

# Simulated N deposition negatively impacts sugar maple regeneration in a northern hardwood ecosystem

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## Summary

1. During the next century, atmospheric nitrogen (N) deposition is projected to more than double, potentially leading to a decline in plant diversity as well as a change in plant community composition and structure.

2. In a decade-long field experiment, simulated atmospheric N deposition has slowed litter decay, resulting in an accumulation of forest floor (i.e. Oi & Oe horizons). We reasoned that a greater forest floor mass under simulated N deposition would impose a physical barrier to sugar maple *Acer saccharum* seedling establishment, thereby reducing seedling populations of an ecologically and economically important tree species.

3. To test this idea, we first quantified sugar maple seedling abundance in replicate northern hardwood forest stands receiving ambient atmospheric N (7–12 kg N ha<sup>-1</sup> year<sup>-1</sup>) and experimental atmospheric N deposition, simulating future amounts in eastern North America (ambient plus 30 kg NO<sub>3</sub><sup>-</sup> N ha<sup>-1</sup> year<sup>-1</sup>). Then, we experimentally manipulated forest floor mass under ambient and simulated N deposition treatments. Finally, we transplanted first-year established seedlings into areas receiving ambient and simulated N deposition and quantified their mortality after 1 year.

4. First-year seedling abundance did not differ under ambient and simulated N deposition; however, there were greater abundances of second- and third-to-fifth-year seedlings under ambient N deposition ( $P < 0.001$ ). In all cases, experimental manipulation to increase forest floor mass, 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, fewer 1-year-old transplanted seedlings survived when grown under simulated N, albeit that result was not statistically significant.

5. *Synthesis and applications.* The slowing of decay and the accumulation of forest floor under anthropogenic N deposition can negatively impact seedling survival and potentially alter stand development and structural diversity. As atmospheric N deposition increases globally, it becomes necessary to understand the mechanisms that lead to population changes for ecologically important tree species. The responses we document should be considered in simulations of future of forest dynamics, as atmospheric N deposition continues to increase, specifically when sugar maple life-history traits are included to simulate regeneration, structural diversity and stand development.

**Key-words:** *Acer saccharum*, atmospheric nitrogen deposition, forest floor accumulation, northern hardwood forest, seedling establishment, seedling survival, sugar maple

## 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, global 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 & Chapin 2000; Vitousek *et al.* 2002), chronic

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N deposition in some forest ecosystems have led to a condition of N saturation, which can culminate in large leaching losses of  $\text{NO}_3^-$  (Aber *et al.* 1989). Moreover, chronic N deposition can decrease plant species diversity even at moderate levels of deposition (e.g.  $10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ; Clark & Tilman 2008; Suding *et al.* 2005; Stevens *et al.* 2004). Sugar maple *Acer saccharum* Marsh., an ecologically important tree species in the Great Lakes and northeastern regions of the United States and southeastern Canada, is often associated with soils of high N availability and  $\text{NO}_3^-$  leaching (Lovett & Mitchell 2004). Forests dominated by this species could be particularly prone to N saturation and the subsequent negative impacts of N saturation on growth and reproduction (Aber *et al.* 1989, 2003).

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 (Bailey, Horsley & Long 2005; Horsley *et al.* 2002; Kolb & McCormick 1993; Lovett & Rueth 1999); 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, Horsley & Lilja 1997) predispose soils to acidification and base-cation loss, especially the leaching loss of  $\text{Ca}^{2+}$ , which can have negative consequences on the growth of sugar maple (Duchesne, Ouimet & Houle 2002; Lovett *et al.* 2004; Zaccherio & Finzi 2007). Indeed, sugar maple decline was more pronounced in unglaciated Ca-poor soils associated with relatively high degrees of cation leaching in the Allegheny Plateau of Pennsylvania, compared with glaciated areas of the same region (Long, Horsley & Lilja 1997). Furthermore, if soils have experienced a high degree of acidification and cation loss, experimental additions of  $\text{Ca}^{2+}$  can enhance the growth, vigour and seed production of sugar maple (Long & Horsley 1997; Moore, Camire & Ouimet 2000; Houle *et al.* 2002; Wargo *et al.* 2002; Juice *et al.* 2006). Additionally, rates of primary productivity, stand health and vigour, and basal area of sugar maple are positively correlated with increased amounts of soil and foliar  $\text{Ca}^{2+}$  (Ellsworth & Liu 1994; Hallett *et al.* 2006; Schaberg *et al.* 2006), providing evidence that  $\text{Ca}^{2+}$  loss initiated by chronic N deposition negatively impacts sugar maple forests on base-poor soils.

