abundance (Figure 2, $F = 24.5$, $p < 0.01$) was an order of magnitude higher in sweetgum logs than in loblolly pine logs. Gene copy numbers in sweetgum wood samples ranged from 5.10×10^8 to 2.97×10^9 (cn g^{-1} fresh wood) whereas the copy numbers in pine logs ranged from 1.01×10^7 to 2.09×10^7 g^{-1} fresh wood (Figure 2) and are similar to abundances found in other studies using these primers in soils (de Graaff *et al.*, 2010; Hawkins *et al.*, 2007).

Discussion

Log species, pine or sweetgum, was a better predictor of fungal community composition, functional gene (CBH-I) richness, and fungal abundance than the

location where the log was decomposed. However, fungal richness did vary among sites; the pine site had higher fungal richness than the mixed pine-sweetgum and the sweetgum sites.

Although we observed no difference in fungal phylogenetic richness between wood types, we observed higher fungal abundance in sweetgum logs compared to pine logs. Additionally, fungal community structure differed between the two log types based on phylogenetic analysis. In fact, while these communities were low in diversity (low richness, high dominance), they were highly variable from sample to sample with none of the dominant phylogenetically defined OTUs overlapping among samples. There are many factors that might contribute to variation in the fungal community between these two log types. Sweetgum logs have higher wood quality (lower C:N and lignin:N) and thus may support more fungal growth and higher fungal abundance than pine logs. Fungal species composition also differed between the two log types. Brown-rot fungi were more common in pine logs while white-rot fungi were more common in angiosperm logs. However, in general, the fungi in our samples were most closely identified as white-rotting species, including those species dominating the pine logs (e.g. *Trechispora confinis, Hyphodontia arguta,* and *Botryobasidium subcoronatum*). This pattern was similar to patterns found by Nakasone (1996); who found that 4% of fungal taxa in sweetgum logs and 19% of fungal taxa loblolly pine logs were brown rot in the southeastern US.

We observed higher fungal richness at the pine only site compared to the common pine-sweetgum site and the sweetgum only site. Sweetgum wood had higher nutrient quality relative to loblolly pine wood; thus, the variation among sites may result from environmental filtering within pine logs allowing the co-existence of more fungal species. Pine wood at the common site may have been colonized by generalist species in addition to the pine specialists. Generalist species colonizing higher quality sweetgum wood may also colonize pine logs via mycelial cords. Although we did not observe any common species in our samples, our survey was by no means exhaustive and we cannot rule out that some species, or even individual organisms, at the common site may have been colonizing both types of wood.

We did not observe a difference in fungal phylogenetic richness between the two wood types; however, functional gene CBH-I richness was higher than fungal OTU richness and it was higher in sweetgum wood compared to pine wood. Higher CBH-I richness compared to fungal phylogenetic richness may be due to multiple orthologous copies of the CBH-I gene family present in many fungal species (Edwards *et al.*, 2008). Since fungal phylogenetic richness was assessed by sequence similarity, not by BLAST matches, higher CBH-I richness in sweetgum wood compared to pine wood cannot be attributed to a single species because many sequences were classified as unidentified. Therefore, while there were more variant copies of the CBH-I gene in the

fungal community in sweetgum wood than in the fungi present in pine wood, we cannot attribute the species identity of these variants and thus determine definitively their orthologous nature/origin within a single genome. Whether the higher richness of functional genes translates to increased functional capability or might explain faster decomposition rates of sweetgum versus loblolly pine remains for future study. In sum, our data indicate that microbial communities with similar phylogenetic diversity measures (richness) may not have similar measures of functional diversity.

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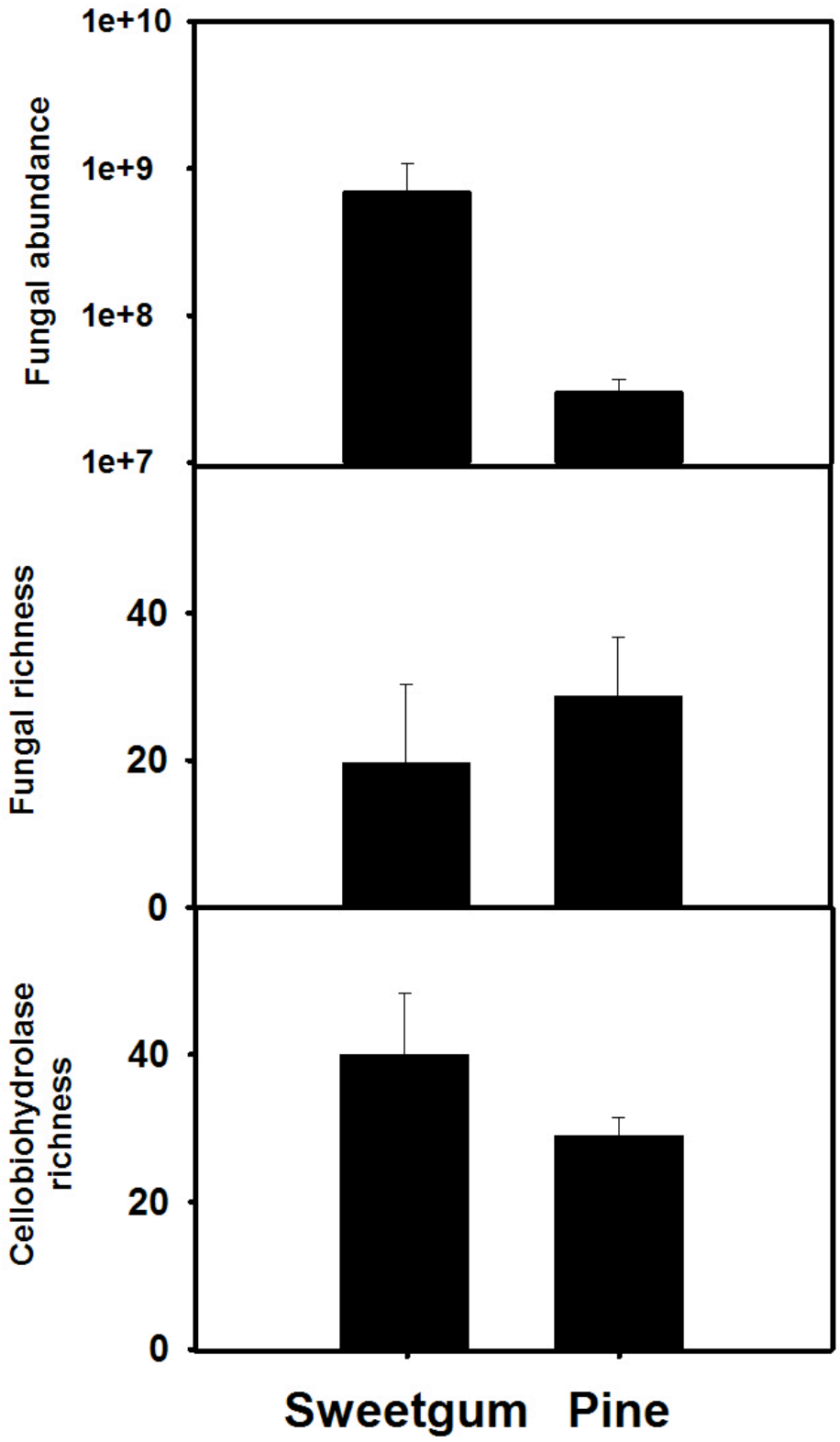
Appendix I: Chapter I figures

Figure 2: Estimated fungal abundance (SSU qPCR, top panel), fungal richness (rRNA gene, middle panel), and cellobiohydrolase richness (CBH-I gene, bottom panel) in decomposing sweetgum and loblolly pine logs.


1e+10
1e+9
1e+8
1e+7
Fungal abundance
40
20
0
Fungal richness
40
20
0
Cellobiohydrolase richness
Sweetgum
Pine

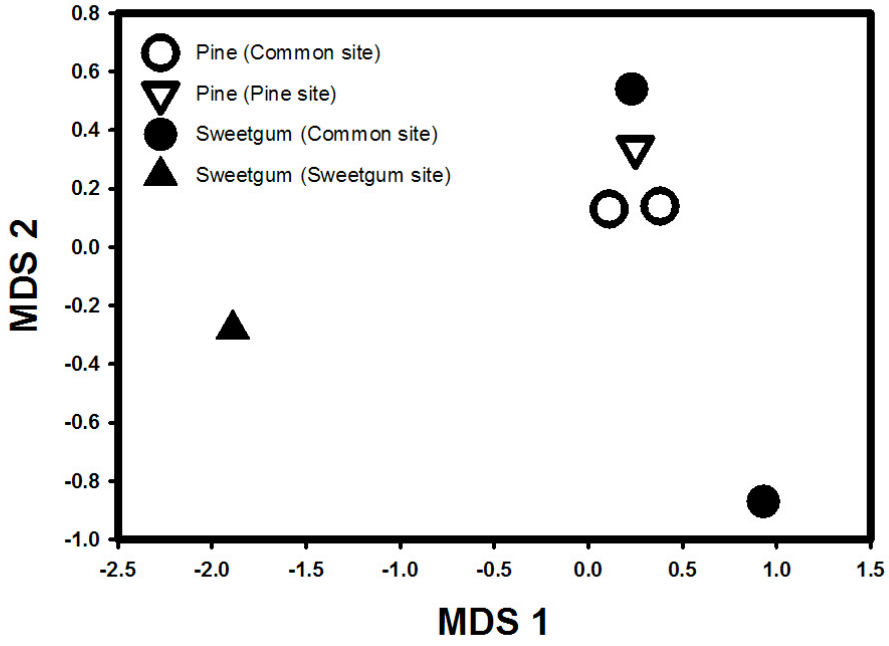
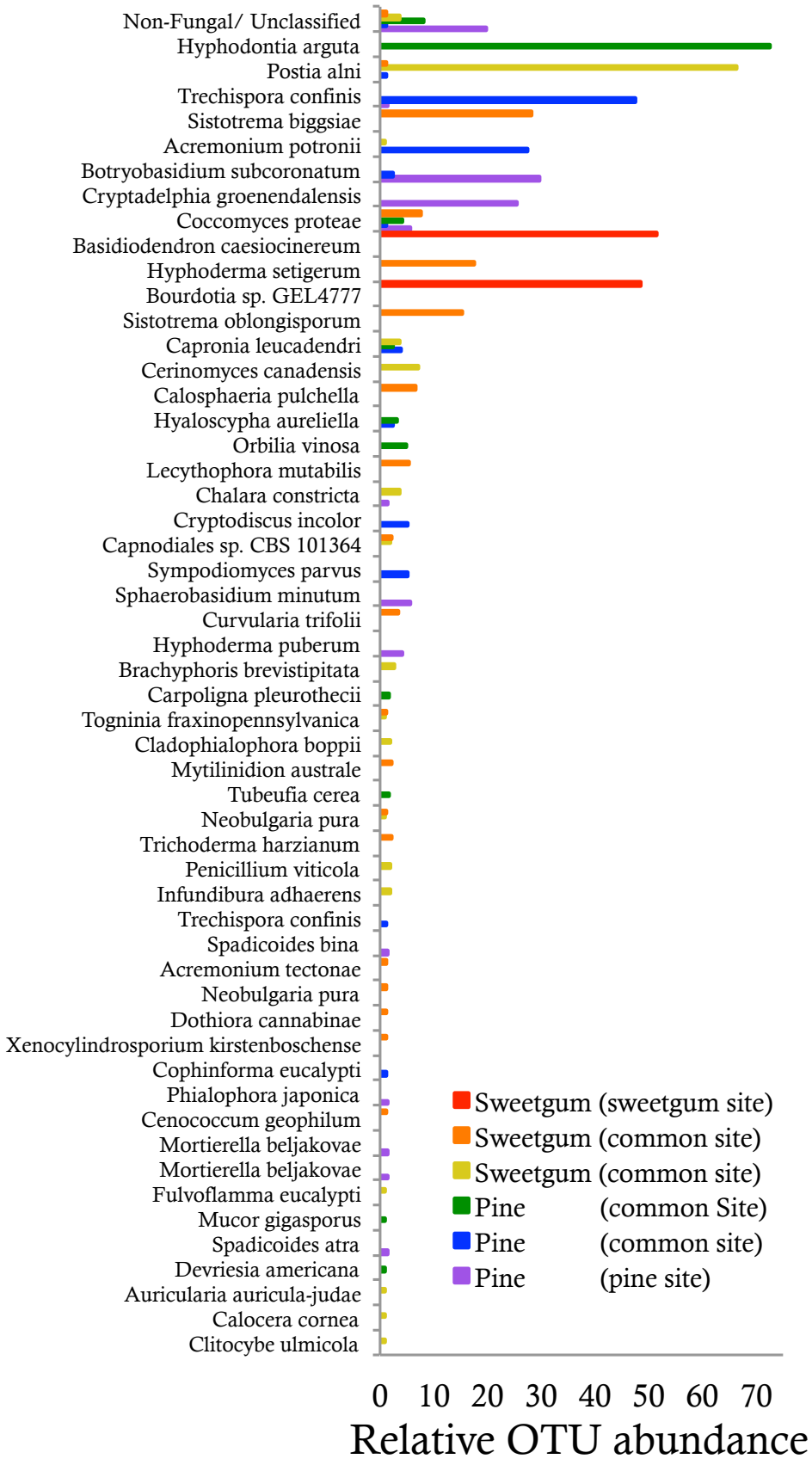


Figure 3: Pine logs fungal communities were more similar to each other than sweetgum log fungal communities. Data are fungal community structure from clone libraries and represented by a NMDS. Filled symbols are sweetgum logs and open symbols represent pine logs. Circles represent samples from the common site, and triangles represent samples from the pine or sweetgum monoculture plots.

