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Nitrogen Deposition Effects on Production and Decomposition of Coarse Woody Debris

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NITROGEN DEPOSITION EFFECTS ON PRODUCTION AND DECOMPOSITION
OF COARSE WOODY DEBRIS

By

Bethany J. Lyons

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

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In Forest Ecology and Management

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Thesis Abstract

Atmospheric nitrogen deposition has the potential to impact forest productivity, microbial associations, nutrient cycling, decomposition and stand dynamics. However, among the least studied aspects of these processes are the production and decomposition of dead woody biomass, or coarse woody debris (CWD). Coarse woody debris is made up of dead woody material on the ground called down dead wood (DDW) and dead standing trees which are often referred to as snags. Observed reductions in decomposition of CWD have been linked to substrate quality and microbial communities. If the decomposition effect is ubiquitous among litter types, CWD density, C:N, and biomass are likely to be impacted by elevated N deposition. Previous research found a slight increase in mortality in the N-amended plots. By assessing CWD volume and biomass, we can conjecture the potential impacts of N-deposition on stand dynamics. This study analyzes the influence of chronic simulated N-deposition on the decomposition of CWD along a latitudinal gradient in Michigan. Methodology included assessing DDW volume and biomass from ambient and elevated N deposition treatments, classifying downed dead wood and snags by their respective visual qualitative decay classes, and calculating wood density and C:N ratios.

Since N deposition treatments began in 1994, DDW biomass has accrued by 16% in N amended plots. Wood density and C:N ratios were significantly and negatively correlated with years since tree death in ambient (control) treatment plots ($P = 0.003$ and 0.005 respectively). N amended wood density and C:N ratios did not significantly decline over times since tree death. Analysis of covariance, with years since tree death as a covariate, indicated that N deposition treatment caused an increase in average DDW density and C:N ratio ($P = 0.063$ and 0.060 respectively). Our analysis indicates that N deposition has the potential to slow rates of wood decomposition and therefore the accumulation of dead woody biomass over time.

Key words: N deposition, decomposition, coarse woody debris, downed dead wood, lignin

Chapter 1: Thesis Introduction

Nitrogen deposition

Atmospheric nitrogen (N) deposition is a natural process that continuously delivers atmospheric N compounds to terrestrial and aquatic ecosystems via precipitation and dry particle deposition. During the process of fossil fuel combustion, nitrogen oxides (NO_x) are produced that are deposited on surrounding regions in the form of aerosol nitrate (NO₃⁻) and ammonium (NH₄⁺) (Barnes *et al.* 1998). To support the demand for food, N-containing fertilizers must be applied to agricultural soils to maximize productivity. Excess N can occur from over-fertilization; this N is then cycled through aquatic and terrestrial ecosystems and can have significant effects on ecosystem functioning and biological processes. As the human population continues to grow, the rate of N addition is expected to increase as well. Scientists expect a 25% increase in N deposition for developed countries in the coming decades (Galloway *et al.* 1994). Asia alone contributes roughly 27% of global NO_x emissions and 43% of ammonia emissions (Galloway *et al.* 2008).

North America is expected to receive an extra 3 g NO₃ m⁻² yr⁻¹ for northern temperate forests (Galloway 2004). Nitrogen is thought to be the most important and often the most limiting nutrient for terrestrial ecosystems (Brady and Weil, 2008). Increases in available nitrogen can have broad implications for a number of forest processes, including productivity (Aber *et al.* 1995), decomposition (Johannes *et al.* 2007), and subsequent carbon storage (Barford *et al.* 2001). In N limited terrestrial ecosystems, experimental N additions can rapidly create N saturated conditions, where leaching nearly equals additions (Aber *et al.* 1989; Fenn *et al.* 1998; Zak *et al.* 2004). High N treatments have also been found to cause soil acidification; up to 70% reduction of base exchangeable cations (Högberg *et al.* 2006). Both increases in N mineralization and N mobilization have been observed under simulated N deposition (Magill *et al.* 1997; Garten 1999).

Forest response to N deposition

Globally there is an unidentified carbon sink that sequesters 15-30% of the world's carbon (C) emissions (Myneni *et al.* 2001). Several recent articles have speculated that northern temperate forests could represent a percentage of this unaccounted C sink (White *et al.* 2000; Myneni *et al.* 2001; Burton *et al.* 2004; Zak *et al.* 2008; Pregitzer *et al.* 2008) although other studies have debated this (Currie *et al.* 2004; Hogberg, 2007;). Magnani *et al.* (2007) speculates that “mankind is ultimately controlling the carbon balance in temperate and boreal forests directly through forest management and indirectly through N deposition.” Because of the potential of a significant C sink within temperate forests, it is important to understand the mechanisms behind C storage in forests. A meta-analysis of forest inventory data along a natural N deposition gradient in the United States reported that aboveground woody biomass increased by 61 kg of C per kg of N deposited, accompanied by increased tree growth in species with mycorrhizal associations (Thomas *et al.* 2009). In a long-term simulated N deposition experiment, Pregitzer (2008) noted an increase in woody biomass production (live + dead woody biomass pools) over a decade of N additions. However, other studies have correlated N additions to decreased tree growth and vigor (Thomas *et al.* 2009), increased mortality (Shen *et al.* 2000), as well as reduced seedling survival (Patterson *et al.* 2012; Catovsky *et al.* 2010). Thus it appears that future levels of N deposition may accelerate stand dynamics by influencing competitive interactions at the individual and species level.

Several studies have described a positive correlation between N deposition and productivity (Nave *et al.* 2009; Pregitzer *et al.* 2008). Nave *et al.* (2009) reported that up to 15% of net primary productivity (NPP) in northern hardwoods is a result of atmospheric N inputs. Increased NPP due to N deposition can result from reduced C allocation to mycorrhizae (van Diepen *et al.* 2007) and elevated foliar N. When N becomes more available under elevated N deposition conditions, plants and trees may shift C allocation away from N seeking mycorrhizal associations. Although N deposition can increase productivity, captured NPP must remain sequestered on site (not lost through mortality and decomposition) in order to have an impact on the degree of C

stored. The primary process that returns soil carbon from a “storage” state to the atmosphere is through heterotrophic respiration- the central process within decomposition.

Decomposition

Decomposition is the process by which organic matter is broken down into successively smaller components and metabolized by heterotrophs to produce energy and biomass. The process of decomposition releases CO₂ through heterotrophic respiration. Recent long term N deposition studies have found a significant “slowing” in the process of litter decomposition. Some studies have observed this result indirectly through reduced soil respiration (Burton *et al.*, 2004; Cusack *et al.*, 2010;) and heterotrophic respiration (Janssens *et al.*, 2010), while others have found correlations with litter quality (Berg and Meentemeyer, 2002; Mansson and Falkengren-Grerup, 2003) enzyme activity (Carreiro *et al.*, 2000; DeForest *et al.*, 2004; Waldrop *et al.*, 2004; Sinsabaugh, 2010; Wang *et al.*, 2010), microbial genetic analyses (Blackwood *et al.*, 2007; Hofmockel *et al.*, 2007; Hassett *et al.*, 2009;), and microbial biomass (Zeglin 2007; Treseder, 2008;). The explanation for reduced decomposition due to elevated N additions has been linked to (I) the substrate being decomposed and (II) the community of decomposers.

Substrate

It has long been known that litter quality is an important factor in determining rates of decomposition (Meetenmeyer 1978). Most decomposition studies are done in leaf litter and soil organic matter mediums. Litter substrates include sugars and starches (simple carbohydrates), hemicellulose and cellulose, and lignin-like (recalcitrant) molecules (Brady and Weil 2008). Within the succession of decomposition, simple carbohydrates are easily attainable and often utilized quickly by microbial decomposers. Cellulose is a polysaccharide chain made up of repeating glucose molecules held by

covalent (glycosidic) bonds. Hemicellulose (a matrix of cellulose chains) is harder to break down compared to simple carbohydrates but is still considered relatively labile (Horwath 2007). Lignin is made up of repeated phenyl-propane units with several types of bonding. It is the primary structural macromolecule within plant cell walls (Horwath 2007). The term “lignin” can be misleading as it was (and often still is) used to define a “waste group” of highly recalcitrant molecules, that were either formed because of harsh chemical washes or were the bulky macromolecules left over after several chemical extractions (Schmidt *et al.* 2011). True lignin has been renamed “acid unhydrolyzable residue,” or “Klason lignin.” For the purposes of this thesis we will use ‘lignin’ for simplicity. Excessive soil N has been found to modify the structure of the lignin molecule, accentuating its recalcitrance (Berg and Matzner 1997). Low molecular weight N containing compounds are adsorbed into lignin’s covalent bonds essentially enhancing lignin’s resistance to decomposition (Stevenson 1982). Many researchers have observed that under elevated N deposition the decomposition of cellulose and hemicellulose components was accelerated, while the decomposition of lignin-like (recalcitrant) components was reduced (Berg and Matzner 1997; Li *et al.* 1994; Johannes *et al.* 2007; Carreiro *et al.* 2000; Waldrop and Zak 2006; Wang *et al.* 2010; Sinsabaugh 2010, DeForest *et al.* 2004; Cusack *et al.* 2010). Researchers have attributed an elevated N effect on microbial decomposer communities and function to this broadening gap between the decomposition of labile and recalcitrant molecules.

