TEMPERATE FOREST CARBON CYCLING:

THE IMPORTANCE OF TREE SPECIES IN A CHANGING GLOBAL ENVIRONMENT

A dissertation

submitted by

Minda R. Berbeco

In partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

in

Biology

TUFTS UNIVERSITY

February, 2011

ADVISER: Colin M. Orians

Abstract

Forest carbon cycling is a political and scientific challenge. From a policy perspective, carbon storage is important to climate mitigation, with the goal of sequestering carbon dioxide (CO_2) in forests. From a scientific perspective, the environmental variables that control storage and loss of carbon are of central interest. This dissertation looks at carbon cycling from both perspectives: carbon storage in live biomass to offset emissions and the mechanisms that determine carbon loss from dead biomass. First we compared Massachusetts' forest carbon sequestration in live biomass to energy-sector CO_2 emissions. We found that over 10% of the state's energy-sector CO_2 emissions were sequestered within the state's forest and nearly half of these forests were at risk of deforestation. From there we looked at the history of a Massachusetts forest to see how a changing composition altered carbon storage in live and dead biomass. We found that this forest increased in above-ground woody biomass carbon storage during the first 40 years, sequestering 3.80 Mg C/ha/yr, but sequestration decreased over the next 20 years. In mature stands, we found that total coarse woody debris biomass in the forest was 13.52 Mg/ha and lignin was 23% of the total biomass. With the knowledge of the woody debris composition, we then asked what would happen to this carbon pool with climate change. We found that soil warming increased mass loss of woody debris by as much as 30%, but that more recalcitrant and larger debris decomposed much slower. We also found that the most lignin-rich species lost lignin the fastest. Last, we asked how a change in woody inputs to the soil from a shifting forest composition would influence soil dynamics under

current and future environmental scenarios of warming and nitrogen deposition. We found that a shift from recalcitrant to more labile woody inputs would only increase microbial respiration and activity under future scenarios of warming and nitrogen addition. The research in this dissertation suggests that forests may be able to mitigate CO_2 emissions under current forest composition and environmental scenarios, but this could shift dramatically in the next 100 years with a changing environment.

Acknowledgements

There are many people that need to be acknowledged for their help over the past 6 years. First and foremost, I would like to thank my advisor, Colin Orians, for his continual support and advice throughout my graduate career. I couldn't think of a more patient, kind and wise person to advise me through my graduate experience and I am grateful for the many opportunities he gave me while at Tufts. I would also like to acknowledge my committee members, George Ellmore, Adrien Finzi and Michael Reed, for their thoughtful contributions to my research. Committee meetings became times to get new angles on my research while in a supportive environment and I am thankful for their willingness to challenge me with questions and new ideas.

Of course, I would be remiss if I did not acknowledge my lab mates: Colleen Butler, Tegan Morton, Alex Thorn, Coco Gomez and the many undergraduates who came through the lab. Their feedback on my research ideas, comments on presentations and ground support with physical labor were invaluable to my success at Tufts.

Many undergraduates at Tufts contributed to the research in this dissertation. They included Tim Korpita, Ian McClellan, Lily Glidden, Robbie Moore, Xin Cao, Dan Brady and Josh Gordon. I enjoyed working with each of them and learned how to be a better mentor through them.

None of my work at Tufts could have ever happened without the help of Michael Grossi, Eileen Magnant, Liz Palmer, Karin Murphy and Tony Keevan. Their help and organization created a wonderful, easy and friendly environment to work in.

The research in this dissertation was the result of collaboration with several institutions and researchers. First, Barbara Parmenter in the Tufts GIS lab was central to defining the first chapter of this dissertation. At Hopkins Forest, David Dethier, Jay Racela, Henry Art, Drew Jones and the Parks family all assisted me in my research and successful stay in Williamstown. At Harvard Forest, Jerry Melillo's team of researchers and post docs were incredibly helpful and supportive of my research there. I am also indebted to Jerry for allowing me to complete my research in his soil warming plots at Harvard Forest. In addition, the people at Harvard Forest were extremely helpful and supportive of my work there, allowing me to borrow equipment, stay on their premises and converse with the world class scientists who work there. Teri Burdett at Harvard Forest and Leszek Bledzki at Mt. Holyoke both were integral in helping with the arduous labor of working with wood samples. I'm not sure my research could have moved forward without them.

While at Tufts I was given funding for my research, travel and education by Tufts Institute for the Environment, Tufts Graduate Student Research Award, Strategic Environmental Research and Development Program Travel Grant, Graduate Women in Science Travel Grant, Graduate Institute for Teaching, Donana

National Park, NSF Ethics of Science Program and of course the Tufts Biology Department. I am grateful for all of the opportunities this funding granted me and am indebted to them for their support. In addition, it should be noted that half of my dissertation took advantage of datasets that were originally set up through tax payer money and large government initiatives. The data from Hopkins Forest was originally collected by the Civilian Conservation Corp during the Great Depression and then continued later by the US Forest Service and last, Williams College. I also took advantage of data sets collected by US satellites and made free to the public. Such datasets are extremely valuable and I feel fortunate to have worked with them.

Last, I would like to acknowledge my friends and family who supported me through this process along the way. My friends Priya Sundararajan, Mike Simon, Dave DesRochers, Meghan Guitry, Ayron Strauch, Dot Baisley, Hallie Lee and of course my sweetie, Will Fertman. You all supported me immensely during this process and saw me through some of my worst moments. I would have been completely lost without you. My secret advisor, Linda Tropp, gave me crucial advice on how to manage my graduate career. My parents, their mates, my many full, step, half and sideways brothers and sisters all contributed to my success while at Tufts. But in particular I owe my success at Tufts to my brothers Steven and Ross, who showed me through their own amazing intellectual pursuits that nothing is impossible, just sometimes really, really, really, really difficult. After the past 6 years of graduate school, I might have to agree.

Table of Contents

Index of Tables

Index of Figures

TEMPERATE FOREST CARBON CYCLING:

THE IMPORTANCE OF TREE SPECIES IN A CHANGING GLOBAL ENVIRONMENT

Chapter 1

Introduction

This past September I went to a talk by a leading climatologist presenting the ins and outs of climate change. He argued that the metaphor of a greenhouse to explain the greenhouse effect was actually a misrepresentation: unlike in a real greenhouse, obstructing convective heat loss was not what warmed the earth. After the talk, I asked him in person what a better metaphor would be. His response was at first that he couldn't think of one, but he relented under pressure. Maybe the atmosphere could be considered more of a "space-age greenhouse" (my term with his approval) with multiple glass layers capturing heat on its way back to outer space. I then asked him if we could use the metaphor of multi-leveled disco balls in the atmosphere, reflecting light and knocking back gases. He said he didn't think so.

Finding an appropriate metaphor for the carbon cycle in forests is equally as challenging. There is of course the clichéd metaphor of the forest as an animal, breathing in carbon and out oxygen. This metaphor turns a dynamic cycling system into a place where only trees live. Boring. Inaccurate. Shallow. But much like the "space-aged greenhouse" arising from the bad greenhouse metaphor, perhaps we can expand the idea of the forest as an animal to the forest

as a “super-animal” embedded with a series of organisms using and discarding carbon in a continual cycle. First in the trees, then to the ground as leaf litter or woody debris, to the soil, through bacteria and fungi and then out again to the atmosphere, only to be recaptured again by photosynthesizing trees. It is this cycle of carbon moving in and out of the forest that I focused my dissertation. It is the movement and pace of this carbon cycling that is the cornerstone of my research.

It should be noted that carbon has implications outside of the forest as well. It is an important part of our atmosphere, responsible in part for the greenhouse effect and as a result global climate change. As a result, it has become an important discussion for both scientists and policy makers, as one group tries to understand this process and the other tries to control it. Though it is with the first group that I identify myself, it is with this second group where my research began.

In early 2007, Massachusetts joined several other Northeastern states in the Regional Greenhouse Gas Initiative. The goal of this policy initiative was to mitigate greenhouse gas emissions from coal-fired power plants within the region. If a power plant exceeded its allotted carbon dioxide emissions, it was required to offset them. One option for emissions offsets was the use of forests to sequester carbon, as trees take in carbon dioxide as part of photosynthesis. Surprisingly, though, no one had actually looked at whether or not using forests as offsets for

energy-sector emissions was possible in a place like Massachusetts. In the first chapter of this dissertation, I sought to answer the question of whether Massachusetts forests could be used in this way. I used calculations of changing forest carbon storage in live trees over time and compared it to state-wide energy-sector emissions.

Live biomass, though popular to study, is not the only carbon pool in forests. When a tree dies, the carbon it has sequestered is not immediately released into the atmosphere. Rather it is broken down slowly over time by microbes. This makes the woody debris pool an important carbon pool in temperate forests, as it is currently a carbon sink contributing minimally to total stand respiration (Liu *et al.* 2006). Due to its value to temperate forest carbon storage, the remainder of my dissertation focused on this carbon pool.

When woody debris carbon is broken down by microbes, it is either released into the atmosphere as carbon dioxide or mixed into the soil where it is stored for extended periods of time. Mattson *et al.* (1987) found that, in fine woody debris, approximately 40% of the carbon released over 7 years is emitted into the atmosphere, while the rest is mixed into the soil. In coarse woody debris this amount jumped to 75% of the carbon released. It appears from this research that size matters when it comes to carbon storage. But does it really? While some studies have found that size will influence decomposition (Mackensen *et al.*

2003), others have not (Chen *et al.* 2001). Similarly, some studies have found that the species of the debris can have an effect on decomposition (Brown *et al.* 1996), while others have found that it is only important in much later decomposition stages (Liu *et al.* 2006). This slow, complex and often inconsistent decomposition process has made the woody debris pool a perplexing and unpopular carbon pool to study.

Decomposition patterns in the woody debris pool become even more complex when considering this process under future environmental scenarios for New England forests. These scenarios include both climate warming and nitrogen deposition. The literature on leaf litter is clear and could be indicative of what to expect from the decomposition of woody debris. Labile leaf litter decomposes faster than recalcitrant litter (Cross & Grace 2010), but recalcitrant litter has been shown to respond more strongly to temperature increases (Fierer *et al.* 2005). Similarly, nitrogen addition slows the decomposition of more recalcitrant leaf litter, whereas it speeds up decomposition of more labile materials (Knorr *et al.* 2005). One would imagine that woody debris would respond to warming and nitrogen addition in a similar manner to leaf litter as they are both carbon components of trees. However, given the inconsistencies within the woody debris literature under normal environmental conditions, it is not clear that woody debris would follow the same patterns as leaf litter in a changing global environment.

This dissertation is split into two parts, answering both practical and theoretical questions. The first half of the dissertation looks at carbon storage in applied terms: quantifying carbon storage and how it could be used. Though the suggestion of using forests for carbon offsets may be controversial, the basic science of how much carbon can be stored in a given forest is not. In chapter 2, I ask the question of how much of Massachusetts' energy sector emissions can be offset by the current forest carbon sequestration. In chapter 3, I look at carbon storage in a deciduous forest in Western Massachusetts over the past 70 years to see how carbon storage changed as the forest aged.

The second half of my dissertation attempts to answer questions regarding the mechanisms that allow for carbon storage and loss from the forest, specifically in the woody debris pool. I chose woody debris in particular because of the many conflicting studies on decomposition in this pool. This lead me to believe that unlike decomposition in other carbon pools, such as soil or leaf litter, there were complex mysteries to be unlocked within the woody debris pool. In the second half of chapter 3, I looked at the woody debris pool to see how and where carbon is stored after the lifespan of the trees. In chapter 4, I looked at the effect of warming of decomposition of woody debris to see how carbon storage will shift in this pool under future scenarios of warming. In chapter 5, I studied the effect of a shifting environment, including changing carbon inputs, temperature and nitrogen deposition, on soil carbon dynamics.

This dissertation was meant to answer questions about carbon storage in both live and dead wood to help us understand these pools further and the mechanisms that drive storage there. It is with this knowledge that we can start to determine how long carbon can be stored in temperate forests and integrate this into our understanding of carbon cycling globally.

Works Cited

Brown S, Mo J, McPherson JK, Bell DT (1996) Decomposition of woody debris in Western Australian forests. *Canadian Journal of Forest Research*, **26**, 954-966.

Chen H, Harmon ME, Griffiths RP (2001) Decomposition and nitrogen release from decomposing woody roots in coniferous forests of the Pacific Northwest: a chronosequence approach. *Canadian Journal of Forest Research*, **31**, 246–260.

Cross A, Grace J (2010) The effect of warming on the CO2 emissions of fresh and old organic soil from under a Sitka spruce plantation. *Geoderma*, **157**, 126-132.

Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. *Ecology*, **86**, 320-326.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252-3257.

Liu WH, Bryant DM, Hutyra LR, Saleska SR, Hammond-Pyle E, Curran D, Wofsy SC (2006) Woody debris contribution to the carbon budget of selectively logged and maturing mid-latitude forests. *Oecologia*, **148**, 108-17.

Mackensen J, Bauhus J, Webber E (2003) Decomposition rates of coarse woody debris—a review with particular emphasis on Australian tree species. *Australian Journal of Botany*, **51**, 27-37.

Mattson KG, Swank WT, Waide J B (1987) Decomposition of woody debris in a regenerating, clear-cut forest in the Southern Appalachians. *Canadian Journal of Forest Research*, **17**, 712-721.

Pachauri RK, Reisinger A (Eds.) (2007) Climate Change 2007: Synthesis Report. IPCC, Geneva, Switzerland.

Wigley TML, Jain AK, Joos F, Nyenzi BS, Shukla PR (1997) Implications of Proposed CO2 Emissions Limitations, Technical Paper 4. IPCC, Geneva, Switzerland.

Chapter 2

Carbon Sequestration in Massachusetts Forests as an Offset for Energy Sector Carbon Dioxide Emissions

Minda R. Berbeco & Colin M. Orians

Presented at the National Association for Utility Commissioners, December 2009

Abstract

Greenhouse gases are both a regulatory and scientific challenge. Policymakers are developing new legislation to utilize forests to mitigate the greenhouse gas, carbon dioxide. The Regional Greenhouse Gas Initiative (RGGI) uses afforestation, the planting of new forests, for this purpose. Using afforestation alone, however, ignores current sequestration due to growth, which is already happening within the Northeastern United States. In addition, it does not take into account carbon that is preserved due to avoided deforestation. In this chapter, we looked at carbon dioxide sequestration due to growth and land-use change within Massachusetts between 1998 and 2005, and compared it to state-wide energy-sector emissions. We then looked at the amount of the state's forest that is at risk for deforestation. We estimated that over 10% of the state's energy-sector carbon dioxide emissions were sequestered within the state's forest. In addition, using the Maine Forest Service's guidelines for identifying forests at risk for deforestation, 52% of the state's forest was categorized as having a high likelihood of conversion to development, which would substantially reduce the state's carbon sequestration abilities. This suggests that only including

afforestation in emission-mitigation policy ignores an additional and current carbon pool and should be protected for future use.