Figure 4: Each log sample was dominated by 1 -2 fungal OTUs and overall richness was low in decomposing loblolly pine and sweetgum wood. Relative abundance of different fungal OTUs in each of the six logs.


Non-Fungal/ Unclassified
Hyphodontia arguta
Postia alni
Trechispora confinis
Sistotrema biggsiae
Acremonium potronii
Botryobasidium subcoronatum
Cryptadelphia groenendalensis
Coccomyces proteae
Basidiodendron caesiocinereum
Hyphoderma setigerum
Bourdotia sp. GEL4777
Sistotrema oblongisporum
Capronia leucadendri
Cerinomyces canadensis
Calosphaeria pulchella
Hyaloscypha aureliella
Orbilia vinosa
Lecythophora mutabilis
Chalara constricta
Cryptodiscus incolor
Capnodiales sp. CBS 101364
Sympodiomyces parvus
Sphaerobasidium minutum
Curvularia trifolii
Hyphoderma puberum
Brachyphoris brevistipitata
Carpoligna pleurothecii
Togninia fraxinopennsylvanica
Cladophialophora boppii
Mytilinidion australe
Tubeufia cerea
Neobulgaria pura
Trichoderma harzianum
Penicillium viticola
Infundibura adhaerens
Trechispora confinis
Spadicoides bina
Acremonium tectonae
Neobulgaria pura
Dothiora cannabinae
Xenocylindrosporium kirstenboschense
Cophinforma eucalypti
Phialophora japonica
Cenococcum geophilum
Mortierella beljakovae
Mortierella beljakovae
Fulvoflamma eucalypti
Mucor gigasporus
Spadicoides atra
Devriesia americana
Auricularia auricula-judae
Calocera cornea
Clitocybe ulmicola
Sweetgum (sweetgum site)
Sweetgum (common site)
Sweetgum (common site)
Pine (common Site)
Pine (common site)
Pine (pine site)
0 10 20 30 40 50 60 70
Relative OTU abundance

Chapter II: Fungal community shifts during wood decomposition do not alter the response of respiration to temperature

Abstract

Warming increases microbial activity and decomposition rates for many substrates including dead wood, an important carbon sink in forests. Warming clearly increases wood decomposition activity, however we know less about how the decomposer community and physical properties of the wood influence the response of decomposition to temperature. Both fungal communities and physical and chemical properties of wood vary over the course of decomposition. We incubated Pinus taeda wood across a range of decomposition stages at four different temperatures in the lab. We measured the microbial community as fungal community composition, fungal and bacterial abundance, decomposer activity as wood respiration rate and potential enzyme activity, and wood physical and chemical properties including nitrogen content, lignin content, and wood density. We observed variation in the decomposer community and activity, physical and chemical properties of wood across the four stages of wood decomposition, and increased activity under warming, but no interactive effects between wood decomposition stage and temperature on respiration rates.

Introduction

Projected warming will increase microbial activity, decomposition, and respiration rates in forested ecosystems leading to a positive carbon (C) feedback to the atmosphere. Microbial respiration usually increases with warming, but the magnitude of this response may vary between microbial communities with variable life strategies, extracellular enzymes, and metabolic pathways. Despite increasing recognition of the important role microbial decomposition plays in the global C cycle, the response of different decomposer groups to warming remains under-explored. (Bradford *et al.*, 2008; Kirschbaum, 2004).

Wood and bark comprise over 90% of the aboveground C pool in forested ecosystems (Cooke & Rayner, 1984) and increasing wood production and tree mortality due to global change pressures may lead to an increase in the woody C pool (Harmon *et al.*, 2013; Kirschbaum *et al.*, 2012; Allen *et al.*, 2010; Gitlin *et al.*, 2006; Norby *et al.*, 2002)). Fungi are the primary decomposers of wood and, in most cases, the only organisms with the enzymatic capability to break down lignocellulose. Both observational and experimental data show that wood-inhabiting fungal communities respond to warming (A'Bear *et al.*, 2012; Gange *et al.*, 2007); however different fungal communities, such as those occurring during different stages of wood decomposition, may mediate or accentuate the direct effects of warming on respiration.

Projected atmospheric warming will likely increase microbial activity, decomposition, and respiration rates in forested ecosystems leading to a positive carbon (C) feedback to the atmosphere. Microbial respiration usually increases with warming, but the magnitude of this response may vary between microbial communities with variable life strategies, extracellular enzymes, and metabolic pathways. Despite increasing recognition of the important role microbial decomposition plays in the global C cycle, the response of different decomposer groups to warming remains under-explored. (Bradford et al., 2008, Kirschbaum, 2004).

Wood and bark comprise over 90% of the aboveground C pool in forested ecosystems (Cook and Rayner 1984) and increasing wood production and tree mortality due to global change pressures may lead to an increase in the woody C pool (Harmon *et al.*, 2013; Kirschbaum *et al.*, 2012; Allen *et al.*, 2010; Gitlin *et al.*, 2006; Norby *et al.*, 2002). Fungi are the primary decomposers of wood and, in most cases, the only organisms with the enzymatic capability to break down lignocellulose complexes. Both observational and experimental data have recently shown that wood-inhabiting fungal communities respond to warming (A'Bear *et al.*, 2012; Gange *et al.*, 2007); however different fungal communities, such as those occurring with succession during different stages of wood decomposition, may mediate or accentuate the direct effects of warming on respiration in divergent ways.

During decomposition, wood changes in both physical and biological characteristics. “Sugar fungi” which decompose the most available substrates, common in the inner bark, dominate early stages of decomposition. Penetration into inner wood can be hindered by density and oxygen limitation in early stages until boring insects create passages. Once established, corticoid fungi immobilize nitrogen from the surrounding environment, increasing nutrient quality and speeding decomposition of more recalcitrant substrates such as lignocellulose by brown-rot fungi or hemi-cellulose by white-rot fungi. Wood decomposition occurs fastest during the middle stages of decay (Freschet *et al.*, 2012) when substrate quality is increasing and decomposition of structural compounds leads to wood collapse and increased water holding capacity. During these stages, fungal richness increases as the substrate becomes more heterogeneous (Heilmann-Clausen & Christensen, 2004) and combative fronts are established between species (Boddy, 2000).

Fungal communities present at different stages of decomposition may alter the response of wood decomposition to temperature. We know that changing fungal richness can alter enzyme activity and decomposition rates (LeBauer, 2010; Duarte *et al.*, 2006; Tiunov & Scheu, 2005; Setälä & McLean, 2004) and fungal species have different maximum growth temperatures (Humphrey & Siggers, 1933). We can expect that if growth is an indication of potential

activity, the fungal communities present at the different stages of decomposition may have varying responses of temperature.

To explore how wood decomposition stage and warming directly and interactively alter microbial activity, microbial community structure and decomposition rates, we incubated loblolly pine (*Pinus taeda*) logs at different stages of decomposition in growth chambers under different temperature treatments (10, 25, 30 and 35 °C) for 5 weeks. We predicted that: (1) Fungal abundance, richness, and respiration would be highest in logs in the middle stages of decomposition, when decomposition rates are predicted to be highest. (2) Across all decomposition classes, warming would increase fungal abundance, respiration, and richness of fungi relative to logs decomposed at ambient temperatures, (3) Warming would interact with wood decomposition stage so that increased fungal community abundance, richness, and respiration in response to warming would be greatest the middle stages of decomposition because there is more available substrate during the middle stages of decomposition.

Methods

Site Description and Experimental Design

Lololly pine (*Pinus taeda)* log segments were collected from a single stand in Oak Ridge National Environmental Research Park in Oak Ridge, TN (35°58'N, 84°20'W). Climate in this area is characterized by cold winters and long

summers, mean annual temperature is about 14°C and precipitation is about 1300 mm per year. Soils in the research park are generally acidic and well weathered; at this site soil is classified as Armuchee silt loam (USDA, National Resource Conservation Services web soil survey http://websoilsurvey.nrcs.usda.gov/app/). We chose *Pinus taeda* stand including a variety of decomposition stages of wood in a stand with a single species of tree, insuring logs originated from the species of the wood because fungal communities and decomposition rates differ between wood of different tree species.

In fall of 2010, 30 logs of decomposition stages 1-4 were selected for use in this study. The decomposition stage of down logs was visually assessed using factors identified as important indicators of decomposition stage across several studies (Sollins, 1982; Maser *et al.*, 1979). Five segments of 20-25 cm were cut from each log using a chainsaw and logs were transported to the lab in paper bags. The fresh mass and volume was assessed for each segment and four segments from each log were placed in individual incubation containers. One initial segment from each log was retained and dried at 100°C for 14 days to correct for initial water content, and sawdust subsamples were obtained for chemical analysis. Ninety-four samples were incubated in total, 23 log sections in two chambers and 24 log sections in two chambers (a few segments were too large to fit in the individual incubation containers).

Individual incubation containers were constructed using 3-gallon buckets, Gamma Seals ™ lids and nylon bulkhead connectors. Two holes were drilled into the top of each Gamma Seals ™ lid, and the bulkhead fitting was attached using a rubber washer and nitrogen-free silicone sealant. A 6-inch segment of latex tubing was attached to each bulkhead fitting for use as a septum. The containers were closed and placed in growth chambers set to constant temperatures 10°C, 25°C, 35°C, and 40°C. Actual log temperatures, measured with a Raynger ST infrared thermometer (Raytek, Santa Cruz, CA) ranged from 7.4°C to 39.8°C and averaged 9.2°C, 24.1°C, 32.5°C, and 38.1°C in each respective chamber.

All segments were removed from incubation chambers after 5 weeks. Upon removal, samples were weighed and volume measurements were taken as follows: For intact logs two measurements of length were taken to the nearest 0.1cm and two measurements of diameter were taken on each end of the log (total 4 diameter measurements). Volume was calculated as a fulcrum for each log. For logs in decomposition stage 4 where the log shape was not intact, the log was crumbled and distributed evenly in the container, and 6-8 measurements of wood height inside the bucket were taken. Volume was then calculated using the area of the bottom of the bucket and the area at the average height of the wood, wood height and the area equation for a fulcrum.

Subsamples were taken for molecular methods as sawdust, the outer bark from each incubated sample was removed and five holes were drilled along the length of the sample with a 10mm drill bit to the depth of the center of the sample. Sawdust subsamples from the 5 drill holes were homogenized and immediately frozen at -40 and transferred to -80 within 1 week. The drill bit was fire sterilized between samples to prevent cross-contamination of fungal mycelia or spores. Sawdust was collected from three additional holes drilled along the length of the log for enzyme analysis, weight of this enzyme subsample was recorded, and ~ 40mL of MilliQ water was added to the sawdust subsample in a specimen cup. The cups were then shaken for 2 hours, and pressed through a 2mm sieve. The resulting liquid was centrifuged in a 50mL tube for 10 minutes, the supernatant was transferred to a new 50mL tube, and the volume was recorded before the sample was frozen at -20 for 2 weeks prior to analysis.

Log segments were allowed to air dry in paper bags for several weeks before air dry mass was measured and sawdust subsamples were acquired as above, excluding fire sterilization of the drill bits, then ball ground for chemical analysis and ashing. Whole logs were dried at 100°C for two weeks and oven-dry mass was measured. Water content was calculated for ground subsamples (200mg) dried in an oven at 100°C for 48 hours and the same subsamples were

then combusted in a muffle furnace for 6 hours at 550°C to calculate inorganic ash content.

Physical, chemical, and functional wood characteristics

Relative density of wood was measured on subsamples of initial log sections using water displacement. Small pieces of dried wood with known mass were dipped in hot paraffin wax and allowed to cool prior to submergence in water in a graduated cylinder where water displacement was calculated to the nearest 0.5cm^3. Wood pH was measured following Freschet et al., 2012. Briefly, sawdust was shaken in deionized water for 1 hour then centrifuged at 9000g for five minutes, pH of the supernatant was measured using benchtop pH meter (Denver Instruments, Bohemia, NY). Ball- ground wood (~25mg) was enclosed in aluminum capsules and analyzed on a CHN analyzer (Costech analytical technologies, Valencia, CA). Total lignin was analyzed using an acid digest in a fiber analyzer (ANKOM, Macedon, NY).

Fungal and Bacterial Abundance and Fungal Community Structure

To assess the microbial community we used quantitative polymerase chain reaction (qPCR) as a proxy for fungal and bacterial abundance and created fungal community fingerprints using terminal-restriction fragment length polymorphism (T-RFLP). DNA of each sample was extracted from sawdust collected using sterile techniques described above. A MoBio soil DNA extraction kit was used according to protocol (MoBio Laboratories, Carlsbad,

CA) with the following modifications; 0.25g of sawdust was placed in bead beating tubes, filters were washed with an ethanol solution twice instead of once as described in the protocol, and 30 rather than 50µl of elution buffer was used in the final step to recover DNA from the filter. The concentration of DNA was assessed at 260 & 280 nm using a Biotek synergy microplate reader and Gen5 Take 3 module and software (Biotek Instruments, Winooski, VT).

Fungal community fingerprints were obtained using T-RFLP (Singh *et al.*, 2006). Polymerase chain reaction (PCR) was performed to amplify the ribosomal ITS region from fungi using primers ITS1f: 5' (6-FAM) CTTGGTCATTTAGAGGAAGTAA -3' (Gardes & Bruns, 1993) and ITS4r: 5' TCCTCCGCTTATTGATATGC -3'(Singh, 2006). 50µl PCR mixtures contained 5 µl 10x KCL reaction buffer, 2 µl 50 mM MgCl2, 1 µl 10 mM dNTPs (Bioline, Tauton, MA), 1 µl 20 mg/ml BSA (Roche, location), 0.5 µl (2.5 Units) Taq DNA polymerase (Bioline, Tauton, MA), and 2 µl of each fungal primer (operon, Huntsville, AL). The PCR reaction was carried out under the following conditions: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and finally 72 °C for 10 minutes on a 96-well Tgradient thermocycler (Biometra, Goettingen, Germany). Upon completion of PCR reaction successful amplification was confirmed with 1% agarose gel electrophoresis.

