

Simulated N Deposition Negatively Affects
Sugar Maple (*Acer saccharum* Marsh.) Regeneration in a Lake
States Northern Hardwood Ecosystem

by

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Abstract

During the next century, atmospheric nitrogen (N) deposition is projected to more than double, potentially leading to a decline in biodiversity of plant assemblages and community structure. We quantified sugar (*Acer saccharum* Marsh.) maple seedling abundance in replicate northern hardwood forest stands ($n = 4$) receiving ambient atmospheric N ($0.7 - 1.18 \text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) and experimental atmospheric N deposition simulating future amounts in eastern North America (ambient plus $3 \text{ g NO}_3^- \cdot \text{N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$). First-year seedling abundance did not differ under ambient and simulated N deposition ($P = 0.961$); however, there were greater abundances of second- and third-fifth-year seedlings under ambient N deposition ($P < 0.001$). In this experiment, simulated atmospheric N deposition has slowed litter decay, resulting in an accumulation of forest floor. We reasoned that a greater forest floor mass would impose a physical barrier to sugar maple seedling establishment, thereby reducing populations of seedlings. To test this idea, we experimentally manipulated forest floor mass over sugar maple seeds under ambient and simulated N deposition. In all cases, a greater forest floor, equivalent to that under simulated N deposition, resulted in significantly ($P = 0.001$) fewer established individuals, regardless of whether the greater forest floor mass occurred under ambient or simulated N deposition. Finally, to assess the effect of simulated N deposition on established seedlings, we transplanted first-year established seedlings into areas receiving ambient and simulated N deposition and quantified their mortality after one year. Fewer seedlings survived when grown under simulated N, albeit that result was not significant ($P = 0.059$). Our results indicate that levels of atmospheric N deposition similar to levels found in many terrestrial ecosystems around the Earth, have the potential to negatively affect stand dynamics in sugar maple-dominated forests, which further has the potential to elicit ecosystem change in regards to overstory carbon storage.

Keywords: northern hardwoods forest, sugar maple (*Acer saccharum*), forest floor accumulation, atmospheric N deposition, seedling establishment, seedling survival

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Introduction

Atmospheric deposition of biologically available nitrogen (N) has doubled in the past century, a phenomenon largely driven by anthropogenic processes associated with modern agriculture and the combustion of fossil fuels (Galloway et al. 2004). Furthermore, atmospheric N deposition is projected to more than double in the next century (Galloway et al. 2004). Although soil N availability broadly limits plant productivity (Aerts and Chapin 2000, Vitousek et al. 2002), chronic N additions in some forest ecosystems have led to a condition of N saturation, which has culminated in large leaching losses of NO_3^- (Aber et al. 1989, Pregitzer et al. 2004). Moreover, chronic N deposition can decrease plant species diversity even at low levels of deposition (e.g., $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$; Clark and Tilman 2008, Suding et al. 2005, Stevens et al. 2004). Sugar maple (*Acer saccharum* Marsh.), an ecologically important tree species in the northeastern United States and southeastern Canada, often occurs on soil with high net nitrification rates in which chronic N deposition could elicit N saturation (Aber et al. 1989) and subsequently negatively impact the growth and reproduction of this dominant forest tree.

During the past four decades, sugar maple abundance has declined in some regions of the northeastern United States and southeastern Canada, especially in areas of high atmospheric N deposition (Kolb et al. 1993, Lovett and Rueth 1999, Horsley et al. 2002, Bailey et al. 2005); this response is consistent with the N saturation hypothesis. It has been argued that the low cation exchange capacity and low base saturation of granitic-derived soils in these regions (Johnson et al. 1968, Long et al. 1997a) predispose these soils to acidification and base cation loss, especially the leaching loss of Ca^{2+} which can have negative consequences on the growth of sugar maple (Duchesne et al. 2002, Lovett et al. 2004, Zaccherio and Finzi 2007). Indeed, sugar maple decline was much more pronounced in unglaciated Ca-poor soils associated with relatively high

degrees of cation leaching in the Allegheny Plateau of Pennsylvania, compared to glaciated areas of the same region (Long et al. 1997a). Furthermore, if soils have experienced a high degree of acidification and cation loss, experimental additions of Ca^{2+} can enhance the growth, vigor, and seed production of sugar maple (Long et al. 1997b, Moore et al. 2000, Wargo et al. 2002, Houle et al. 2002, Juice et al. 2006). Therefore, when Ca^{2+} is added to soils derived from low- Ca^{2+} -containing parent material, sugar maple abundance should respond positively. Additionally, rates of primary productivity, stand health and vigor, and basal area of sugar maple are positively correlated with increased amounts of soil and foliar Ca^{2+} (Ellsworth and Liu 1994, Schaberg et al. 2006, Hallett et al. 2006).

In contrast, sugar maple forests in the Upper Lakes State region occur on soils formed from calcareous glacial drift, in which Ca^{2+} is abundant (Albert et al. 1986, Barnes and Wagner 1996). Notwithstanding, atmospheric N deposition could exert a negative effect on sugar maple in this region via a very different and unstudied mechanism effecting seedling establishment. Forests in this region exposed to simulated N deposition, comparable to rates expected by 2050 ($3 \text{ g NO}_3^- \text{ m}^{-2} \text{ yr}^{-1}$; Galloway et al. 2004), have experienced a decline in plant litter decay and a significant increase in forest floor mass (Zak et al. 2008). Seedling recruitment, a vulnerable and essential component of the life cycle of all plants (Grubb 1977), could plausibly be suppressed by a significant accumulation of forest floor. For example, seeds should have higher energetic requirement to penetrate a thicker forest floor to reach mineral soil (Facelli and Pickett 1991). Oak germinants, when grown over dense mats of litter, had higher incidence of mechanical damage to their hypocotyls and higher rates of mortality compared with those grown in the absence of litter (Barrett 1931). In addition, smaller-sized seeds with lower amounts of stored carbohydrates and fats are more likely to experience increased mortality, because it should take

greater amounts of stored energy to penetrate a deeper forest floor (Hamrick and Lee 1987, Tao et al. 1987, Molofsky and Augspurger 1992, Metcalfe and Grubb 1997, Dzwonko and Gawronski 2002). Sugar maples seeds are small in size (~7-9mm in diameter; ~ 460 mg seed⁻¹; [Gabriel 1978, Godman et al. 1990]), suggesting an accumulation of forest floor induced by atmospheric N deposition could impose a physical barrier for establishment. Despite a high germination capacity of sugar maple seeds (95%), low establishment success due to an accumulation of forest floor could negatively affect the recruitment of sugar maple seedlings into the overstory (Godman et al. 1990).