Introduction

Regulating greenhouse gases is both a political and scientific challenge. Policymakers and researchers alike are examining ways to control these pollutants. One method that is gaining popularity through regional policy initiatives is the sequestration (the fixation and storage) of carbon dioxide in forests. The northeastern states, well known sinks for carbon (Barnes *et al.* 1998), have developed a policy that uses forests to help offset emissions. The Regional Greenhouse Gas Initiative (RGGI) requires states to minimize their emissions by 10% by the year 2018. To help achieve this goal, carbon sequestered in forests can be calculated and then sold to greenhouse gas polluters in order to meet 3.3% of their compliance obligation. Currently, the plan is to use afforestation, the planting of new forests, alone as a carbon offset. Massachusetts and some other states, however, do not have significant amounts of open land that could be converted to forest, and some of this forested land is under constant threat from developers. In these states, young and productive forests sequester significant amounts of carbon and we suggest that these forests should be protected and included in regional policies.

Extensive research has already been completed calculating biomass and carbon storage of forests within specific regional parameters. The difference between fixation and loss over an established period of time is used to estimate biomass and carbon stocks among and within large regions (Schroeder *et al*. 1997; Brown & Schroeder 1999; Mickler *et al.* 2002; Jenkins *et al.* 2001). The primary goal of

such studies has been to quantify changes in carbon stocks in different regions under different management practices. Southern New England forests in particular are important sinks for carbon dioxide, as forests are where the majority of terrestrial carbon is sequestered (Uriarte & Papaik 2007). This type of information can be utilized for larger models looking at carbon inputs and outputs with the goal of eventually informing policy. These studies, however, do not address these carbon stores in practical terms in relation to offsetting actual energy emissions in the local region.

In July 2009, the Maine Forest Service and others released a series of recommendations to RGGI regarding forestry offsets. These recommendations suggested including other types of forest offsets in the RGGI policy. These were avoided deforestation, forest management and urban forestry. Though each of these has great potential for carbon storage and sequestration, we chose to focus on avoided deforestation due to the vast forest coverage in Massachusetts. In this paper we calculate carbon storage in Massachusetts forests over an 8-year period and compare it to statewide energy-sector emissions. From this we estimate the degree to which these forests are currently offsetting carbon emissions, and calculate the amount that would be lost through deforestation. We argue that avoided deforestation should be included in future RGGI policy due to the ability of Massachusetts' forests to offset a substantial proportion of its own energy sector emissions.

Methods

Study Area:

The history of Massachusetts forests, and other New England forests, is directly linked to their carbon sequestration capacity today. In general, these forests experienced severe deforestation followed by remarkable preservation and regeneration. From the colonial era up until the turn of the 20th century, most Massachusetts forests were converted to pasture, timberland and farmland. With the movement of agriculture further west and the push towards other fuel sources aside from wood, forests began to regenerate in the state. Today 64% of the state is forested again (Foster *et al.* 2006), with the majority of the forest found in the western counties (McDonald *et al.* 2006). Of the 3.2 million acres of forest in the state, 99% are regenerating secondary growth forests with only 3,000 acres of old-growth (Foster *et al.* 2006).

Massachusetts' success in reforesting the landscape is impressive in the face of it being the 3rd most densely populated state in the nation (Foster 1998). Due to preservation it has become the 8th nationwide in percent forest cover (Foster *et al.* 2006). Because the forests are young, growing rapidly, diverse and plentiful they have the potential to sequester significant carbon.

Inventory Data:

Data for this study was taken from the Forest Inventory and Analysis (FIA) dataset provided by the US Forest Service using the Forest Inventory Database

Online (FIDO) (available at http://fiatools.fs.fed.us/fido/index.html). This dataset includes information on tree counts in forestland by both tree species and size (diameter at breast height) in plots randomly distributed across the states. In Massachusetts, one plot per 5,933 acres of land was sampled repeatedly and data are available for all mainland and island counties, except for Suffolk County. For more information regarding the sampling protocol see Bechtold & Patterson (2005). The FIDO program allows the user to estimate changes in tree cover over time. We used data collected in 1998 and 2005 to estimate forest gain and loss for each county. A change in forest biomass within these forestlands is due to the growth of existing trees or to tree mortality. Tree mortality could be due to both natural sources, such as storms, or human activities, such as harvesting.

Analysis of Forest Carbon:

To estimate carbon, we first determined biomass. General biometric equations for hardwood and softwood tree biomass were combined with tree diameter (DBH) and species information from the FIA database (Tritton & Hornbeck 1982). This was then multiplied by 50%, the estimated carbon content of tree biomass (Pettersen 1984) to estimate the change in carbon content of above-ground woody biomass in forestland within each county from 1998 to 2005.

Analysis of Carbon Emissions:

Carbon dioxide (CO_2) emissions data was collected from the Environmental Information Administration for the years 1998-2005 for the energy sector in

Massachusetts (Energy Information Administration 2006). This dataset quantifies the carbon dioxide emissions in metric tons from coal, natural gas, petroleum and minority power sources, including biomass and wood burning. CO_2 emissions were then converted to metric tons of carbon in order to compare to the forest sequestration using the following equation based on the atomic weights of carbon (12) and oxygen (16):

Carbon = Carbon Dioxide/(44/12)

(Energy Information Administration 2000)

Risk of Carbon Loss from Deforestation:

We based our deforestation risk analysis on the recent release of a policy framework recommendation from the Maine Forest Service and others (Maine Forest Service *et al.* 2009). Outlined in this paper were factors that indicated the likelihood of forests to be converted to development. These factors included distance to population centers, already developed parcels, and major and local roads, as well as the slope of the land (Table 1). We chose to omit distance to population centers, as the majority of Massachusetts is within 3 hours of Boston, a population center with over 500,000 people, which was the cut off for high likelihood of conversion. Because over 90% of the land in Massachusetts fell into this category, it may be an unreliable indicator of development. We also omitted forests that are currently being protected or are considered conservation land. GIS layers for roads were taken from the MassGIS website. The road map layer was published in 2007. The GIS layer for elevation from which slope was

calculated was taken from the MassGIS website. The elevation map layer had a cell size of 5 x 5 meters and was published in 2005. The layers for development and forest cover were taken from the National Land Cover Database 2001 with a 30 x 30 meter cell size. Layers were analyzed and reclassified according to the Maine Forest Services recommendations with associated point values, in order to decipher the areas with high, medium and low risk of deforestation. We assigned high risk to the areas of forest with the highest values in each category.

Results

Carbon storage for the entire state of Massachusetts in 1998 and 2005 was 76,877,134 and 84,517,647 metric tons of carbon respectively, resulting in an increase in carbon storage of 7,640,513 metric tons. This represents an average of 955,064 per year. Though there was an increase in carbon storage overall for the state, carbon storage in some counties actually decreased during this time period (Figure 1). Overall, there was more carbon stored in the western part of Massachusetts then the east. The low storage in the eastern part of the state was in large part due to poor soil quality and development that reduced forest productivity and cover.

Between 1998 and 2005, the state's energy sector released 57,319,484 metric tons of carbon, with an average of 7,164,935 metric tons of carbon per year (Figure 2). As a result, the state's forests are estimated to sequester 13.33% of the state's emissions in above-ground woody biomass.

We found that 52 % of the state's forests are at high risk for deforestation. These high risk forests have a slope less than 25%, are less than 1 mile from a local road, are less than 5 miles from a major road and are less than 1 mile from an already developed parcel of land. In addition, forests in this high risk category are not currently protected or considered conservation land, meaning that if the owner chose, these forests could be open to development. The region with the highest carbon sequestration, Worcester County, had the most forest at the highest risk for deforestation (Figure 3).

Discussion

Overall, Massachusetts forests were able to sequester over 10% of the state's energy-sector emissions in above-ground woody biomass. This is an impressive amount, considering that these forests are not currently being managed for carbon stores. The increase in sequestration over this time period is mostly due to the young age of the forests in this state, allowing them to be a carbon sink. Deforestation resulting from development could, in the future, reduce this sequestration by as much as 52% given that so much of the state's forests fall into the "high risk for deforestation" category.

It should be noted that this paper only looked at above-ground woody biomass. Carbon soil stores below-ground are more than twice that of vegetative stores in some mid-latitude forests (Dixon *et al.* 1994). While we cannot be sure that all

forest types within the state would have sequestered 50% or more belowground, this research suggests that Massachusetts forests may have offset significantly more than our calculations show during this time period.

Massachusetts forests offset substantial amounts of energy-sector emissions but are at high risk for deforestation and land conversion due to development. We feel that our research shows a clear argument for the inclusion of avoided deforestation in RGGI policy. Preserving Massachusetts forests will preserve the carbon sequestration within the state and could continue to offset some of the state's energy-sector emissions provided that emissions do not increase. RGGI's current plan of using solely afforestation is insufficient, as it is missing an opportunity to protect forests and carbon stores as well as continue carbon sequestration to offset energy-sector emissions. An expanded RGGI initiative should include sequestration by existing forests and give credits to landowners to protect their forests from deforestation.

Figures and Tables

Figure 1. Change in carbon storage in Massachusetts forestland by county for the time period 1998-2005. Carbon content was calculated by integrating FIA data for the state with general allometric equations for above-ground woody biomass and multiplying by 50%. Note that not all counties increase in carbon content over this time period.

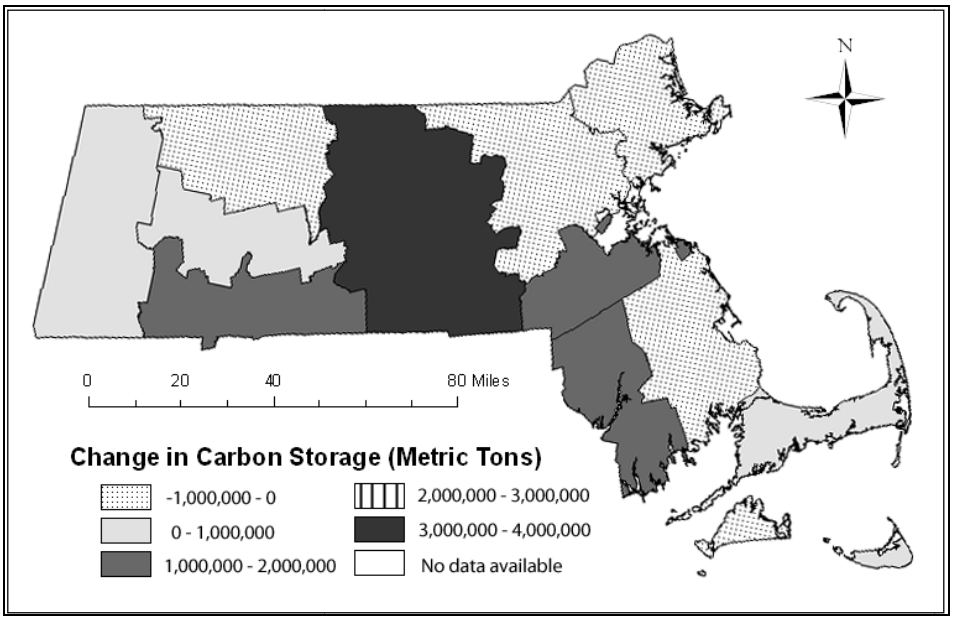


Figure 2. Carbon dioxide emissions converted to carbon from the energy sector for the state of Massachusetts, 1998-2005. The majority of the energy-sector emissions are from the burning of coal, natural gas and petroleum. Carbon dioxide emissions data was obtained from the Energy Information Administration and then converted to carbon using the formula: Carbon = $CO_2/(44/12)$.

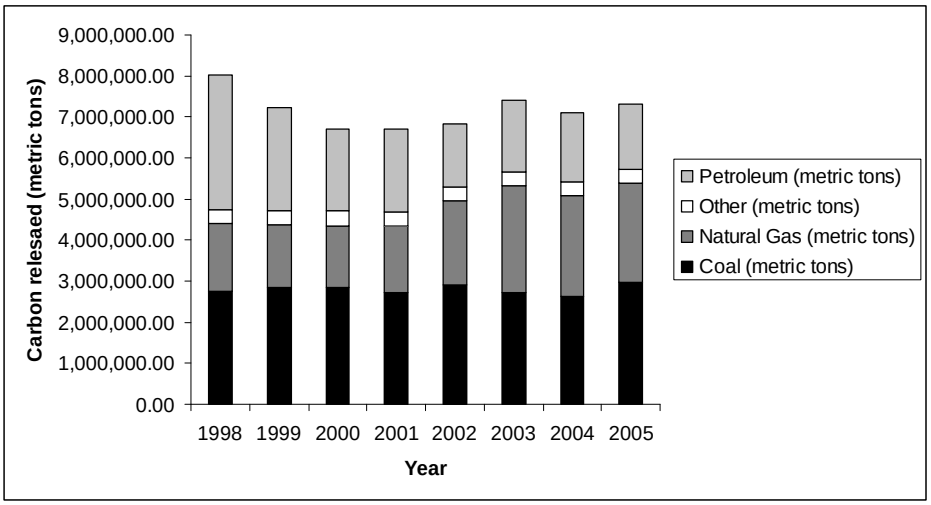


Figure 3. Risk of deforestation by county. This analysis is based on the Project Score for Likelihood of Conversion from a Policy Framework released by the Main Forest Service and others in July, 2009.

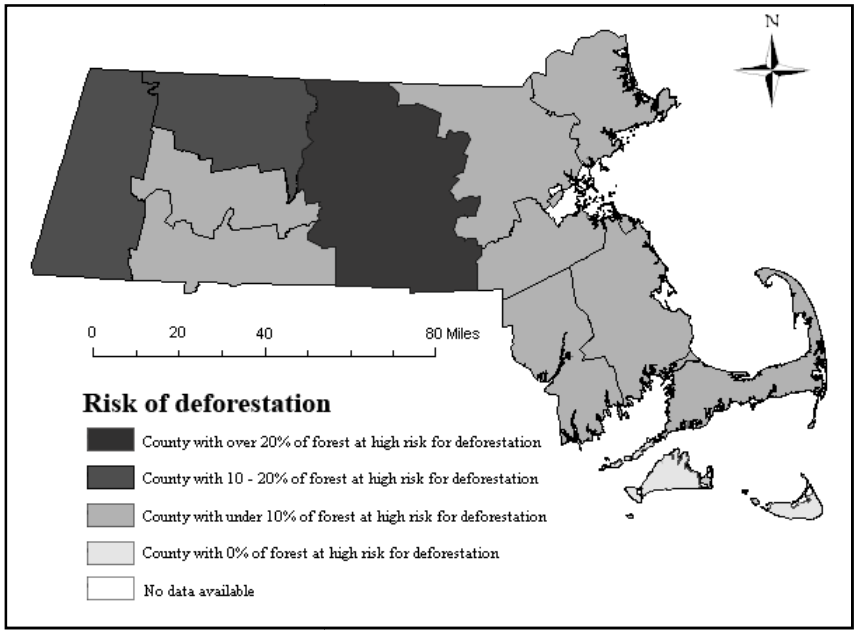


Table 1. Project score likelihood of conversion from forest to development. This is taken from "A Policy Framework for Including Avoided Deforestation and Forest Management Practices as Forest Offset Types in the Regional Greenhouse Gas Initiative" released by the Main Forest Service and others in July 2009. Distance to population center was omitted from our analysis, as the majority of Massachusetts is within 3 hours of Boston which has a population of over 500,000.