Amplicons were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and quantified using a Synergy HT microplate reader (Biotek, Winooski, Vermont, USA). Each PCR product was individually digested with *Msp*I in the following 20 µl reaction: 14 µl template, 2 µl 10X buffer B, 2 µl MSP1 (Fisher Scientific, USA), 2 µl 10 mg/ml acetylated BSA (Promega, Madison, WI). Samples were incubated at 37 °C for 3 hours followed by a deactivation step at 95 °C for 10 min. After digestion, a cocktail was made containing 0.5 µl LIZ labeled GeneScan 1200 internal size standard (Applied Biosystems, Grand Island, NY), 12.5 µl Hi-Di formamide (Applied Biosystems, Grand Island, NY), and 1 µl of digested product. Samples were centrifuged and incubated at 94 °C for 4 min then at 4 °C for 5 min. Fragments were analyzed on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Grand Island, NY).

T-RFLP profiles were produced using the GeneMapper software (Applied Biosystems, Grand Island, NY). Terminal restriction fragments (TRFs) at under 62 bp were assumed to be caused by primer-dimers and. Abundance of a TRF in each sample's T-RFLP profile was calculated relative to total peak height of all TRFs in the profile (Singh *et al.*, 2006).

We assessed fungal and bacterial gene copy number as a proxy for abundance by running quantitative polymerase chain reaction (qPCR) on the small subunit of the 18s (fungi) and 16S (bacteria) ribosomal DNA gene after methods described previously (Castro *et al.*, 2010). PCR mixtures for 18S rRNA gene

amplification contained 15 µl of SYBR green master mix (Invitrogen, Life Technologies, Grand Island, NY), 5 µmol of each primer (Borneman & Hartin, 2000) (Eurofins mwg operon, Huntsville, AL), and 0.1 µl of DNA template, brought to a final volume of 30 µl with sterile water. Amplification protocol consisted of an initial denaturing cycle of 95 °C for three minutes followed by 39 cycles of 95 °C for 15 s, 53 °C for 15 s, and 70 °C for 30 s. Fungal rRNA gene abundance in each sample was quantified by comparing unknown samples to serial dilutions of 18S or 16S rRNA genes from *Saccharomyces cerevisiae* or *Escherichia coli* in each PCR run. A melting curve analysis was conducted to ensure purity of amplification product. PCR amplification was performed on a 96-well Chromo4 thermocycler (Bio-Rad Laboratories, Hercules, CA).

Decomposer Activity

We assayed microbial activity by measuring potential activity of three relevant extracellular enzymes using methylubelliferone (MUB) linked substrates of: xylosidase (hemicellulose degreadation), β glucosidase (degradation of cellulse and other -1,4 glucans), and cellobyohydrolase (cellulose degradation), and phenol oxidase (lignin degradation) using 3,4 Dihydrophenylalanine (L-DOPA). We prepared 96 well plates with blanks, boiled-cell controls, and samples replicated eight times each. Xylosidase, β glucosidase, and cellobiohydrolase plates were incubated at room temperature in the dark for two hours, phenol oxidase plates were incubated for twenty-four hours. Floresence was read at an

excitation of 365 nm and emission of 450 nm for MUB-linked substrates and absorbance was read at 460nm for phenol oxidase plates on a Synergy HT microplate reader (Biotek, Winooski, VT). Potential enzyme activity is presented as nmol $h^{-1} g^{-1}$ (Saiya-Cork *et al.*, 2002; Sinsabaugh & Moorhead, 1994).

Respiration was measured from headspace samples extracted from the chambers using a syringe and injected into a Li-Cor 6400 IRGA. Calibration gases spanning the range of observed carbon dioxide levels were injected to calibrate the Li-Cor. CO_2 was calculated based on a linear relationship between peak CO_2 observed and actual CO_2 concentration.

Statistical Analysis

Respiration rates, fungal abundance, % nitrogen, and wood quality (lignin:nitrogen) data were log transformed, and phenol oxidase activity and bacterial abundance were square root transformed to meet the assumptions of normality. Normality of all response variables was tested using a Shapiro-Wilks test.

To assess whether the stage of wood decomposition or chamber temperature influenced wood respiration rates, fungal or bacterial abundance, pH, % lignin, % nitrogen, lignin: nitrogen, or phenol oxidase activity, we used a two way ANOVA with chamber temperature and decomposition stage as

predictor variables. Mean respiration and rates and wood temperature were calculated across weeks to avoid pseudo-replication (Crawley, 2007). Where we observed a significant effect, we used Tukey's HSD post- hoc test to reveal significant differences between treatments. To test whether wood density varied between decomposition stages, we used a one-way analysis of variance (ANOVA).

To assess the effects of temperature treatment on fungal community structure, we used PRIMER (Plymouth Marine Laboratory, UK) to conduct permutational multivariate analysis of variance (PERMANOVA) using decomposition stage as a covariate in our models. We performed PERMANOVA tests on Bray-Curtis dissimilarity triangular matrices (Bray & Curtis, 1957) on T-RFLP relative peak height data. In order to illustrate the multivariate patterns of species composition, non-metric multidimensional scaling (nMDS) ordinations were performed using the Bray-Curtis dissimilarity triangular matrix. To test whether wood density affected initial fungal community structure T-RFLP fragment lengths were analyzed using distance based linear models with density as a continuous covariate.

Results

The log segments differed across the four decay stages both chemically and biologically. Nitrogen content (% nitrogen) was very low in these samples, and

increased from 0.28% and 0.27% in early decomposition stages one and two, to nearly double, 0.40% and 0.50%, in later stages of decomposition three and four ($F = 14.15$, $p < 0.0001$). Lignin content, as a percentage of total wood mass, ranged from 32% to 38% and there was a trend toward higher lignin content in the latest stage of decomposition ($F = 2.26$, $p = 0.09$). Considered together, the strong increase in nitrogen over the course of decomposition led to increased wood quality (decreased lignin: nitrogen) in the later stages of decomposition ($F = 12.22$, $p < 0.0001$). Wood density decreased over the course of wood decomposition, with highest densities in stage one and lowest densities in stage four ($F = 16.19$, $p < 0.0001$). pH ranged from 3.5 5 but did not vary between decomposition stages.

Fungal community composition in the initial samples co-varied with wood density, although there was no direct effect of the original classifications of decomposition stage on fungal community composition. Fungal OTU richness did not differ between logs in different stages of decomposition ($F = 0.24$, $p = 0.63$), or vary with wood density ($F = 0.29$, $p = 0.59$) although the log origin did affect fungal community structure ($F = 3.22$, $p < 0.0001$). Fungal abundance was lowest in stage four of decomposition compared to stages one through three ($F = 7.51$, $p < 0.001$), but bacterial abundance showed no pattern across the stages of wood decomposition ($F = 0.50$, $p = 0.64$).

Respiration rate showed a bowl shaped pattern across the stages of decomposition, respiration rate was higher in stage one than in stage two, but then increased again in stages three and four ($F = 8.05$, $p < 0.001$). There was a hump- shaped pattern in enzyme activity (phenol oxidase) across the four stages of decomposition ($F = 3.81$, $p = 0.01$) although this direct effect was confounded by an interaction between chamber temperature and decomposition stage ($F = 2.50$, $p = 0.01$). Specifically, phenol oxidase activity in wood in the middle decomposition stages at several temperature treatments (stage three at 10°C and 25°C, and stage two at 40°C) was higher than phenol oxidase activity in wood in the earliest stage of decay (stage one) incubated at 25 °C, although this pattern did not hold across all temperatures.

Generally, temperature treatment had no effect on wood chemical quality. Nitrogen did not vary with wood temperature ($F = 0.97$, $p = 0.33$), neither did lignin ($F = 0.491$, $p = 0.49$), and there was no change in lignin: nitrogen with wood temperature ($F = 0.22$, $p = 0.65$).

Respiration increased with increasing temperature treatment ($F = 4.42$, $p < 0.01$), but there was no interaction between the effects temperature and decomposition stage on wood respiration rate ($F = 0.52$, $p = 0.85$). Fungal abundance was highest in the warmest treatment (40°C) compared to either the coolest (10°C) or second warmest (30°C) treatment, however fungal abundance in wood in the 30°C treatment was lower than any of the other treatments ($F =$

28.11, $p < 0.0001$). Bacterial abundance showed a hump-shaped pattern across the four temperature treatments with the highest bacterial abundance in the 30°C treatment, and bacterial abundance in the 10°C treatment was lower than in any other treatment ($F = 11.27$, $p < 0.0001$). Potential activity of xylosidase β-glucocidase and cellobiohydrolase were below the limit of detection. Enzyme activity (phenol oxidase) did not vary across the four temperature treatments, although the effect of decomposition stage on phenol oxidase activity varied between decay stages (see above) ($F = 0.06$, $p = 0.98$).

Fungal community composition following the incubation period differed between the temperature treatments, and each temperature treatment was different than the fungal communities in the initial samples (pseudo-$F = 6.01$, $p < 0.001$). But fungal OTU richness did not differ by treatment ($F = 0.43$, $p = 0.78$) or by decomposition stage. Fungal abundance showed a bowl- shaped pattern across temperature, with the lowest fungal abundance at the 30°C treatment and highest fungal abundance in the 40°C treatment (Figure 7, $F = 28.11$, $p < 0.0001$). Bacterial abundance showed a hump-shaped pattern across the stages of wood decomposition with highest bacterial abundance in the 30°C treatment (Figure 7, $F = 11.27$, $p < 0.0001$).

Discussion

Whether different communities will influence the response of decomposition to warming has important implications for carbon cycling. Fungal richness and community structure has been shown to alter fungal community function in wood, leaf litter, and soil (Dickie *et al.*, 2012; LeBauer, 2010; Dang *et al.*, 2005; Setälä & McLean, 2004). Since fungal communities and wood physical and chemical properties differ across the stages of wood decomposition, and since fungi are the dominant decomposers of wood, we predicted that wood in different stages of decay would vary in the response of wood respiration rate (decomposition) to altered temperature. While wood respiration rate did increase with temperature, we did not observe a difference in the rate of increase between logs at different stages of decomposition despite the different fungal communities present. Additionally, we did not observe correlation between fungal abundance and wood respiration rate.

Decomposition stage effects on fungal communities and wood respiration

Initial fungal community structure did not differ between different stages of decomposition, but there was a correlation between fungal community structure and wood density. Since there was no effect of density on fungal richness this can be difficult to visualize but it seems that the less dense (more decomposed) logs had more similar fungal communities. Since density

decreased across the four stages of wood decomposition, we can assume that there was some variation in fungal community structure across wood age, which was not captured by the relatively subjective measure classifications used for accessing decomposition stage (Sollins, 1982; Maser *et al.*, 1979).

Contrary to what has been observed in some fruiting body surveys, density or decomposition stage did not influence fungal OTU richness these samples. These different community assessment measures can reveal different fungal community members even within the relatively simple communities of decomposing wood. In fact in a recent study, pyrosequencing, community fingerprinting by denaturing gradient gel electrophoresis (DGGE), and fruiting body surveys each observed some fungi that were not observed by other methods in the same samples (Ovaskainen *et al.*, 2010). However, we may expect similar patterns regardless of which specific OTUs are observed. Another study showed DGGE could not detect variation in fungal richness across stages of decomposition (Kebli *et al.*, 2011). This may be due to primer biases or lack of saturation of rarefaction curves although fungal communities in wood are much less rich than those in soil. Differences in the observed community between fruiting body surveys and molecular techniques may also be due the "snapshot" effect when a single molecular sampling time is used and or the inconsistent and ephemeral nature of fungal fruiting. Temporal variation in fungal communities is more likely to be observed in fruiting body surveys, although it

is often assumed that all fungi are present as hyphae in a relatively small sample when not producing fruiting bodies, molecular analysis has most often in the past been performed on a sample or samples taken at a single given time whereas the nature of fruiting body surveys require repeated measures over the time frame of a single season up to multiple years.

Although there were no consistent changes in fungal community composition with decomposition stage in our samples, we did observe variation in fungal abundance across the fours stages of decomposition. Fungal abundance was highest in the middle stages of decomposition and significantly lower in the final decomposition stage (stage four) compared to all other stages. There was no counteracting increase in bacterial abundance. This indicates that something unmeasured may have been limiting the growth of the microbial communities given that both oxygen and nitrogen content were shown to be highest in stage four. Therefore, there may have been a limited supply of available carbon; indeed % lignin was higher, though not significantly so, in stage four than in all other stages.

However, although fungal abundance was lowest in stage four, wood respiration rate was highest in decomposition stage four indicating higher specific activity (activity per unit biomass). We observed a decrease in respiration rate between stages one and two that may indicate the depletion the nutrient rich inner cambium. The inner bark is thought to be the first part of

decomposing wood colonized by "sugar fungi" because this is the most nutrient rich area of the log, however it comprises only a small component of the total mass. Respiration rate increased in stages three and four, which may be related to increases in nitrogen content and reduced oxygen limitation as indicated by decreasing wood density (Figure 6).

Potential phenol oxidase activity showed a hump- shaped pattern across the stages of decay with the highest potential activity in stage three. While this pattern differs from that of wood respiration rates, degradation of lignocellulose would presumably not be required for the consumption of the nutrient rich portion of the inner bark. Thus, if "sugar fungi" consuming the inner bark explains the decrease in respiration rate between stages one and two, we would not observe the same pattern in phenol oxidase activity. Phenol oxidase activity decreased in stage four compared to stage three along with fungal abundance, while respiration rate was higher in stage four than stage three. However, neither phenol oxidase activity or respiration rate showed a significant difference between stages three and four in posthoc analysis.