In contrast, sugar maple forests in the Upper Great Lakes region occur on soils formed from calcareous (i.e. base rich) glacial drift, in which  $\text{Ca}^{2+}$  is abundant (Schaetzl, Darden & Brandt 2009). Notwithstanding, atmospheric N deposition could exert a negative effect on sugar maple in this region via a very different and unstudied mechanism affecting seedling establishment. Forests in this region exposed to simulated N deposition, comparable to rates occurring across large areas of Europe ( $30 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ; Holland *et al.* 2005), have experienced a decline in plant litter decay and a significant increase in forest floor mass (Pregitzer *et al.* 2008; 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 a higher energetic requirement to

emerge through forest floor to reach light and penetrate a thicker forest floor to reach mineral soil (Facelli & Pickett 1991). *Quercus montana* germinants, when grown under dense mats of litter, had a 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 they may not have sufficient amounts of energy for their hypocotyl to emerge, or radicle to penetrate, through a deeper forest floor (Hamrick & Lee 1987; Tao, Xu & Li 1987; Molofsky & Augspurger 1992; Metcalfe & Grubb 1997; Dzwonko & Gawronski 2002). Sugar maple seeds are modest in size (c. 7–9 mm in diameter; c. 460 mg seed<sup>-1</sup>; Gabriel 1978; Godman, Yawney & Tubbs 1990) and germinate within the Oe horizon (S. Patterson and D. Zak, personal observation). Despite a high germination capacity (c. 90%), seeds in the forest floor only remain viable for c. 1 year in a 'winter transient' seed bank (Houle & Payette 1991). Consequently, seedling recruitment into the overstorey is dependent on a 'seedling bank' (Marks & Gardescu 1998), rather than the maintenance of a 'seed bank' in the forest floor. Therefore, an accumulation of forest floor (Oe horizon), induced by atmospheric N deposition, could impose a physical barrier for seedling hypocotyl emergence and, hence, seedling establishment (Godman, Yawney & Tubbs 1990). Over the long term, such a response to atmospheric N deposition would alter the structural diversity of forests.

Our 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. Because of the modest size of sugar maple seeds, and consequentially low amounts of stored energy, we reasoned that a thicker forest floor generated under simulated N deposition would impose a barrier to both hypocotyl emergence and root radicle penetration, thereby decreasing sugar maple seedling abundance. To test this idea, we 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, we experimentally manipulated forest floor mass over sugar maple seeds placed in plots receiving ambient and simulated N deposition. Finally, to evaluate whether simulated N deposition affects sugar maple seedling mortality following establishment, we transplanted first-year seedlings into plots receiving ambient and simulated N deposition.

## Materials and methods

### STUDY SITES

In 1987, four sites were selected from 31 candidate sites to be as ecologically and edaphically identical as possible across the sugar maple-dominated northern hardwood forests in Great Lakes Region of the USA (Burton *et al.* 1991; Table 1). These sites are located on a north-south climatic and atmospheric N deposition gradient,

**Table 1.** Climatic, 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)*	879	874	913	824
Mean annual temperature (°C)†	4.9	6.2	7.0	7.7
Wet + dry total N deposition (g N m <sup>-2</sup> year <sup>-1</sup> )‡	0.68	0.91	1.17	1.18
Vegetation				
Overstorey age (2008)	101	95	96	100
Soil chemistry§				
Exchangeable calcium (cmol(+) per kg)	1.14	3.43	1.31	2.36
Exchangeable magnesium (cmol(+) per kg)	0.27	0.49	0.27	0.44
Exchangeable aluminium (cmol(+) per kg)	0.86	0.19	0.79	0.63
Base saturation (%)	66	69	96	82
pH (10 cm mineral soil)	4.63	4.92	4.40	4.60

\*Mean annual precipitation, for the years 1994–2008, was recorded using weighing rain gages (Model 5-780; Belfort Instrument Co., Baltimore, MD, USA) located in open areas within 5 km of each site.