Likelihood of conversion	Slope	Distance to population centers	Distance to local road	Distance to major road	Distance to already developed parcel
High (2 points)	<25%	< 3 hours to a population >500,000	<1 mile	<5 miles	<1 mile
Moderate (1 point)	25-40%	< 3 hours to a population > 50,000	1-5 miles	5-15 miles	1-5 miles
Low (0 points)	>40%	> 3 hours to a population > 50,000	> 5 miles	> 15 miles	> 5 miles

Works Cited

Barnes BV, Zak DR, Denton SR, Spurr SH (1998) Forest Ecology. New York: John Wiley and Sons.

Bechtold WA, Patterson PL (2005) The enhanced forest inventory and analysis program – national sampling design and estimation procedures. *General Technical Report SRS-80.*

Brown SL, Schroeder PE (1999) Spatial patterns of aboveground production and mortality of woody biomass for Eastern U.S. forests. *Ecological Applications*, **9**, 968-980.

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J (1994) Carbon pools and flux of global forest ecosystems. *Science*, **263**, 185-191.

Energy Information Administration (2000) "Carbon Dioxide Emissions from the Generation of Electric Power in the United States." Available from http://www.eia.doe.gov/cneaf/electricity/page/co2_report/co2emiss.pdf Retrieved on 1 June 2007.

Energy Information Administration (2006) "U.S. Electric Power Industry Estimated Emissions by State (EIA-767 and EIA-906)." Available at

http://www.eia.doe.gov/cneaf/electricity/epa/emission_state.xls Retrieved on 1 June 2007.

Foster CW ed. (1998) Stepping back to look forward: a history of Massachusetts forests. Cambridge: Harvard University Press.

Foster D, Kittredge D, Donahue B, Motzkin G, Orwig D, Ellison A, Hall B, Colburn B, D'Amato A (2006) Wild lands and Woodlands: a vision for the forests of Massachusetts. Petersham, MA: Harvard Forest, Harvard University.

Jenkins JC, Birdsey RA, Pan Y (2001) Biomass and NPP estimation for the Mid-Atlantic region (USA) using plot-level Forest Inventory Data. *Ecological Applications*, **11**, 1174 – 1193.

Liu G, Han S (2009) Long-term forest management and timely transfer of carbon into wood products help reduce atmospheric carbon. *Ecological Modeling*, **220**, 1719-1723.

Maine Forest Service, Environment Northeast, Manomet Center for Conservation Sciences, Maine Department of Environmental Protection. A Policy Framework for Including Avoided Deforestation and Forest Management Practices as Forest Offset Types in the Regional Greenhouse Gas Initiative. 15 July 2009.

McDonald RI, Motzkin G, Bank MS, Kittredge DB, Burk J, Foster DR (2006) Forest harvesting and land-use conversion over two decades in Massachusetts. *Forest Ecology and Management*, **227**, 31–41.

Mickler RA, Earnhardt TS, Moore JA (2002) Regional estimation of current and future forest biomass. *Environmental Pollution,* **116**, S7-S16.

Pettersen RC (1984) The Chemical Composition of Wood. In: Rowell R M ed. The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: American Chemical Society; Chapter 2.

Schroeder P, Brown S, Mo J, Birdsey R, Cieszewski C (1997) Biomass estimation for temperate broadleaf forests of the United States using inventory data. *Forest Science,* **43**, 424-434.

Tritton LM, Hornbeck JW (1982) Biomass equations for major tree species of the Northeast, *General Technical Report NE-69,* United States Department of Agriculture.

Uriarte M, Papaik M (2007) Hurricane impacts on dynamics, structure and carbon sequestration potential of forest ecosystems in Southern New England, USA, *Tellus*, **59A**, 519-528.

Chapter 3

Carbon storage in live and dead woody biomass in a New England temperate forest over 60 years

Minda R. Berbeco, Colin M. Orians & Henry W. Art

Abstract

Woody debris is an important carbon storage component in temperate forest ecosystems because it contains recalcitrant carbon molecules that slow the loss of carbon. Deciduous temperate forests in particular have unique carbon storage capacities because of the varying levels of recalcitrant carbon in their diverse species. In this paper we looked at a New England temperate forest's carbon sequestration history and addressed the potential of carbon storage in the woody debris carbon pool after sequestration by live trees had approached steady-state conditions. We found that the intermediate-aged forest increased carbon storage in above ground woody biomass during the first 40 years of monitoring, sequestering 3.80 Mg of C ha^{-1} yr^{-1}, but that with forest maturation, this sequestration leveled off and even decreased over the next 20 years losing 0.09 Mg C ha^{-1} yr^{-1}. This trend suggests the transfer of carbon to the woody debris pool. In mature stands, we found that total coarse woody debris biomass in the forest was 13.52 Mg ha^{-1} and lignin was 23% of the total biomass. Through a better understanding of carbon sequestration in live biomass and carbon storage in woody debris, we can better understand and manage forest carbon dynamics.

Introduction

In the last chapter we showed that temperate forest carbon sequestration in above-ground woody biomass is of central importance to mitigation policy. As a forest ages though, its sequestration capabilities are reduced, as carbon fixation slows and trees die and turn into woody debris on the forest floor. These older forests then become carbon storage pools with larger trees of a more recalcitrant quality (Liptzi & Ashton 1999; Naesett 1999; Janisch *et al.* 2005; Chambers *et al.* 2000). This shift in forest composition over time is important to understanding how carbon sequestration has changed in the past and how carbon may be stored in the future.

It is estimated that world-wide temperate forests store 159 Gigatons of carbon (Intergovernmental Panel on Climate Change 2000), with less than 10% stored in the woody debris pool (Barnes *et al.* 1998). There has been considerable research regarding the amount of carbon that can be stored in the woody debris carbon pool in different forest types (Woodall *et al.* 2008; Gough *et al.* 2007; Carmona *et al. 2002*). However, little attention has been paid to carbon quality, i.e. how the carbon is stored (in labile or more recalcitrant molecules). Carbon quality is central to the woody debris pool's carbon storage capabilities, since recalcitrant carbon molecules are more difficult to breakdown and therefore longer lasting than more labile carbon molecules (Harmon *et al.* 1986).

One of the challenges of studying carbon storage in New England forests is the differing carbon qualities in the diversity of species found there (Table 1). In low-diversity forests where there are one or two dominant species, carbon quality in the coarse woody debris pool is more homogenous and decay constants will be more easily constructed through chronosequences. However the diverse forests of New England have varying levels of carbon quality, making decay constants more challenging to construct. By focusing on the levels of recalcitrant carbon in the dominant species growing in temperate forests, we can still looked at long term carbon storage and predict which trees will best store carbon long after death. In addition, we can start to manage forests not only for their short-term sequestration ability, but also for the more recalcitrant species that will hold carbon over the longer term.

Forest composition is not only important to carbon sequestration, but it also defines how carbon is stored in the woody debris pool (i.e. recalcitrant lignin). In this study, we looked at both carbon sequestration in live biomass and the proportion of recalcitrant carbon in the woody debris pool to see how carbon storage changes over time in a temperate New England forest.

Methods

Site information:

The Hopkins Memorial Forest is a 1,052 hectare mixed-hardwood forest located on the eastern slopes of the Taconic Range in the northwest corner of

Massachusetts (42^{o} 42’ 40’N 73^{o} 15’ 00”W). This forest is deciduous mixed, dominated by *Acer saccharum, Fagus grandifolia, Quercus rubra,* and other Northern hardwood species. The mean winter temperature is -5° C, the mean summer temperature is 22°C, and the average precipitation for the area is approximately 110 cm annually. In the early 1800’s, this land was used as agriculture, resulting in 75% of it being cleared by 1830. Over the next 70 years over half of this land was abandoned and returned to forest and by 1924, the remaining agricultural land was abandoned as well. Since the mid-1930s human disturbance in this forest has been minimal as it has been protected from harvesting, and there have been no natural fires. In 1935, the US Forest Service established the Hopkins Memorial Forest, gridding the land into 5-acre cells, each containing a ¼ acre permanent plot. In 1936-1937 all trees with a diameter at breast height over 2.54 cm were recorded by species in each of the 330 permanent plots. The permanent plots were censused again in 1971-1973 and 1994-1996 by Williams College personnel after the ownership of the land was transferred to Williams. We investigated changes in carbon over these three different time periods: 1930s, 1970s and 1990s.

Two additional, larger-scale inventories were initiated by the US Forest Service in mature forest stands in Hopkins Forest in 1947. The two forest plots are the Beinecke Stand dominated by sugar maple and sampled in 6 strip transects totaling 1.55 ha and the 2000 m^2 IBP plot that is dominated by red oak. Since the early 1970s, H.W. Art and others have inventoried these stands at 2 to 5 year

intervals collecting data on tree species, age, diameter at breast height (DBH), time of death and spatial location of each tree. We used these plots to gather information on the coarse woody debris pool in mature forests.

Carbon Storage in Live Tree Biomass Calculation:

We combined the dataset for live trees from the 1930's, 1970's and 1990's with general biometric equations for hardwood and softwood tree biomass using tree diameter (DBH) and species information (Tritton & Hornbeck 1982; Ter-Mikaelian & Korzukhin 1997). This was then multiplied by 50%, the estimated carbon content of tree biomass (Matthews 1993) to estimate the change in carbon content of above-ground woody biomass in forestland within each county from 1998 to 2005.

Coarse Woody Debris Calculation:

Coarse woody debris samples were collected from the Beinecke stand and the IBP plot in the summer of 2008. Wood core samples were collected from coarse woody debris over 7.5 cm in diameter in three locations (bottom, middle and top) for analysis of density (dry mass/wet volume) and carbon content. The cores were taken from the top-side of the debris down through the fallen bole to the soil below it. We also measured any hollows in the woody debris boles. We measured the length of the debris to calculate volume using Newton's formula for volume (Harmon & Sexton 1996):

$$V = L(A_b + A_m + A_t)/6$$

Where V is the volume, L is the length, A_b, A_m and A_t are the areas of the base, middle and top respectively. We then subtracted out the volume of any hollows measured in the woody debris. Biomass was calculated by multiplying volume by the density of the wood.

Sample Analysis:

Debris was weighed and measured and then dried at 70º C until mass became constant. Samples were reweighed and ground to a fine powder using a Wiley Mill and then a Ball Mill. Carbon was assumed to be 50% of dry biomass (Matthews 1993). To obtain total carbon per tree, we multiplied the biomass by carbon content for each sample. Lignin content was analyzed using the acetyl bromide method (developed from Johnson, 1961), using species specific wood standards of known lignin content. The acetyl bromide method was chosen over other methods due to it being a faster and more reliable method than others (Fukushima & Hatfield 2004). Briefly, 20 mg of dried sample was combined with 1ml of 25% Acetyl Bromide in Glacial Acetic Acid in Teflon topped tubes. The tubes were capped and placed in a water bath at 50°C for 2 hours. The samples were then cooled on ice. We then added 2ml 2M NaOH, 2.4 ml acetic acid and 0.35 0.5 M hydroxylamine to the samples. Glacial acetic acid was then added to bring the volume up to 10ml. Sample UV absorption was measured against a blank using a spectrophotometer (HP 8452A Diode Array Spectrophotometer) at 280nm.

Results

Carbon storage in live tree biomass:

We found that in the 1930s the Hopkins Memorial Forest as a whole contained on average 236.03 Mg C ha^{-1} and increased in carbon storage to 388.10 Mg C ha^{-1} by the 1970s, but leveled out and actually lost 2 Mg C ha^{-1} between the 1970s and 1990s. It should be noted though that though the forest stopped net carbon increases to the above ground woody biomass pool, there was still 386 Mg C ha^{-1} stored there in the 1990s, showing it is still a significant carbon store. In addition, between the 1930s and 1990s there was a shift from species such as *Betula papyrifera* with less lignin to species such as *Q. rubra*. In addition, we found that as expected there were larger trees in the older forest, which eventually will contribute large boles to the woody debris pool (Table 2).

Carbon storage in woody debris biomass:

We found that the mature forests had 13.52 Mg ha^{-1} of biomass in the woody debris pool, comprised of 6.76 Mg C ha^{-1} and 3.06 Mg lignin ha^{-1}. The majority of the biomass and therefore carbon and lignin was stored in *Acer saccharum*. However, on average the less common but more lignin-rich *Q. rubra* held the same amount of lignin forest-wide as *A. saccharum* (Figure 1). In addition, the Q. rubra debris was also the largest in diameter (Figure 2).

Discussion

In this study, carbon storage in above ground woody biomass increased between the 1930's and 1970's and then leveled off, even losing some carbon suggesting a shift of carbon to the woody debris pool over the next 20 years. This however is not consistent with other regions, where carbon continues to be sequestered long after the 60 years we saw in our study (Gough *et al.* 2007; Peichl & Arain 2006). In fact, in a coniferous old-growth forest in the pacific northwest, carbon storage did not level off for 200 years, at which point storage in aboveground woody biomass had reached 451 Mg C ha^{-1} (Janisch & Harmon 2002). This may have to do with the land use history of the given forests, as our forest was not old growth, but rather growing back undisturbed from agricultural land.

Our findings for coarse woody debris carbon storage are higher than research from the region (Currie & Nadelhoffer 2002; Tritton 1980). We found 6.76 Mg C ha^{-1} in our maple dominated forest, while Woodall *et al.* (2008) found that forests at similar latitudes had 4.27 Mg C ha^{-1} and Currie & Nadelhoffer (2002) found 3.7 Mg C ha^{-1} in a mixed *Quercus* forest further east from our forest. The latter study did not address the quality of carbon found in the woody debris pool. Our results suggest that if they had, they may have had a proportionally larger recalcitrant carbon pool than our study due to the dominance of the lignin-rich oak in their forest.

We found that the abundance of lignin in the woody debris followed the abundance of a given species, i.e. *A. saccharum* had the highest biomass and

therefore the largest pool of lignin. However, we also found that on average the more lignin-rich *Q. rubra* actually held more lignin in the forest than other species. Furthermore *Q. rubra* was also the largest diameter debris in the forest. As larger diameter debris has been shown in some cases to have slower decomposition than smaller material (Naesett 1999; Brown *et al.* 1996), this suggests that carbon could have the longest proportional storage, not in the species with the highest abundance, but in the species with the largest diameter and highest starting lignin content. *Q. rubra* may have the slowest loss of carbon and therefore, be more important in the long-term storage of carbon than more abundant species such as *A. saccharum*.

Conclusions

Our research shows that though this forest appears to have reached a plateau in its sequestration, the forest composition can have valuable implications for carbon storage in the woody debris pool. One of the challenges of temperate deciduous forests is the high species diversity, making it challenging to complete species-specific decomposition studies. Due to the value of woody debris to carbon storage, it should be studied in greater detail to understand carbon dynamics in temperate forests. Future studies should look into the dominant species of New England forests to see how the carbon quality of these species influences decomposition and how they interact with other major drivers of decomposition, moisture and temperature.

Figures and Tables

Figure 1. Average coarse woody debris biomass (kg) broken down by carbon quality for the dominant species in the mature forest plots. Note that though *Q. rubra* is less common than other species, it overall has the same amount of lignin stored in the forest as the more common *A. saccharum*.