My objective was to determine whether a greater forest floor mass, induced by the slowing of plant litter decay caused by simulated N deposition, has negatively impacted the establishment of sugar maple seedlings. Due to the relatively small size of sugar maple seeds and consequentially low amounts of stored energy, I hypothesized that a thicker forest floor generated under simulated N deposition would decrease seedling establishment and thereby decrease sugar maple regeneration. To test this idea, I surveyed sugar maple seedling abundance in four sugar maple dominated hardwood forest stands in the Upper Lakes States region that have been receiving ambient and simulated N deposition for the past 17 years. Next, to determine the influence of forest floor thickness on sugar maple establishment, I experimentally manipulated forest floor mass over sugar maple seeds placed in plots receiving ambient and chronic N deposition. Finally, to evaluate whether chronic N deposition affects sugar maple seedling mortality following establishment, I transplanted first-year seedlings into plots receiving ambient and chronic N deposition.

Methods

Study Sites

I assessed the influence of a greater forest floor mass under simulated N deposition on the establishment of sugar maple seedlings in four sugar maple-dominated northern hardwood stands in Lower and Upper Michigan, USA (Fig. 1). The stands are located on a north-south climatic and atmospheric N deposition gradient wherein the southern-most site receives the greatest amount of ambient N deposition (NO_3^- composes $\sim 60\%$ of wet and dry deposition) and the northern-most site receives the least (~ 12 and $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ respectively). All four stands are occur on well-drained Kalkaska sand and are similar in floristic composition and stand structure, with the dominant overstory trees being *A. saccharum* with a mean age of 94 yrs ($\sim 82\%$ basal area sugar maple [Pregitzer et al. 2008]). Understory and ground cover species are sparse; sugar maple seedlings and saplings are the dominant plants in these structural layers of vegetation.

Since 1994, three 30-m by 30-m plots in each stand have been receiving experimental amounts of NO_3^- deposition in the form of NaNO_3^- pellets applied six times throughout the growing season ($0.5 \text{ g N m}^{-2} \text{ month}^{-1}$). Three other 30-m by 30-m plots receive ambient amounts of atmospheric N deposition. Each plot is surrounded by a 10-m wide buffer, also subjected to the experimental treatments. The Oi horizon is composed primarily of sugar maple leaf litter.

Species of Interest

Acer saccharum is a late-successional overstory tree common to hardwood forests of the northeast. Trees bear seed at 30-40 years of age, with maximum seed production at 60 years of age. Following wind pollination, seeds ripen into double samaras containing either one, or more rarely, two viable seeds (Godman et al. 1990). Seeds ripen and fall two weeks after maturation, concurrent with leaf fall in mid- to late September (Houle and Payette 1991). In order to

germinate the following spring, seeds require temperatures slightly above freezing for 35 to 90 days, along with ample moisture (Godman et al. 1990). Newly produced seeds have 95% germination capacity, though they seldom remain viable in the forest floor following one year (Godman et al. 1990). Germination occurs before the overstory canopy develops, which typically occurs during mid-May at our study sites. Establishment of germinating seeds is contingent upon the hypocotyls reaching mineral soil and the emergence cotyledons through the forest floor (Godman et al. 1990). Once established, sugar maple seedlings and saplings are extremely shade tolerant and can survive prolonged periods of low light in the understory until being recruited to the overstory (~ 20 yrs; Marks and Gardescu 1998).

Field Sampling: Seedling Survey

I conducted three sugar maple seedlings surveys in all four forest sites to determine whether simulated N deposition had influenced sugar maple seedling density. The first survey was conducted in May 2009; I then repeated the survey in mid-August 2009 to account for mortality during the growing season. In May of 2010, when seedlings were at the same phenological stage as the previous May, I again repeated the survey. In each 30-m x 30-m plot, three 1-m wide transects were randomly placed and all sugar maple seedlings within the boundary of the transect were counted. The age of each seedling was determined by examination of the terminal bud scale scar on the apical branch (Taylor and Aarssen 1989); they were grouped into first-year seedlings, second-year seedlings, and seedlings between three and five years old. Reproductive litter did not differ between forest areas (Table 2). I used a two-way ANOVA with a site by treatment interaction term to determine whether seedling density (stems m⁻²) differed between ambient and simulated N deposition treatments.

Forest Floor Salinity

Sodium nitrate was used in our simulated N deposition treatment because Na^+ is not an essential plant nutrient. However, we did consider that Na^+ could adversely affect the vitality of sugar maple, a non-salt tolerant plant (Richards 1954). To quantify the effect of Na^+ additions associated with our simulated N deposition treatment, I compared the salinity of forest floor under ambient and simulated N deposition in all four sites using the conductivity (EC) of a forest floor and water slurry (Richards 1954). Soil with an EC of 4 dS m^{-1} or greater are considered saline soils (Lal and Shukla 2004). Differences in salinity between forest floor in ambient and N simulated treatment were assessed by using a two-way ANOVA with a site by treatment interaction.

Does Forest Floor Accumulation Inhibit Seedling Establishment?