†Mean annual temperature, for the years 1994–2008, was recorded on site at 2 m using thermistors, which were read every 30 min throughout the year, with averages recorded every 3 h using data loggers (EasyLogger Models 824 and 925; Data Loggers, Inc., Logan, UT, USA).

‡MacDonald *et al.* 1992.

§D. R. Zak, unpublished data.

wherein the southernmost site receives the greatest amount of ambient N deposition and the northernmost site receives the least (*c.* 12 and 4 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively; NO<sub>3</sub><sup>-</sup> comprises *c.* 60% of wet and dry deposition). All four stands occur on well-drained sandy soils (Typic haplorthods) and are similar in floristic composition and stand structure, with the dominant overstorey trees being sugar maple with a mean age of 94 years (*c.* 82% basal area sugar maple; Pregitzer *et al.* 2008). The groundcover is composed primarily of sugar maple seedlings (*c.* 90% of all stems; A. Talhelm, unpublished data), and the Oi horizon is primarily sugar maple leaf litter.

Since 1994, three 30 × 30 m plots in each site have been receiving experimental additions of NO<sub>3</sub><sup>-</sup> in the form of NaNO<sub>3</sub> pellets applied six times throughout the growing season, which is designed to simulate increased atmospheric N deposition (5 kg N ha<sup>-1</sup> month<sup>-1</sup>; 30 kg N ha<sup>-1</sup> year<sup>-1</sup>). Three other 30 × 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. Averaged over our four study sites, chronic N deposition has increased forest floor mass from 1700 to 2580 g m<sup>-2</sup>, a significant *c.* 50% increase in mass over a decade (Zak *et al.* 2008).

#### FIELD SAMPLING: SEEDLING SURVEY

In May 2009, August 2009 and May 2010, we conducted seedling surveys in each forest site to determine whether a thicker forest floor under simulated N deposition had imposed a physical barrier to sugar maple seedlings. We used three randomly located 1 × 30-m transects in each plot to estimate sugar maple seedling abundance. Seedling age was determined by examination of the terminal bud scale scar on the apical branch (Taylor & Aarssen 1989). Seedlings were grouped into first-year seedlings, second-year seedlings and seedlings between 3- and 5-year old. Reproductive litter mass did not differ between forest study sites (Table 2); therefore, any differences in seedling abundance could not result from differential

seed production. We used a two-way ANOVA with a site by treatment interaction term to test whether simulated N deposition had decreased seedling density (stems m<sup>-2</sup>).

#### FOREST FLOOR SALINITY

Sodium nitrate was used in our simulated N deposition treatment, because Na<sup>+</sup> is not an essential plant nutrient. However, we did consider that Na<sup>+</sup> could adversely affect the vitality of sugar maple, a nonsalt tolerant plant (Richards 1954). To quantify the effect of Na<sup>+</sup> additions associated with our simulated N deposition treatment, we compared the salinity of forest floor under ambient and simulated N deposition in all four sites using the conductivity (EC) of a forest floor-water slurry (Richards 1954). Soils with an EC of 4 dS m<sup>-1</sup> or greater are considered saline (Lal & Shukla 2004). Differences in salinity between forest floor in ambient and N-simulated treatments were assessed with a two-way ANOVA with a site by treatment interaction ( $\alpha = 0.05$ ).

#### DOES FOREST FLOOR ACCUMULATION INHIBIT SEEDLING ESTABLISHMENT?

To test the effects of litter accumulation on the emergence and establishment of sugar maple seedlings, we experimentally manipulated forest floor mass in Site D. In our experiment, sugar maple seeds occur throughout the Oe horizon, but probably remain viable for only 1 year (Godman, Yawney & Tubbs 1990). Three 1 × 0.5 m subplots were randomly located in the buffer surrounding each of the ambient N deposition plots in Site D. Forest floor (Oe) was completely removed to expose surface the Oa/A horizon in each 0.5 m<sup>-2</sup> plot, and 120 sugar maple seeds were then evenly distributed across the plot. This density of seeds (480 seeds m<sup>-2</sup>) was derived using average seed rain in Michigan (*c.* 100–1000 seeds m<sup>-2</sup> depending on climatic conditions and mast years; Gabriel 1978). One half of the

**Table 2.** Overstorey and soil characteristics under ambient N deposition and simulated N deposition with their associated level of significance