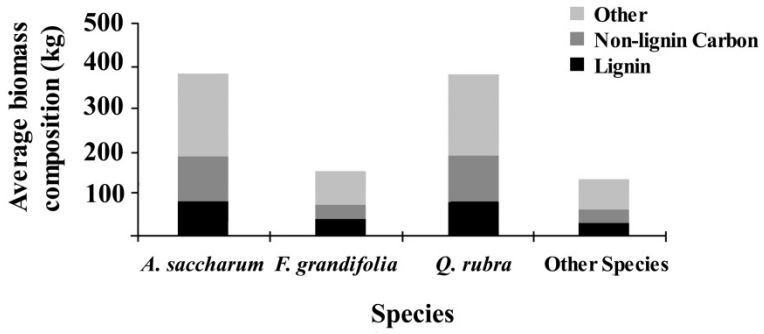


Figure 2. Average diameter of coarse woody debris in the mature stands for the three dominant species in the forest with all other species pooled. Note the larger recalcitrant *Q. Rubra.*

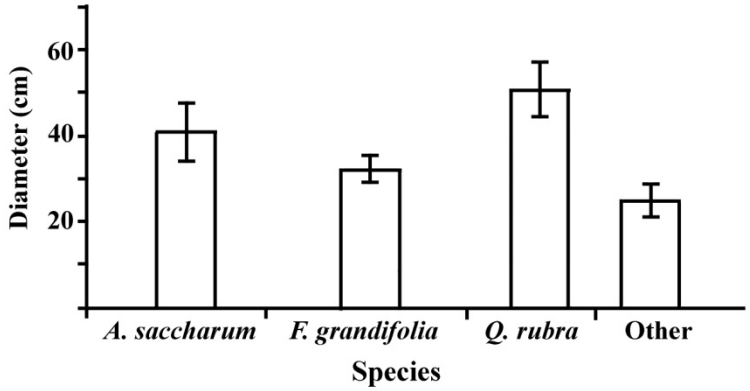


Table 1. Percent lignin in four dominant species in New England forests common to Hopkins Forest. Data taken from Pettersen (1984).

Species	Lignin(%)
Acer species	22-27
Betula species	18-21
Fagus grandifolia	22
Quercus species	24-28

Table 2. Diameter and density of live trees in Hopkins Forest in the 1930's, 1970's and 1990's for the four dominant species and combined other species in the forest.

Species	Diameter		Density	
	Mean	standard error	#/ha	Stems (%)
1930's				
Acer species	13.12	0.18	144	31.1
Betula papyrifera	19.30	0.25	70	15.2
Fagus grandifolia	13.39	0.27	55	11.9
Quercus rubra	20.30	0.40	49	10.5
Other	12.74	0.18	145	31.2
Total			463	100.0
1970's				
Acer species	16.46	0.20	180	34.0
Betula papyrifera	24.07	0.31	52	9.9
Fagus grandifolia	14.71	0.25	76	14.4
Quercus rubra	28.44	0.48	57	10.8
Other	13.96	0.19	163	30.8
Total			528	100.0
1990's				
Acer species	18.94	0.23	171	34.4
Betula papyrifera	26.90	0.46	26	5.2
Fagus grandifolia	15.74	0.24	94	19.0
Quercus rubra	34.18	0.56	55	10.9
Other	16.35	0.24	152	30.5
Total			498	100.0

Works Cited

Barnes BV, Zak DR, Denton SR, Spurr SH (1998) Forest Ecology, 4th edition. Wiley, New York.

Brown S, Mo J, McPherson JK, Bell DT (1996) Decomposition of woody debris in Western Australian forests. *Canadian Journal of Forest Research*, **26**, 954-966.

Carmona MR, Armesto JJ, Aravena JC, Perez CA (2002) Coarse woody debris biomass in successional and primary temperate forests in Chiloe Island, Chile. *Forest Ecology Management*, **164**, 265-275.

Chambers JQ, Higuchi N, Schimel JP, Ferreira LV, Melack JM (2000) Decomposition and carbon cycling of dead trees in tropical forests of the central Amazon. *Oecologia*, **122**, 380-388.

Currie WS, Nadelhoffer KJ (2002) The imprint of land-use history: patterns of carbon and nitrogen in downed woody debris at the Harvard Forest. *Ecosystems*, **5**, 446-460.

Gough CM, Vogel CS, Kazanski C, Nagel L, Flower CE, Curtis PS (2007) Coarse woody debris and the carbon balance of a north temperate forest. *Forest Ecology Management*, **244**, 60-67.

Harmon ME, Franklin JF, Swamson FJ, Sollins P, Gregory SV, Lattin JD, Andersen NH, Cline SP, Aumen NG, Sedell JR, Lienkaemper GW, Cromack K, Cummins KW (1986) Ecology of coarse woody debris in temperate ecosystems. In Advances in Ecological Research. Edited by A. MacFadyen and E.D. Ford. Harcourt Brace Jovanovich, New York, 133–302.

Harmon ME, Sexton J (1996) Guidelines for measurements of woody detritus in forest ecosystems. Available from US LTER Network Office, College of Forest Resources, University of Washington: Seattle. LTER Network publication 20.

Hatfield RD, Grabber J, Ralph J, Brei K (1999) Using the acetyl bromide assay to determine lignin concentrations in herbaceous plants: some cautionary notes. *Journal of Agricultural and Food Chemistry*, **47**, 628-632.

Intergovernmental Panel on Climate Change (2000) IPCC Special Report on Land Use, Land-Use Change And Forestry. Chapter 1: Global Perspective. Available from: http://www.ipcc-nggip.iges.or.jp/public/gpglulucf/gpglulucf_contents.html Retrieved on 5 July 2010.

Janisch JE, Harmon ME, Chen H, Fasth B, Sexton J (2005) Decomposition of coarse woody debris originating by clearcutting of an old-growth conifer forest. *Ecoscience*, **12**, 151-160.

Matthews G (1993) The carbon content of trees. Forestry Commission Technical Paper 4. Forestry Commission, Edinburgh.

Naesett E (1999) Decomposition rate constants of Picea abies logs in southeastern Norway. *Canadian Journal of Soil Science*, **29**, 372-381.

Peichl M, Arain MA (2006) Above- and belowground ecosystem biomass and carbon pools in an age-sequence of temperate pine plantation forests. *Agricultural and Forest Meteorology*, **140**, 51-63.

Pettersen RC (1984) The Chemical Composition of Wood. In: Rowell R M ed. The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: American Chemical Society; Chapter 2.

Ter-Mikaelian MT, Korzukhin MD (1997) Biomass equations for sixty-five North American tree species. *Forest Ecology and Management*, **97**, 1-24.

Tritton LM (1980) Dead wood in the northern hardwood forest ecosystem. PhD thesis, Yale University, Connecticut.

Tritton LM, Hornbeck JW (1982) Biomass Equations for Major Three Species on the Northeast: General Technical Report NE-69. Available from United States Department of Agriculture, Northeastern Forest Experiment Station.

Woodall CW, Heath LS, Smith JE (2008) National inventories of down and dead woody material forest carbon stocks in the United States: Challenges and opportunities. *Forest Ecology and Management*, **256**, 221-228.

Chapter 4

Soil warming differentially accelerates decomposition of woody debris species

Minda R. Berbeco, Jerry M. Melillo, Colin M. Orians

Abstract

Although the woody debris pool is currently a carbon sink in temperate forests, it could change to a source in the next 100 years in response to soil warming from global climate change. Fine woody debris typically takes years to decompose. It is unclear, however, how soil warming will alter decomposition in temperate forests or how debris size and quality differentially alter patterns of decomposition. To look at the effect of soil warming on the woody debris decomposition of varying size and quality, we placed fine woody debris of two size classes (2 x 20 cm and 4 x 40 cm) and four species (*Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis*) in a soil warming and ambient area at Harvard Forest in central Massachusetts. We collected the debris once a year for two years and measured mass loss and lignin concentration from the debris collected. We found that warming increased mass loss for all species and size classes over two years (by as much as 30%), but that more recalcitrant and larger debris decomposed much more slowly. Surprisingly, lignin degradation did not follow the same trends as mass loss. Lignin loss from the most lignin-rich species, *T. canadensis*, was highest despite the fact that it decomposed the slowest. Prior research has demonstrated that soil warming increases soil

respiration and decreases carbon stores belowground, and our results indicated that soil warming will also increase decomposition of woody debris. Faster decomposition of species with higher quality wood, such as *Acer* and *Betula*, will further increase carbon dioxide released into the atmosphere. Future models and policy efforts must account for this change.

Introduction

The stability of the woody debris carbon pool is at risk from global climate change. Currently contributing minimally to total stand respiration (Liu *et al.* 2006), warming increases decomposition rates (Moore *et al.* 1999; Mackenson *et al.* 2003), and therefore could shift the woody debris pool from a sink to a source (Woodall and Liknes 2008; Yin 1999). The magnitude of this mass loss under warming, however, may be mitigated by quality, as determined by the biochemical complexity of the debris (Aber & Melillo 2001), and the size of woody debris. The interaction between these has not been studied before in temperate forests. As a result, we cannot yet predict how much warming will speed the decomposition from this pool or what variables could mitigate this loss.

Debris quality is critical to our understanding of woody debris decomposition (Melillo *et al.* 1984; Cornelissen 1996; Yang *et al.* 2010). Characterized from the microbe's perspective, quality is defined by the accessibility of carbon molecules (Coûteaux *et al.* 1995). Plant material with more labile carbon is easier to break down and therefore is considered higher quality, while material with more recalcitrant carbon is considered lower quality (Aber & Melillo 2001; Chapin *et al.* 2002). Lignin is a common recalcitrant carbon molecule in wood and it varies among species (Weedon *et al.* 2009). As a result, species are often used as a substitute for quality. Although some studies have found a difference in species decomposition rates (Brown *et al.* 1996) and others have connected those differences to lignin concentration (Mellilo *et al.* 1983; Melillo *et al.* 1984), still

others found an effect of species only in later stages of decomposition (Liu *et al.* 2006). As a result, the role of quality in woody debris decomposition is still unclear.

Similarly, debris size is another variable with conflicting effects on decomposition. Size can often act as a buffer to extreme temperature and moisture changes, as larger debris will be able to hold onto moisture longer. It is not surprising then to see that several studies have found an inverse relationship between debris diameter and decomposition (Abbott *et al.* 1982; Stone *et al.* 1998; Mackensen *et al.* 2003, Liu *et al.* 2006). In addition to moisture content, larger debris may reduce access to decomposers through a low surface to volume ratio and a lower rate of gas and water exchange per unit volume (Mackensen *et al.* 2003). Not all studies found this same relationship between size and decomposition though, as Chen *et al.* (2001) found no effect at all and Guo *et al.* (2006) found a curvilinear relationship between decay rate and size. As a result, the effect of size on woody debris decomposition may be species specific or secondary to external factors.

In this study, we looked at the effect of soil warming on woody debris decomposition as a function of quality and debris size. We hypothesized that decomposition would increase in response to warming but that debris quality would influence decomposition, with more recalcitrant debris decomposing slower under ambient and warmed conditions. We also predicted that the extent of

decomposition would differ by the size of the debris, with smaller debris decomposing faster.

If woody debris decomposition is increased by soil warming, carbon storage in this pool will be disrupted, potentially leading to significant amounts of additional carbon being released into the atmosphere. Woody debris is able to store carbon for extended periods of time (Mattson *et al.* 1987). However, if this storage is disrupted by external environmental variables such as warming, significant amounts of additional carbon may be released into the atmosphere. Such a pulse of additional carbon entering the atmosphere has consequences for the larger global carbon cycle and could shift our understanding of global climate change in the future.

Materials and Methods

Site information:

Barre Woods is located in the Harvard Forest Research Station in Central Massachusetts (Mellillo *et al.* 2010). The stand is an even-aged, mixed-hardwood forest that was destroyed in a 1938 hurricane and grew back naturally. Historical documents suggest that in earlier years it was pastureland. The climate in this region is cool, temperate and humid, with a mean air temperature of -6º C in winter and 20º C in the summer. Annual precipitation is 108 cm, distributed evenly throughout the year.

Soil Warming Treatment:

In the Summer of 2001, 5.47 km of heating cables were buried at a depth of 10 cm, 20 cm apart across a 30 x 30 m area. A 30 x 30 meter ambient area was delineated next to the warming area. Baseline measurements were taken in 2002 showing no difference between the two areas. Cables were not buried in the ambient area, because previous research found no temperature, moisture or soil respiration differences between the areas with no cables and those with cables that had never been turned on (Melillo *et al.* 2002). The power was then turned on in the Spring of 2003. Areas are controlled automatically to keep a 5ºC differential between the ambient and warming areas. Moisture level was measured using Time Domain Reflectometer probes set at 5 cm below the soil surface. In 2007, moisture data was collected every 6 hours. From 2008 onward, moisture data was collected every hour. Soil moisture was on average 6% higher in the ambient area over the heated area for all of the years that our debris was decomposing (Figure 1).

Data Collection:

Initial Survey of Woody Debris:

Each treatment area was separated into 10 x 10 m areas. Within each smaller area, we used the line-intercept method starting from the center of each area in a random direction to collect information on woody detritus (coarse and fine). Two-inch long pieces were cut from the smaller end of each piece of debris for density measurement. Decay class was split into 3 groups based on the work of

Currie *et al.* (2002): sound (no evidence of decay), intermediate (missing some bark, density is soft to the touch, wood is missing) and rotten (wood is rotten the whole way through).

Soil Warming Experiment:

In the Fall of 2007, fresh saplings were cut from 4 species of trees: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis*. These four species were chosen due to their forest abundance and difference in quality. From the natural experiment earlier in the Summer, it was determined that the majority of woody debris within the site was less than 5 cm in diameter. Since Harmon and Sexton (1996) recommend having the length ten times longer than the diameter, as the radial colonization rate by decomposers is 10% of the longitudinal rate, we cut the wood into two size classes keeping the ratio of diameter to length constant: 2 cm diameter by 20 cm long and 4 cm diameter by 40 cm long. We cut an additional 2.54 cm piece of wood from each tree to do an initial analysis of moisture and used a subset of those pieces for lignin analysis. We weighed the debris and placed it in debris bags with a 5 mm mesh to allow decomposers access. Two pieces of debris (one of each size) from the same species were put into individual bags. The bags were then anchored under the leaf litter in subareas within each treatment area.

We divided each 30 x 30 m treatment area into nine 10 x 10 m subplots. Within each of the nine subplots two 1 x 1 m mini-plots were randomly located and

served as the locations for this experiment. The experiment was set up in the Fall of 2007 and each Fall since we removed 18 debris bags per species from each treatment area for a total of 288 pieces of woody debris (4 species * 2 size classes * 2 treatment areas * 9 subplots * 2 mini-plots=288). From the collected debris we determined mass loss and moisture content.