Although bacteria cannot breakdown lignocellulose they consume the bi-products of fungal extracellular enzymes and the resulting decrease in product concentration may increase the activity of these enzymes (de Boer *et al.*, 2005) or promote substrate availability via breakdown products. However, the disconnection we observed between overall respiration and microbial

abundance can't be explained by the bacterial abundance, which remained constant across all four stages of decomposition. Rather it seems more likely that the increase in nitrogen abundance, decrease in density, and thus potentially relief from oxygen limitation could have allowed fewer fungi to have higher levels of activity.

Literature shows that fungal communities shift in their functional capabilities during the course of wood decomposition. Fungal communities in wood have high rates of competition (Boddy, 2000), and competitive exclusion over the course of wood decomposition that could result in more efficient fungal communities at late stages of decomposition. Therefore it is possible that the communities present at later stages of decomposition may be more efficient, explaining the disconnection between higher wood respiration rates and lower fungal abundance in stage four of wood decomposition. We also observed a strong effect of warming on fungal community structure, which may have overwhelmed the effects of decomposition stage on fungal community structure.

Warming alters fungal community structure and wood respiration rate, but not abundance or OTU richness

Fungal communities restructured under our warming treatments, which may have overwhelmed overwhelmed of decomposition stage on fungal community

structure, by the end of the incubation period. Fungal communities differed with density at the start of the experiment, and many studies have observed changing fungal community structure over the course of wood decomposition, it appears the fungal communities from the different decay state logs converged based on temperature treatment over the course of the incubation. Although it has been established that fungal community structure responds to temperature in the field and in the lab, we did not expect this effect to overpower the effects of decomposition stage on fungal community structure. Other studies have shown that in a common environment, priority effects can structure fungal communities and result in functional differences (Fukami *et al.*, 2010). Therefore we predicted the trajectory of fungal community development would be different based on the initial fungal communities present across the different stages of decomposition at the start of the experiment. In fact, we did see an effect of source log on final fungal community composition. The effect of source log could be related to priority effects structuring the communities similarly across the different chambers, or of different habitats within the different logs. While the individual wood sample containers were sealed, the respiration measurements were not taken in a sterile environment and to reduce temperature fluctuation from opening and closing chambers, samples from the same treatment chamber were taken on the same day. Therefore it is possible that spores traveled between the samples. But because samples from

the same logs were present in each treatment, this effect was probably not a factor within the experiment. Additionally, the logs were isolated from the outside environment where new spores or cord- forming fungi could have established in the logs had they been within natural systems. This may have affected the range of potential communities to develop and change over time in each log.

We did not observe increased fungal abundance as a response to warming. Fungal cultures generally grow faster in warmer temperatures until temperature exceeds physiological constraints of the fungi (Kåårik, 1974). We therefore expected fungal abundance, measured as gene copy number, to increase under warming. However, we observed a higher fungal abundance in the warmest chamber, but the lowest fungal abundance in the 30°C chamber compared to all other temperatures. Although in a controlled laboratory setting, fungi in our samples were not functioning in isolation. We did not remove any organisms from our log segments; therefore mycophagous fungi or arthropods may have been exerting top-down controls on fungal abundance. Similarly, the resources were not unlimited in our samples, and increased temperature does not necessarily result in increased acquisition of recalcitrant resources if another component is limiting. However, if nitrogen or oxygen were limiting the response of fungal abundance to warming, we would predict there would have been an interaction where fungal abundance would increase under warming in

logs in the later stages of decomposition, but would show no response to temperature in the earlier stages. Finally, competition with bacteria or bacterial modification of the log environment may have been limiting fungal growth in the log segments in these chambers. Bacterial abundance, although low overall compared to abundance in soil, was significantly higher in the 30°C chamber. Wood respiration increased under warming, as we expected. Enzymes, including those that break down wood, are often temperature limited, and generally respiration increases at higher temperatures (Davidson & Janssens, 2006). Previous work in decomposing wood has shown evidence for a temperature response of wood respiration such as higher rates of respiration at warmer sites (Hicks *et al.*, 2003; Paim & Beckel, 1963). We observed respiration rates ranging from 0.5 2 mg CO_2 (0.13-0.54 mg C) per g dry wood each hour. This is within the same range as other studies of wood respiration (Progar *et al.*, 2000; Boddy, 1983).

Density (decomposition stage) did not influence the response of wood respiration to temperature

Although we did observe different fungal communities and wood chemical and physical characteristics across the different stages of wood decomposition, these differences did not appear to result in physiological differences significant enough to alter the response of wood decomposition to temperature.

We observed no interaction between decomposition stage and temperature on respiration rate. We expected freshly fallen logs to be limited by low fungal colonization and more decomposed logs to be limited by substrate quality resulting in the strongest temperature response at the middle stages of decomposition as had been observed previously (Hicks *et al.*, 2003). In addition wood at the middle stages of decomposition is considered a more heterogeneous environment (Pyle & Brown, 1999) and greater fruiting body richness has been observed in mid decomposition stage logs (Heilmann-clausen & Christensen, 2003). Fungal richness can be correlated with decomposition rates especially at low levels of fungal richness (Tiunov & Scheu, 2005; Setälä & McLean, 2004). Although we did not observe differences in fungal richness, heterogeneity in environmental conditions can lead to increased trait and functional diversity; we therefore anticipated the fungal communities present in mid stages of decomposition would have more functional diversity and more potential to take advantage of warming and increase activity. Our samples were small compared to intact logs, however. The range of heterogenous environments present in our log subsamples of 20-25 cm used in the chambers, are likely much less than that present along a full log of 20-25m in length, for example. Additionally, the fungal communities converged based on temperature treatment, which may have altered the potential to see differences in the response of wood decomposition to temperature. The significant

differences in wood chemical composition and structure did not influence the rate of increased respiration with warming.

In conclusion, our results suggest that differing fungal communities in wood at different stages of decomposition do not influence the response of wood decomposition to temperature but rather restructure into new assemblages in response to warming. These results suggest that while fungal community structure responses to warming may be important for community assemblages, different communities may not always alter the response of respiration to temperature in wood and therefore influence carbon evolution from wood under a warming climate.

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Appendix II: Chapter II figures

Figure 5: Wood chemical and physical properties varied over the course of decomposition from stage one to stage four. Nitrogen content increased over the course of decomposition (top), lignin was highest in decay stage four although this trend was not significant (middle), and density (g dry wood cm^{-3}) decreased, increasing oxygen availability as decomposition proceeded (bottom).

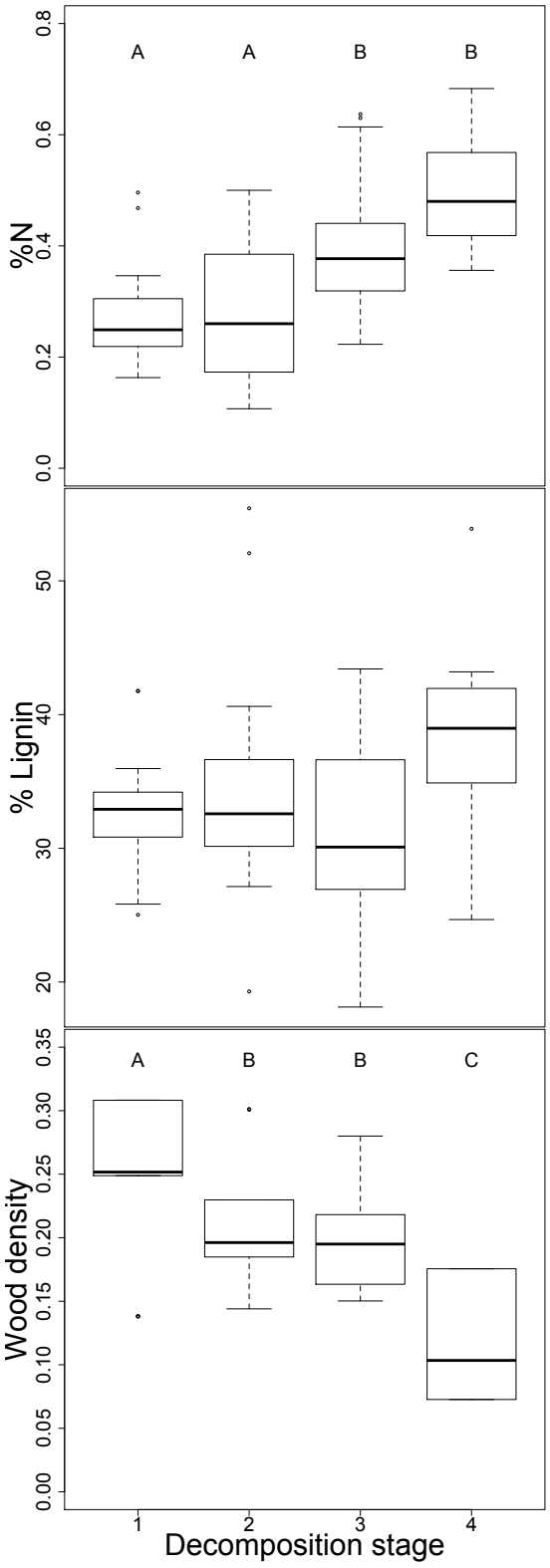
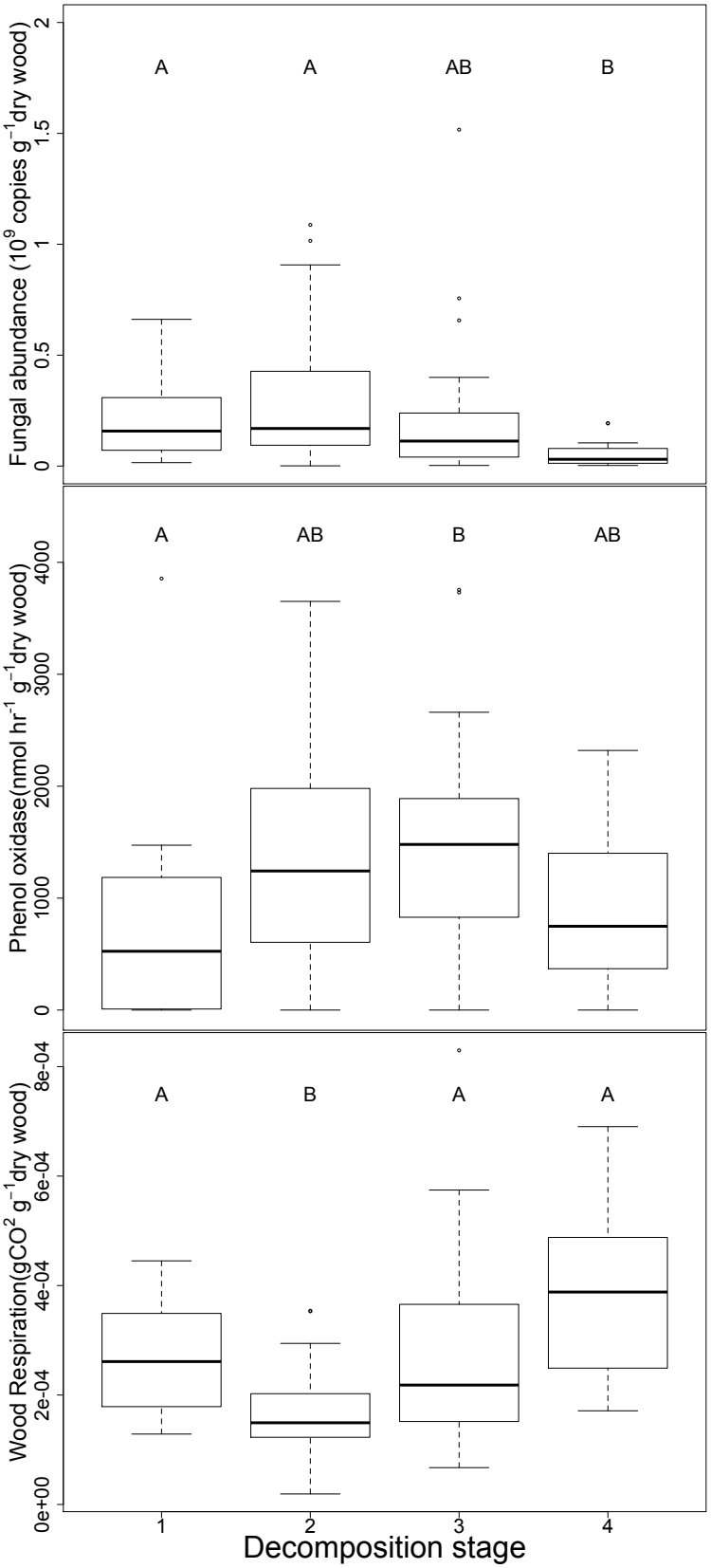

%N
A
A
B
B
0.0
0.2
0.4
0.6
0.8
% Lignin
20
30
40
50
Wood density
A
B
B
C
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
1
2
3
4
Decomposition stage

Figure 6: Fungal abundance and activity varied over the course of decomposition from stage one to stage four. Fungal abundance (top) and phenol oxidase (middle) were highest in the middle stages of decomposition, while wood respiration rate (bottom) was lowest in the middle stages, decomposition stages two and three.