Characteristic	Treatment		
	Ambient	NO <sub>3</sub> <sup>-</sup>	<i>P</i>
Vegetative			
LAI (m <sup>2</sup> m <sup>-2</sup> ; from 1994–2006)	7.0 (1.34)	7.1 (1.40)	0.403
Reproductive litter mass (g m <sup>-2</sup> ; 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 matric potential (mPa)*	-0.207	-0.183	< 0.001
Exchangeable Ca <sup>2+</sup> (cmol(+) per kg)	2.05	2.13	0.864
Base saturation (%)	80	77	0.622
Conductivity of forest floor (dS m <sup>-1</sup> )	0.636 (0.058)	0.537 (0.161)	0.290

Values are means; standard deviation is shown in parenthesis. Data in this table are available at [http://www.webpages.uidaho.edu/nitrogen-gradient/Archived\\_data.htm](http://www.webpages.uidaho.edu/nitrogen-gradient/Archived_data.htm).

\*Average of daily soil moisture at the first 15 cm: values taken eight times a day from 2004 to 2008.

subplot served as the control treatment; the original forest floor mass was replaced over the 120 seeds. To test whether greater forest floor depth would negatively affect seedling emergence and establishment, forest floor mass was increased to 2579 g m<sup>-2</sup> on the other subplot half to simulate the average amount of forest floor mass that occurs under simulated N deposition at this site (Zak *et al.* 2008). Netting (10 mm mesh) was secured over the entire 0.5 m<sup>2</sup> plot to ensure the forest floor treatments would remain in place over the sugar maple seeds.

Similarly, in the surrounding buffer of the simulated N deposition plots, three 1 × 0.5 m plots were also randomly located ( $n = 3$ ). Forest floor (Oe/A) was again removed to expose surface soil, 120 sugar maple seeds were evenly distributed across the 0.5 m<sup>2</sup> area and the original forest floor mass was replaced. To test whether a decrease in forest floor mass would positively affect seedlings, forest floor depth was decreased to 1708 g m<sup>-2</sup> on the other subplot half to simulate the average forest floor mass under ambient N deposition (Zak *et al.* 2008). In each forest floor manipulation treatment, we kept the forest floor intact as possible when decreasing or increasing its depth; additional forest floor material was gathered from areas in the buffer surrounding each plot. 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), we quantified the proportion of germinates in all plots and again in June to assess establishment. We defined establishment as the first set of fully expanded true leaves.

Our predictions were as follows: (i) sugar maple seedling establishment would be greater beneath forest floor under ambient N deposition compared with simulated N deposition, (ii) seedling establishment would be lower under a greater mass of forest floor, regardless of N deposition treatment, and (iii) seedling establishment would be greater under a smaller mass of forest floor, regardless of the level of N deposition. To test these hypotheses, we used a two-way ANOVA with both date and treatment as a factor ( $\alpha = 0.05$ ).

#### TRANSPLANTED SEEDLINGS

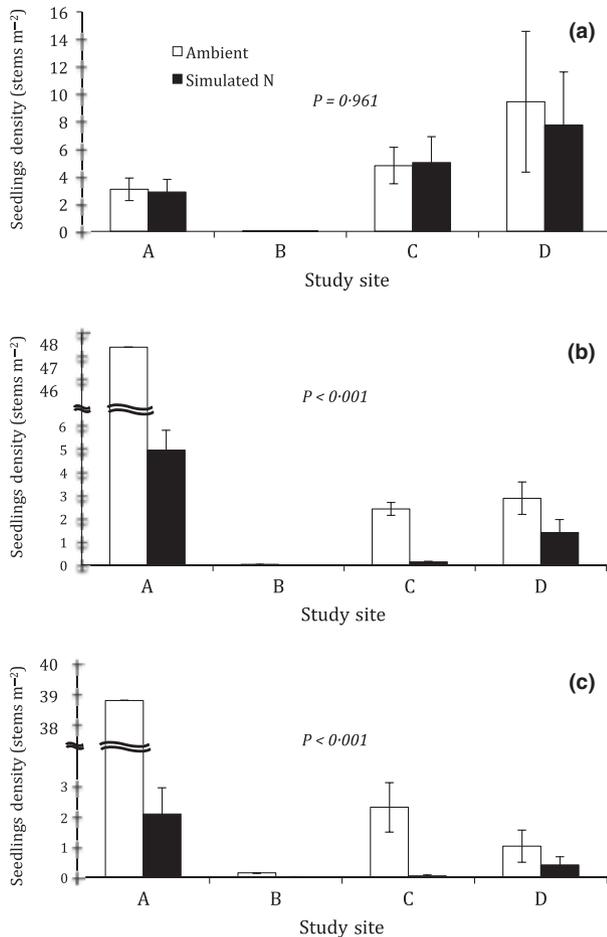
In August 2009, 288 first-year sugar maple seedlings, which had been derived from seeds collected in central Michigan, vernalized and germinated in a greenhouse, were planted in Site D to assess mortality between N deposition treatments. Half were planted in the buffer area surrounding plots receiving ambient N deposition, and the remaining