Mass Loss:

To evaluate mass loss we measured debris before and after being put in the field. Because mass loss can also increase with moisture content (Wang *et al.* 2002; Garrett *et al.* 2007), and because soil moisture content varied by temperature treatment (Figure 1), we factored that into our analysis in two ways. First, we calculated the initial percent moisture content for each species and used this to determine if species with higher moisture content decomposed faster. Since our woody debris was placed out as fresh wood, moisture concentration was calculated by weighing wet subsamples, drying them in an oven at 70ºC until weight did not change (approximately 48 hours) and then weighing them again. Second, we determine if moisture content after year 1 predicted patterns of decomposition in year 2. When decayed debris was pulled from our site, we calculated moisture in the same manner, weighing samples wet, drying them in an oven at 70ºC until weight did not change and then weighing them again.

Decay Constants:

Using mass loss we calculated the decay constants for each species and size under warming and ambient conditions using the following single exponential model which assumes that decomposition rate is proportional to the amount of mass left (Swift *et al.* 1979):

$$k = \frac{(\ln M_0 - \ln M_t)}{t}$$

Where k is the decay constant, M_t is mass at time t, M_0 is mass at time 0. We chose to use mass rather than density to look at decay, because our debris volume did not change over our experiment. To look at time to decompose 95% ($t_{0.95}$) of matter we used the equation (Makensen & Bauhus, 2003):

$$t_{0.95} = -\ln(0.05)/k$$

Lignin Analysis:

Lignin concentration was analyzed from samples ground to 40 mm using a Thomas Model 4 Wiley Mill. All samples from each year of decomposition (year 1 and 2) were analyzed for lignin, but a subsample of 10 samples per treatment, per size class were used for initial lignin concentration (year 0). Lignin concentration was analyzed using the acetyl bromide method (developed from Johnson, 1961), using species specific wood standards of known lignin concentration. The acetyl bromide method was chosen over other methods due to it being a faster and more reliable method than others (Fukushima & Hatfield 2004). Briefly, 20 mg of dried sample was combined with 1ml of 25% Acetyl Bromide in Glacial Acetic Acid in Teflon topped tubes. The tubes were capped and placed in a water bath at 50°C for 2 hours. The samples were then cooled on

ice. We then added 2ml 2M NaOH, 2.4 ml acetic acid and 0.35 0.5 M hydroxylamine to the samples. Glacial acetic acid was then added to bring the volume up to 10ml. Sample UV absorption was measured against a blank using a spectrophotometer (HP 8452A Diode Array Spectrophotometer) at 280nm.

Statistical Analysis:

We analyzed the effects of warming, debris species and debris size on (1) mass loss and (2) change in lignin concentration using a series of 3-way ANOVAs with treatment, species and size as our main effects. Each year was analyzed separately. A Levine's test failed to find homogeneous variances in many of our data sets and typical transformations did not correct for this. As a result, we rank-transformed all of our data before running the 3-way ANOVA. Rank transformations have been found to be a useful statistical tool that bridge non-parametric and parametric tests when other transformations do not correct violated assumptions of the ANOVA test (Conover & Iman 1981). As we found that size interacted with both temperature and species in our year 1 mass loss data, we broke this data into the two size classes and ran two-way ANOVAs to look more specifically at the effect of size and temperature within each size class.

To look at the relationship between mass loss and moisture content, we ran a 2-tailed Pearson Correlation for each size class for average moisture content in year 0 and average mass loss in year 1, and between average moisture content in year 1

and average mass loss in year 2. We analyzed our data using SPSS (GradPack v 17.0).

Results

Initial Survey:

Total woody biomass in the initial survey was 11,928 g/m^2 in the ambient area and 8,802 g/m^2 in the soil warming area. The difference between the two sites was driven by the presence of a single large downed tree in the ambient area. We found no differences between the two areas for other debris size classes or in decay class. In addition, there was no difference in tree mortality since the experiment started (personal correspondence with Jacqueline Mohan, Spring 2008). In both sites, the majority of the woody debris was under 5 cm in diameter and the total number of woody debris sampled was equitable. The decay classes for both sites were also very similar, with sound wood being the most prevalent followed by the intermediate decay class and then rotten wood.

Soil Warming Experiment:

Mass Loss:

In year 1, size interacted with both temperature and species to significantly influence mass loss (Table 1a; Figure 2a). As a result, we split the data by size class and performed a two-way ANOVA with temperature and species as the main effects. We found that in the smaller debris, temperature and species interacted to influence mass loss (Table 2a), with *B. lenta* decomposing the fastest

losing 25% of its mass independent of temperature. The other species were more strongly affected by temperature, increasing mass loss by approximately 10% with warming. Meanwhile, only temperature had a significant effect on mass loss in the larger debris (Table 2b).

In year 2, only the main effects of temperature, species and size had significant effect on mass loss (Table 1b; Figure 2b). Warming appeared to increase mass loss across all species and the smaller debris decomposed the fastest. We found that *T. canadensis* decomposed slower than the other 3 species.

Debris Moisture:

Moisture content in year 0 was negatively correlated to mass loss in year 1, but this was only significant for smaller debris (large debris: r=-0.47, n=8, p=0.24; small debris: r=-0.78, n=9, p=0.02). Although moisture content after year 1 also appeared to be negatively correlated to mass loss in year 2, this was not statistically significant for large (r = -0.43, n=8, p=0.29) or small (r=-0.38, n=8, p=0.36) debris. Similarly, mass loss for all debris was greater under warming (Figure 2), even though moisture level was higher in the ambient area (Figure 1).

Decay Constants:

Similar to our findings for mass loss, we found that the smaller and more labile debris had the higher decay constants under the warming treatment (Table 3a).

Warming shortened the time until 95% of the sample was decayed across all species and size classes, reducing the time by 20-30% in most cases (Table 3b).

Lignin:

Temperature had a significant effect on lignin concentration after two years of decomposition, showing an increase in lignin concentration under the warming treatment (Table 4c; Table 5, year 2). Meanwhile, species had a significant effect on lignin concentration throughout the experiment, but concentration did not remain consistently higher in the same species (Table 4). For example, in year 0, *T. canadensis* debris of both size classes had the highest lignin concentration, but by year 2 it had lost the greatest amount of lignin (Table 5b). In the smaller debris, all of the other species increased in lignin concentration. Meanwhile in the larger size class, lignin concentration diverged by year 2, with *A. saccharum* increasing in lignin concentration and *B. lenta* and *Q. rubra* staying close to initial levels. Lignin concentration was significantly higher in the larger size class debris before decomposition and after two years (Table 4a, c). For the effect of decomposition on lignin content and how this differed by treatment, species and size, see Figure 1 and 2 in the Appendix.

Discussion

In our study, we found that although there was no difference between the two treatment areas in regard to debris accumulation, there was an effect of warming on woody debris decomposition. Warming significantly shortened the debris

residence time on the forest floor, while species and size appeared to dictate the magnitude of this response. For example, warming shortened the time to 95% mass loss for the more recalcitrant *T. canadensis* debris of the larger size class by 12 years, while shortening the time for the other 3 species by only 3-7 years. This research demonstrates that although temperature increases woody debris decomposition, substrate accessibility will dictate the level of the response.

Initial lignin concentration could control decomposition through two methods: controlling substrate accessibility or by dictating the type of microbial community that can colonize the debris. The former has been shown before in the literature (Melillo *et al.* 1982) and explains the slow mass loss of the more lignin-rich debris. The latter has not been as well-explored. Although many studies have looked at the size and type of the microbial community that colonizes wood (Norden *et al.* 2004, Küffer *et al.* 2008), little research has gone into how this influences the type of carbon being consumed. In our study, the relationship between mass loss and initial lignin concentration would suggest lignin is slowing decomposition, thereby accounting for the lignin-rich *T. canadensis* decomposing slower than all other species. However a closer look at lignin concentration over time shows that lignin in the larger *T. canadensis* debris is being consumed in higher proportion than in the other species. This is counterintuitive if we presume that lignin concentration slows decomposition of this species. Perhaps initial lignin concentration is determining long-term decomposition rates by dictating the community that can initially colonize the debris. If true, this would not be a

unique to wood debris. In a study on leaf litter, Cox *et al.* (2001) found that inoculating leaf litter with different microbial communities (lignin vs. cellulose degrading) influenced the type of carbon consumed while not changing overall mass loss. Without further data on the microbial community in our samples, we are left to speculate that the same thing may have occurred in our woody debris. This is an obvious topic for further study.

Decomposition was much faster for smaller debris than larger debris. Two non-mutually exclusive factors might explain this difference. First, we found that larger size classes had a higher initial lignin concentration which would slow decomposition of this debris. Moreover, Mackensen *et al.* (2003) suggested that quality goes down as size goes up in large woody debris, as larger logs have a greater proportion of low quality heartwood. Second, larger debris can buffer the effects of extreme temperature changes (Laiho & Prescott 2004), which again could slow decomposition in the larger debris. As a result, perhaps size was only a correlate for quality or temperature in our study, as the larger debris had higher lignin, more recalcitrant heartwood and the potential to buffer against the effects of extreme changes in climate.

Several studies found that both temperature and moisture availability (through internal wood moisture content or precipitation) increase decomposition (Wang *et al.* 2002; Garret *et al.* 2007). Our data however suggests that even though moisture level in the debris was negatively correlated with decomposition

independent of treatment (warmed or ambient), it was not a good predictor of mass loss with the exception of small debris after 1 year of decomposition. Similarly, the soil moisture was higher in the ambient area than the warming area, which would suggest a higher decomposition in the ambient area. This was not the case though, leading us to believe that warming, not moisture, was driving increased decomposition.

Conclusions

Our findings of a 20-30% increase in fine woody debris decomposition with soil warming matches a study by Melillo *et al.* (2002) on the effect of warming of the decomposition of soil organic matter. We expect that the woody debris pool will always have an elevated response to warming as it will have constant new inputs over time. These findings are important for future carbon models, because of the increased speed in which organic matter will enter the soil carbon pool and the atmosphere.

Our study demonstrates that soil warming will increase decomposition of fine woody debris in temperate forests. Although the decay rate may be mitigated by the debris species and size, the exact mechanisms driving woody debris decomposition are still not clear. More research into the microbial communities and the type of carbon consumed is required to understand how warming may interact with debris quality to alter decomposition patterns. This increase in decomposition due to warming and the mechanisms that drive them needs to be

included in future climate models (Ostle *et al.* 2009) as the temperate forest woody debris carbon pool could shift from a sink to a source in the next 100 years (Yin, 1999).

Figures and Tables

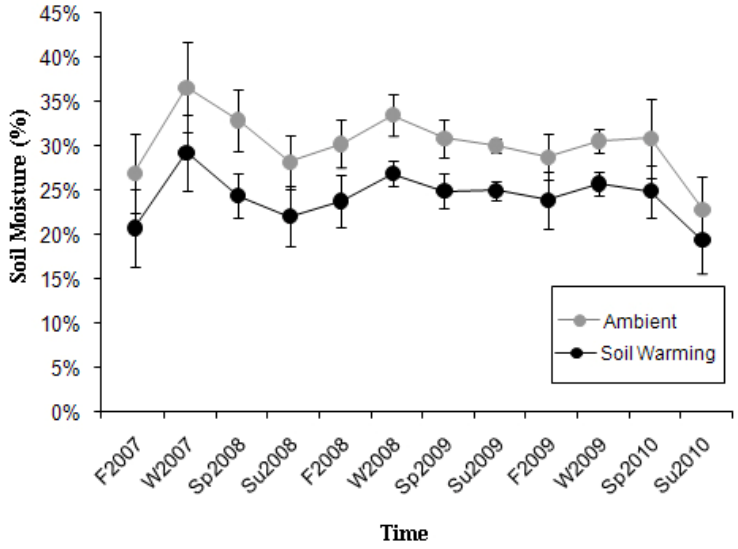


Figure 1. Mean percent soil moisture (+/- SE) in the ambient and heated areas from Fall 2007 to the Summer 2010. The soil moisture in the ambient area was on average 6% higher than the heated area throughout our study.

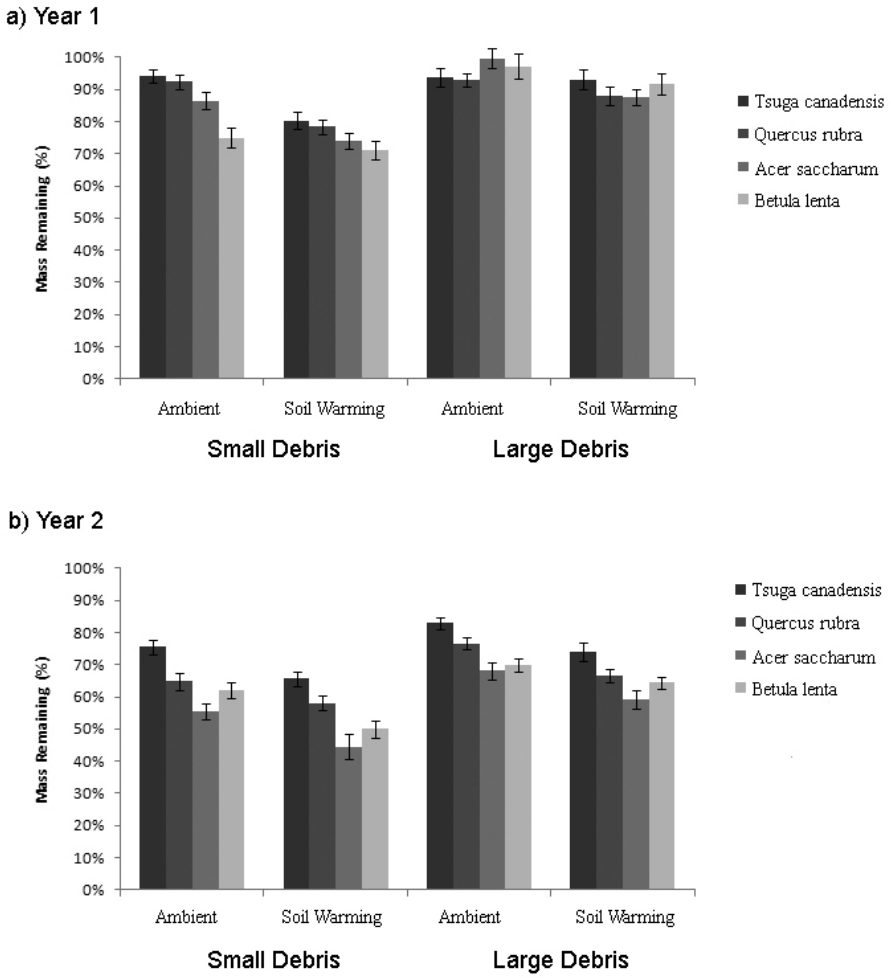


Figure 2. Percent woody debris mass remaining of small debris (20 x 2 cm) and large debris (40 x 4 cm) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* after a) one and b) two years under either the ambient or the soil warming treatment.