Fungal abundance (10^9 copies g^{-1}dry wood)
A
A
AB
B
Phenol oxidase(nmol hr^{-1} g^{-1}dry wood)
A
AB
B
AB
Wood Respiration(gCO^2 g^{-1}dry wood)
A
B
A
A
Decomposition stage

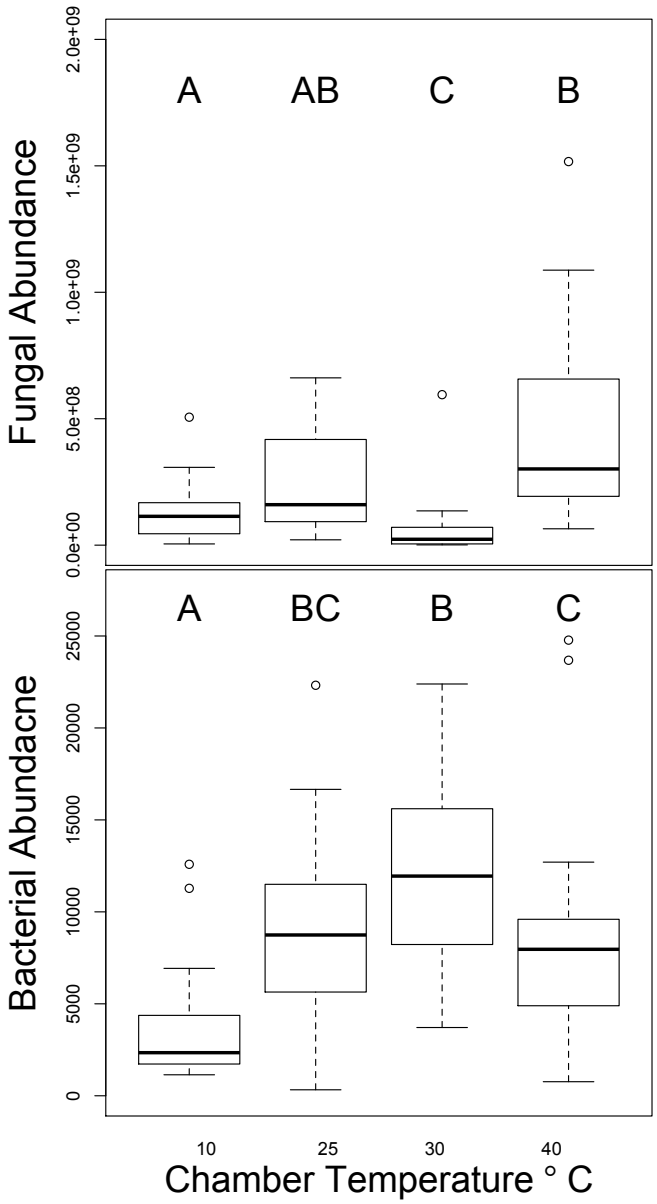


Figure 7: Fungal (top) and bacterial (bottom) abundance, measured as gene copy number g^{-1} dry wood, varied with temperature treatment. Fungal abundance was lowest in the 30°C treatment, where bacterial abundance was highest.

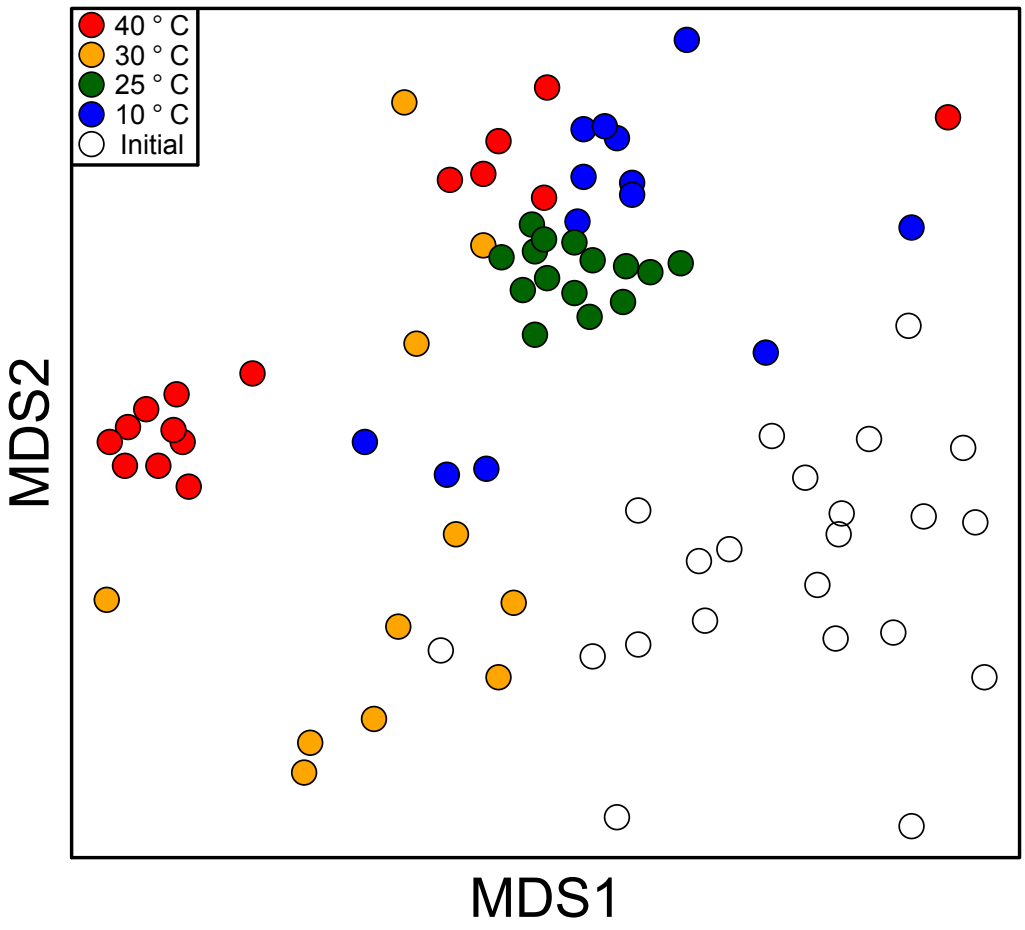


Figure 8: Fungal communities differed by temperature treatment at the conclusion of the experiment, and differed from initial fungal community structure. Each point represents the fungal community in a given log section, colors indicate different incubation temperatures and open circles are initial fungal community. Non-metric multi- dimensional scaling is used for data representation, and permutational multivariate analysis of variance was used to identify significance.

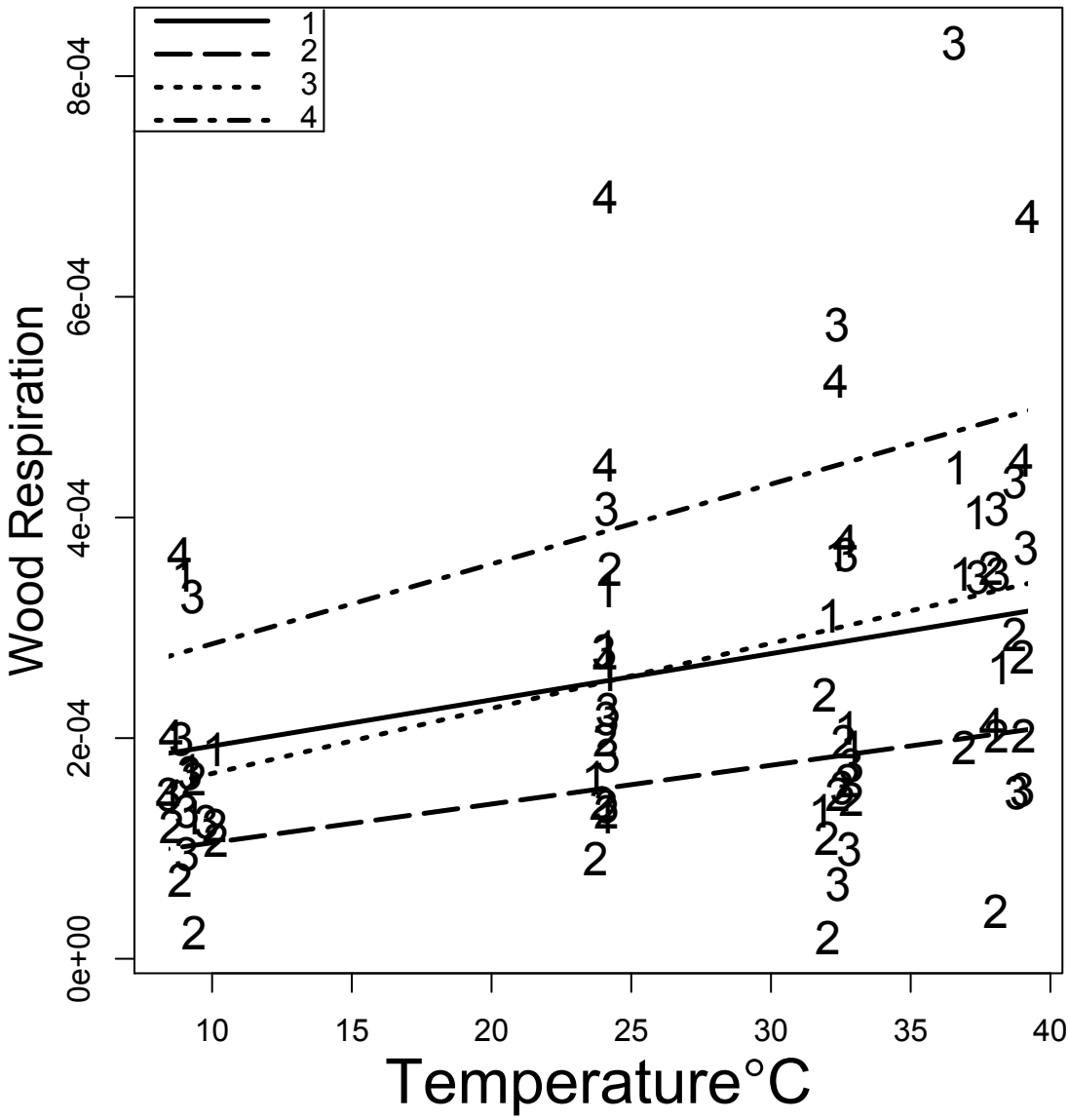


Figure 9: Wood respiration rates increased with temperature, but we observed no interaction between decomposition stage and warming on respiration rate. Wood respiration rate is reported as mg CO^2 g^{-1} dry wood $hour^{-1}$

Chapter III: Experimental warming influences decomposer communities and wood decomposition, but the effects vary geographically

Abstract

Climatic warming is altering the community composition and activity of decomposers such as fungi and arthropods. In turn, the activity of these organisms could alter important ecosystem functions such as wood decomposition. Decomposition increases with temperature, but the impact of temperature might vary at sites with different climatic régimes and decomposer communities. We experimentally examined wood decomposition and decomposer communities using open-top chambers warmed in a regression design at two sites in the eastern United States. We predicted that wood decomposition rates would be higher at the warmer site and that wood decomposition would increase with experimental warming at both sites. Additionally, we predicted that experimental warming would have larger impacts on fungal community composition at the warmer site than the cooler site because communities at the warmer southern site may already be under heat stress. As expected, coarse woody debris tended to decompose faster at the warmer southern site and in the warmest treatments, although not significantly. However, fine woody debris showed an unexpected pattern. Fine woody debris decomposed slower at the warmer southern site compared to the cooler northern site and slowed further under warming at the southern site, whereas at the northern site fine- woody debris decomposition increased with warming.

Experimental warming had larger effects on arthropod and fungal community composition at the southern site relative to the northern site. The disparity between predicted and observed rates of mass loss in fine- woody debris may have been related to shifts in the decomposer community, or high temperatures limiting decomposer activity.

Introduction

Temperature is the main driver of organic matter decomposition, and global surface temperatures are projected to exceed historical norms between 2047 and 2069 (Muro, 2013). Additionally, the taxa that regulate decomposition, fungi and macro-arthropods, may shift their activity or community composition in response to warming (Dang *et al.*, 2009; Iglesias Briones *et al.*, 2009; Gange *et al.*, 2007). Warming directly increases microbial enzyme activity, and correspondingly may increase soil carbon flux to the atmosphere in many ecosystems, but the effects of warming on decomposer community and activity may vary between ecosystems (Frey *et al.*, 2008; Aerts, 2006; Zogg *et al.*, 1993). Variation in the decomposer community response to warming across sites can influence decomposition rates and influence the global carbon cycle.

Wood, primarily made up of recalcitrant lignoceullosic compounds, is slow to degrade and is an important long-term carbon reservoir in forests. Indeed, dead and decomposing wood comprise 20% of carbon stored in forests (Harmon et al. 1986). Enzymes degrading recalcitrant compounds such as

lignocellulose tend to have higher activation energy and thus more potential to be accelerated by warming (Suseela *et al.*, 2012; Davidson & Janssens, 2006). Unlike soil, which may be buffered against short-term temperature and moisture changes, coarse- and fine-woody debris are exposed to daily and seasonal changes in temperature and moisture (Boddy, 1983a). Thus, wood decomposition rates may be influenced by changing climate to a greater extent than soil carbon decomposition, especially if seasonal variability increases with climate change.

Despite the importance of wood in global carbon cycling, relatively little is know about the response of wood decomposition to warming. However, average annual temperature explains most of the variation in wood mass loss in comparisons across multiple sites (Trofymow & Moore, 2002; Preston *et al.*, 2000). Several studies show that wood decomposing community activity increases in controlled laboratory environments (A'Bear *et al.*, 2012; Boddy, 1983b) and that mass loss can increase within field-based warming experiments (Berbeco *et al.*, 2012). However, other factors such as moisture and the decomposer community affect wood decomposition rates and may influence the response of wood decomposition to warming.