Decomposer community

Within forests, bacteria and fungi mediate the process of decomposition (Barnes *et al.* 1998). Certain fungal groups occupy specific niche spaces determined by their preferred substrate. The range of tolerance of a substrate also varies by species. For example, lignin decomposition often occurs during the final stages of litter decay and is mediated by a specialized group of lignin degraders called white-rot fungi (basidiomycetes) (Campbell, 1930; Hammel, 1997; Osono, 2007). Several articles have speculated that microbial community shifts could at least partially explain the stimulation

and inhibition of enzyme activities under elevated soil N (Hofmockel *et al.* 2007; Treseder 2008; Weand *et al.* 2010; van Diepen *et al.* 2007; Lucas and Casper 2008; Waldrop *et al.* 2004; Zak *et al.* 2011). Much of this research has yielded varying results that cannot be compared across sites based on differing methodologies (time, forest type, specific microbial community, and analysis). Methodological issues include whether the authors used microbial biomass, phospholipid fatty-acid analysis, or genetic testing as a means of estimating changes in microbial community composition. When it appeared that lignin-degrading basidiomycete activity was low due to N inputs, Zak *et al.* (2011) hypothesized that basidiomycete competitors like lignin-degrading Actinobacteria, would fill in an apparently open niche space and decomposition activity (production of enzymes) would resume. Their results showed otherwise; elevated N inputs did not significantly alter competitive interactions among soil bacteria and fungi. Determining microbial community shifts poses a problem because several different microbial species carry out similar functions with similar enzymes (Osono 2007). With more research using transcriptomic analyses, researchers will not only be able to identify microbial species abundance but they will also be able to separate microbial abundance/biomass from genetic activity (i.e. function).

Microbial function

Microbial decomposers produce enzymes that can oxidize specific macromolecular substrates to obtain C and other nutrients for growth and respiration (Thorn and Lynch 2007). Excess N (NO_3^- and NH_4^+) is thought to interfere with the fungal metabolism of lignin, thus inhibiting decomposition (Sinsabaugh *et al.*, 2002; Frey *et al.*, 2004). On the other hand, other microbial decomposers specialized on cellulose-based compounds seem to receive a beneficial boost in activity (Berg and Matzner, 1997). Under elevated N deposition, cellulase activity increased, illustrating the accelerated decomposition associated with more labile C macromolecules. Laccase (phenol oxidase) activity decreased with high levels of soil N. Some research has considered that excess N has a direct effect on fungal metabolism (Hofmockel 2007),

however, the effect of N deposition on laccase was found at the genetic level as well (Blackwood *et al.* 2007; Lauber *et al.* 2009). Nearly all literature published on N deposition and decomposition focuses on leaf litter, soil organic matter, or culture experiments, which can have varying concentrations of labile and recalcitrant substances.

Michigan Gradient Study

This study follows research from a Long Term Research in Environmental Biology (LTREB) project that has been simulating chronic elevated N deposition along a latitudinal gradient in Michigan. An additional $3 \text{ g NO}_3^- \text{ m}^{-2} \text{ y}^{-1}$ has been broadcasted every growing season on treatment plots since 1994. Researchers noted that leaf biomass, root biomass, and root turnover remained constant (Burton *et al.* 2004), but surface soil organic matter (0-10 cm) increased (690 g C m^{-2}) (Pregitzer *et al.* 2008) under elevated N deposition. This indicates that N deposition did not increase soil organic matter by producing more leaf or root biomass but by slowing the decomposition of organic matter. Zak *et al.* (2008) reported that after a decade of experimental N deposition, organic matter increased (12%) in the forest floor and surface mineral soil layers. Soil respiration has decreased (Burton *et al.* 2004) as has sugar maple (*Acer saccharum* Marsh.) seedling survival (Patterson *et al.* 2012). These findings indicate that elevated N deposition has altered the process of decomposition and potentially influenced stand dynamics at the Michigan Gradient sites.

To our knowledge, there is little known about the effect of N deposition on CWD decomposition. Large pieces of CWD have long residence times and play an important role in carbon and nitrogen storage and cycling (Creed *et al.* 2004; Ganjegunte *et al.* 2004). To determine if the N deposition treatment has altered decomposition rates, I analyzed CWD wood densities and C:N ratios for CWD produced by tree death during the project (see Chapter 2). I also assessed the effects of chronic N addition on CWD biomass and volume, as well as the differences due to site, decay class, and time. Chapter 3 contains biomass and volume analysis and a discussion on stand dynamics.

The objectives of this study were to determine if CWD decomposition was altered under elevated N deposition, to discuss the possible accumulation of DDW volume and biomass under elevated N deposition, and to promote the inclusion of CWD in carbon storage predictions under future levels of N deposition. I hypothesized that N additions would reduce CWD decomposition due to an inhibition of white-rot fungi and that enhanced tree growth due to N addition would accelerate stand dynamics, increasing tree mortality through stem exclusion (*i.e.* increasing the production of CWD). I also hypothesized that reduced decomposition and accelerated stand dynamics would foster greater C storage in CWD under elevated N deposition.

Chapter 2: Chronic Simulated Nitrogen Deposition and the Decomposition
of Coarse Woody Debris

Introduction

Due to anthropogenic activities, ecosystems around the world are currently experiencing increasing levels of inorganic atmospheric nitrogen (N) deposition (Detener *et al.* 2006). To meet the world's demand for energy and food, the combustion of fossil fuels and the excessive use of fertilizer have significantly influenced the global cycling of nitrogen. Research suggests a 25% increase in total N deposition by the year 2020 for developed countries like North America (Galloway *et al.* 1994). Within North America, this increase translates to an extra $3 \text{ g NO}_3^- \text{ and NH}_3 \text{ m}^{-2} \text{ yr}^{-1}$ for northern hardwood forests (Galloway *et al.* 2004).

In temperate forest ecosystems, N is considered one of the most limiting factors for forest productivity (Brady and Weil 2008). Not only is it needed by plants to build amino acids (proteins, enzymes, and genetic material) and is an integral element of chlorophyll, soil bacteria and fungi also actively seek N-containing compounds for their own growth and metabolism (Thorn and Lynch 2007). Because of this dependence on N, forest communities and the processes that maintain them can be significantly altered by rising levels of N deposition (Magill *et al.* 1997; Myneni *et al.* 2001; Magnani *et al.* 2007).

Increased experimental N deposition has been found to influence a suite of forest processes including net primary productivity (NPP) (Nave *et al.* 2009; Pregitzer *et al.* 2008), soil respiration (Burton *et al.*, 2004; Cusack *et al.*, 2010; Janssens *et al.*, 2010), microbial enzyme activities (Waldrop *et al.*, 2004; Carreiro *et al.*, 2000; Wang *et al.*, 2010; Sinsabaugh, 2010; DeForest *et al.*, 2004), and leaf litter decomposition (Johannes *et al.* 2007; Prescott 1995). Research on the effects of elevated N on microbial and fungal decomposers has had varying results (Knorr 2005). Excess N availability has been found to increase the activity of some microbial and fungal decomposers; in contrast, other research has documented a marked decline in decomposition under elevated soil N. Based on a review of the literature it appears these conflicting accounts are a result of two

factors: the substrates being decomposed, and the makeup of the microbial “decomposer” community.

Nitrogen mediated declines in the decomposition of lignin have been attributed to an elevated N response in specialized soil fungi that degrade lignin: basidiomycetes (Boominathan *et al.* 1990). These specialized fungi produce enzymes including phenol oxidase, manganese peroxidase, and lignin peroxidase (only found in wood-degraders) that can breakdown the bulky lignin molecule and obtain the cellulose hidden within the lignin shell (Osono 2007). A group of bleaching fungi known as “white rot” basidiomycetes are, “the most abundant degraders of wood in nature” and the primary wood degraders in hardwood systems (Hammel 1997).

A culture study done on a nutrient deregulated lignin-degrader found that the nitrogen deregulated mutant *Phanerochaete chrysosporium*, a white rot basidiomycete, produced four-fold the amount of lignin peroxidase compared to that produced by wild types in a low N medium. In contrast, wild types produced no detectable amount of lignin peroxidase in high N media while the N deregulated mutants continued to produce excess peroxidase enzymes (Boominathan *et al.* 1990). Carreiro *et al.* (2000) demonstrated that elevated N deposition increased the activity of microbial and fungal cellulases but decreased the activity of laccases (phenol oxidase). Other research has documented the same decrease in phenol oxidase activity due to N additions (Berg and Matzner 1996; Li *et al.* 1994; Johannes *et al.* 2007; Carreiro *et al.* 2000; Waldrop and Zak 2006; Wang *et al.* 2010; Sinsabaugh 2010, DeForest *et al.* 2004; Cusack *et al.* 2010). The inhibition of enzymatic activity at high-N concentrations is present across several forest types (Gallo *et al.* 2004). These findings and several other publications confirm that elevated N specifically reduces the decomposition of the macromolecule lignin through the inhibition of specialized lignin-degrading enzymes.

The unique lignin-specific response to elevated N inputs has been explained in a number of ways. The most comprehensive mechanism states that elevated N conditions uncouple the decomposition of polysaccharides (*i.e.* cellulose) and polyphenols (*i.e.* lignin). At the genetic level, N regulates the transcription of lignin degrading enzymes,

where low N stimulates the production of lignin-degrading enzymes. There also is evidence that excess N affects decomposition through shifts in the decomposer community (Frey *et al.* 2005; DeForest *et al.* 2004; Waldrop *et al.* 2003; Zak *et al.* 2011). DeForest *et al.* (2004) observed a 35% decrease in phenol oxidase activity and an 18% reduction in microbial biomass with N addition in northern hardwood forests, relative to a control treatment. Waldrop *et al.* (2003) demonstrated the widening gap in substrate decomposition by finding a greater microbial response to maple litter and a reduced response to oak litter, where oak litter had relatively larger concentrations of recalcitrant, lignin-like molecules. These findings illustrate that reductions in enzymatic activity may not be entirely responsible for the reduction in decomposition. Elevated N deposition appears to affect each component of decomposition: substrate, microbial community, and microbial function (Sinsabaugh *et al.* 2002; Knorr *et al.* 2005).