In order to test the effects of litter accumulation on the establishment of sugar maple seedlings, I experimentally manipulated forest floor in Site D, in which sugar maple seedlings were more abundant under ambient atmospheric N than those under stimulated atmospheric N (S. Patterson, *personal observation*). Three 1.0-m x 0.5-m subplots were randomly located ($n = 3$) in the buffer surrounding ambient N deposition plots in Site D. Forest floor (Oe/a) was completely removed to expose mineral soil in each 0.5 m^2 plot and 120 sugar maple seeds were then evenly distributed across the plot. This density of seeds (480 seeds m^{-2}) was derived using average seed rain in Michigan ($\sim 100 - 1000 \text{ seeds m}^{-2}$ depending on climactic conditions and mast years; Gabriel 1978). One half of the subplot served as the control treatment, the original forest floor mass was replaced atop the 120 seeds. In order to test if greater forest floor would negatively affect seedling establishment, on the other half, forest floor mass was increased to 2579 g m^{-2} to simulate average forest floor accumulation observed under simulated N deposition

(Zak et al. 2008). Netting (10 mm mesh) was secured over the entire 0.5 m^{-2} plot to ensure placement of the forest floor over the sugar maple seeds.

Similarly, in the surrounding buffer of the simulated N deposition plots, three 1.0-m x 0.5-m plots were also randomly located ($n = 3$). Forest floor (Oe/a) was again removed to expose mineral soil and 120 sugar maple seeds were evenly distributed across the 0.5 m^{-2} area. The original forest floor mass was replaced atop the 120 seeds in each subplot. In order to test whether a decrease in forest floor mass would positively affect seedlings, on the other half, forest floor was decreased to 1708 g m^{-2} to simulate the average accumulation of forest floor under ambient N deposition (Zak et al. 2008). Seeds were placed in the field in October 2009, consistent with the phenology of sugar maple seed production and dispersal. The following spring (i.e., May), I surveyed the proportion of germinates in all plots and again in June to assess establishment. I defined establishment as the first set of fully expanded true leaves.

I had three predictions for this experiment: 1) sugar maple seedling establishment would be greater beneath forest floor under ambient N deposition compared to simulated N deposition, 2) seedling establishment would be lower under a greater mass of forest floor, regardless of N deposition treatment, and 3) seedling establishment would be greater under a smaller mass of forest floor, regardless of the level of N deposition. To test these hypotheses, I used a two-way ANOVA with both date and treatment as a factor.

Transplanted Seedlings

In August of 2009, 288 first-year germinate sugar maple seedlings derived from the same parent tree, were planted in Site D to compare mortality between N deposition treatments. Half were planted in the buffer area surrounding plots receiving ambient N deposition and remaining seedlings were planted in the buffer surrounding plots receiving simulated N deposition. In May

2010, seedling survival was quantified in the field. I tested differential mortality of returning second year seedlings using a one-way ANOVA.

Results

Seedling Survey

Simulated N deposition did not influence the abundance of first-year sugar maple seedlings, wherein mean first-year abundance was 4.3 ± 3.9 stems m^{-2} under ambient N deposition and was 3.9 ± 3.3 stems m^{-2} under simulated N deposition (data not shown; $P = 0.759$). There also was no significant interaction between site and treatment for first-year seedlings (Figure 2A; $P = 0.961$). However, averaged across site and time, the abundance of second-year seedlings significantly declined under simulated N deposition, wherein mean abundance of second-year seedlings was 13.1 stems m^{-2} under ambient N deposition and 1.6 stems m^{-2} under simulated N deposition. These results were consistent in all four sites; however, the magnitude of this decline differed among sites, resulting in a significant site by treatment interaction (Figure 2B; $P < 0.001$). For example, in Site A under ambient N deposition, second-year seedling abundance was ten-times greater than seedling abundance under simulated N deposition. The mean abundance of seedlings between 3 and 5 years of age also significantly declined under simulated N deposition; 10.6 stems m^{-2} grew under ambient N deposition compared with 0.6 stems m^{-2} under simulated N deposition ($P < 0.001$). The magnitude of this decline also differed between sites, resulting in a site by treatment interaction (Figure 2C; $P < 0.001$).

One could argue that this difference in seedling densities is due to local site characteristics such as light availability, water availability, soil quality, and seed production between ambient and simulated N treatments. However, I have no evidence to support such an assertion (Table 2). I found no difference in leaf area indices (LAI) under ambient and simulated N to indicate differential light availability; seed production also did not differ between treatments

(Table 2). Furthermore, the application of NaNO_3 did not increase forest floor conductivity in any site, or across all sites, indicating that Na^+ has not accumulated in forest floor. No samples displayed $\text{EC} > 1 \text{ dS m}^{-1}$, well below levels that could potentially affect sugar maple germination (Lal and Shukla 2004). Using these variables as covariates in an ANOVA of seedling densities did not alter our results; none of them were significant covariates of seedling density.

Does Forest Floor Accumulation Inhibit Seedling Establishment?

When forest floor mass was not manipulated in ambient and simulated N deposition treatments, almost five times as many seeds established in the ambient N deposition treatment, relative to the simulated N deposition treatment (Fig. 3A; $P = 0.001$), consistent with our expectation that great forest floor can inhibit seedling emergence. Similarly, a greater forest floor mass decreased sugar maple seedling establishment, regardless of whether the greater litter mass occurred under ambient or simulated N deposition ($P = 0.004$ and $P = 0.002$ respectively). We manipulated forest floor mass in each treatment to determine whether greater forest floor mass diminished seedling establishment in the simulated N deposition treatment, which consistently had a greater forest floor mass due to the slowing of decomposition (Zak et al. 2008). Increasing forest floor mass in the ambient N deposition (2579 g m^{-2}) treatment to that of the simulated N deposition treatment (1708 g m^{-2}) resulted in a 65% decline in seedling establishment, which was statistically significant (Fig. 3B; $P = 0.004$). In contrast, when forest floor mass under simulated N deposition was decrease to match that under ambient N deposition, seedling establishment significantly increased by 80% (Fig. 3C; $P = 0.002$).