Similar to leaf or root litter decomposition, initial wood chemical quality and wood surface area can alter decomposition rates (Freschet *et al.*, 2012; Weedon *et al.*, 2009; Meentemeyer, 1978) and geographic differences other than climate

(e.g. soil type) can influence wood decomposition rates (van der Wal *et al.*, 2007). These factors may also influence the response of wood decomposition to temperature. High quality wood (low C:N ratio) tends to decompose faster with warming, while low-quality wood is relatively unresponsive to temperature changes (Yatskov *et al.*, 2003). Recent studies that experimentally manipulated warming in the northern and southern ranges of eastern deciduous forests found that soil bacterial and ant communities responded to warming in the southern range of eastern deciduous forests, but not in the northern range (Stuble *et al.*, 2013) (Cregger et al *in review*). Because fungal community structure can influence function (LeBauer, 2010; Setälä & McLean, 2004), variation in the response of the decomposer community to temperature across sites may alter the response of wood decomposition to temperature. Taken together, these findings suggest that more experiments manipulating warming and wood quality across multiple locations are needed.

Here, I take advantage of a large-scale warming experiment at the southern and northern rage of eastern deciduous forests to explore how coarse- and fine-woody debris, as well as the decomposer community, respond to experimental warming. At each site, we decomposed red maple woody debris in open-top chambers that heated the air in a regression design from ambient to +5.5°C in 0.5°C steps. Because decomposition is often limited by temperature, we predicted that mass loss of both the fine- and coarse-woody debris would be

greater in warm treatments, and mass loss would be higher at the warmer southern site. Similarly, we predicted the fungal and arthropod community would be more active, abundant, and diverse (higher richness) at the southern site and abundance would decrease with experimental warming.

Materials and Methods

The open-top chamber warming experiments were established in 2009 at two sites: a northern site at Harvard Forest in Petersham, MA [42° 31' 48" N, 72° 11' 24" W] and a southern site at Duke Forest in Durham, NC [35° 52' 0" N, 79° 59' 45" W]. Mean annual temperature and precipitation are 7.1°C and 1066mm at the northern site, Harvard Forest, and 15.5°C and 1140mm at the southern site, Duke Forest. Temperature ranged from -21.9°C (minimum January air temperature) to 43.2°C (maximum July air temperature) at the northern site, and -7.7°C to 40.2°C at the southern site. The experimental design includes 12, 5-m diameter chambers. Each chamber is constructed around a hardwood tree, and air is pumped into the chambers at the following temperatures: +0, +1.5, +2.0, +2.5, +3.0, +3.5, +4.0, +4.5, +5.0, and +5.5° C above current ambient temperature (for details see, Pelini *et al.*, 2011). Mean annual Δ- air temperature was calculated for each chamber (hourly chamber air temperature − hourly outside air temperature). Vapor pressure deficit was calculated according to the ASCE standardized reference evapotranspiration equation (Allen, 2005).

During the summer of 2009, we felled 6 young red maples *(Acer rubrum)*, near each site ranging in DBH between 12.7cm and 15cm, as red maple is an abundant hardwood present at both sites. Fine-woody debris (20-25cm long; < 2 mm in diameter) was cut from the trees at each site and placed in the chamber from the site from which it was harvested. We deployed eight fine-woody debris samples per chamber in 5cm × 20 cm decomposition bags constructed from 3-mm mesh on top and 1.3-mm mesh on bottom, the larger mesh size on top allowed access to arthropods and smaller mesh on bottom prevented fragments from being lost. We collected one fine-woody debris sample from each chamber annually for three years (2010, 2011, and 2012); the remaining samples will continue to be collected in future years.

We cut each harvested tree into 40-cm long coarse-woody debris segments; the mass of the segments ranged from 0.7 6.0 kg (corrected oven dried mass of 0.5 3.4kg). Some coarse-woody debris segments at the southern site exceeded the limits of our scale (6kg) at the time of harvest, and were cut to 30cm to maintain a fresh mass < 6kg. The cut ends of coarse-woody debris were painted with paraffin wax before being deployed into the chambers to limit the effect experimental cutting may have had on drying or increased inoculation potential. Coarse-woody debris samples were tagged with an individual ID number (4 per chamber) and collected after 1 and 2 years in 2010 and 2011. Future collections of coarse-woody debris are planned for 2014 and 2018.

We measured fresh mass within 48 hours of collection, air dry mass after samples were dried at room temperature for at least 14 days, and oven dried mass after drying at 100°C for at least seven days. For molecular analysis of fungal communities, we collected sawdust subsamples from fresh coarse-woody debris by drilling holes into the center of the coarse-woody debris using a 10mm stainless steel drill bit which we fire- sterilized between each sample to avoid cross- contamination. For chemical analysis, we used a similar process for sawdust collection, from air-dried coarse-woody, although drill bits were cleaned with deionized water rather than fire-sterilized. We ground fine-woody debris samples in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to 2mm mesh and further ground both sawdust chemical subsamples and fine-woody debris samples to a fine powder using a ball mill grinder (Pica Blender mill 2601, Cianflone of Pittsburgh, PA). To determine inorganic ash content, we then combusted oven- dried samples in a muffle furnace (550°C for six hours). We calculated mass loss based on initial and final ash-free oven-dried mass to correct for differences in mineral or water content. To determine initial water and ash content, we collected separate initial samples at the start of the experiment.

Arthropods from each log were collected in the lab. Each log was placed in a wax coated cardboard tube (quickrete) and the tube was encased in fine mesh (1.3 mm) for transport to the lab. While the logs were being processed (fresh

mass measured and subsamples removed for molecular analysis) an insect aspirator was kept on hand and any visible insects on the surface of the logs were aspirated and placed in 70% ETOH. In the lab, one end of the mesh was closed over a specimen cup filled with 70% ethanol and logs encased in the mesh were suspended over the cup for two weeks. Arthropods were later identified to order and morphotype using a dissecting microscope.

We developed fungal community fingerprints to assess fungal community structure in coarse- woody debris samples after two years of decomposition (2011) using fungal terminal-restriction fragment length polymorphism targeted at the ITS section of the rRNA gene (T-RFLP) (Singh *et al.*, 2006). The sawdust subsamples we collected for molecular analysis were immediately frozen and stored at -80 °C. We extracted DNA templates using the Mo-Bio Powersoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) with 0.25g of sawdust placed in bead beating tubes. We added an extra filter-washing step (repeating the filter- washing step twice) and used 30 µl, rather than 50µl of elution buffer, but otherwise followed the kit protocol. To amplify the ITS region of the rRNA gene, we used the following reagents and polymerase chain reaction (PCR) protocol: 50 µl PCR mixtures contained 5 µl 10x KCL reaction buffer, 2 µl 50 mM MgCl2, 1 µl 10 mM dNTPs (Bioline, Tauton, MA), 1 µl 20 mg/ml BSA (Roche, location), 0.5 µl (2.5 Units) Taq DNA polymerase (Bioline, Tauton, MA), and 2 µl of each fungal primer (ITS1f: 5' (6-FAM)

CTTGGTCATTTAGAGGAAGTAA -3' (Bruns, 1993) and ITS4r: 5' TCCTCCGCTTATTGATATGC -3'(Singh, 2006). We carried out the PCR reaction under the following conditions: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and finally 72 °C for 10 minutes on a 96-well Tgradient thermocycler (Biometra, Goettingen, Germany). To confirm successful amplification, we used 1% agarose gel electrophoresis.To clean amplicons, we used the QIAquick PCR purification kit (Qiagen, Valencia, CA) and quantified the template using a Synergy HT microplate reader (Biotek, Winooski, Vermont, USA). We digested each PCR product individually with *Msp*I in the following 20 µl reaction: 14 µl of purified PCR products, 2 µl 10X buffer B, 2 µl MSP1 (Fisher Scientific, USA), 2 µl 10 mg/ml acetylated BSA (Promega, Madison, WI) by incubation at 37 °C for three hours followed by a deactivation step at 95 °C for 10 min. After digestion, we made a cocktail containing 0.5 µl LIZ labeled GeneScan 1200 internal size standard (Applied Biosystems, Grand Island, NY), 12.5 µl Hi-Di formamide (Applied Biosystems, Grand Island, NY), and 1 µl of digested product. We briefly centrifuged then incubated samples at 94 °C for four min then at 4 °C for five min prior to analysis. For fragment analysis we used an ABI Prism 3100 genetic analyzer (Applied Biosystems, Grand Island, NY). We produced T-RFLP profiles using the GeneMapper software (Applied Biosystems, Grand Island, NY) assuming apparent terminal restriction fragments (TRFs) at fewer than 62 bp to be caused

by primer-dimers. To calculate the relative abundance of a TRF, we calculated the TRF peak height relative to total peak height of all TRFs in the T-RFLP profile (Singh *et al.*, 2006).

To test whether mass loss or % moisture of fine- woody debris or coarse-woody debris differed between removals (years 1,2, and 3 for fine-woody debris or years 1 and 2 for coarse- woody debris), site (northern or southern site) and temperature treatment we used ANCOVAs where site and removal year were discrete variables and Δ- temperature or vapor pressure deficit were a continuous predictor variables. Separate ANCOVAs were performed with mass loss of fine- woody debris, mass loss of coarse- woody debris, % moisture of fine- woody debris and % moisture of coarse woody debris as the response variable. Initially, we included Δ- soil moisture as a continuous predictor variable, but after observing no direct or interactive effects of soil moisture on wood mass loss or moisture content, we removed soil moisture from the model. All statistics were performed using the R statistical program v2.06. Mass loss of coarse-woody debris and fine- woody debris were square root transformed to meet the assumptions of normality. Normal distributions of all data sets were confirmed using a Wilkes-Lambda test.

We calculated species richness, abundance, evenness, and Simpsons Diversity Index in the Primer statistical package (Plymouth Marine Laboratory, UK) for both arthropod communities and fungal OTUs. To test the effects of

temperature treatment and site on fungal richness, evenness and Simpsons Diversity Index, we used separate ANCOVAs for each response with site as a discrete and Δ- temperature was a continuous variable. Arthropod richness, abundance, evenness and diversity were not normally distributed and we performed Monte Carlo analysis with 9999 resamples of the data using the coin package v. 1.0-21 in R and a one-way Wilcox test was performed 10 times with no change in significant results, to test whether species richness or abundance varied between the northern and southern sites. To test whether environmental variables altered arthropod species richness or abundance within sites, spearman's test was used with 9999 Monte Carlo resamples and repeated 10 times where significant or trending results were observed.

For the purposes of fungal community fingerprint analysis, we considered each T-RFLP peak as an operational taxonomic unit (OTU) and peak height relative to total sample peak height as relative abundance of each OTU. To assess the difference in fungal community similarity between sites, we used the betadisper function in R (Anderson, 2006). To assess the effects of environmental factors on fungal and arthropod community structure, we used Constrained Correspondence Analysis (CCA) and environmental vectors for 10 environmental variables (mean air temperature, mean soil temperature, mean soil moisture, mean relative humidity, Δ- air temperature, Δ- soil temperature, Δ- soil moisture, Minimum January air temperature, maximum July air

temperature and vapor pressure deficit) were fit to the ordinations for the fungal and arthropod communities using the envfit function in the vegan package v. 2.0 - 7 (Oksanen, 2008).

Results

Fine-woody debris lost 39% of mass after three years, and decomposed 1.4× faster at the northern site than at the southern site ($F_{1,64} = 17.93$, $p < 0.0001$, Figure 10). The effect of warming treatment on mass loss also differed between the two sites, such that warming tended to increase mass loss from fine woody debris at the northern site but decrease mass loss from fine-woody debris at the southern site ($F_{1,64} = 7.00$, $p = 0.01$, Figure 1). There was a similar interactive pattern between vapor pressure deficit and site where chambers with a greater vapor pressure deficit at the northern site tended to have more mass loss whereas chambers with greater vapor pressure deficit at the southern site tended to have less mass loss ($F_{1,64} = 3.99$, $p = 0.05$). As expected, mass loss of fine-woody debris increased over time ($F_{1,64} = 5.83$, $p = 0.02$, Figure 10). Moisture content of fine-woody debris was 2× higher at the northern site than at the southern site ($F_{1,64} = 23.14$, $p < 0.0001$), and varied from year to year ($F_{1,64} = 23.14$, $p < 0.0001$). However, there was an interaction between site and removal year on moisture in fine-woody debris ($F_{1,64} = 4.275$, $p = 0.04$) such that in year one, moisture was higher at the southern site than the northern site. However, in years two and three we observed a wide range of moisture content

in fine-woody debris at the northern site, whereas moisture remained relatively stable at the southern site. Moisture content of fine-woody debris also decreased with warming ($F_{1,64} = 23.14$, $p = 0.02$).

Coarse woody debris decayed about half as fast as fine- woody debris. After two years, approximately 15% mass loss from coarse- woody debris occurred, whereas 33% mass loss from fine- woody debris occurred over the same period. Although there were no significant effects of removal year, site, vapor pressure deficit, or warming treatment on mass loss from coarse-woody debris, there was a trend towards increasing mass loss over time ($F_{1,40} = 3.29$, $p = 0.08$) and more mass loss from coarse-woody debris at the southern site ($F_{1,40} = 3.52$, $p = 0.07$) than at the northern site. Moisture was 10% higher in coarse-woody debris at the northern site (25% moisture) compared to the southern site (15% moisture), ($F_{1,40} = 42.12$, $p < 0.0001$) and decreased with warming ($F_{1,40} = 4.43$, $p = 0.04$), but coarse-woody debris moisture was similar between removal years ($F_{1,40} = 0.11$, $p = 0.75$).

Fungal communities in coarse-woody debris differed between the two sites, and were more similar in composition among treatments at the northern site than at the southern site (Figure 12) ($F = 4.67$, $p < 0.05$). At the northern site, moisture strongly corresponded to fungal community composition (soil moisture content $R^2 = 0.96$, $p = 0.001$, Δ- soil moisture $R^2 = 0.96$, $p = 0.001$, Figure 12) whereas at the southern site, both temperature (average air temperature $R^2 = 0.72$, $p = 0.01$,

average soil temperature $R^2 = 0.63$, $p = 0.03$, Δ- air temperature $R^2 = 0.68$, $p = 0.02$, minimum January air temperature $R^2 = 0.58$, $p = 0.04$) and moisture factors (relative humidity $R^2 = 0.79$, $p < 0.01$, and vapor pressure deficit $R^2 = 0.72$, $p = 0.01$) were significant in correlated with fungal community composition (Figure 12).