Interestingly, nearly all decomposition studies, either short term N fertilization studies or chronic elevated N deposition studies, utilized leaf litter as the substrate. Leaf litter is composed of several types of macromolecules which vary in concentration based on tree species and environmental conditions (Weedon *et al.* 2009). These macromolecules include water-soluble substances like simple carbohydrates, holocellulose, glycoproteins, hydrophobic lipids and waxes, and acid unhydrolyzable residues (lignin) (Brady and Weil 2008). Lignin concentration in leaf litter varies widely among species (Melillo 1982; Meetenmeyer 1978), but wood litter or coarse woody debris (CWD) is more consistent, containing approximately 25% lignin (this can vary somewhat between gymnosperms and angiosperms) (Sjostrom 1993). Weedon *et al.* (2009) states the necessity for more research on wood traits that affect CWD decomposition patterns including N and P content as well as C:N ratios. Based on a review of the literature there have not been any elevated N deposition studies that assessed wood decomposition.

Objective and hypotheses

The objective of this study was to determine whether the inhibitory effect of elevated N availability on the decomposition of plant litter is ubiquitous in coarse woody debris. We determined the degree of decomposition by assessing wood density (g cm^{-3}) and C:N ratios in CWD produced over a seventeen year period in northern temperate forests receiving ambient and experimentally elevated ($+ 3 \text{ g N m}^{-2} \text{ y}^{-1}$) N deposition. We hypothesized (1) that chronic elevated N deposition inhibits the decomposition of CWD, and (2) that this inhibition is reflected in higher wood density and higher wood carbon to nitrogen (C:N) ratios.

Methods

Site description

The four study sites used in this research are located across a 500 km latitudinal gradient in Michigan (Figure 1). All four sites contain three 30 m x 30 m treatment plots and three 30 m x 30 m control plots. Since 1994, elevated N-deposition has been simulated by broadcasting $0.5 \text{ g N m}^{-2} \text{ month}^{-1}$ of solid NaNO_3 pellets in 6 equal applications during the growing season (3 g N m^{-2} annually). The sites receive total ambient N deposition ranging from 0.7 to $1.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Table 1). All sites contain similar flora, being primarily composed of *Acer saccharum* (80% of basal area) with *Acer rubrum*, *Quercus rubra*, *Fagus grandifolia*, and *Prunus serotina* components (Table 1). The four sites are representative of northern hardwood forests (see Burton *et al.* 1991 for further information on site characteristics) with Kalkaska sand (Typic haploorthod) soils, similar age, and similar stand structure.

Coarse Woody Debris Density and C:N

All trees within each plot have been numbered, with diameter at breast height (dbh) measured annually at permanent marks since 1987 for ambient plots and since 1994 for N amended plots. Tree death has been recorded during annual measurements. Every tree within the study plots has known coordinates which allows them to be easily located. Wood from every third downed dead tree dying between 1994 and 2011 on all plots was sampled during the summer of 2011. Decay classes 1 through 5 were used to classify down dead wood (DDW), or trees that died and are now lying on the forest floor, while decay classes 11, 22, and 33 were used to classify dead standing trees (snags). Decay class 1 indicated the tree was freshly fallen and often still had fine branches and possibly leaves. Decay class 2 was separated from decay class 3 by the presence of bark and secondary branches. Decay class 4 appeared slightly ellipsoidal with some fragmentation as well as evidence of insect and fungal attack. Decay class 5 was highly fragmented,

often sunken in soil and moss covered. Decay classes 11, 22, and 33 are the same as decay classes 1, 2, and 3 except that they were applied to dead trees that were still standing (snags). The use of different codes for snags aided in identifying and tracking samples in the laboratory. Each decay class is a relative measurement of decomposition for debris pieces (methods adapted from Duvall and Grigal 1999).

Wood sampling was done using either a chainsaw for decay classes 1-3 (sometimes 4), or steel core (5 cm diameter) for decay classes 4 and 5. A hole-saw or chisel was used to collect wood samples from every third dead standing tree. Sample volume was calculated manually with dimension measurements from steel core samples and by water displacement on a balance (for unbroken wood) for all other samples. Samples were then placed in a drying oven at 65 °C until a constant mass was achieved. Wood density (g cm^{-3}) was obtained from volume and dry mass measurements. The samples then were prepped for C and N analysis by grinding in a Spex CertiPrep ball mill to a fine powder. All samples were analyzed for C and N using a Fisons NA 1500 elemental analyzer.

Statistical analysis

Analysis of Variance (ANOVA), with site and treatment as factors was used to assess treatment effects on wood density. Decay class was not used as a factor due to the limited number of wood samples from each decay class, especially decay class 5. Analysis of covariance (ANCOVA), with years since death as a covariate, was also used. Linear regression models were used to compare changes over time in wood density for the ambient and N amended plots, with years since death as a predictor. Wood C:N ratios were tested for effects of N deposition treatment with analysis of covariance (ANCOVA) with site and treatment as factors and years since death as a covariate. The relationship between C:N ratios and time was tested with a linear regression model, with years since death being the predictor variable for the C:N ratio response.

Results

Wood density

In ambient plots, wood density decreased with higher decay classes. In N amended plots, wood density remained higher relative to ambient plots, throughout all decay classes (Figure 2). There was no detectable trend in snag wood density. Dead standing wood density fluctuated with visual decay classes (Figure 2). Because of the variability in snag wood density for snag decay classes, we assessed wood density in relation to years since tree death.

A two-way ANOVA revealed that site and N addition treatment affected wood density (Table 2). However, with years since death added as a covariate in an ANCOVA the effect of N addition treatment on wood density was reduced (Table 3). Years since death made a difference in ANCOVA results, *i.e.* higher density in more recently dead woody material. In other words, older CWD in N-amended plots had lost less mass than the older CWD in ambient plots. With a linear regression model, wood density in ambient plots significantly decreased with years since death (Figure 4): $\text{density} = 0.610 - 0.0256 \times \text{years since death}$ ($r = -.530$, $P = 0.003$). However, wood density in the N amended treatment did not exhibit a similar relationship. In fact, the relationship between wood density and years since death in N amended plots was non-significant ($r = -0.010$, $P = 0.562$; Figure 4).

Wood C:N ratios

Results for the C:N ratios were analogous to those for wood density. ANCOVA results indicated that wood C:N ratio was significantly affected by site and N addition treatment with years since death as a covariate (Table 4). Like wood density, linear regression analysis indicated the decrease with time since death in wood C:N ratios was significantly greater for ambient than for N-amended plots (Figure 5). For ambient plots

wood C:N ratio = $368.232 - 11.866 \times \text{years since death}$ ($r = 0.34$, $P = 0.005$). N-amended plots did not have a significant linear relationship between C:N ratio and years since death ($r = 0.153$, $P = 0.226$).

Discussion

Our findings support the hypothesis that chronic elevated N deposition suppressed the decomposition of CWD and this effect was seen reduced rates of mass loss over time and higher wood C:N ratios for the experimental N deposition treatment along a latitudinal gradient in Michigan's northern hardwood forests.

Wood density

Site had a significant effect on wood density because of a much higher average wood density at site A (0.50 g cm^{-3}) versus sites B, C, and D (from $0.29 - 0.31 \text{ g cm}^{-3}$). The site difference could be attributed to species or environmental factors at site A which causes higher wood density and higher subsequent C:N ratios. Site A has the lowest mean annual temperature (Table 1). Cooler conditions at site A could contribute to lower overall decomposition rates as well as reduced live tree growth. Site A also has the highest percentage of sugar maple, which has higher wood density than the other major species on the study sites. Across decay class 2, site A had the highest average wood density therefore, CWD decomposition at site A starts with a higher wood density.

Within the plots that received ambient N deposition, wood density decreased with time (Figure 2). As decomposition proceeds, soil microbes and fungi break covalent bonds between polymers and wood becomes aerated, light, and highly fragmented. Plots receiving chronic experimental additions of NO_3^- did not exhibit this trend. In the later stages of decay (classes 3 and 4 in Figure 1 and years 8-17 in Figure 2), wood from the N deposition plots exhibited higher densities than ambient plots. This indicates that overall, older pieces of coarse woody debris are less dense in ambient plots; in other words older CWD in N amended plots is less decomposed than in ambient plots.

Wood C:N ratios

Differences in wood C:N ratios were similarly explained by the effects of site, treatment, and years since death (Table 2). Wood C:N ratios exhibited practically the same response as wood density. Wood C:N ratios in ambient plots displayed a significant negative linear response to years since death (Figure 3). In contrast, the relationship between wood C:N ratios and years since death from N amended plots was non-significant (Figure 3). As wood C:N decreases in ambient plots, either N is being imported via the life cycles of microbial decomposers, *e.g.* dead fungal hyphae releasing N into the decaying wood, or C is being lost via respiration. In N amended plots, excess soil N did not influence C:N ratios as they remained relatively high in new CWD (Figure 3). The wood density and wood C:N data sets are independent. Errors in wood volume measurements could have affected wood density estimates. However wood volume errors would not have altered C:N results.

Decomposition effect: substrate, enzymes, and carbon storage

The effects of elevated N deposition on decomposition are two-fold: it increases the production of cellulases *i.e.* it accelerates the decomposition of more labile substrates and it reduces the production of phenol oxidases and peroxidases, *i.e.* it slows the decomposition of lignin (Waldrop *et al.* 2004; Carreiro *et al.* 2000; Wang *et al.* 2010; Sinsabaugh, 2010; DeForest *et al.* 2004). The decomposition of lignin is a naturally slow process (Osono 2007; Horwath 2007). This “widening” of the gap between decomposition of labile and recalcitrant macromolecules has led researchers to question the ability of forest ecosystems under elevated N deposition to sequester soil carbon (Myneni *et al.* 2000; White *et al.* 2000). The addition of N to leaf litter has been found to “lead to greater accumulations of humified organic matter” (Prescott 2010). Several studies have observed decreases in soil respiration (Burton *et al.*, 2004; Cusack *et al.*, 2010), some even specifically heterotrophic respiration (Janssens *et al.* 2010). Taken together, N deposition increases carbon fixation (increased NPP); decreases C lost

(decreased soil/heterotrophic respiration, and increases the amount of C retained (reduced decomposition/humification of organic matter) (Saiya-cork *et al.* 2002; Pregitzer *et al.* 2008; Berg and Matzner 1997). In sum, we can surmise that future levels of N deposition will have the potential to increase carbon storage in northern hardwood forests and that future forest C storage models must consider the role of accumulating C in CWD, due in part to reduced decomposition.