Transplanted Seedlings

Seedlings planted in the ambient N deposition treatment returned as second-year seedlings the following spring in greater numbers than those planted in the simulated N deposition treatment (21.7 ± 3.8 individuals vs. 9.3 ± 2.7 individuals; Figure 4). This difference was not statistically significant ($P = 0.059$), but it likely is of ecological significance.

Discussion

The atmospheric deposition of anthropogenic N has altered forest biogeochemistry and growth throughout the northeastern U.S. and Canada, especially in sugar-maple-dominated northern hardwood forests, in which N export has increased and tree vigor have declined (Magill et al. 1997, Wallace et al. 2007). For example, as a result of chronic N deposition, sugar maple populations in the northeast U.S. have been negatively effected by soil acidification, base cation loss, and increased Al^{3+} availability (Duchesnes et al. 2002, Lovett et al. 2004, Zaccherio and Finzi 2007). While soils of this region are often derived from granitic parent materials, which have low base saturation and cation exchange capacity, soils in the Upper Great Lakes region developed from calcareous glacial drift and have a have high base saturation; they also have high available Ca^{2+} , a particularly important nutritional requirement for sugar maple growth and development (Long et al. 1997b, Duchesnes et al. 2002; Lovett et al. 2004; Zaccherio and Finzi 2007). Simulated N deposition on these calcareous soils has led to the slowing of plant litter decay and an accumulation of forest floor (Pregitzer et al. 2008; Zak et al. 2008). We have argued that the slowing of decay and a subsequent accumulation of forest floor could negatively affect sugar maple populations at early stages of development by imposing a physical barrier to seedling emergence and establishment. We have also argued that even if emergence of seedlings is not different, N deposition has the potential to negatively affect sugar maple seedlings further on in development. If seeds can penetrate through a thick mass of forest floor and establish, the affects of simulated N deposition are perhaps continuing later in their growth. This mechanism differs from the negative impact of anthropogenic N deposition on sugar maple nutrition and growth in the northeastern U.S. We predicted that sugar maple seedlings will be less abundant under simulated N deposition; secondly, that a greater mass of forest floor would inhibit seedling

establishment; and finally, that simulated N deposition will negatively effect sugar maple saplings following establishment. The results of our field survey and experiments are consistent with these predictions. The degree to which anthropogenic N deposition can impact the population dynamics of sugar maple by decreasing their establishment in the understory has not been previously considered and clearly will impact stand age structure and the potential of these forests to store C as atmospheric N deposition globally increases (Galloway et al. 2004).

After 17 years of simulated atmospheric N deposition at future levels expected in northeastern North America, second-year and third-fifth year sugar maple seedlings were consistently less abundant than under ambient atmospheric N deposition (Fig. 2B & C), indicating that chronic N deposition can affect the livelihood of this dominant species. Although first-year seedling abundance was not altered by simulated N deposition, our observations suggest this agent of global change can affect sugar maple at very early stages of establishment (i.e, 2-5 years). Accumulating evidence supports the assertion that under high N deposition and on soils of low base saturation, sugar maple adult tree survivorship declines (Duchesnes et al. 2002, Lovett et al. 2004, Zaccherio and Finzi 2007). However, survivorship of sugar maple seedlings following establishment has also been previously found to decline under chronic N deposition, albeit not significantly (Catovsky and Bazzaz 2002, Zaccherio and Finzi 2007). These results are not consistent among different tree species. For example, survivorship of red maple seedlings (*A. rubrum*) was greater under chronic N addition (Zaccherio & Finzi 2007), suggesting that the impact of chronic N deposition may be species specific.

Our results indicate that sugar maple populations are affected at a very different stage of development in the Upper Great Lakes region compared with forests in the northeastern U.S. The mechanism for our observed mortality of sugar maple seedlings under simulated N

deposition could potentially be unique to these forests, in which, concurrent with this increase in seedling mortality, decomposition has slowed thereby fostering an accumulation of forest floor (Pregitzer et al. 2008, Zak et al. 2008). An accumulation of forest floor has been found to decrease establishment of both herbaceous and woody plant species (Facelli and Pickett 1991, Ibanez and Schupp 2002). If establishment of sugar maple seedlings is affected by greater forest floor mass, it further supports the hypothesis that chronic N deposition has the potential to impact the population dynamics of ecologically important tree species populations in the Upper Great Lakes region.

Greater forest floor mass, due to 17 years of simulated N deposition, did inhibit the establishment of sugar maple seedlings, evidence that supports our hypothesis. Regardless of whether seeds experienced simulated N or ambient N deposition, seedling establishment decreased significantly when forest floor mass atop seeds was experimentally increased (Fig. 3A-C). Because seeds were placed directly on the surface of mineral soil, it appears that cotyledon emergence was unsuccessful under the thick O horizon in the simulated N deposition treatment, rather than due to the desiccation of the root radicle entering the mineral soil. Germination was not different in our forest floor manipulation experiment (S. Patterson, *unpublished data*); therefore, it appears that greater forest floor mass does not inhibit germination, but did affect seedling emergence through forest floor. These results indicate that decomposition has slowed to the point where organic matter accumulating in the forest floor is a physical barrier for seedling establishment. Our findings are not particularly novel in this regard, because seedling establishment of many tree species has been found to decline under a greater litter or forest floor mass (Facelli and Pickett 1991, Houle et al. 1992 and 1990, Ibanez and Schupp 2002); indeed, establishment is generally limited by the root radicle penetrating mineral

soil (Facelli and Pickett, 1991). Sugar maple, however, is a late-successional shade-tolerant species, with little to no reliance on the seed bank (seeds rarely remain viable following one year in the forest floor; Godman et al. 1990). Instead, recruitment of sugar maple trees into the overstory is dependent upon the dense population of understory seedlings. If forest floor mass continues to accumulate and sugar maple seedlings in the Upper Lakes State region behave in the fashion we have reported, the seedling bank of this tree will decline and overstory recruitment of these trees will be impacted. In our experiment, forest floor mass under simulated N deposition had accumulated to 3757 g m^{-2} (Site D; $\pm 1466 \text{ g m}^{-2}$), twice the amount than under ambient N deposition. Simulated N has slowed decomposition, by changing soil microbial communities, to double the amount of forest floor consistently in all other forest stands, relative to forest floor in the ambient N treatment. If sugar maple seeds in other forests respond in a similar manner to our observations, then simulated N induced forest floor accumulation could have ecosystem level implications by reducing seedling recruitment.