Arthropods were 6 × more abundant at the southern site than at the northern site ($Z = 2.38$, $p < 0.003$), but there was no difference in arthropod richness between sites. At the southern site, arthropod abundance increased with soil temperature ($Z = 2.11$, $p = 0.03$), and there was a trend towards lower species richness at higher soil moisture ($Z = -1.73$, $p = 0.09$), although there was also a trend towards lower soil moisture in plots with higher soil temperature ($R^2 = 0.13$, $p = 0.08$). However, none of the measured environmental variables altered arthropod abundance or richness at the northern site. Arthropod community structure differed between the two sites, and none of the measured environmental factors correlated with arthropod community structure at the northern site, although arthropod community structure was correlated with maximum July air temperature at the southern site ($R^2 = 0.61$, $p = 0.01$).

Discussion

The effects of temperature on wood decomposition in our study were site specific and varied with the type of woody debris (coarse- vs. fine- woody debris). We predicted both fine-woody debris and coarse-woody debris would

decompose faster at the warmer, southern site relative to the cooler, northern site and similarly, that decomposition rates at each site would increase with experimental warming. As predicted, coarse-woody debris decomposed more quickly at the southern site, however, fine-woody debris decomposed more quickly at the northern site. While warming accelerated decomposition of fine-woody debris at the northern site, warming slowed decomposition of fine-woody debris at the southern site. The observed disconnect between the response of mass loss to warming at the southern and northern sites was unexpected but might be explained by either substrate moisture limitations or decomposer organisms operating above their thermal optima.

Wood retains moisture longer than surrounding litter and soil, and high moisture levels can inhibit decomposition during early stages (Boddy, 1983b). However, some studies show that high moisture and oxygen limitation are more prevalent in the tropics, and wood decomposition is more likely to be limited by low moisture levels in the temperate and boreal regions (Torres & González, 2005; Abbott & Crossley Jr, 1982). At low temperatures, moisture is unlikely to limit decomposition but with increasing temperature, moisture limitation increases; similarly, wood decomposition increases with temperature to a greater degree with increasing levels of moisture (Boddy, 1983a). Since warming decreased moisture in fine- woody debris and slowed decomposition of fine-woody debris at the warmer southern site, moisture limitation could be a

possible mechanism linking warming to slower decomposition rates at the southern site. Generally speaking, moisture levels below 30% are considered limiting to basidiomycetes, a major group of wood decomposing fungi (Kåårik, 1974). Moisture levels in fine-woody debris were lower at the southern site (below 18% at all removals) compared to the northern site (25% average), despite higher annual precipitation at the southern site. Mean moisture levels at both sites are below the 30% threshold considered limiting to basidiomycetes, thus if moisture limitation explains the negative correlation between warming and mass loss in fine-woody debris at the southern site, we should observe a similar pattern at the southern site. However, these measurements represent wood moisture at the time of removal and moisture may have varied during the incubation, either becoming so low as to limit fungal growth at the northern site or so high as to support optimum fungal growth at the southern site. Although there was no effect of vapor pressure deficit on mass loss of coarse- or fine- woody debris, there was a reversal in the trend in coarse woody debris at the southern site (Figure 13), in fine woody debris there was a pattern of more mass loss from fine- woody debris in plots with a greater vapor pressure deficit at the northern site and less mass loss from fine- woody debris in chambers with a greater vapor pressure deficit at the southern site. While this pattern is not significant it reflects the same trends as those observed between % mass loss and Δ-temperature. However, at the southern site, coarse woody

debris tended to lose more mass in warmer chambers but less mass in chambers with a high vapor pressure deficit, indicating that moisture may be playing a role (Figure 11, Figure 13). While extreme temperatures may have led to drying of the fine- woody debris at the southern site, limiting the decomposer activity, there was no effect of either Δ- soil moisture or Δ- relative humidity on mass loss from fine- or coarse- woody debris in our models. Thus, moisture was probably not the dominant factor limiting fine- woody debris decomposition in warmer chambers at the warmer, southern site.

Alternatively, extreme temperatures may have directly limited decomposer activity. Controlled laboratory experiments show that wood respiration increases with warming as high as 25 - 30°C (Pietikäinen *et al.*, 2005; Boddy, 1983b), the same temperature range for optimum basidiomycete fungal growth (Cartwright & Findlay, 1934). While mean temperatures at these sites (northern site = 10.7°C, southern site = 17.3°C) were well below thermal maxima for wood decomposing fungi, maximum temperatures were much higher at both sites (43.2°C at northern site and 40.2°C at southern site). Previous research at these sites showed temperature affects soil bacterial richness and abundance at the southern site, but not at the northern site. This suggests that the high temperatures observed at the southern site may cross a threshold for microbial growth or survival (Cregger et al., 2013). This threshold could be reached more quickly in fine- woody debris where fluctuations in temperature are less

buffered by surrounding wood compared to coarse- woody debris where we observed increased mass loss with warming, although not a statistically significant pattern. This could explain why we saw slower rates of mass loss with increasing temperature in fine- woody debris at the southern site and increasing rates of mass loss with temperature in fine- woody debris at the northern site, but no effect of temperature on mass loss of coarse woody debris. Fungal communities in coarse-woody debris differed between the two sites (Figure 12), and communities were more dissimilar at the southern site ($F = 4.7$, $p < 0.05$). Within sites, environmental factors explained sizable portions of the variation in fungal community structure. At the southern site both temperature and moisture were important factors influencing wood inhabiting fungal community composition (Figure 12), and soil moisture structured fungal community at the northern site (Figure 12). Differences in the ranges of species providing potential inoculum, or arthropod grazing could also explain differences in the fungal community or co-vary with climatic variables. Arthropods may be more important to decomposition in hot, dry environments compared to cool or humid environments, but can also be limited by moisture and temperature (Whitford & Parker, 1989; Abbott & Crossley Jr, 1982). Arthropods were 6× more abundant in coarse- woody debris at the southern site than coarse- woody debris at the northern site. Therefore it is not surprising that we observed a relationship between mass loss and arthropod

communities only at the southern site. Mass loss was highest in coarse-woody debris with high arthropod abundance and richness at the southern site despite arthropod abundance being negatively correlated with arthropod richness. We found that maximum July air temperature was highly correlated with arthropod community structure at the warmer southern site, which is in congruence with earlier findings at these sites. Previous research in this experiment has shown that temperature effects on arthropod foraging is site specific, and temperature increased forager abundance at the southern site, but not at the northern site (Stuble *et al.*, 2013). Arthropods play important roles in early stages of wood decomposition as they tunnel into wood, increasing the surface area, reducing oxygen limitation, and introducing fungal spores to the inner wood. Higher arthropod abundance at the southern site may have reduced fungal abundance or altered the fungal community through selective grazing (A'Bear *et al.*, 2012). Clearly, climatic warming can alter the community structure and activity of decomposers, thus altering carbon release from woody debris a large carbon pool. Our study reveals several nuances to this generally accepted pattern. First, the relationship between warming and decomposition varied between sites. Second, the sign and magnitude of decomposition responses to increased temperature may be highly confounded by changes in substrate type, moisture and/ or other variables. The direct effects of temperature on decomposition rates are increasingly understood, well measured, and modeled; however, they

appear idiosyncratic. Incorporating direct effects of warming and drying as well as indirect effects of drying via warming, on decomposer community structure may be important to accurately predict future carbon cycling.

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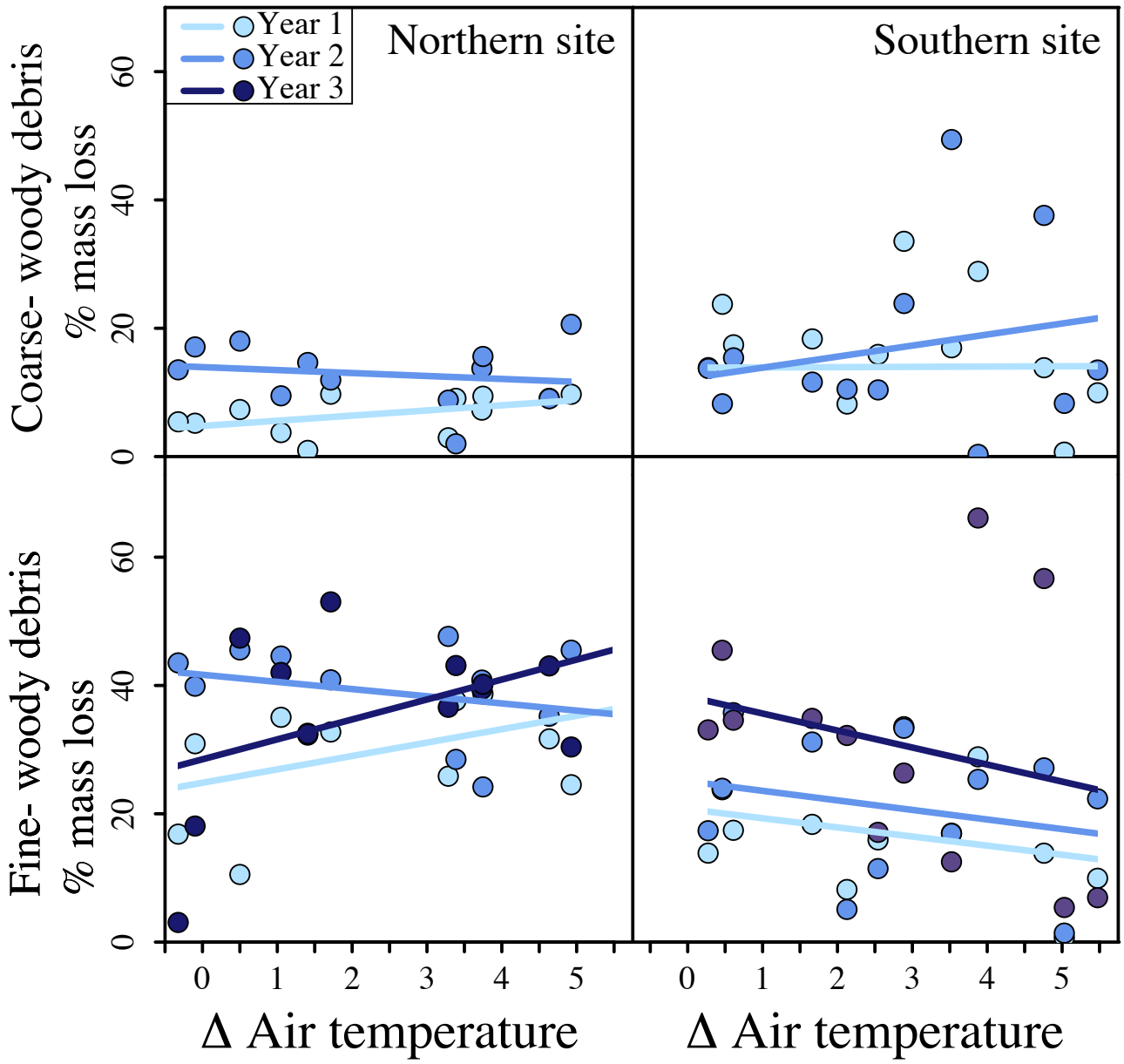
Apendix III: Chapter III figures

Figure 10: Mass loss of fine-woody debris (bottom) increased over time ($F_{1,64}$ = 5.83, p = 0.02), and more mass was lost from fine woody debris at the northern site (left) than the southern site (right) ($F_{1,64}$ = 17.93, p < 0.0001). However, there was an interaction between the effects of site and chamber temperature on mass loss where warming tended to increase mass loss from fine- woody debris at the northern site, but decrease mass loss from fine-woody debris at the southern site ($F_{1,64}$ = 7.00, p = 0.01). Mass loss from coarse woody debris tended to increase over time, ($F_{1,40}$ = 3.29, p = 0.08) and we observed a trend towards more mass loss from coarse- woody debris at the southern site ($F_{1,40}$ = 3.52, p = 0.07).