In a recent finding from the same Michigan Gradient study, Thomas *et al.* (2012) concluded that although organic matter has accumulated under chronic simulated N deposition and extracellular enzyme activity is correlated with treatment, the relative oxidation state of lignin was not related to the activity of lignin-degrading enzymes. Instead, “microbial transformations of root detritus may underlie the accumulation of organic matter.” Therefore the authors concluded that within the N amended forest floor and mineral soil layers, lignin-like molecules were not accumulating and that the biochemical composition of soil organic matter was not correlated with extracellular enzyme activity.

Because we found direct evidence of reduced decomposition of CWD (density and C:N), the effect of N deposition on decomposition appears to be ubiquitous among litter types (leaf litter, soil organic matter, and CWD). In contrast with the conclusions of Thomas *et al.* (2012), accumulations of CWD are clearly not root-derived. Because C:N is correlated with the concentration of lignin-like molecules (Weedon *et al.* 2009), C does in fact appear to be accumulating, at least within CWD. Therefore the relationship between fungal communities, extracellular enzyme activity, relative oxidation of lignin, and substrate composition, all under the effect of elevated N deposition, requires further understanding and refinement. It may be the case that several different mechanisms underlie the response of decomposition to N deposition, which might include substrate variability and changes in microbial community composition. If several mechanisms do underlie this response, then the most striking finding is that all mechanisms seem to lead to an accumulation of organic matter and therefore an accumulation of C.



Figure 1. Four replicate sites along a north-south climatic gradient in Michigan have been receiving chronic elevated N additions since 1994. N amendments are in the form of solid NaNO_3 pellets, applied in 6 equal increments during the growing season (3 g N m^{-2} annually).

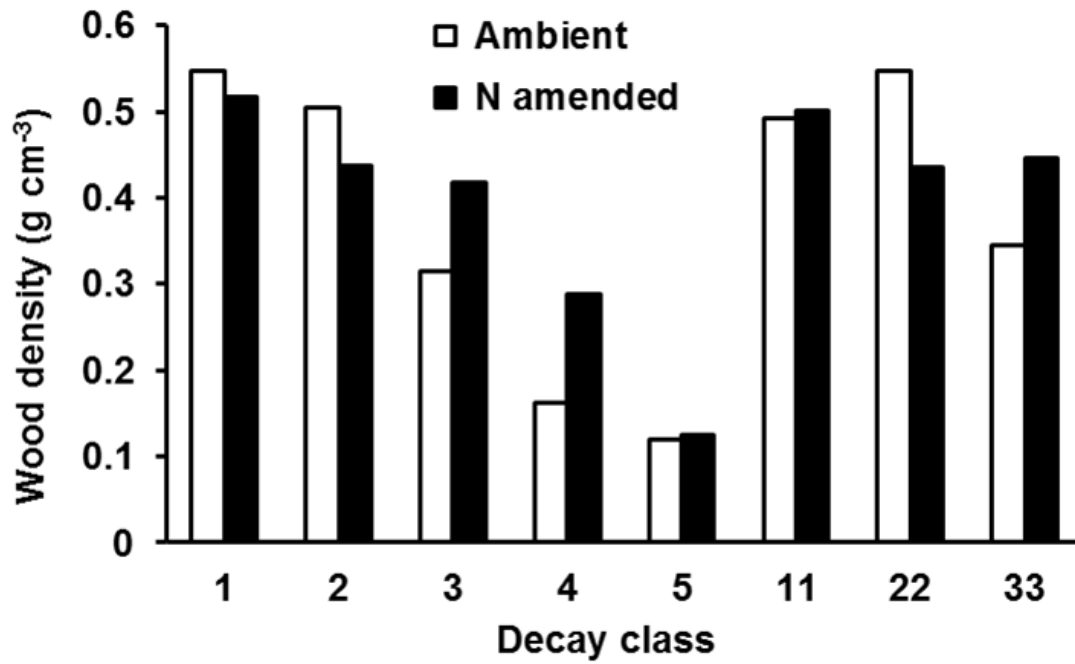


Figure 2. CWD density for visual decay classes in ambient and N amended treatments

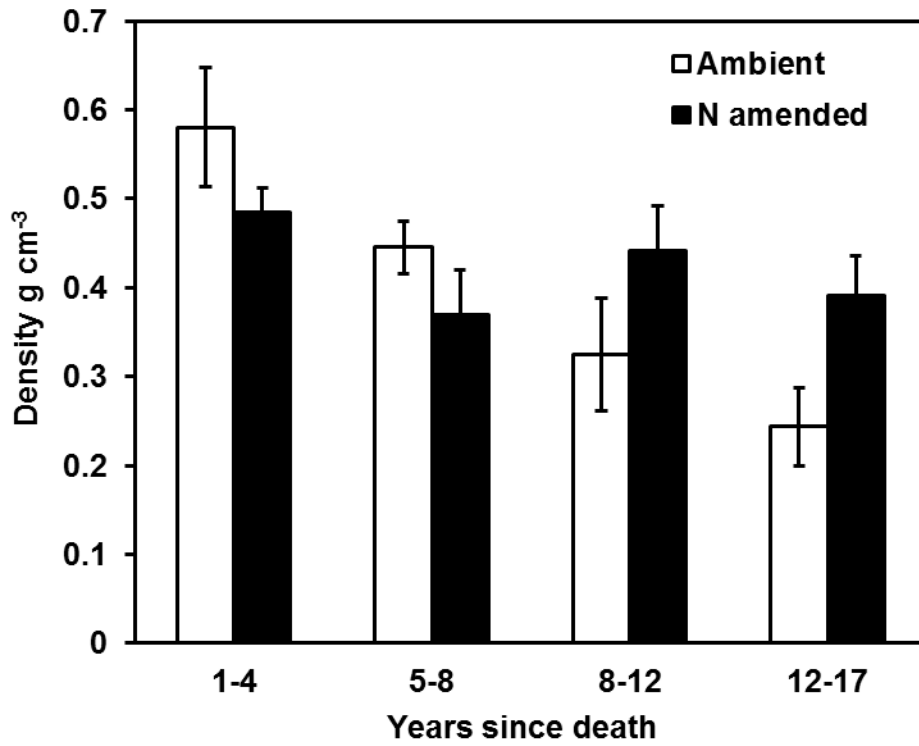


Figure 3. Wood density for the ambient and N amended treatments by *years since death* classes. Error bars indicate ± 1 standard error of the mean within each treatment for years since death classes.

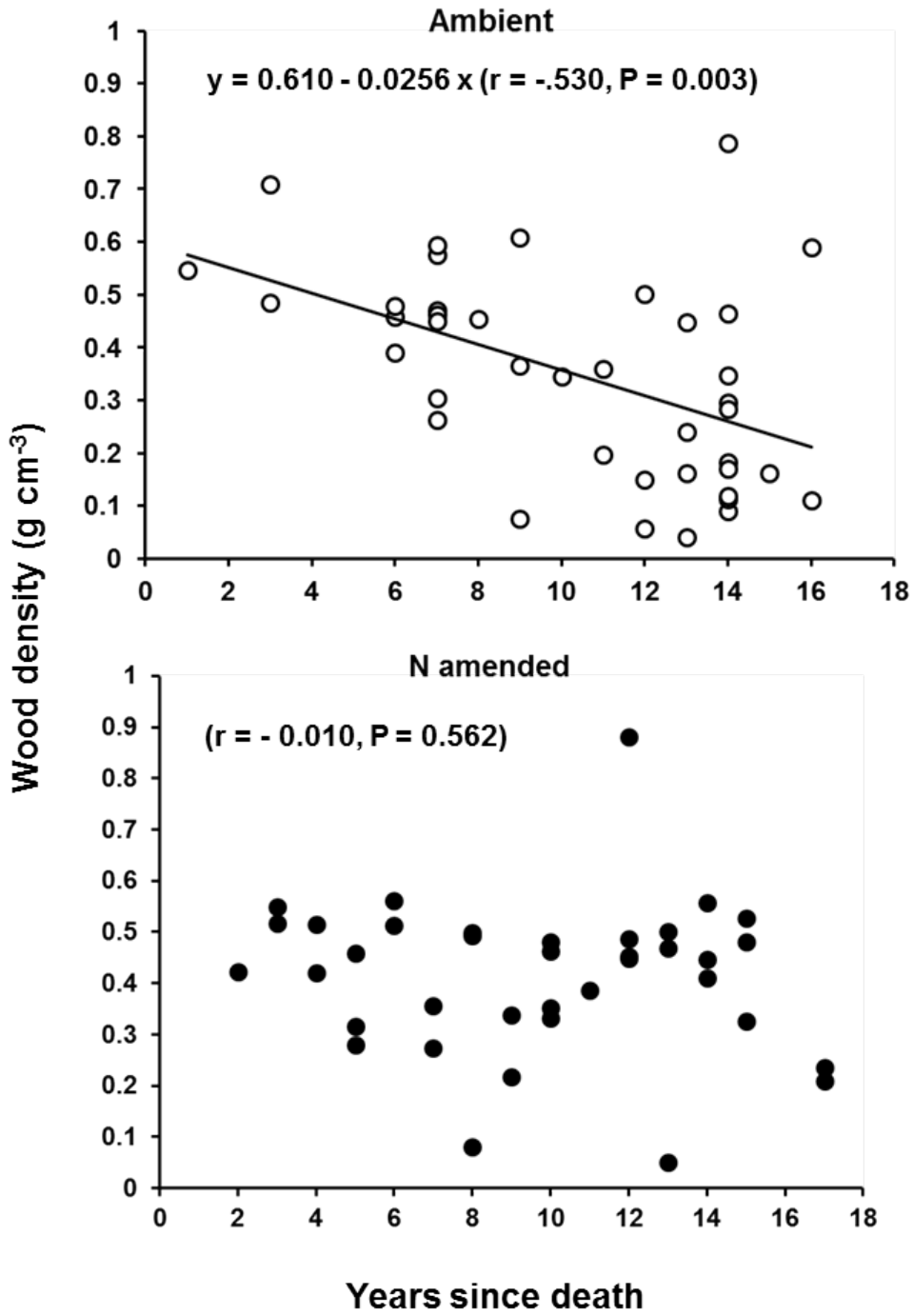


Figure 4. Scatter plot of CWD density by years since tree death for ambient and N amended treatments. Data is from wood sampling in 2011 field season.