One could argue that preferential deer browsing or insect herbivory of seedlings resulting from our simulated N treatment could elicit results consistent with our observations. Indeed, it is plausible that herbivores have a greater propensity to consume woody plant seedlings of higher foliar N or that there would be a greater abundance of insect seed predators under simulated N deposition (Throop and Lerdau 2004, Haase et al. 2008, Jones et al. 2008). However, several lines of evidence dispute this idea. First, seedling damage was noted at three of the four replicate sites under both ambient and simulated N (S. Patterson, *personal observation*). If herbivore damage was present, then herbivores homogeneously affected seedlings in each stand, regardless of N deposition treatment. Second, if seedlings were consumed by herbivores at greater proportions under simulated N deposition, we would not have found a difference in seedlings

establishment between seeds under ambient forest floor or manipulated forest floor. What is more, the difference we did find between sugar maple establishment was consistent throughout the experiment: as we increased the mass of forest floor, sugar maple establishment declined (Fig 3A-C), providing further support for our hypothesis. Forest floor mass affects seedling establishment whether the increase in forest floor mass occurred under ambient or simulated N deposition.

Another caveat we must address is the unintended effect of Na^+ associated with the simulated N deposition treatment, which in large enough quantity, could desiccate or damage seeds and inhibit sugar maple establishment (Zang et al. 2008; Chen et al. 2003), thereby leading us to incorrectly support our hypothesis. However, using electrical conductivity as a metric for salt concentration, we did not find a significant difference between the electrical conductivity of a forest floor under simulated N deposition or the ambient treatment ($0.64 \pm 0.06 \text{ S m}^{-1}$ and $0.43 \pm 0.16 \text{ S m}^{-1}$ [mean \pm SD] respectively). Salt intolerant plants, like sugar maple, experience salt-related stress at EC levels greater than 2 S m^{-1} (Richards 1954), well above that in our experiment. If Na^+ did have a negative effect on early establishment of seedlings, then we would have observed similar seedling mortality when we experimentally removed forest floor in our simulated N deposition treatment; this did not occur. The evidence we have accumulated clearly indicates that an accumulation of Na^+ was not a factor influencing seedling mortality in our experiment.

Of the seedlings transplanted into forests under ambient and simulated N, more seedlings survived the following summer under ambient N deposition (Fig. 4), which supports our hypothesis that simulated N deposition can affect sugar maple seedlings beyond their first year of growth. Several factors could explain why sugar maple seedlings are less successful under

greater atmospheric N deposition, but a decline in mycorrhizal fungi biomass appears most plausible. Sugar maple forms an association with arbuscular mycorrhizal fungi, a group of fungi that have been found to decline under simulated N deposition (Treseder 2004; Van Diepen 2007; Van Diepen et al. 2010). Both within root colonization and extraradical abundance of AM fungi can decrease as soil N availability increases under simulated N deposition, indicating there is a decline in nutrient uptake by sugar maple exposed to simulated N deposition (Van Diepen et al. 2010). The reason for a decline in AM fungi is not known, but it has been suggested that P limitation under simulated N could be a cause or a subsequent decrease in pH following NH_4^+ uptake (Van Diepen et al. 2010). However, these mechanisms have not been observed in our study sites. Because the availability of base cations is high in our soils (Table 2), focus should now be directed towards a lack of AM fungal abundance under simulated N. It is plausible that the association between AMF and sugar maple could limit sugar maple seedling growth in very early stages of establishment, thereby decreasing its survival under simulated N.

Another plausible mechanism leading to greater seedling mortality under simulated N deposition are the higher amounts of phenolic dissolved organic carbon (DOC) produced in forest floor exposed to simulated N deposition (Pregitzer et al. 2004), which are known to decrease the leaf area, biomass and development of sugar maple seedlings (Hanes et al. 2003). For example, DOC production and leaching losses have increased remarkably under simulated N deposition, a response that is consistent across all study sites. In our experiment, large amounts of DOC are produced and then leached from forest floor following snowmelt in spring and during autumnal senescence. Because high concentrations of phenolic DOC have a negative effect on sugar maple seedling survival (Hanes et al. 2003), it is possible that the greater production of phenolic DOC could negatively affect seedlings post establishment. First year

seedlings may escape this effect, because they would not be exposed to higher amounts of phenolic DOC until the autumn and the subsequent spring. Clearly, further investigation into the effect of phenolic DOC produced under simulated N deposition on sugar maple seedlings would help resolve if it is a mechanism of mortality following initial establishment.

Our observed decline in sugar maple seedling abundance under simulated N has led us to conclude that future rates of atmospheric N deposition have the potential to alter stand dynamics in sugar-maple-dominated northern hardwood forests in the Upper Great Lakes region of the U.S. Under simulated N deposition, trees in these forests have higher rates of NPP and the largest overstory trees are dying at a rate 29% greater than those under ambient N deposition (Pregitzer et al. 2008). If adult sugar maple trees are dying at earlier ages, in order for stand dynamics to remain unchanged, an established seedling must be recruited to the understory and subsequently replace each dead individual in the overstory. We are unaware of a critical population of seedlings that must be established in order for recruitment of sugar maple trees to the overstory to be unaffected. Yet, we are aware that the populations of sugar maple seedlings are indeed negatively affected by simulated N deposition via the slowing of decay and the accumulation of forest floor. This mechanism will be useful to include in simulations of ecosystem dynamics when exposed to higher N deposition, specifically when sugar maple life history traits are included to estimate regeneration, as in the SORTIE ecosystem model (Pacala et al. 1996).