Table 1. Summary of 3-way ANOVAs of woody debris mass loss, for small debris (20 x 2 cm) and large debris (40 x 4 cm) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* after a) one and b) two years under either the ambient or the soil warming treatment. All data was rank transformed before analysis

Mass Loss

a) Year 1

Source of variation	**df**	**F**	**p**
Temperature	1	45.36	**<0.0001**
Species	3	5.10	**0.002**
Size	1	67.27	**<0.0001**
Temperature x Species	3	1.73	0.16
Temperature x Size	1	5.72	**0.02**
Species x Size	3	5.61	**0.001**
Species x Size x Temperature	3	2.15	0.09
Error	272		
Total	288		

b) Year 2

Source of variation	**df**	**F**	**p**
Temperature	1	55.57	**<0.0001**
Species	3	35.55	**<0.0001**
Size	1	74.15	**<0.0001**
Temperature x Species	3	0.36	0.78
Temperature x Size	1	0.00	0.99
Species x Size	3	0.46	0.71
Species x Size x Temperature	3	0.65	0.58
Error	272		
Total	288		

Table 2. Summary of 2-way ANOVAs of woody debris mass loss for a) small debris (20 x 2 cm) and b) large debris (40 x 4 cm) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* after one year under either the ambient or the soil warming treatment. All data was rank transformed before analysis

Mass Loss

a) Small

Source of variation	**df**	**F**	**p**
Temperature	1	49.40	**<0.0001**
Species	3	12.56	**<0.0001**
Temperature x Species	3	2.91	0.04
Error	136		
Total	144		

b) Large

Source of variation	**df**	**F**	**p**
Temperature	1	8.15	**0.005**
Species	3	0.10	0.96
Temperature x Species	3	1.23	0.30
Error	136		
Total	144		

Table 3. Decomposition patterns for fine woody debris showing a) decay constants and b) years to decompose 95% of debris for small debris (20 x 2 cm) and large debris (40 x 4 cm) across four species: *Acer saccharum*, *Betula lenta*, *Quercus rubra* and *Tsuga canadensis* and under either the ambient or the soil warming treatment.

a) Decay Constants

	Ambient		Soil Warming	
Species	**Large**	**Small**	**Large**	**Small**
Acer saccharum	0.20	0.30	0.27	0.45
Betula lenta	0.18	0.25	0.22	0.36
Quercus rubra	0.14	0.22	0.21	0.29
Tsuga canadensis	0.10	0.14	0.16	0.22

b) Years to decompose 95% of debris

	Ambient		Soil Warming	
Species	**Large**	**Small**	**Large**	**Small**
Acer saccharum	15	10	11	7
Betula lenta	16	12	13	8
Quercus rubra	22	13	15	10
Tsuga canadensis	31	21	19	14

Table 4. Summary of a 3-way ANOVA of woody debris lignin concentration, for small debris (20 x 2 cm) and large debris (40 x 4 cm) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* under either the ambient or the soil warming treatment. Samples were measured a) initially and then after b) one and c) two years of decomposition in the field. All data was rank transformed before analysis

a) Year 0

Source of variation	**df**	**F**	**p**
Species	3	15.32	**<0.0001**
Size	1	4.60	**0.035**
Species x Size	3	1.13	0.34
Error	72		
Total	80		

b) Year 1

Source of variation	**df**	**F**	**p**
Temperature	1	2.94	0.09
Species	3	35.46	**<0.0001**
Size	1	0.003	0.95
Temperature x Species	3	1.86	0.14
Temperature x Size	1	0.001	0.98
Species x Size	3	0.59	0.62
Species x Size x Temperature	3	0.04	0.99
Error	272		
Total	288		

c) Year 2

Source of variation	**df**	**F**	**p**
Temperature	1	11.79	**0.001**
Species	3	24.98	**<0.0001**
Size	1	25.45	**<0.0001**
Temperature x Species	3	0.93	0.43
Temperature x Size	1	1.91	0.17
Species x Size	3	0.96	0.41
Species x Size x Temperature	3	0.66	0.58
Error	272		
Total	288		

Table 5. Lignin concentration (mg/g) for a) small and b) larger debris from before decomposition (subsamples analyzed for year 0) through 2 years of decomposition (year 1 and 2) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* under either the ambient or the soil warming treatment. Standard error is in parenthesis.

a) Small Debris

	Year 0	Year 1		Year 2	
Species		**Ambient**	**Soil Warming**	**Ambient**	**Soil Warming**
Acer saccharum	177 (14.80)	255 (16.30)	242 (10.95)	289 (23.11)	365 (23.97)
Betula lenta	195 (13.14)	232 (13.40)	215 (23.70)	243 (16.38)	305 (20.97)
Quercus rubra	217 (18.79)	165 (11.55)	186 (5.63)	223 (12.54)	233 (10.84)
Tsuga canadensis	279 (19.14)	268 (15.96)	247 (17.91)	219 (11.17)	245 (15.20)

b) Large Debris

	Year 0	Year 1		Year 2	
Species		**Ambient**	**Soil Warming**	**Ambient**	**Soil Warming**
Acer saccharum	201 (13.63)	260 (11.37)	252 (12.65)	257 (12.65)	261 (14.11)
Betula lenta	232 (13.21)	214 (8.87)	205 (19.09)	230 (16.76)	257 (17.01)
Quercus rubra	205 (9.63)	174 (8.58)	184 (9.41)	199 (13.45)	199 (7.40)
Tsuga canadensis	334 (21.25)	277 (15.41)	265 (23.94)	174 (13.66)	198 (10.67)

Works Cited

Abbott DT, Crossley DA (1982) Woody litter decomposition following clear-cutting. *Ecology*, **63**, 35-42.

Aber J D, Melillo JM (2001) Terrestrial Ecosystems. Saunders College Publishing, Philadelphia.

Brown S, Mo J, McPherson JK, Bell DT (1996) Decomposition of woody debris in Western Australian forests. *Canadian Journal of Forest Research*, **26**, 954-966.

Chapin FS, Matson PA, Mooney HA (2002) Principles of Terrestrial Ecosystem Ecology. Spring-Science, NY.

Chen H, Harmon ME, Griffiths RP (2001) Decomposition and nitrogen release from decomposing woody roots in coniferous forests of the Pacific Northwest: a chronosequence approach. *Canadian Journal of Forest Research*, **31**, 246–260.

Conover WJ, Iman R (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician*, **35**, 124-129.

Cornelissen JHC (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology*, **84**, 573-582.

Coûteaux M-M, Bottner P, Berg B (1995) Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution*, **10**, 63-66.

Cox P, Wilkinson SP, Anderson JM (2001) Effects of fungal inocula on the decomposition of lignin and structural polysaccharides in Pinus sylvestris litter. *Biology and Fertility of Soils*, **33**, 246-251.

Currie WS, Nadelhoffer KJ (2002) The imprint of land-use history: patterns of carbon and nitrogen in downed woody debris at the Harvard Forest. *Ecosystems*, **5**, 446-460.

Fukushima RS, Hatfield RD (2004) Comparison of the acetyl bromide spectrophotometric method with other analytical lignin methods for determining lignin concentration in forage samples. *Journal of Agricultural and Food Chemistry*, **52**, 3713–3720.

Garrett L, Davis M, Oliver G (2007) Decomposition of coarse woody debris, and methods for determining decay rates. *New Zealand Journal of Forestry Science*, **37**, 227-240.

Guo LB, Bek E, Gifford RM (2006) Woody debris in a 16-year old *Pinus radiata* plantation in Australia: mass, carbon and nitrogen stocks, and turnover. *Forest Ecology and Management*, **228**, 145-151.

Harmon ME, Sexton J (1996) Guidelines for measurements of woody detritus in forest ecosystems. US LTER Network Publication 20. University of Washington, Seattle, Washington.

Johnson DB, Moore WE, Zank LC (1961) The spectrophotometric determination of lignin in small wood samples. *TAPPI*, **44**, 793-798.

Küffer N, Gillet F, Senn-Irlet B , Aragno M, Job D (2008) Ecological determinants of fungal diversity on dead wood in European forests. *Fungal Diversity*, **30**, 83-95.

Laiho R, Prescott CE (2004) Decay and nutrient dynamics of coarse woody debris in northern coniferous forests: a synthesis. *Canadian Journal of Forest Research*, **34**, 763–777.

Liu WH, Bryant DM, Hutyra LR, Saleska SR, Hammond-Pyle E, Curran D, Wofsy SC (2006) Woody debris contribution to the carbon budget of selectively logged and maturing mid-latitude forests. *Oecologia*, **148**, 108-17.

Mackensen J, Bauhus J (2003) Density loss and respiration rates in coarse woody debris of Pinus radiata, Eucalyptus regnans and Eucalyptus maculate. *Soil Biology and Biochemistry*, **35**, 177-186.

Mackensen J, Bauhus J, Webber E (2003) Decomposition rates of coarse woody debris—a review with particular emphasis on Australian tree species. *Australian Journal of Botany*, **51**, 27-37.

Mattson KG, Swank WT, Waide J B (1987) Decomposition of woody debris in a regenerating, clear-cut forest in the Southern Appalachians. *Canadian Journal of Forest Research*, **17**, 712-721.

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology,* **63**, 621–626.

Melillo JM, Naiman RJ, Aber JD, Eshleman KN (1983) The influence of substrate quality and stream size on wood decomposition dynamics. *Oecologia*, **58**, 281-285.

Melillo JM, Naiman RJ, Aber JD, Linkins AE (1984) Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. *Bulletin of Marine Science*, **35**, 341-355.

Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, Catricala C, Magill A, Ahrens T, Morrisseau S (2002) Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, **298**, 2173-2176.

Melillo J, Kicklighter DW, Tian H, Butler S (2010) Fertilizing Change: Carbon-Nitrogen Interactions and Carbon Storage in Land Ecosystems. In: Handbook of Climate Change and Agroecosystems: Impacts, Adaptation, and Mitigation. Eds Hillel D, Rosenzweig C. Imperial College Press.

Moore TR, Trofymow JA, Taylor B, Prescott C, Camiré C, Duschene L, Fyles J, Kozak L, Kranabetter M, Morrison I, Siltanen M, Smith S, Titus B, Visser S, Wein R, Zoltai S (1999) Litter decomposition rates in Canadian forests. *Global Change Biology*, **5**, 75–82.

Nordén B, Ryberg M, Götmark F, Olaussona B (2004) Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. *Biological Conservation*, **117**, 1-10.

Ostle NJ, Smith P, Fisher R, Woodward FI, Fisher JB, Smith JU, Galbraith D, Levy P, Meir P, McNamara NP, Bardgett RD (2009) Integrating plant–soil interactions into global carbon cycle models. *Journal of Ecology*, **97**, 851–863.

Stone JN, MacKinnon A, Parminter JV, Lertzman KP (1998) Coarse woody debris decomposition documented over 65 years on southern Vancouver Island. *Canadian Journal of Forest Research*, **28**, 788–793.

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in Terrestrial Ecosystems. University of California Press, Berkeley.

Wang C, Bond-Lamberty B, Gower ST (2002) Environmental controls on carbon dioxide flux from black spruce coarse woody debris. *Oecologia*, **132**, 374-381.

Weedon JT, Cornwell WK, Cornelissen JHC, Zanne AE, Wirth C, Coomes DA (2009) Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? *Ecology Letters*, **12**, 45-56.

Woodall CW, Liknes GC (2008) Climatic regions as an indicator of forest coarse and fine woody debris carbon stocks in the United States. *Carbon Balance and Management*, **3**, 1-8.

Yang F-F, Li Y-L, Zhou G-Y, Wenigmann KO, Zhang D-Q, Wenigmann M, Liu S-Z, Zhang Q-M (2010) Dynamics of coarse woody debris and decomposition rates in an old-growth forest in lower tropical China. *Forest Ecology and Management*, **259**, 1666-1672.

Yin X (1999) The decay of forest woody debris: numerical modeling and implications based on some 300 data cases from North America. *Oecologia,* **121**, 81-98.

Chapter 5

Shifting woody inputs drive decomposition in forest soil under warming and nitrogen addition

Minda R. Berbeco & Colin M. Orians

Abstract

Climate warming and nitrogen deposition will alter the soil carbon cycle in temperate forests over the next 100 years. It is not known how a change in carbon inputs from a shift in dominant tree species will alter carbon storage and emissions from the soil carbon pool. Using microcosms we investigated the interactive effects of carbon quality input, temperature and nitrogen addition on soil respiration and the activity of two lignin degrading enzymes, phenol oxidase and peroxidase. We focused on the change in carbon inputs caused by the shift from the more recalcitrant species, *Tsuga canadensis*, to the more labile species, *Betula lenta*, which is currently occurring in New England forests due to the invasion of the hemlock woolly adelgid. This species shift will alter the type of woody litter regularly entering the forest soil, through sapling thinning and the downing of branches. Though our focus was on one species shift, we expect that our findings could translate to any place where a similar shift in quality of material entering the forest soil may change. We found that an introduction of the recalcitrant *T. canadensis* to the soil increased soil respiration only under warming alone. However adding the more labile *B. lenta* increased soil

respiration under warming alone and warming+nitrogen addition. We found a similar pattern for the activity of the lignin-degrading enzyme, phenol oxidase. Peroxidase activity, in contrast, only increased in activity with an increase in temperature, independent of the woody material added. These findings suggest that a change in the quality of woody material input could increase carbon dioxide emissions under future warming and nitrogen addition scenarios.

Introduction

In the next one hundred years, increasing nitrogen deposition and higher temperatures due to climate change will alter soil carbon cycling in temperate forests (Melillo *et al.* 2002; Frey *et al.* 2004). These environmental changes, along with pests, are likely to shift species composition, influencing the quantity and quality of woody material entering the forest floor (Boggs *et al.* 2005; Mohan *et al.* 2009; Gandhi & Herms 2010). It is not known, however, how a shift in the type of woody materials mixing into the soil from sapling thinning and branch mortality will change carbon emissions from the soil carbon pool (Janssens *et al.* 2010). A shift from a more recalcitrant carbon input to a more labile one could greatly increase carbon emissions from the soil depending on future scenarios of climate warming and nitrogen fertilization.

There is already evidence that a change in above-ground land cover alters below-ground carbon dynamics. Hartley *et al.* (2010) investigated this in an arctic system, adding labile carbon to a variety of soils to see how they would respond to future scenarios where plants were able to move into regions previously not colonized. They found that this labile carbon addition increased carbon emissions from the soil, which they attributed to a "priming" of the soil by the addition of labile compounds. Burton *et al.* (2010) found that a change in land-use from native forest to 1st and 2nd rotation pine altered carbon storage and microbial biomass in the soil. They attributed this to shifts in the quality and quantity of organic inputs. To our knowledge, no one has looked at the effect of a natural

shift in the dominant forest species on carbon emissions from the soil. This is especially important in a region, where not only is the dominant species changing, but also the environment around it.

A shift in the dominant tree species in the forest would result in a shift in carbon quality entering the soil carbon pool from the influx of woody litter. Quality, characterized from the microbial perspective, is determined by the accessibility of both carbon and nitrogen molecules (Chapin *et al.* 2002). Organic matter, with labile carbon molecules and high nitrogen content, such as leaf litter, has been shown to break down easily (Melillo *et al.* 1982; Edmonds 1987) and is therefore considered higher quality. Meanwhile, organic matter with more recalcitrant carbon molecules and lower nitrogen, such as woody litter, is considered lower quality (Moore *et al.* 1999; Adair *et al.* 2008). Lignin, a recalcitrant carbon molecule, is often used as a gauge for carbon quality, as it slows decomposition (Chapin *et al.* 2002) and varies consistently among species (Matthews 1993).