seedlings were planted in the buffer surrounding plots receiving simulated N deposition. The roots of all seedlings, regardless of treatment, were placed in the surface mineral soil (A and E horizons) by hand excavating a small hole *c.* 10–15 cm deep. In May 2010, seedling survival was quantified in the field. We tested differential mortality of returning second-year seedlings using a one-way ANOVA ( $\alpha = 0.05$ ).

## Results

#### SEEDLING SURVEY

Simulated N deposition did not influence the abundance of first-year sugar maple seedlings. Mean first-year abundance was  $4.3 \pm 3.9$  stems m<sup>-2</sup> (mean  $\pm$  SE) under ambient N deposition and  $3.9 \pm 3.3$  stems m<sup>-2</sup> under simulated N deposition (data not shown;  $P = 0.759$ ). There also was no significant interaction between site and treatment for first-year seedlings (Fig. 1a;  $P = 0.961$ ). 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<sup>-2</sup> under ambient N deposition and 1.6 stems m<sup>-2</sup> 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 (Fig. 1b;  $P < 0.001$ ). 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 high seedling density in Site A, relative to the other sites, is the likely result of higher light levels at the forest floor, due to a relatively lower leaf area in this stand (A. Talhelm, personal observation). The mean abundance of seedlings between 3 and 5 years of age also significantly declined under simulated N deposition; 10.6 stems m<sup>-2</sup> grew under ambient N deposition compared with 0.6 stems m<sup>-2</sup> under simulated N deposition ( $P < 0.001$ ). The magnitude of this decline also differed between sites, resulting in a site by treatment interaction (Fig. 1c;  $P < 0.001$ ).

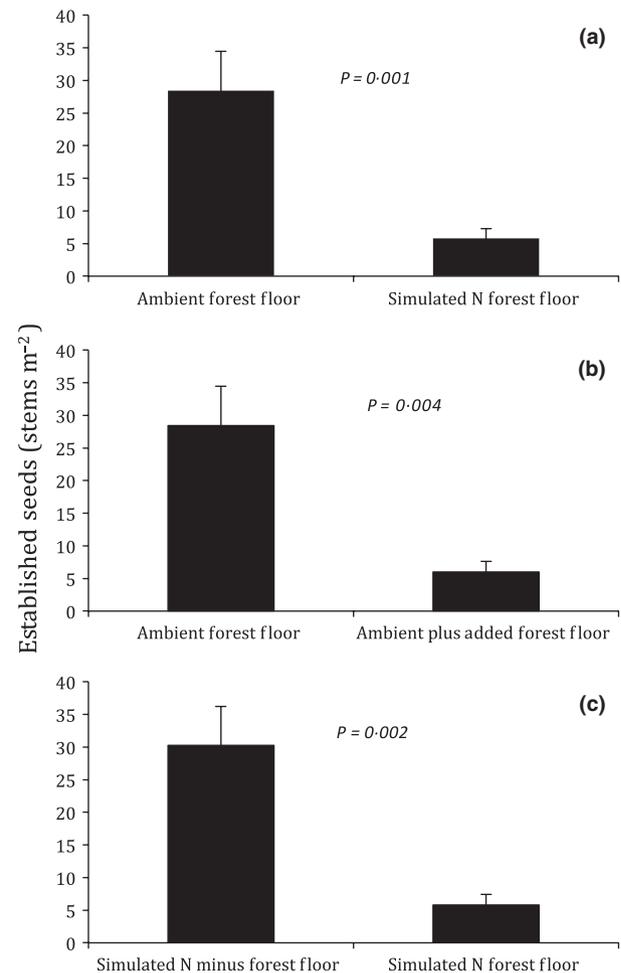


**Fig. 1.** Seedling densities (stems m<sup>-2</sup>) of first-year seedlings (a), second-year seedlings (b) and seedlings 3–5 years (c) in all four study sites. Values are means of three surveys conducted in spring 2009, autumn 2009 and spring 2010. Error bars represent standard error.