Although simulated N deposition has increased soil C storage in this forest ecosystem (Pregitzer et al. 2008; Zak et al. 2008), the effect of organic matter accumulation in forest floor can indirectly affect the potential C stored in juvenile sugar maple trees being recruited to the overstory. If simulated N negatively affects decomposition rates as observed (Zak et al. 2008;

Pregitzer et al. 2008), then C storage is enhanced through an accumulation of organic matter. However, if decomposition rates have slowed to create a mass of forest floor which will inhibit the establishment of dominate tree species, C storage in northern hardwood forests has the potential to decline. Our results have elucidated an indirect effect of atmospheric N deposition on stand dynamics and ecosystem function across sugar maple forests in the Upper Great Lakes region of the U.S. As atmospheric N deposition increases globally, it becomes necessary to understand and predict the mechanisms that can lead to population change of this ecologically and economically important tree species.

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Table 1. Climactic, floristic, and edaphic properties of four sugar maple dominated hardwood sites receiving experimental N deposition.

Characteristic	Site			
	A	B	C	D
Location				
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°52'	85°50'	86°09'
Climate				
Mean annual precipitation (mm)	873	871	888	812
Mean annual temperature (°C)	4.7	6.0	6.9	7.6
Wet + dry total N deposition (g N m ⁻² yr ⁻¹)	0.68	0.91	1.17	1.18
Vegetation				
Overstory age (2004)	97	91	92	96
Soil Chemistry				
Exchangeable calcium (cmol(+)/kg)	1.14	3.43	1.31	2.36
Exchangeable magnesium (cmol(+)/kg)	0.27	0.49	0.27	0.44
Exchangeable aluminum	0.86	0.19	0.79	0.63
Base Saturation (%)	66	69	96	82
pH	4.63	4.92	4.40	4.60

Data collected from 1994-2004

Table 2. Overstory and soil characteristics under ambient N deposition and simulated N deposition with their associated level of significance. Values are means; standard deviation is shown in parenthesis.

Characteristic	Treatment		<i>P</i>
	Ambient	NO ₃ ⁻	
Vegetative			
LAI (m ² m ⁻² ; from 1994 -2006)	7.0 (1.34)	7.1 (1.40)	0.403
Reproductive Litter Mass (g m ⁻² ; from 1994 – 2006)	33.6 (30.90)	34.6 (32.13)	0.657
Soil & Forest Floor			
pH	4.56 (0.259)	4.71 (0.327)	0.149
Soil Moisture (mPa)*	-0.207	-0.183	< 0.001
Exchangeable Ca ²⁺ (cmol(+)/kg)	2.05	2.13	0.864
Base Saturation (%)	80	77	0.622
Conductivity of forest floor (dS/m)	0.636 (0.058)	0.537 (0.161)	0.290

*Average of daily soil moisture at the first 10cm: values taken four times a day from 2004 to 2008.

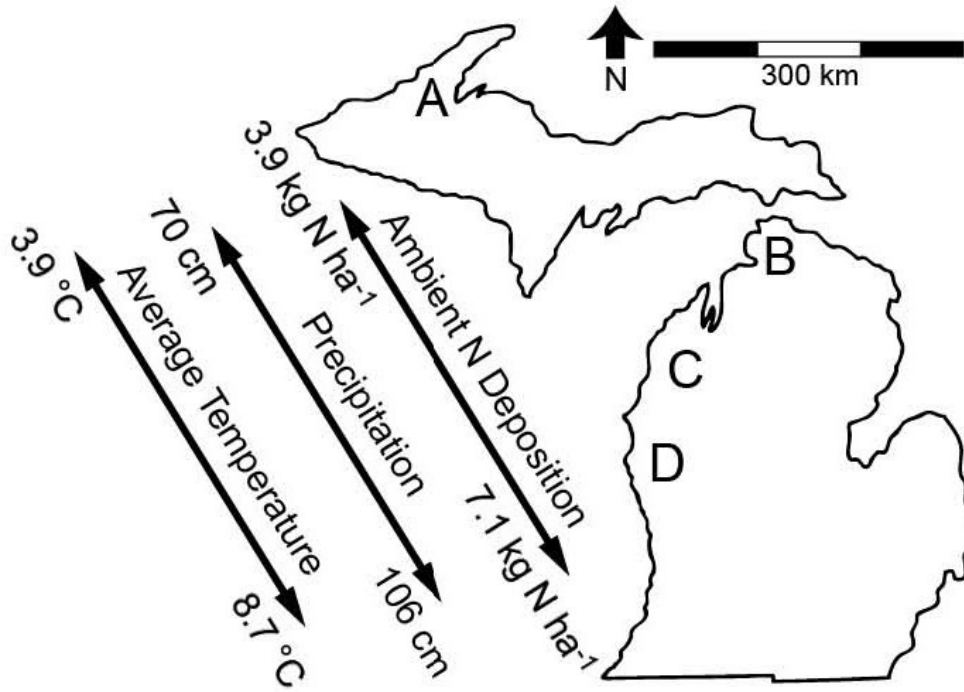


Figure 1. Location of four study sites, floristically and edaphically similar, but differing in N deposition and climactic characteristics.