In New England forests, the invasion of the hemlock woolly adelgid is causing a shift in forest composition from eastern hemlock, *Tsuga canadensis*, (35% lignin) to black birch, *Betula lenta*, (18-22% lignin in *Betula* species) (Orwig *et al.* 2002; Pettersen 1984). Clearly a shift in species composition will generate a shift in the quality of woody material landing on the forest floor and being mixed into the soil. Based on the aforementioned studies that looked at the influence of input

changes on soil dynamics, a shift from *T. canadensis* to *B. lenta* could cause an increase in carbon emissions from the soil.

Both warming and nitrogen addition differentially influences litter decomposition of varying carbon quality. While more labile litter decomposes faster under ambient conditions (Cross & Grace 2010), recalcitrant litter has been shown to respond more strongly to temperature increases (Fierer *et al.* 2005). This differentiation has been attributed to enzyme kinetics, making clear the importance of enzymes in dictating decomposition patterns.

A similar division in response is seen with the addition of nitrogen. In leaf litter, nitrogen addition slows the decomposition of more recalcitrant materials, whereas it speeds up decomposition of more labile materials (Knorr *et al.* 2005). Two mechanisms have been proposed to explain the divergence in how different quality material responds to nitrogen addition. First, it may be that low quality substrates influence the community structure directly, allowing lignin-decomposing fungi to flourish as the primary decomposers of wood over bacteria (Sinsabaugh 2010). With the addition of nitrogen, fungi might be out-competed by bacteria which lack ligninases. Bacteria are well known to flourish in nitrogen-rich environments, which would suppress wood decomposition (Brant *et al.* 2006). Second, and perhaps in addition, nitrogen could alter the community's activity without changing the overall community structure (Chapin *et al.* 2002). For example, fungi could be changing their enzyme production to increase lignin-

degradation depending on the availability of nitrogen, but keeping the overall fungi:bacteria ratio the same (Manning *et al.* 2008). Since the activity of lignin degrading enzymes is so closely tied to fungal activity, measuring ligninases is a means of looking at how the treatments might be influencing the microbial community.

In this study, we examined how a shift in species composition may influence soil dynamics under current and future warming and nitrogen fertilization scenarios. We measured carbon emissions and lignin-degrading enzymes from a series of constructed microcosms. The microcosms had either soil alone or soil mixed with woody material. They were then incubated under different levels of warming and nitrogen fertilization to simulate current and future scenarios of climate and nitrogen deposition. We hypothesized that microcosms incubated at ambient and elevated temperatures would increase in microbial respiration and ligninase activity with the addition of woody material, particularly the labile *B. lenta*. Moreover, we anticipated that nitrogen-fertilized microcosms would increase in respiration and ligninase activity only when the more labile *B. lenta* woody material was added. For microcosms exposed to both warming and nitrogen fertilization, we did not expect the addition of recalcitrant *T. canadensis* to cause an increase in respiration or ligninase activity, but that adding *B. lenta* would increase respiration. Though our research focuses on the shift from *T. canadensis* to *B. lenta* from the invasion of the hemlock woolly adelgid, we anticipate that

our findings could be representative of any temperate forest where there will be a shift from a recalcitrant to labile dominant tree species.

Methods

Substrate:

To look at the effect of adding woody material of varying quality on carbon mineralization, we compared a soil-only to two soil+woody debris treatments. Soils contain large pools of recalcitrant carbon (Vancampenhout *et al*. 2009), but adding woody materials will change the levels of labile and recalcitrant carbon available depending on the quality of the plant species added (Chapin *et al*. 2002). We used two tree species of varying quality: the high lignin *Tsuga canadensis* (33% +/-6% lignin) and the low lignin *Betula lenta* (23% +/-4%) (Berbeco, unpublished data). Though both are native to New England forests, *T. canadensis* is being killed by the invasive hemlock woolly adelgid, and is being replaced by primarily by *B. lenta* (Orwig *et al.* 2002).

Experimental Protocol:

We harvested 10 saplings of size 2 cm dbh of *T. canadensis* and *B. lenta* from forest stands in Harvard Forest located in Petersham, Massachusetts. We used saplings in our study because we were interested in the effect of thinning or branch fall on carbon mineralization. Though saplings have lower lignin content than their older counterparts (Berbeco, unpublished data), we still found a difference in initial lignin content between the *T. canadensis* and *B. lenta* woody

material. The saplings were then chopped into wood shavings using a Model 4 Wiley Mill and left wet in order to allow immediate access by the microbial community. Forest mineral soil was obtained from several mixed stands in Harvard Forest where both *B. lenta* and *T. canadensis* are present. The soil was mixed, sieved through 1-cm mesh to break apart soil aggregates, and placed in 250-ml experimental microcosms for a total of 130 grams of dry soil in each container. The control treatment contained only soil and no woody debris.

The two experimental treatments contained shavings of either *T. canadensis* or *B. lenta* at a ratio of 1 part wood shavings to 3 parts soil. The wood shavings consisted of sapwood, heartwood and bark. Half of the microcosms were incubated in a growth chamber set to ambient summer temperature for Harvard forest (20°C) and half were incubated in a growth chamber set at 25°C (5°C above ambient). The global mean surface temperature is predicted to increase 1-6°C by the year 2100 (Pachauri & Reisinger 2007), but many studies looking at the effect of temperature on carbon cycling use an increase of 5°C as a standard (Frey *et al.* 2008; Melillo *et al.* 2002). Nitrogen was added to half of the pots once a week as a solution 5 g/m^2/year nitrogen as NH_4NO_3 (ammonium nitrate), which again is standard for nitrogen addition in decomposition studies in New England forests (Frey *et al.* 2004; Magill & Aber 1998). There were 10 replicates per treatment (substrate x temperature x nitrogen). The soil's pH was 4.0 and was reduced slightly by the addition of nitrogen to approximately 3.70. Moisture was

monitored using a moisture meter (Draper 3 in 1 soil tester) and kept constant at field moisture levels through regular watering (between 30-40%).

Respiration:

Microcosm respiration was measured using a closed chamber system with a CIRAS-2 Portable Photosynthesis System Infrared Gas Analyzer once a week for 2 months. The change in carbon dioxide was measured over 2 minutes to calculate the soil respiration from each microcosm. The system was flushed between each reading.

Enzyme activity:

Two lignin degrading enzymes, phenol oxidase and peroxidase, were measured at the end of our study according to DeForest (2009) and Frey *et al.* (2004) adapted for a 96-well plate (Hendel *et al*. 2005). For phenol oxidase activity, two grams of substrate were suspended in 80 ml of 25 mM acetate buffer pH 5 and stirred constantly over ice to maintain temperatures below 20°C. We combined 200 microliters of slurry with 50 microliters of 25 mM L-DOPA (L-3,4-dihydroxyphenylalanine) solution in 25 mM acetate buffer. 50 mM EDTA (ethylenediaminetetraacetic acid**)** was added to the L-DOPA solution previous to adding to the sample homogenate to keep the L-DOPA from binding with metals in the slurry.

For peroxidase, the same reagents were added together with the addition of 10 microliters 0.3% hydrogen peroxide. The samples were incubated at 20°C for 1.5 hours. We pipetted 100 microliters of the supernatant from each well to a new microplate to measure the absorbance of the samples at 460 nm in a spectrophotometer (Biorad Benchmark plus microplate spectrophotometer, version 5.2.1). Enzyme activities were calculated using the following formulas:

Phenol oxidase activity: $A_{\text{phenol oxidase}} = \frac{Abs_{460} * v}{k * s * t * dw}$

Peroxidase activity: $A_{\text{peroxidase}} = \frac{Abs_{460} * v}{k * s * t * dw} - A_{\text{phenol oxidase}}$

Where v=volume of sample slurry, k=7.9 the extinction factor of L-DOPA, s=the amount of solution measured, t=incubation time and dw=dry weight of the samples. Activity was expressed as µmoles $hour^{-1}$ gram organic $matter^{-1}$. The peroxidase assay measures a summation of peroxidase activity and phenol oxidase activity, requiring the subtraction of phenol oxidase activity from the total value to get peroxidase activity.

Data Analysis:

Since we were interested in the effect of adding woody material of varying quality to soil carbon dynamics under current and future environmental scenarios, we

analyzed the difference between species within warming and nitrogen treatments. For respiration, we used one-way repeated-measure ANOVAs with time as the repeated measure. For enzymes (measured at the end of the experiment), we used one-way ANOVAs (SPSS Grad Pack 17.0). If no effect existed with the addition of woody material, we looked for main effects and interactions of the nitrogen and soil warming treatments using 3-way ANOVAs with nitrogen addition, warming and substrate as the main effects.

Results

Microbial Respiration:

There was no effect on respiration of adding woody material to soil under ambient temperature with nitrogen added ($F=0.17$, $df=2$, $p=0.85$) or without nitrogen added ($F=-0.50$, $df=2$, $p=0.61$). When temperature was increased, we started to see an effect of adding woody material to the soil with nitrogen addition ($F=3.13$, $df=2$, $p=0.06$) and without nitrogen addition ($F=6.16$, $df=2$, $p=0.0006$). Under warming and nitrogen, it was the addition of *B. lenta* that increased microbial respiration while under warming alone the addition of both *T. canadensis* and *B. lenta* increased respiration (Figure 1).

Enzyme activity:

Adding woody material to soil had no effect on phenol oxidase activity under ambient temperature with nitrogen addition ($F=0.17$, $df=2$, $p=0.84$) or without nitrogen addition ($F=1.70$, $df=2$, $p=0.20$). When temperature was increased, we

started to see an effect of adding woody material with nitrogen (F=5.90, df=2, p=0.007) and without nitrogen addition (F=8.70, df=2, p=0.001). Under warming and nitrogen addition, both *B. lenta* and *T. canadensis* increase phenol oxidase activity, while under warming only adding *B. lenta* increased activity (Figure 2a). Adding woody material did not influence peroxidase activity under any environmental scenario. Rather warming appeared to increase peroxidase activity across all substrates, independent of whether woody material was added (Figure 2b; F=12.09, df=1, p=0.001).

Discussion

Effect on Microbial Respiration:

Our study suggests that a shift in the quality of woody material added to the forest soil will have significant effects on respiration under future warming and nitrogen deposition scenarios, but not under current environmental scenarios. We found a 12% increase in carbon dioxide emissions under warming alone between the addition of the *T. canadensis* and *B. lenta* to the microcosms. Moreover, respiration increased nearly 30% between species when exposed to both warming and nitrogen addition combined. We did not see the same striking increase in carbon dioxide emissions from our microcosms that were incubated at lower temperature and nitrogen levels. This suggests that under current conditions, a shift from *T. canadensis* to *B. len*ta woody material may not immediately change carbon storage on the forest floor. Rather, a warmer, nitrogen-rich environment was required to increase respiration in these microcosms.

Our results match the literature for longer field experiments that look at the effect of both warming and nitrogen addition on soil respiration, suggesting that if our experiment had run longer we would have similar effects. Melillo *et al.* (2002) found that warming increased soil respiration from a hardwood forest soil in the first few years of treatment, but then reverted to control levels. They suggested that this was due to the early microbial consumption of labile carbon under warming. As we found a similar increase in respiration from our study microcosms in response to warming, we could speculate that over time this effect may have lessened as the labile carbon was consumed. Bowden *et al.* (2004) found that nitrogen addition continued to suppress soil respiration after 13 years in both pine and hardwood stands, suggesting that nitrogen might have had a more significant role suppressing respiration if our study had lasted longer.

Effect on Enzyme Activity:

Phenol oxidase activity followed a similar pattern as microbial respiration when *T. canadensis* and *B. lenta* were added to the microcosms. Since phenol oxidase is responsible for lignin consumption, we had anticipated that adding woody material to the microcosms would increase phenol oxidase activity, especially when the lignin-rich *T. canadensis* was added. Perhaps the short time scale of our experiment did not allow for the microbes to access the carbon and nitrogen required to synthesize new enzymes. Or perhaps enough labile carbon was

available in the soil and woody material so that consuming lignin was not necessary.

Though our study was not designed to look at specific mechanisms of decay, the shift in phenol oxidase activity with warming and nitrogen addition suggests that the microbial community is influenced by these changing environmental factors. Overall, phenol oxidase activity tended to be higher in the absence of nitrogen within each temperature treatment. Though not statistically significant, this trend matched our expectations of nitrogen suppressing phenol oxidase activity. Adding nitrogen may have altered the microbial community, favoring bacteria over fungi (Brant *et al.* 2006). As fungi are the primary decomposers of wood and creators of phenol oxidase, adding nitrogen may have shifted the community away from fungal domination thereby reducing phenol oxidase activity. Further investigation into the microbial community is required to find the specific mechanisms that are driving our results.

Overall, higher temperature was the only factor that increased peroxidase activity. This short term effect of temperature on peroxidase regulation has been seen before (Singh & Chen 2008). He *et al.* (2007) found a strong relationship between peroxidase activity and lignin degradation under higher temperatures that became even more pronounced over time. They speculated that peroxidase was responsible for lignin degradation at higher temperatures, while phenol oxidase was the primary lignin-degrading enzyme at lower temperatures. Our study

followed a similar pattern, with reduced phenol oxidase and elevated peroxidase activity under increased temperature for when *T. canadensis* was added to the soil and for soil alone. This demonstrated that the two enzymes may have different optimum temperatures, allowing for continued degradation of lignin independent of climate.

Implications of a Changing Forest Structure:

Hemlock woolly adelgid invasion will lead to an unprecedented decline in *T. canadensis* trees (Orwig *et al.* 2002), resulting in accelerated branch and sapling inputs onto the forest floor (Nuckolls *et al.* 2009). Our research suggests that this movement of *T. canadensis* woody material to the soil may not immediately result in a pulse of carbon leaving the forest from decomposition. Rather it appears that this woody material may initially be a carbon store. Under future scenarios of warming, though, an influx of *T. canadensis* woody material could increase soil respiration, while both warming and nitrogen deposition might suppress it. A shift to a *B. lenta* dominated forest would have even more drastic results, as it would no longer be a carbon sink under warming alone or coupled with nitrogen addition. Rather this shift of dominate species in conjunction with warming and nitrogen addition could radically increase the carbon emissions from the soil.

Conclusions

Though this experiment focused on a species shift that is going on in New England forests from the more recalcitrant *T. canadensis* to more labile *B. lenta*

tree species, these results should be considered in any location where the dominant tree species are changing. In our study, we did not see an effect of the species shift under current ambient conditions, however, this changed when the environment was altered in combination with the addition of more labile woody debris. A better understanding of how carbon is being stored once in the soil and how the microbial community is changing in response to these factors is needed to predict how carbon dynamics may change in the future with shifting dominant forest species, nitrogen deposition and climate warming.

Figures

Figure 1. Respiration (g CO_2 m^{-2} hr^{-1}) of soil alone, *T. canadensis*+soil and *B. lenta*+soil for two temperature treatments (20°C and 25°C) and two nitrogen treatments (no nitrogen and nitrogen addition). Samples were averaged over 8 weeks. Data shown are means +/- standard error (n=10).