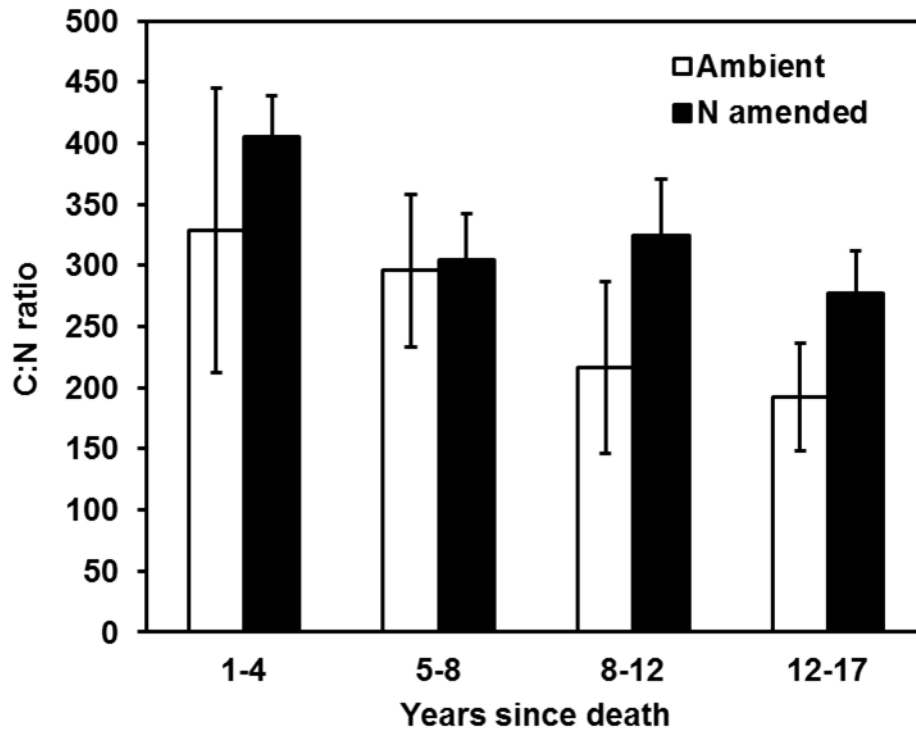


Figure 5. Comparison of carbon to nitrogen (C:N) ratios in wood samples from N amended and ambient treatments for each *years since death* class. Error bars indicate standard error within each treatment for years since death classes.

Table 1. Climatic, floristic, and edaphic characteristics of four northern hardwood sites in Michigan

Location	Site			
	A	B	C	D
Latitude, N	46°52'	45°33'	44°23'	43°40'
Longitude, W	88°53'	84°52'	85°50'	86°09'
Climate				
Mean Annual Temperature (°C)	4.7	6	6.9	7.6
Mean Annual Precipitation (mm)	873	871	888	812
Wet + Dry NO ₃ --N Deposition (g m ⁻² y ⁻¹)	0.38	0.58	0.78	0.76
Wet + Dry Total N Deposition (g m ⁻² y ⁻¹)	0.68	0.91	1.17	1.18
Vegatation				
Overstory Biomass (Mg/ha)	261	261	274	234
<i>Acer saccharum</i> Biomass (Mg/ha)	237	224	216	201
Soil (0-10 cm)				
% Sand	85	89	89	87
pH (1:1 soil:H ₂ O)	4.83	5.03	4.47	4.66

Table 2. Two-way ANOVA results for site and N addition treatment as factors affecting wood density (g cm^{-3})

Source	df	Sum-of-Squares	Mean-Square	F-ratio	P
Site	3	0.327	0.109	3.9	0.012
Treatment	1	0.125	0.125	4.485	0.038
Site*Treatment	3	0.028	0.009	0.333	0.801
error	71	1.986	0.028		

Table 3. Two-way analysis of covariance (ANCOVA) results for the effects of *site* and *treatment* on wood density with *years since death* as a covariate

Source	df	Sum-of-Squares	Mean-Square	F-ratio	P
Site	3	0.402	0.134	5.848	0.001
Treatment	1	0.082	0.082	3.569	0.063
Site*Treatment	3	0.057	0.019	0.822	0.486
Years since death	1	0.382	0.382	16.662	0.000
error	70	1.604	0.023		

Table 4 Two-way analysis of covariance (ANCOVA) results for the effects of *site* and *treatment* on the carbon to nitrogen (C:N) ratio with *years since death* as a covariate

Source	df	Sum-of-Squares	Mean-Square	F-ratio	P
Site	3	249520.698	83173.566	4.66	0.004
Treatment	1	64282.303	64282.303	3.602	0.060
Site*Treatment	3	109050.48	36350.16	2.037	0.112
Years since death	1	203350.687	203350.687	11.394	0.001
error	122	2177274.102	17846.509		

Chapter 3: An Assessment of Forest Stand Dynamics and Coarse Woody
Debris Biomass Accumulation under Chronic Simulated Nitrogen
Deposition

Introduction

North American forests are expected to receive a 25% increase in atmospheric nitrogen (N) deposition in the coming decades (Galloway *et al.* 1994). Due to the combustion of fossil fuels and the use of N containing fertilizer, humans have significantly influenced the global N cycle. Both experimental additions of N in short-term studies and chronic additions in long-term studies have found similar patterns in forest response to N amendments. In an analysis of 23 atmospheric chemistry transport models, Detener *et al.* (2006) states, “Currently 11% of the world’s natural vegetation receives N deposition in excess of the ‘critical load’ threshold of 1000 mg (N) m⁻² yr⁻¹,” the United States is considered one of the most affected regions.

A study from the University of Michigan’s Biological Station (UMBS) in northern lower Michigan calculated atmospheric N deposition from canopy N retention and soil N mineralization and found that atmospheric N inputs contribute up to 15% of NPP (Nave *et al.* 2009). Elevated N deposition has been found to increase aboveground net primary productivity (ANPP) (Nave *et al.* 2009; Pregitzer *et al.* 2008; Aber *et al.* 1995) in forests. This increase in productivity has resulted in an increase in aboveground live plus dead woody biomass (Pregitzer *et al.* 2008). Several studies have found that N amendments lead to an accumulation in forest floor organic matter (Berg and Matzner 1996; Franklin *et al.* 2003; Mansson and Falkengren-Grerup 2003). Pregitzer *et al.* (2008) recorded a 50% increase in forest floor (Oi and Oe horizons) mass over a decade of N amendments. The increased mass of organic matter was concluded to be a physical barrier for sugar maple seedlings, effectively reducing sugar maple regeneration (Patterson *et al.* 2012). In N limited ecosystems, elevated N inputs have led to soil N saturation, an occurrence when soil solution N leaching nearly equals ecosystem N inputs (Pregitzer *et al.* 2004; Wallace *et al.* 2007; Aber *et al.* 1998; Garten 1999). Prolonged N saturation has been related to increases in tree mortality (Shen *et al.* 2001; Thomas *et al.* 2010; Wallace *et al.* 2007). Nissenan *et al.* (1999) speculated that initially tree growth would increase with N additions, but eventually it would decrease as other nutrients

become limiting. Another pathway to increased mortality is that initial enhanced growth rates due to elevated N deposition could lead to increased competitive interactions within individuals. Those interactions would eventually result in some tree mortality through stem-exclusion, which can be accounted for via an assessment of CWD volume and biomass. The consequence of accelerated stand dynamics and associated self-thinning is a potential increase in the production of CWD.

Elevated N deposition has been correlated with reduced decomposition in leaf litter. Several studies have observed this result indirectly through reduced soil respiration (Burton *et al.*, 2004; Cusack *et al.*, 2010) and reduced heterotrophic microbial respiration (Janssens *et al.*, 2010). Pregitzer *et al.* (2008) documented an accumulation of soil organic matter under experimental N deposition. Other research has found correlations with litter quality (Berg and Meentemeyer, 2002; Mansson and Falkengren-Grerup, 2003) enzyme activity (Waldrop *et al.*, 2004; Carreiro *et al.*, 2000; Wang *et al.*, 2010; Sinsabaugh, 2010; DeForest *et al.*, 2004), microbial genetic analyses (Blackwood *et al.*, 2007; Hassett *et al.*, 2009; Hofmockel *et al.*, 2007), microbial biomass (Treseder, 2008; Zeglin *et al.* 2007), and reduced decomposition of coarse woody debris CWD (See Chapter 2).