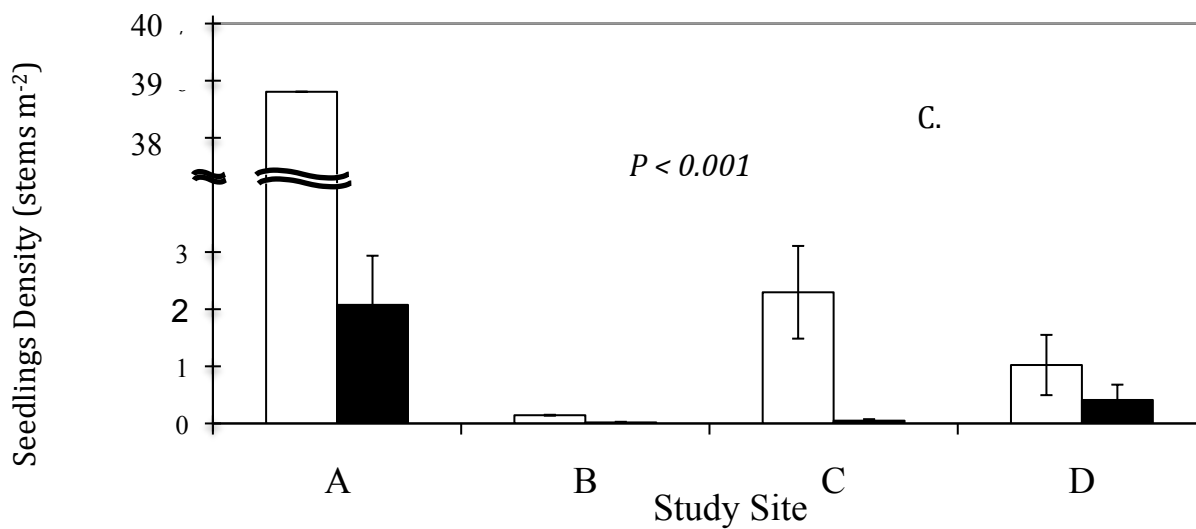
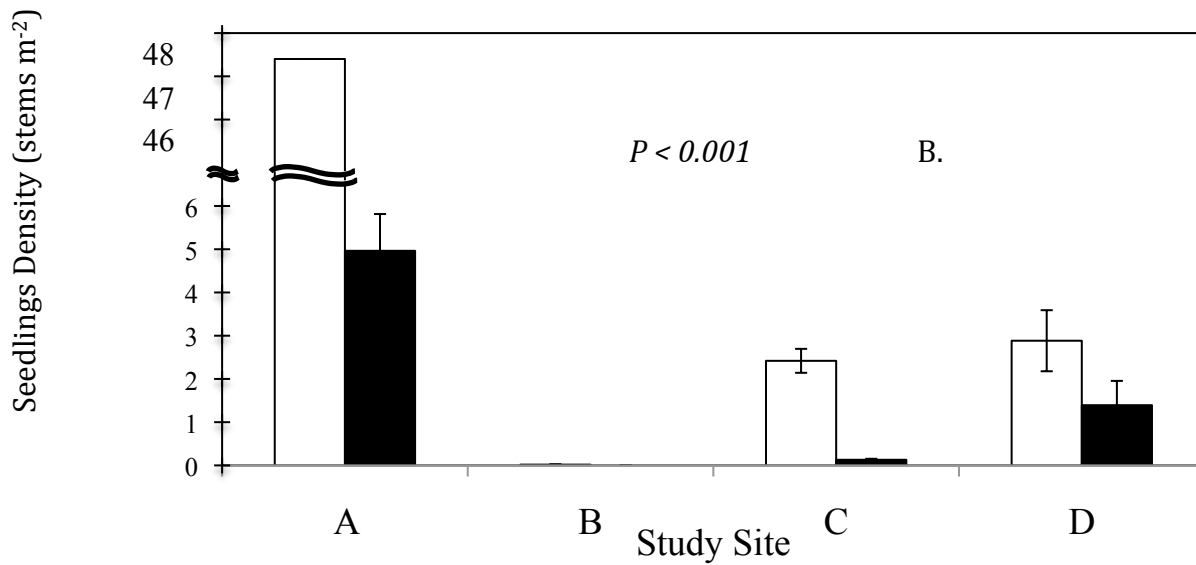
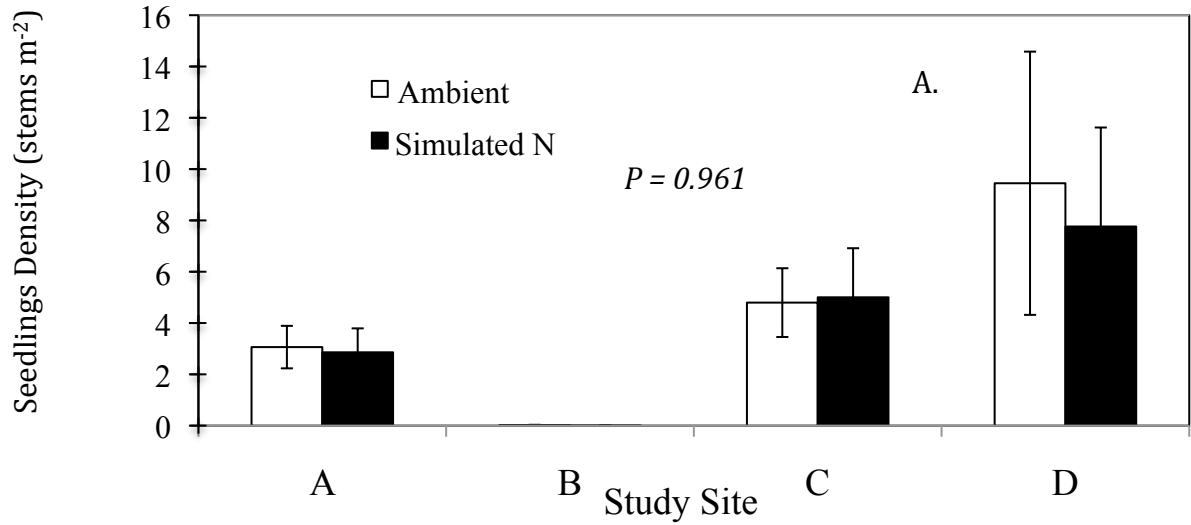


Figure 2. Seedling densities (stems/m²) of first year seedlings (A), second year seedlings (B) and seedlings 3-5 years (C) in all four study sites. Values are means of three surveys conducted in spring 2009, autumn