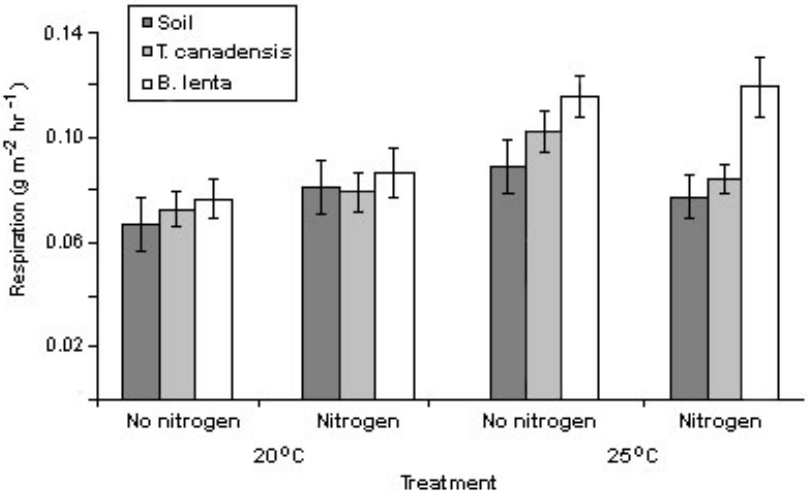
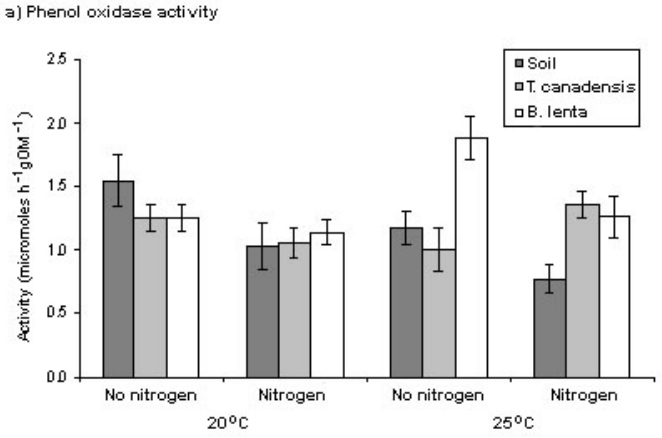
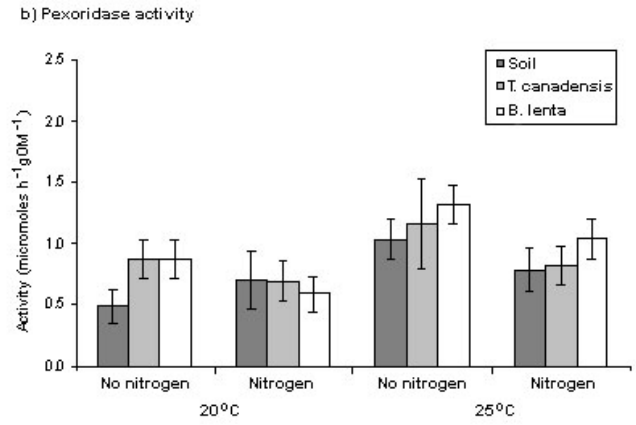


Figure 2. Average a) phenol oxidase and b) peroxidase activity for soil alone, *T. canadensis*+soil and *B. lenta*+soil for two temperature treatment (20°C and 25°C) and two nitrogen treatments (no nitrogen and nitrogen addition). Enzyme activity was measured after 8 weeks of incubation under each treatment.





Works Cited

Adair EC, Parton WJ, Del Grosso SJ, Silver WL, Harmon ME, Hall SA, Burke IC, Hart SC (2008) Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. *Global Change Biology*, **14**, 2636-2660.

Boggs JL, McNulty SG, Gavazzi MJ, Myers, JM (2005) Tree growth, foliar chemistry, and nitrogen cycling across a nitrogen deposition gradient in southern Appalachian deciduous forests. *Canadian Journal of Forest Research*, **35**, 1901-1913.

Bowden RD, Davidson E, Savage K, Arabia C, Steudler P (2004) Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest Ecology and Management*, **196**, 43–56.

Brant JB, Sulzman EW, Myrold DD (2006) Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biology & Biochemistry*, **38**, 2219-2232.

Burton J, Chen C, Xu Z, Ghadiri H (2010) Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. *Journal of Soils and Sediments*, **10**, 1267-1277.

Chapin FS, Mooney HA, Chapin MC (2002) Principles of Terrestrial Ecosystem Ecology, 1st ed. Springer, New York.

Cross A, Grace J (2010) The effect of warming on the CO2 emissions of fresh and old organic soil from under a Sitka spruce plantation. *Geoderma*, **157**, 126-132.

DeForest JL (2009) The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. *Soil Biology and Biochemistry*, **41**, 1180-1186.

Edmonds RL (1987) Decomposition rates and nutrient dynamics in small-diameter woody litter in four forest ecosystems in Washington, U.S.A. *Canadian Journal of Forest Research*, **17**, 499-509.

Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. *Ecology*, **86**, 320-326.

Frey SD, Knorr M, Parrent J, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management*, **196**, 159-171.

Frey SD, Drijber R, Smith H, Melillo, J (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming**.** *Soil Biology and Biochemistry*, **40**, 2904-2907.

Hartley IP, Hopkins DW, Sommerkorn M, Wookey PA (2010) The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils. *Soil Biology and Biochemistry*, **42**, 92-100.

He XB, Song FQ, Zhang P, Lin YH, Tian XJ, Ren LL, Chen C, Li XN, Tan HX (2007) Variation in litter decomposition-temperature relationships between coniferous and broadleaf forests in Huangshan Mountain, China. *Journal of Forestry Research*, **18**, 291-297.

Hendel B, Sinsabaugh RL, Marxsen J (2005) Lignin-degrading enzymes: Phenol oxidase and peroxidase. In: Graca MAS, Barlocher F, Gessner MO eds Methods in Study Litter Decomposition: a Practical Guide. Springer, Netherlands 273-278.

Janssens IA, Dieleman W, Luyssaert S, Subke J-A, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G, Papale D, Piao SL, Schulze E-D, Tang J, Law BE (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315-322.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252-3257.

Gandhi KJK, Herms DA (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions*, **12**, 389-405.

Magill AH, Aber JD (1998) Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. *Plant Soil*, **203**, 301–311.

Manning P, Saunders M, Bardgett RD, Bonkowski M, Bradford MA, Ellis RJ, Kandeler E, Marhan S, Tscherko D (2008) Direct and indirect effects of nitrogen deposition on litter decomposition. *Soil Biology and Biochemistry*, **40**, 688-698.

Matthews G (1993) The carbon content of trees. Forestry Commission Technical Paper 4, Forestry Commission, Edinburgh, Scotland.

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, **63**, 621-626.

Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, Catricala C, Magill A, Ahrens T, Morrisseau S (2002) Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, **298**, 2173 – 2176.

Mohan JE, Cox RM, Iverson LR (2009) Composition and carbon dynamics of forests in northeastern North America in a future, warmer world. *Canadian Journal of Forest Research*, **39**, 213-230.

Moore TR, Trofymow JA, Taylor B, Prescott C, Camiré C, Duschene L, Fyles J, Kozak L, Kranabetter M, Morrison I, Siltanen M, Smith S, Titus B, Visser S, Wein R, Zoltai S (1999) Litter decomposition rates in Canadian forests. *Global Change Biology*, **5**, 75–82.

Nuckolls AE, Wurzburger N, Ford CR, Hendrick RL, Vose JM, Kloeppel BD (2009) Hemlock declines rapidly with Hemlock Woolly Adelgid infestation: impacts on the carbon cycle in Southern Appalachian forests. *Ecosystems*, **12**, 179-190.

Orwig DA, Foster DR, Mausel DL (2002) Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. *Journal of Biogeography*, **29**, 1475-1487.

Pachauri RK, Reisinger A (Eds.) (2007) Climate Change 2007: Synthesis Report. IPCC, Geneva, Switzerland.

Pettersen RC (1984) The Chemical Composition of Wood. In: Rowell R M ed. The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: American Chemical Society; Chapter 2.

Singh D, Chen S (2008) The white-rot fungus Phanerochaete chrysosporium: conditions for the production of lignin-degrading enzymes. *Applied Microbiology and Biotechnology*, **81**, 399-417.

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry*, **42**, 391-404.

Vancampenhout K, Wouters K, De Vos B, Buurmand P, Swennen R, Deckers J (2009) Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions – A pyrolysis–GC/MS study. *Soil Biology and Biochemistry*, **41**, 568-579.

Conclusion

In 1990, the Intergovernmental Panel on Climate Change (IPCC) published its first scientific assessment of global climate change, laying out a strong argument for a human-induced change to the planet perpetuated by the release of greenhouse gases. In this document, the IPCC identified several areas of uncertainty including an incomplete understanding of greenhouse gas sources and sinks, as well as the unknown effect of warming on biological processes. Though there has been substantial research published since that time, there are still many unanswered questions as scientists explore new systems with a focus on different carbon pools.

My research is a piece of this global puzzle. In chapter 2 of my dissertation, I showed that temperate New England forests are currently good carbon stores sequestering over 10% of the state's energy-sector carbon dioxide emissions. In chapter 3, it was shown that when carbon shifts from live to dead biomass, over 20% of that carbon is stored in slowly decaying recalcitrant carbon molecules. The second half of my dissertation showed that this carbon storage in dead biomass may be disrupted by future scenarios of warming. While warming will increase carbon emissions from forests, nitrogen deposition may mitigate this response depending on the type of woody material present.

In terrestrial biogeochemistry models, woody debris is not considered its own carbon pool (Prentice *et al.* 2007); rather it is considered a segment of the soil

carbon pool, acting only to feed fresh inputs into the soil. If woody debris has unique qualities that make it a valuable carbon store as I suggest with my research, is this simplified view of woody debris in models detrimental to carbon emission estimates? Ultimately, I believe it is the response to warming that dictates whether this is an oversight.

In 2002, Melillo *et al.* reported that soil respiration increased in response to warming by 20%. In chapter 4 of my dissertation, I showed that warming increased decomposition of woody debris by a similar amount: 20-30% depending on the species. Due to the similarity in response, one could include fine woody debris in the soil carbon pool with little consequence on an overall model. However, as established in chapter 5 of my dissertation, it is vital that forest structure is considered when doing so as a forest with a labile woody debris pool will respond more strongly to warming than one with a recalcitrant pool.

Though I was able to establish the direction and magnitude of the decomposition response to both external and internal factors, the mechanism is still not clear. My research focused on quantifying carbon moving in and out of the forest, leaving out the microbial community responsible for carbon emissions. Though I started to look at microbial enzymes, we still do not know specifically what organisms were there and how specific communities respond to different treatments. In addition, it is not known how microbial communities will change

with long-term shifts in the environment. These are the next steps that are required to truly understand temperate forest carbon cycling.

Though we have learned a lot since the first IPCC report about the direction and magnitude of system responses to climate change, we need to uncover the mechanism determining that change. It is important that future research starts to look at the microbial community: what organisms are there and how the community will shift in response to environmental factors. By understanding these communities, we will be able to better predict ecosystem responses to climate change.

Works Cited

Intergovernmental Panel on Climate Change (1990) First Assessment Report. Available online at http://www.ipcc.ch/ipccreports/far/IPCC_1990_and _1992_Assessments/English/ipcc-90-92-assessments-overview.pdf. Retrieved on 17 November, 2010.

Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, Catricala C, Magill A, Ahrens T, Morrisseau S (2002) Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, **298**, 2173-2176.

Prentice I, Bondeau A, Cramer W, Harrison S, Hickler T, Lucht W, Sitch S, Smith B, Sykes M (2007) Dynamic global vegetation modeling: quantifying terrestrial ecosystem responses to large-scale environmental change. In Terrestrial Ecosystems in a Changing World. 175–192. Available at http://se-server.ethz.ch/Staff/af/AR4-Ch4_Grey_Lit/Pr72.pdf . Retrieved on 1 November, 2010.

Appendix

Figure 1. Summary of a 3-way ANOVA of woody debris lignin content, for small debris (20 x 2 cm) and large debris (40 x 4 cm) across four species: *Acer saccharum*, *Betula lenta*, *Quercus rubra* and *Tsuga canadensis* under either the ambient or the soil warming treatment. Samples were measured a) initially and then after b) one and c) two years of decomposition in the field. All data was rank transformed before analysis

a) Year 0

Source of variation	**df**	**F**	**p**
Species	3	49.35	**<0.0001**
Size	1	1233.60	**<0.0001**
Species x Size	3	4.23	**0.0006**
Error	72		
Total	80		

b) Year 1

Source of variation	**df**	**F**	**p**
Temperature	1	3.80	**0.05**
Species	3	11.16	**<0.0001**
Size	1	831.37	**<0.0001**
Temperature x Species	3	0.70	0.55
Temperature x Size	1	0.04	0.85
Species x Size	3	1.10	0.39
Species x Size x Temperature	3	0.87	0.46
Error	272		
Total	288		

c) Year 2

Source of variation	**df**	**F**	**p**
Temperature	1	0.39	0.54
Species	3	3.56	**0.02**
Size	1	901.19	**<0.0001**
Temperature x Species	3	1.30	0.27
Temperature x Size	1	0.71	0.40
Species x Size	3	3.87	**0.01**
Species x Size x Temperature	3	0.35	0.79
Error	272		
Total	288		

Figure 2. Lignin content in grams for a) small and b) larger debris from before decomposition (subsamples analyzed for year 0) through 2 years of decomposition (year 1 and 2) across four species: *Acer saccharum, Betula lenta, Quercus rubra* and *Tsuga canadensis* under either the ambient or the soil warming treatment. Standard error is in parenthesis.

a) Small Debris

	Year 0	Year 1		Year 2	
Species		**Ambient**	**Soil Warming**	**Ambient**	**Soil Warming**
Acer saccharum	6.71 (0.22)	7.81 (0.64)	7.19 (0.55)	6.25 (0.38)	6.41 (0.72)
Betula lenta	7.22 (0.19)	5.95 (0.39)	5.69 (0.60)	5.55 (0.42)	5.26 (0.36)
Quercus rubra	8.51 (0.17)	5.85 (0.47)	5.85 (0.32)	5.73 (0.32)	4.95 (0.23)
Tsuga canadensis	8.85 (0.33)	7.22 (0.57)	6.87 (0.59)	5.88 (0.44)	6.16 (0.49)

b) Large Debris

	Year 0	Year 1		Year 2	
Species		**Ambient**	**Soil Warming**	**Ambient**	**Soil Warming**
Acer saccharum	67.77 (1.94)	71.53 (3.42)	82.13 (5.83)	58.95 (4.42)	51.49 (3.93)
Betula lenta	65.51 (2.31)	61.08 (3.41)	52.24 (4.15)	49.82 (3.54)	51.66 (4.37)
Quercus rubra	78.29 (1.01)	64.56 (4.06)	58.10 (3.25)	47.98 (3.88)	44.90 (2.21)
Tsuga canadensis	94.40 (2.92)	76.22 (5.49)	65.11 (5.82)	38.78 (2.63)	43.48 (2.54)