Year 1
Year 2
Year 3
Northern site
Southern site
Coarse- woody debris
% mass loss
Fine- woody debris
% mass loss
0
20
40
60
0
1
2
3
4
5
Δ Air temperature
Δ Air temperature

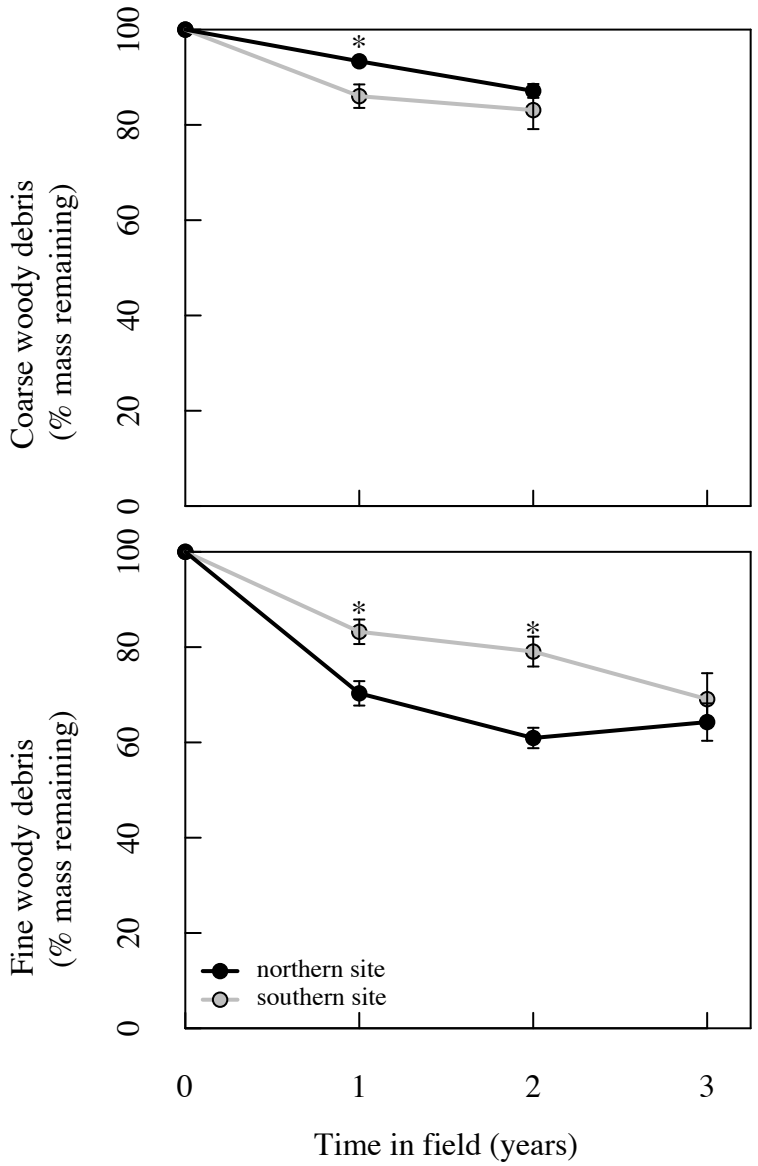
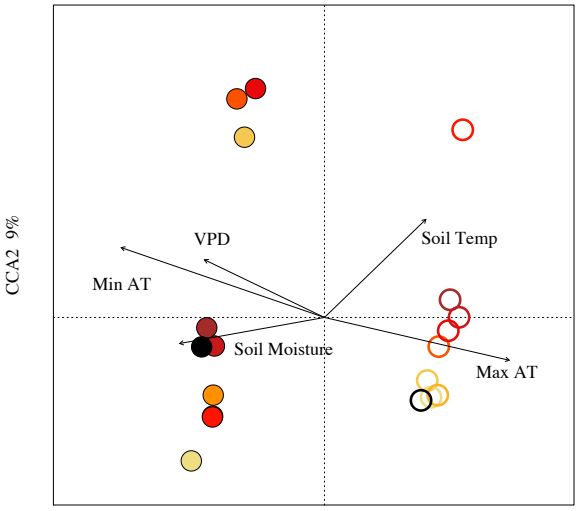
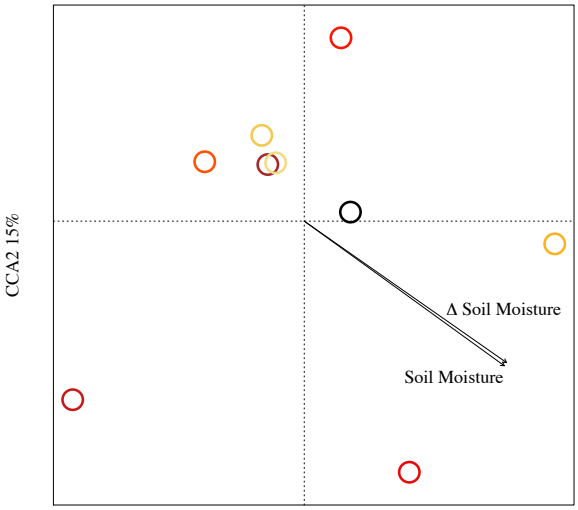
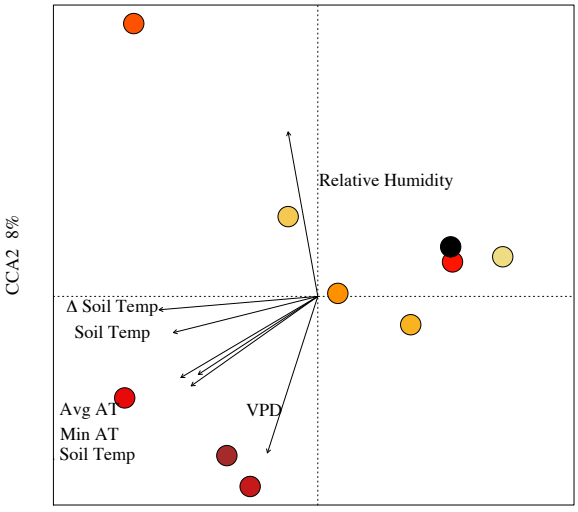


Figure 11: Mass loss of coarse- woody debris (top) was greater at the southern site in year one. Mass loss of fine- woody debris (bottom) was greater at the southern site in years one and two.

Figure 12: Fungal communities differed between sites and were more dissimilar at the southern site (top), fungal communitiey composition at the northern site was associated with soil moisture (middle) and Fungal community composition at the southern site was associated with both temperature and moisture variables(bottom). Data displayed are from a fungal community fingerprint constrained canonical analysis. Each dot represents the fungal community structure in a coarse-woody debris sample at the northern site (open symbols) or the southern site (filled symbols). Colors represent chamber temperature treatments ranging from ambient (black) to +1.5°C (yellow) to +5.5°C (dark red). Vectors are significant ($p < 0.05$) explanatory environmental factors (mean soil temperature, minimum January air temperature, maximum July air temperature, soil moisture, vapor pressure deficit, Δ- soil moisture, Δ- soil temperature, mean air temperature, and relative humidity).


CCA2 9%
VPD
Soil Temp
Min AT
Soil Moisture
Max AT
CCA1 11%


CCA2 15%
Δ Soil Moisture
Soil Moisture
CCA1 16%


CCA2 8%
Relative Humidity
Δ Soil Temp
Soil Temp
Avg AT
Min AT
Soil Temp
VPD
CCA1 11%

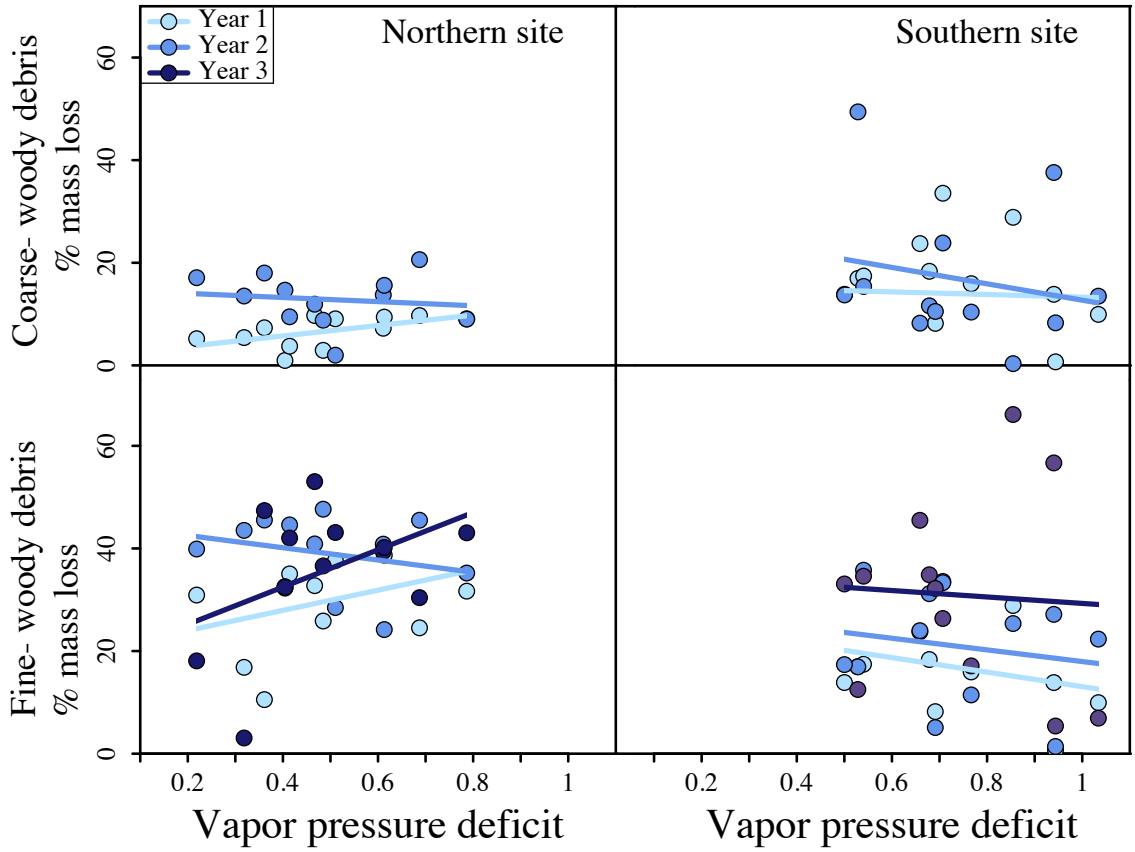


Figure 13: Trends in % mass loss from fine- (bottom) woody debris may be driven by the observed correlation between % mass loss from fine- woody debris and Δ- air temperature (Figure 10). However, the pattern between % mass loss from coarse- woody debris and vapor pressure deficit at the southern site (top right) differs from the pattern between % mass loss from coarse woody debris and Δ- air temperature (Figure 10), indicating that moisture may be playing a role in limiting wood decomposition rate at the southern site.

Conclusions and future directions

My dissertation shows that warming affects wood decomposition rate, and the wood decomposing fungal and arthropod communities. The effect of climatic warming is varied by geography but may be relatively constant across variations in wood physical and chemical properties associated with the changes in wood over the course of decomposition. While my research contributes to a growing body of literature on the effects of climatic change on microbial communities and ecosystem properties, and adds to research on wood decomposition and wood decomposing fungal communities, my work leaves some important questions unanswered.

1. While I did not observe an effect of wood decomposition stage on fungal community structure, many other factors vary across the stages of wood decomposition including the changes I observed in chemical and physical characteristics. In order to test whether fungal community structure affects the response of ecosystem processes such as respiration or decomposition rates to climatic change, the fungal community composition needs to be manipulated in a controlled environment. While several studies have manipulated fungal community structure and found effects on several processes,

to my knowledge none has co-manipulated the effects of fungal community structure and temperature.

2. Wood decomposition is a slow process due to the recalcitrant nature of wood. The time- scale of dissertation research limits the research presented here, however the field- based temperature manipulation is ongoing and will be continued for a total of eight years. Still, wood decomposition can take much longer than 20 years. Many long- term observations of fungal fruiting bodies are underway, and I expect these types of studies will continue with added molecular advances. Another method to consider is the modified chronosequence approach.
3. Finally, our knowledge of what structures fungal communities is still limited. A growing body of research has shown that soil bacterial community structure is not influenced by temperature, latitude, or elevation, as plant communities are but rather by soil pH (Fierer & Jackson, 2006; Rousk et al., 2010). And ectomycorrhizal communities do not follow the common pattern of increasing diversity with decreasing latitude, but rather have higher diversity in temperate regions (Tedersoo & Nara, 2010). In future projects I plan to expand a method I developed during my dissertation using standard wooden substrates as baits, deploying these bates at sites across a range of

environments and environmental gradients then identify the fungal communities and measure rates of mass loss. I have tested this method in sites across a latitudinal gradient from Georgia to Maine and identified different communities using T-RFLP (Figure 14). I will compile a database of fungal communities and environmental variables from sites and manipulations across the world and identify the important variable structuring fungal communities.

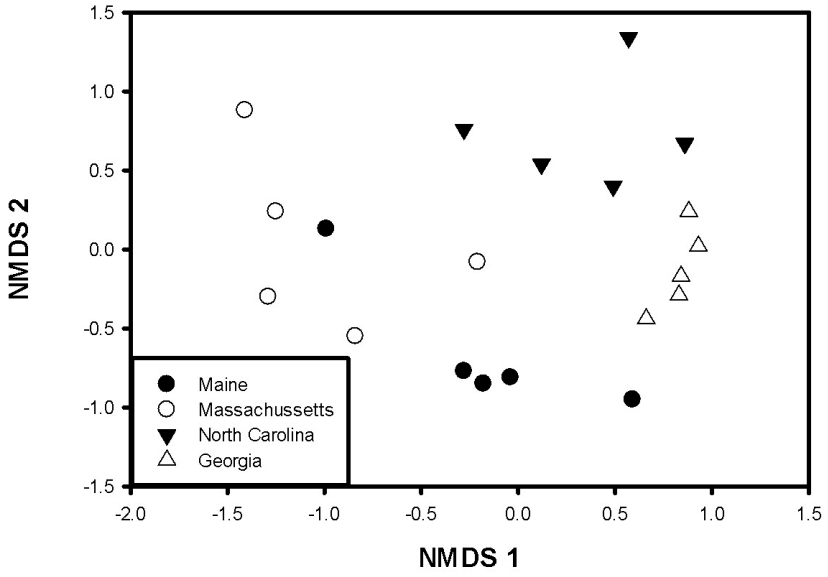


Figure 14: Fungal community structure from baits across a latitudinal gradient from Georgia to Maine USA.

Vita

Emily Elizabeth Austin hails from the great state of Massachusetts where she graduated form Arlington and Beacon High Schools in Arlington and Brookline, MA and Hampshire College in Amherst, MA. She has worked as an intern in the NSF Research Experience for Undergraduates (REU) program with Principal Investigator (PI) Eric Davidson at the Woods Hole Research Center, as an undergraduate researcher with PIs Aaron Ellison and Julian Hadley at the Harvard Forest, as a post-baccalaureate with PIs Chris Schadt and Aimée Classen at Oak Ridge National Laboratory and as a PhD student and candidate at the University of Tennessee. She looks forward to a long career teaching and researching in ecosystem ecology.