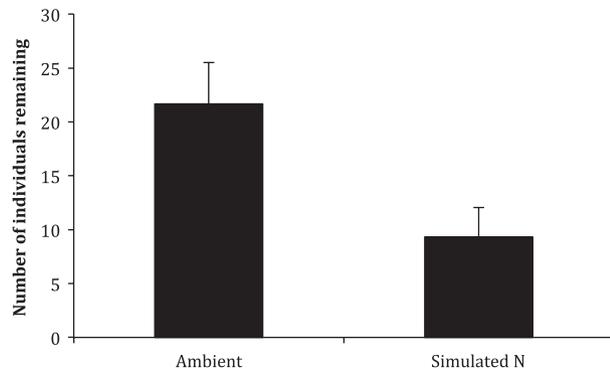
One could argue that this difference in seedling densities resulted from local site characteristics such as light availability, water availability, soil fertility and seed production between ambient and simulated N treatments. However, we have no evidence to support such an assertion (Table 2). We found 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<sub>3</sub> did not increase forest floor conductivity in any site, or across all sites, indicating that Na<sup>+</sup> had not accumulated in the forest floor. No samples displayed EC > 1 dS m<sup>-1</sup>, and all were well below levels that could potentially affect sugar maple germination (Lal & 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. Therefore, our evidence collectively indicates that a thicker forest floor under simulated N deposition imposed a physical barrier for seedling establishment; however, from these surveys, we are unable to discern whether this decline occurred because of the emergence from, or penetration into, a thicker forest floor.

#### DOES FOREST FLOOR ACCUMULATION INHIBIT SEEDLING EMERGENCE AND ESTABLISHMENT?

When forest floor mass was not manipulated in ambient and simulated N deposition treatments, almost five times as many seedlings established in the ambient N deposition treatment than in the simulated N deposition treatment (Fig. 2a;  $P = 0.001$ ), consistent with our seedling density surveys. Increasing forest floor mass in the ambient N deposition treatment (2579 g m<sup>-2</sup>) to equal that of the simulated N deposition treatment (1708 g m<sup>-2</sup>) resulted in a 65% decline in seedling establishment (Fig. 2b;  $P = 0.004$ ). In contrast, when forest floor mass under simulated N deposition was decreased to match that under ambient N deposition, seedling establishment increased by 80% (Fig. 2c;  $P = 0.002$ ).



**Fig. 2.** (a) Established seeds under unmanipulated forest floor in the ambient and simulated N deposition treatments. (b) Established seeds in the ambient treatment under ambient forest floor mass and simulated N forest floor mass. (c) Established seeds in the simulated N deposition treatment beneath the forest floor mass of the simulated N and ambient N deposition treatments. We initially placed 420 seeds m<sup>-2</sup> in each subplot receiving the aforementioned forest floor manipulations.



**Fig. 3.** Transplanted seedling mortality of first-year seedlings planted in both ambient and simulated N deposition plots. Seedlings were planted in July of 2009, and mortality was assessed in May of 2009.

#### 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 ( $21.7 \pm 3.8$  individuals vs.  $9.3 \pm 2.7$  (mean  $\pm$  SE) individuals; Fig. 3). This difference was not statistically significant ( $P = 0.059$ ).