Objectives and hypotheses

Few studies have directly assessed changes in the amount of dead woody biomass produced under conditions of chronic experimental elevated N deposition. We assessed tree mortality in a 17-year experimental N deposition study in northern hardwood forests. The objectives of this study were to determine if rates of biomass mortality were altered by the N deposition treatment and assess whether N deposition treatment led to an accumulation of CWD volume and biomass. We also evaluated changes over time in the average diameter of trees dying. Results were used to assess the influence of elevated N deposition on forest stand dynamics and carbon storage in CWD. We hypothesized that long term N amendments had significantly increased the amount of CWD production through enhanced tree mortality and had increased CWD biomass due to enhanced CWD

inputs and reduced decomposition rates. We expect N amendments to accelerate stand dynamics largely by increasing mortality in the larger diameter at breast height (dbh) classes in recent years.

Methods

Site description

The four study sites used in this research are located across a 500 km latitudinal gradient in Michigan (Figure 6). All four sites contain three 30 m x 30 m treatment plots and three 30 m x 30 m control plots. Since 1994, elevated N-deposition has been simulated by broadcasting $0.5 \text{ g N m}^{-2} \text{ month}^{-1}$ of solid NaNO_3 pellets in 6 equal applications during the growing season (3 g N m^{-2} annually). The sites receive total ambient N deposition ranging from 0.7 to $1.2 \text{ g N m}^{-2} \text{ yr}^{-1}$. All sites contain similar flora, with the overstory dominated by *Acer saccharum* (80% of basal area). *Acer rubrum*, *Quercus rubra*, *Fagus grandifolia*, and *Prunus serotina* are also common (Table 5). The four sites are northern hardwood forests (see Burton *et al.* 1991 for further information on site characteristics) with Kalkaska sand (Typic haploorthod) soils, similar age, and similar stand structure.

Coarse Woody Debris Density

All trees within each plot have been numbered, with diameter at breast height (dbh) measured annually at permanent marks since 1987 for ambient plots and since 1994 for N amended plots. Tree death has been recorded during annual measurements. Every tree within the study plots has known coordinates, which allowed them to be easily located. Wood from every third down dead tree dying between 1994 and 2011 was sampled during the summer of 2011. Decay classes 1 through 5 were used to classify down dead wood (DDW), or trees that died and are now lying on the forest floor. Decay class 1 indicated the tree was freshly fallen with bark and fine branches intact and often still having leaves. Decay class 2 was differentiated from decay class 3 by the presence of bark and secondary branches in decay class 2. Decay class 4 appeared slightly ellipsoidal with some fragmentation as well as evidence of insect and fungal attack. Decay class 5 was highly fragmented, often sunken in soil and moss covered. An extra class was used in addition to the decay classes 1 through 5. Decay class 9 was used to identify CWD

which had been removed from a site by the public, presumably for use as firewood. Evidence of decay class 9 included cut CWD, wood chips from a chainsaw, and cut snag stumps.

Wood sampling for density analysis was done using either a chainsaw for decay classes 1 through 3 (sometimes 4), or steel core (5 cm diameter) for decay classes 4 and 5. A hole-saw or chisel was used to collect from every third dead standing tree (snag). Each visual decay class is intended to represent a relative level of decomposition for debris pieces (methods adapted from Duvall and Grigal 1999). Volume for density samples was calculated using dimension measurements from steel core samples and by water displacement on a balance for solid wood samples collected by the other methods. Samples were then placed in a drying oven at 65°C until a constant mass was achieved. Wood density (g cm^{-3}) was obtained from volume and dry mass measurements.

Coarse woody debris volume and biomass

The “triangular transect” method described in Delisle *et al.* (1988) was employed to estimate DDW biomass at all sites during the 2012 field season. Each transect was an equilateral triangle with 30 m sides. Every piece of DDW ≥ 5 cm dbh was measured for diameter at the line intersection point and decay class. All six plots at each of the four sites were sampled (a total of 24 triangle transects). DDW volume was calculated as:

$$V = (1.234) \times d^2 / L$$

Where V is volume (m^3/ha), d is the diameter (cm) of the piece of DDW measured perpendicular to the length of the log, and L is the length of transect (90 m total in this case). Volume measurements as well as the previously noted density values calculated in the lab were used to estimate average biomass per treatment for each decay class. DDW volume and biomass data were analyzed with a three-way analysis of variance (ANOVA) with site, treatment, and decay class as factors. A two-way ANOVA (site by treatment) was done on total DDW biomass for each plot (sum of the five decay classes).

Mortality basal area

For the 17 years of elevated N additions, tree mortality was recorded annually. That mortality is captured in our CWD analysis. To further examine the structure of forest mortality, we classified the diameters of all trees dying and used regression analysis to determine if the diameters of trees dying were changing over time and, if so, if there were differences among treatments in the rates of such changes.

Results

DDW biomass and volume

Nitrogen additions did not have a significant effect on DDW volume (Table 7) or biomass (Table 8). DDW Volume decreased from site A to D; the relationship was marginally significant ($P = 0.075$, Table 7). Down dead wood biomass declined from site A to site D; although non-significant ($P = 0.155$), this trend is important to note. DDW biomass differed significantly among decay classes (Figure 8). A significant decay class by site interaction also occurred ($P = 0.012$). All treatment effects and interactions were non-significant (Table 6). The largest increase in DDW biomass from ambient to N-amended plots occurred in decay classes 3 and 4 (987 kg ha^{-1} and 714 kg ha^{-1} respectively, Figure 8). Assuming 50% of biomass is carbon content of DDW, total carbon increased by 16% from ambient to N-amended plots.

Mortality and stand dynamics

Across all sites, 52 more trees died in the N deposition treatment between 1994 and 2011 than in the ambient treatment, mostly occurring in sites C and D (Figure 7). The majority of mortality which occurred on both ambient and N-amended plots was found in the 6, 10, and 14 cm dbh classes (Figure 10). There was not a significant relationship between the year of tree death and their diameter. Both ambient and N-amended mortality occurred evenly throughout the period of the Michigan Gradient study with a slight, non-significant increase in mortality in larger trees in more recent years (Figure 11).

Discussion

Biomass and volume

Down dead wood biomass declined from site A to site D. The explanation for this is due in part to human removal at primarily the two southernmost sites (C and D). Although signs are displayed with statements that are meant to deter unnecessary human disturbance, several snags and DDW pieces were removed by the public (for firewood) during the study. Removed CWD accounts for 25% of all dead trees surveyed, with 86% of removed trees taken from the two southern sites (C and D).

Research at the Michigan Gradient sites has indicated that dead wood density significantly differed in ambient and N amended treatments, with higher wood densities found in N amended plots (unpublished data). During 17 years of chronic experimental N deposition, DDW volume and biomass have accumulated in greater amounts in N amended plots (Figure 8). However, even though biomass and volume treatment effects were non-significant, we believe that DDW biomass is consistent with our hypothesis that DDW biomass will increase due to slower decomposition and resultant higher wood density. There was a 7% decrease in DDW volume in N amended plots (Figure 9). When the significant differences in density (See Chapter 2) are taken into account when converting wood volume to biomass, there is a 16% increase in DDW biomass in N amended plots (Figure 8).

DDW volume is a highly variable forest characteristic; therefore we were unable to detect small differences between treatments. With this low power but a significant trend in wood density (see Chapter 2), it remains a possibility that DDW biomass may significantly increase under elevated N deposition. I conclude that as CWD density responds to N additions, biomass increases to a greater degree than volume and therefore may accumulate under N deposition. Due to reduced decomposition rates of DDW, I suspect that the N treatment effect on DDW biomass may become significant in the future.

Stand dynamics and mortality

I hypothesized that chronic elevated N deposition would accelerate stand dynamics. If N limited individuals are exposed to ample amounts of readily available N, they may grow faster. As trees grow faster, self-thinning may proceed more rapidly due to competition. This may result in enhanced mortality of intermediates and co-dominants. Eventually larger trees would begin to die, as they compete with each other. This trend likely wouldn't appear until several years of enhanced aboveground NPP had occurred, as there is likely a lag time in stand structure response to competitive interactions. In our study plots, mortality generally occurred in the smaller dbh classes (Figure 10) as opposed to larger diameter classes. This is most likely a remnant of an earlier stem exclusion stage in stand development. Both N amended and ambient plots exhibited only minor differences in mortality in recent years (Figure 11). Although non-significant, the more recent mortality in both treated and ambient plots has included more individuals from larger dbh classes. This is most likely a result of natural shifts in stand dynamics and perhaps a “lag time” in accelerated stand dynamics for the N deposition treatment will take several more years, or even decades to become distinguishable.

Carbon storage

Several studies have concluded that N deposition increases C storage in forests. Although statistically non-significant, CWD in our N amended plots stored 16% more C than in ambient plots. Elevated N deposition increased C storage by 26% in northern hardwood forests due to enhanced tree growth in combination with reductions in decomposition and associated increases in forest floor and surface soil organic matter (Pregitzer *et al.* 2008). Thomas *et al.* (2009) used forest inventory data to estimate the degree of carbon storage in trees along an N deposition gradient in the United States. The analysis found that “above-ground biomass increment increased by 61 kg of C per kg of N deposited.” When this number is expanded to the global level, N deposition could potentially increase C storage in trees by an extra 0.31 Pg carbon yr⁻¹. Because N

additions have led to increased relative recalcitrance of lignin (Berg and Matzner 1997; Stevenson 1982), increased tree mortality (Shen *et al.* 2001; Thomas *et al.* 2010; Wallace *et al.* 2007), and reduced decomposition of DDW (see Chapter 2), DDW could play a significant role in forest C storage under future levels of N deposition. With this in mind, forest C models should be amended to consider the portion of C retained in CWD.