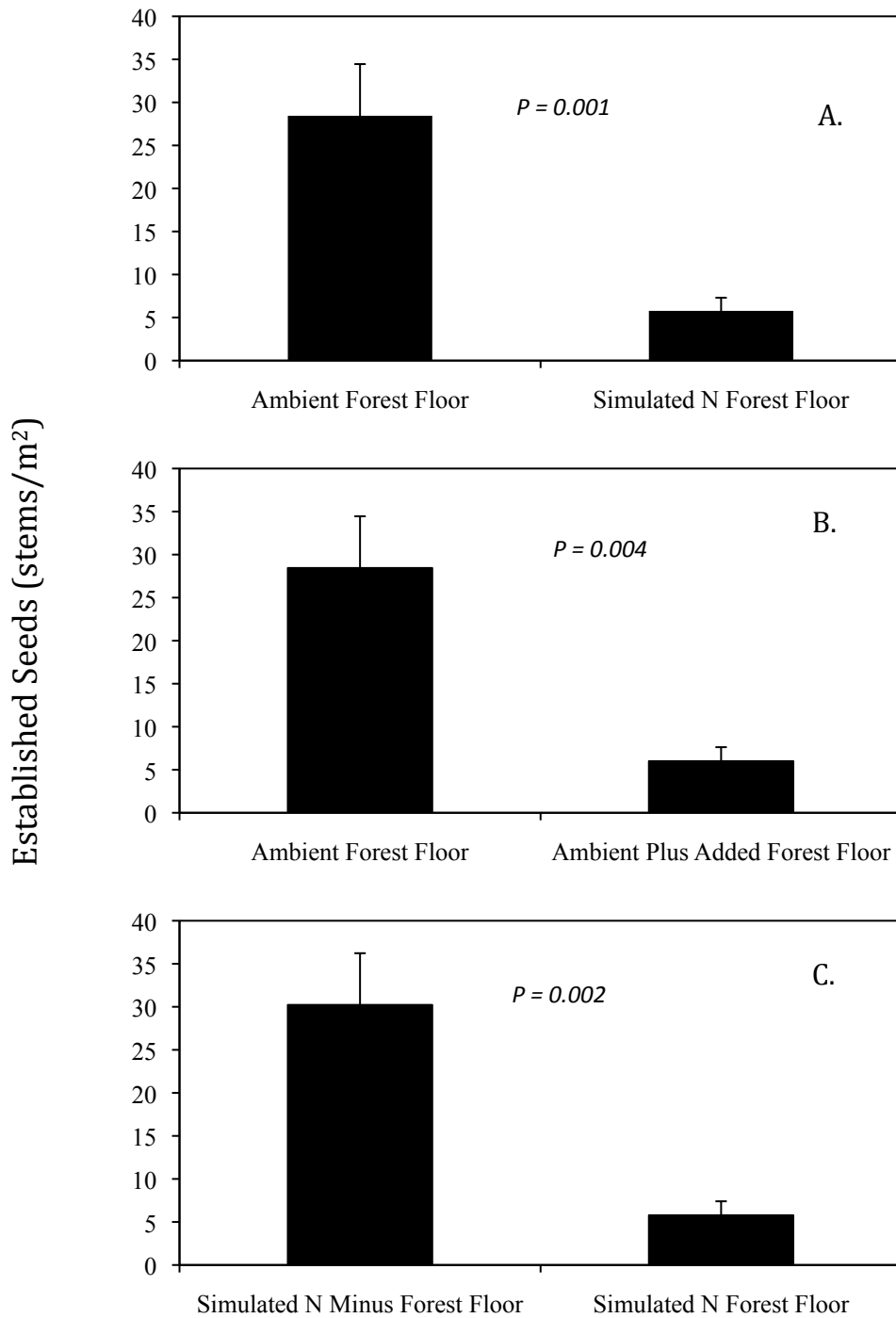


Figure 3. A. Established seeds under ambient and simulated N forest floor. B. Established seeds under ambient forest floor and ambient forest floor plus $871 \text{ g} \cdot \text{m}^{-2}$ forest floor C. Established seeds under simulated N forest floor accumulation minus $871 \text{ g} \cdot \text{m}^{-2}$

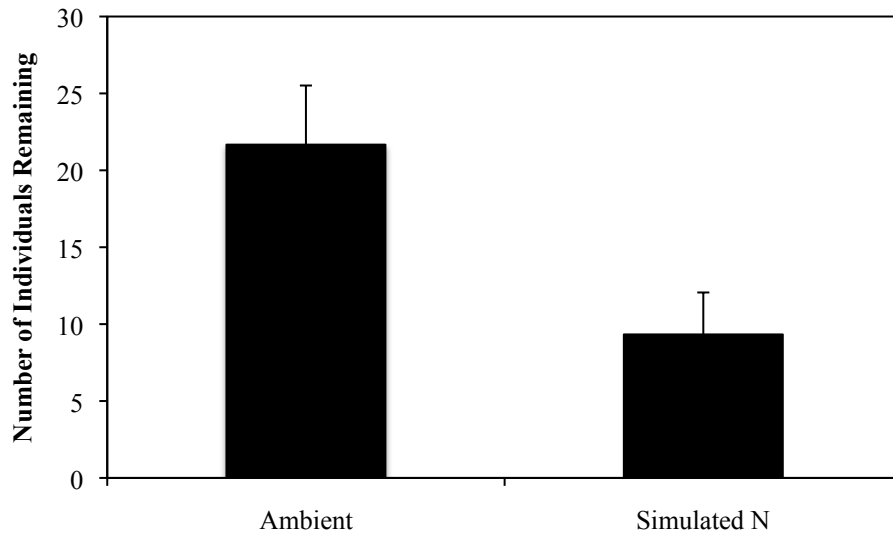


Figure 4. Transplanted seedling mortality of first year seedlings planted in both ambient and simulated N deposition plots. Seedlings planted in July of 2009. Mortality assessed in May of 2009.