Implications for management

Forest managers examine coarse woody debris for a variety of reasons. The U.S. Forest Service uses CWD as part of their estimates of forest carbon storage (Smith *et al.* 2002). Others are concerned with climate change and carbon sequestration. N deposition has the potential to increase carbon stored as CWD by reducing CWD decomposition (unpublished data). Downed dead wood and snags provide habitat and nesting sites for a variety of birds (Rosenberg *et al.* 1988; Swallow *et al.* 1988), small mammals (Zollner and Crane 2003; Smith and Maguire 2004), amphibians (Dupuis *et al.* 1995; Butts and McComb 2000; Moseley *et al.* 2004), and invertebrates (Jonsell and Weslien 2003; Jabin *et al.* 2004). N amendments disrupt the decomposition of CWD (see Chapter 2), which may or may not influence an organism's ability to utilize it for resources. N amendments have in some cases led to greater tree mortality (Shen *et al.* 2001; Thomas *et al.* 2010; Wallace *et al.* 2007). Although research is needed in order to quantify this, perhaps N deposition will increase available wildlife habitat over time. Accumulations of CWD are common in old growth forests. The Michigan gradient plots are unmanaged second-growth forests (Burton *et al.* 1991) currently undergoing understory reinitiation. Over time, N deposition may lead to greater structural complexity in unmanaged second-growth and essentially push the stand closer to old-growth-like characteristics (purely structural), which may be of interest to those managing for the creation of old-growth-like conditions. More research is needed in order to understand if, and to what extent, N deposition increases wildlife habitat, increase carbon storage, and structural complexity in forests.



Figure 6. Four replicate sites along a north-south climatic gradient in Michigan have been receiving chronic elevated N additions since 1994. N amendments are in the form of solid NaNO_3 pellets, applied in 6 equal increments during the growing season (3 g N m^{-2} annually).

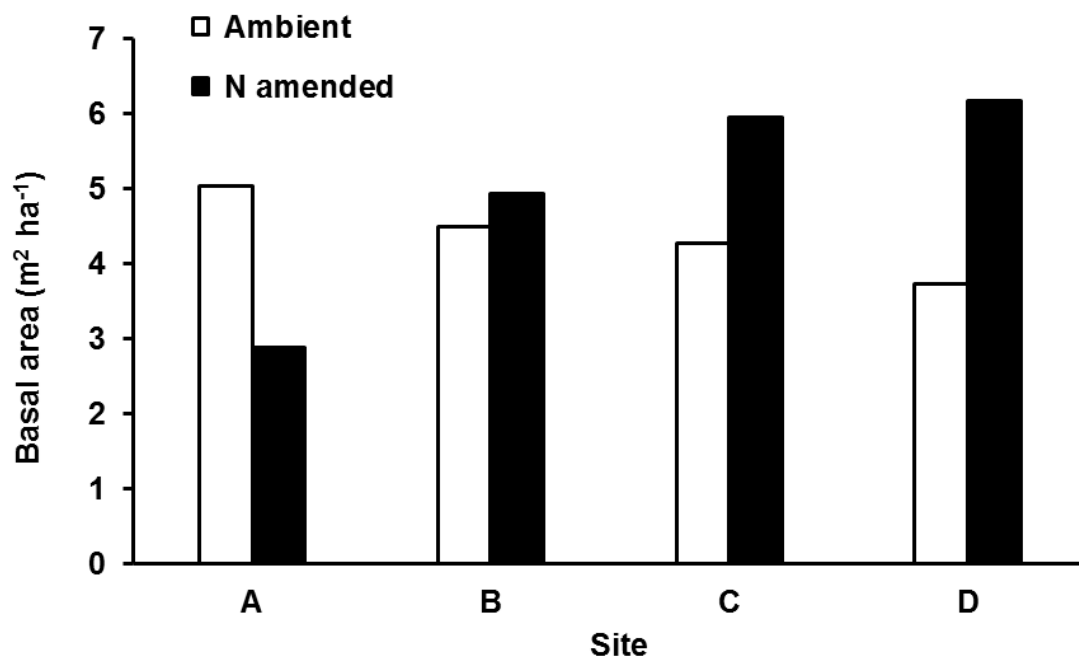


Figure 7. Mortality basal area per hectare at sites A through D. Values are based on the dbhs of all trees dying from 1994 through 2011.

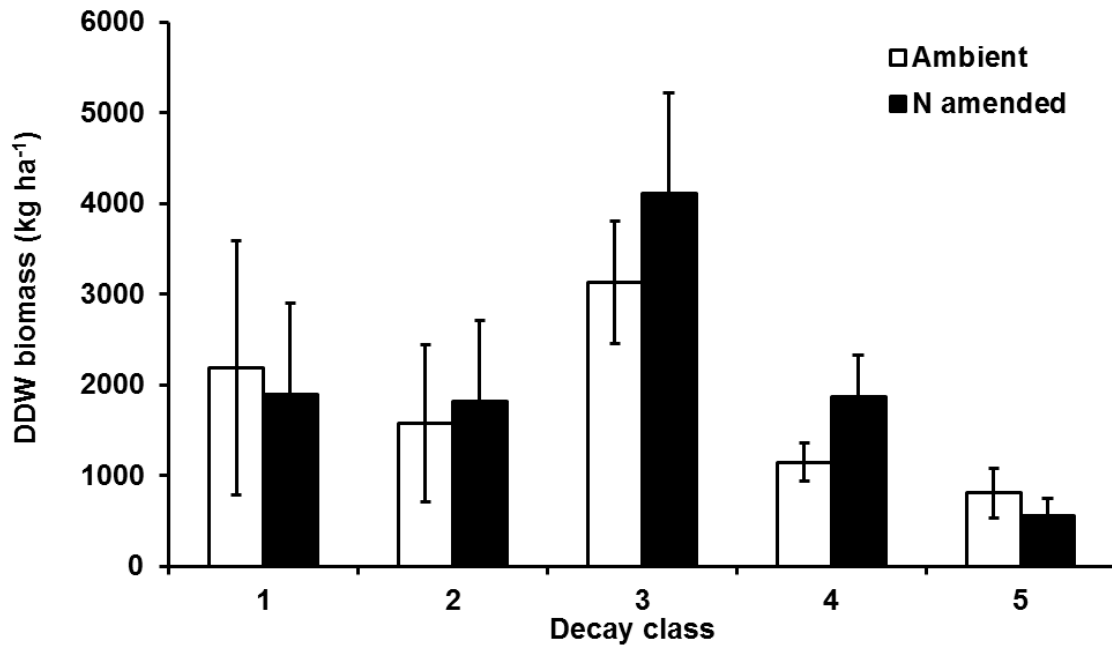


Figure 8. Average DDW biomass per hectare by decay class for N amended and ambient treatments. Error bars indicate ± 1 standard error of the mean.

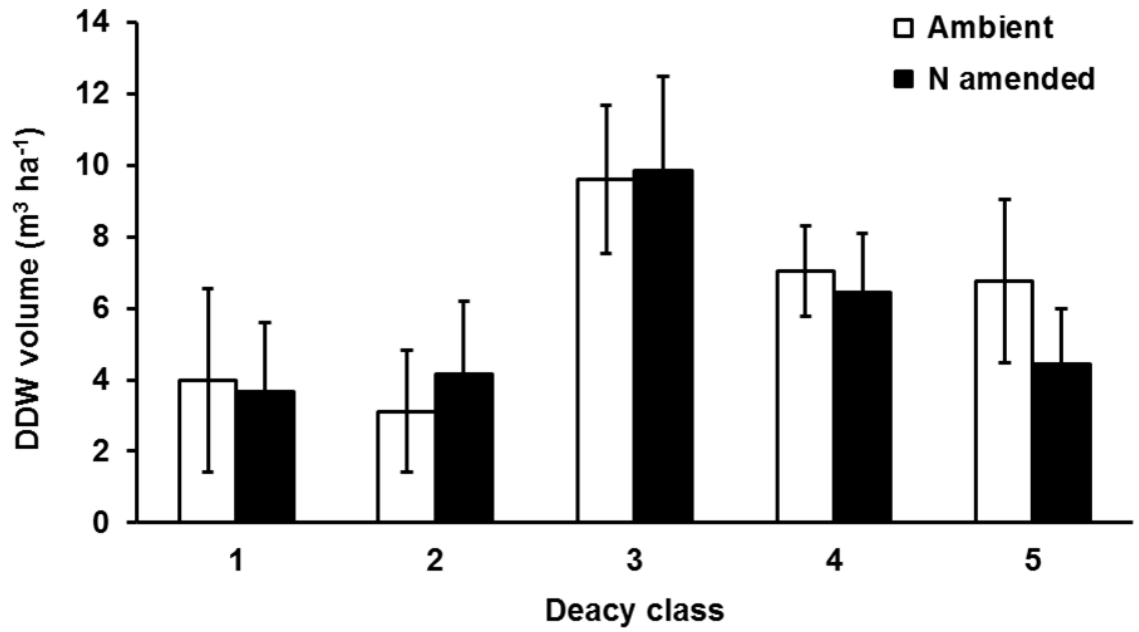


Figure 9. Average DDW volume ($\text{m}^3 \text{ha}^{-1}$) in N amended and ambient treatments by decay classes surveyed in 2012. Error bars indicate ± 1 standard error of the mean.

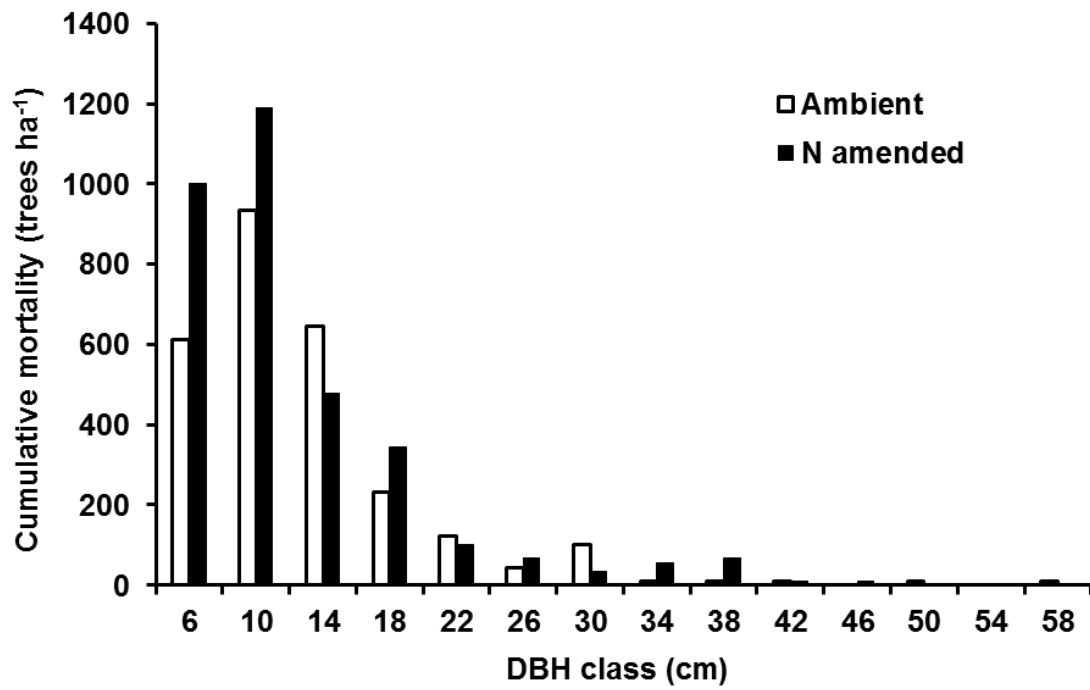


Figure 10. Diameter distribution for cumulative tree mortality from 1994 through 2011 for the control and N deposition treatments

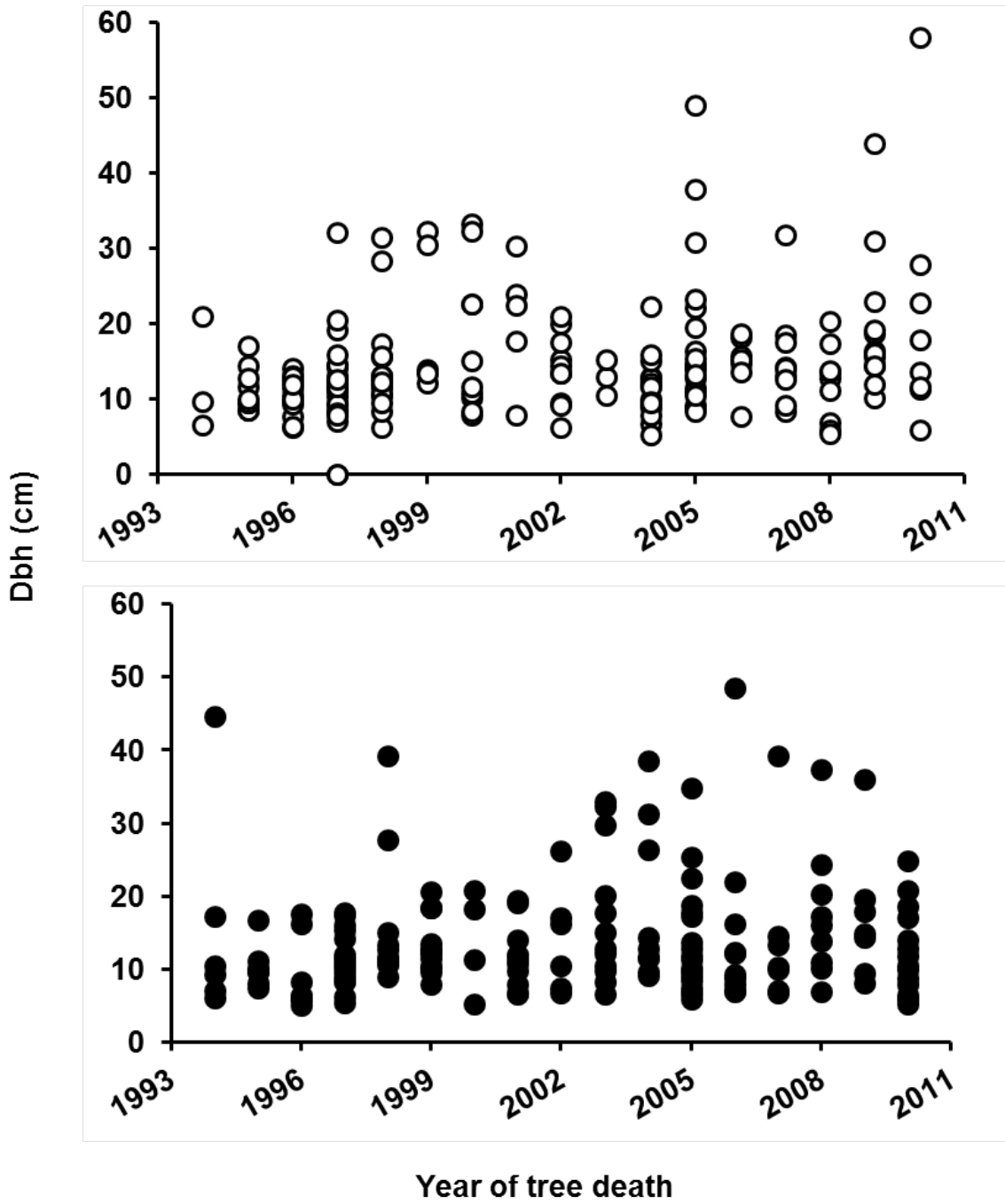


Figure 11. Diameter of trees dying plotted against the year of tree mortality for ambient and N amended treatments.

Table 5. Climatic, floristic, edaphic characteristics of four northern hardwood forests in Michigan

Location	Site			
	A	B	C	D
Latitude, N	46°52'	45°33'	44°23'	43°40'
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Soil (0-10 cm)				
% Sand	85	89	89	87
pH (1:1 soil:H ₂ O)	4.83	5.03	4.47	4.66

Table 6. Three-way ANOVA results for the effects of *site*, *treatment*, and *decay class* on DDW biomass

Source	df	Sum-of-Squares	Mean-Square	F-ratio	P
Site	3	$3.656 \cdot 10^7$	$1.219 \cdot 10^7$	1.793	0.155
Treatment	1	$2.383 \cdot 10^6$	$2.383 \cdot 10^6$	0.351	0.555
Decayclass	4	$1.117 \cdot 10^8$	$2.792 \cdot 10^7$	4.109	0.004
Site*Treatment	3	$2.506 \cdot 10^7$	$8.353 \cdot 10^6$	1.229	0.305
Site*Decay class	12	$1.912 \cdot 10^8$	$4.594 \cdot 10^7$	2.345	0.012
Treatment*Decay class	4	$7.778 \cdot 10^6$	$1.994 \cdot 10^6$	0.286	0.886
Site*Treatment*Decay class	12	$7.167 \cdot 10^7$	$5.972 \cdot 10^6$	0.879	0.571
Error	80	$5.437 \cdot 10^8$	$6.796 \cdot 10^6$		

Table 7. Three-way ANOVA results for DDW volume ($\text{m}^{-3} \text{ha}^{-1}$) with site, treatment, and decay class as factors

Source	df	SS	MS	F-ratio	P
Site	3	316.327	105.442	2.385	0.075
Treatment	1	4.525	4.525	0.102	0.750
Decay class	4	596.286	149.072	3.372	0.013
Site*Treatment	3	69.728	23.243	0.526	0.666
Site*Decay class	12	977.322	81.444	1.842	0.055
Treatment*Decay class	4	37.211	9.303	0.21	0.932
Site*Treatment*Decay class	12	456.685	38.057	0.861	0.589
error	80	353.164	44.215		

Table 8. Two-way ANOVA results for total DDW biomass (summed across decay classes) with *site* and *treatment* as factors

Source	Df	Sum-of Squares	Mean-Square	F-ratio	P
Site	3	$1.828 \cdot 10^8$	$6.093 \cdot 10^7$	1.216	0.336
Treatment	1	$1.191 \cdot 10^7$	$1.191 \cdot 10^7$	0.238	0.632
Site*Treatment	3	$1.252 \cdot 10^8$	$4.176 \cdot 10^7$	0.834	0.495
error	16	$8.016 \cdot 10^8$	$5.010 \cdot 10^7$		

Table 9. Two-way ANOVA for total DDW volume (summed across decay classes) with *site* and *treatment* as factors

Source	Df	Sum-of-Squares	Mean-Square	F-ratio	P
Site	3	1581.635	527.212	1.737	0.200
Treatment	1	22.624	22.624	0.075	0.788
Site*Treatment	3	348.642	116.214	0.383	0.767
error	16	4857.285	303.580		

Thesis Conclusions

Experimental N deposition affected DDW wood density and wood C:N ratios and subsequently reduced the decomposition of DDW in northern temperate forests (Chapter 2). The reduction in decomposition was likely due to an inhibition of white-rot fungi by elevated N additions. Wood density and wood C:N ratio data sets are independent of each other. Any bias or error in wood density resulting from errors in volume estimates would not affect wood C:N ratios, yet both data sets yielded similar results. Long term N deposition studies on wood degrading fungi within DDW are needed to confirm if elevated N inputs inhibited white-rot fungal enzyme activity, shifted community composition, or modified the structure of lignin in DDW. Elevated N deposition has the potential to increase DDW biomass in part by accelerating stand dynamics, ultimately enhancing DDW inputs from mortality, especially that of larger diameter trees. Further research on forest stand dynamics under long-term elevated N deposition is needed to confirm this. Reduced decomposition of DDW and accumulating dead woody biomass could increase C sequestration in northern temperate forests. N deposition effects on DDW dynamics should be included in forest C models, using realistic scenarios for future levels of N deposition.

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