#### Discussion

Simulated N deposition on the calcareous soils of our study has led to the slowing of plant litter decay and an accumulation of forest floor mass (Pregitzer *et al.* 2008; Zak *et al.* 2008). We hypothesized that the accumulation of forest floor mass could negatively affect sugar maple populations at early stages of development by imposing a physical barrier to seedling establishment by either preventing hypocotyl emergence or root radicle penetration. This mechanism differs from the negative impact of anthropogenic N deposition on sugar maple nutrition and growth in the northeastern USA, which is thought to result from acidification, base-cation leaching, as well as increased  $Al^{3+}$  availability. We predicted that sugar maple seedlings would be less abundant under simulated N deposition, because a thicker forest floor would decrease seedling establishment; secondly, that a greater mass of forest floor would inhibit seedling emergence and establishment; and finally, that simulated N deposition would negatively affect sugar maple seedlings following establishment. The results of our field surveys and experiments are consistent with these predictions. The degree to which anthropogenic N deposition can impact the population dynamics of sugar maple by decreasing seedling survival has not been previously considered and potentially could impact stand development as well as the structural diversity of future forests across our region.

After 17 years of simulated increased N deposition, sugar maple seedlings were significantly less abundant than under ambient N deposition (Fig. 1b,c), indicating that chronic N deposition has the potential to alter the regeneration of this dominant species by imposing a physical barrier. Accumulating evidence supports the assertion that, under high N deposi-

tion and on soils of low base saturation, sugar maple adult tree survivorship declines (Duchesne, Ouimet & Houle 2002; Lovett *et al.* 2004; Zaccherio & 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 & Bazzaz 2002; Zaccherio & 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 negatively affected by chronic N deposition during seedling establishment in Upper Great Lakes region, in contrast to the negative effects on the nutrition of mature trees on granitic soils in the northeastern USA. 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 mass (Pregitzer *et al.* 2008; Zak *et al.* 2008). An accumulation of forest floor mass has been found to decrease establishment of both herbaceous and woody plant species (Facelli & Pickett 1991; Ibanez & 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 negatively impact the population dynamics of ecologically important trees in the Upper Great Lakes region.

Greater forest floor mass, because of 17 years of simulated N deposition, decreased the establishment of sugar maple seedlings, evidence that supports our hypothesis. Regardless of whether seeds were placed in plots receiving simulated N or ambient N deposition, seedling emergence and establishment decreased significantly when forest floor mass was experimentally increased (Fig. 2a–c). Because seeds were placed directly on the surface of Oa/A horizon, it appears that cotyledon emergence was unsuccessful under the thickened Oe horizon under simulated N deposition treatment. 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 barrier to seedling establishment, especially when sugar maple seeds have migrated downward into the forest floor. 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 & Pickett 1991; Houle & Payette 1991; Ibanez & Schupp 2002); however, our results are novel in regard to the links we have established among chronic N deposition, the slowing of litter decay, the accumulation of forest floor mass and a decline in seedling establishment.

Sugar maple is a late-successional shade-tolerant species, with little to no reliance on the seed bank. Instead, recruitment

of sugar maple trees into the overstorey is dependent upon the dense population of understorey seedlings (i.e. a seedling bank). If forest floor mass continues to accumulate and sugar maple seedlings in the Upper Lakes State region behave in the fashion we have observed, then the seedling bank of this tree will decline and overstorey recruitment of these trees could be negatively impacted. If sugar maple seeds in other forests respond in a similar manner, then the accumulation of forest floor mass induced by simulated N deposition 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 & Lerdau 2004; Haase *et al.* 2008; Jones, Paine & Fenn 2008). However, several lines of evidence dispute this idea. First, seedling damage was noted at three of the four 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. Secondly, 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. 2a–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  $\text{Na}^+$  associated with the simulated N deposition treatment, which in large enough quantity could desiccate or damage seeds and inhibit sugar maple establishment (Chen *et al.* 2003; Zang, Song & Liu 2008). However, using EC as a metric for salinity, we did not find a significant difference between the salinity 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  $\pm$  SE) respectively]. Salt-intolerant plants, like sugar maple, experience salt-related stress at EC levels  $> 2 \text{ S m}^{-1}$  (Richards 1954), well above the levels observed in our experiment. If  $\text{Na}^+$  did have a negative effect on early establishment of seedlings, then we would have observed similar seedling mortality when we experimentally removed forest floor mass in our simulated N deposition treatment; this did not occur. The evidence we have accumulated clearly indicates that an accumulation of  $\text{Na}^+$  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. 3). Several factors could explain why sugar maple seedlings are less successful under simulated N deposition, but a decline in mycorrhizal fungi

biomass appears most plausible. Sugar maple forms an association with arbuscular mycorrhizal (AM) fungi, a group of fungi that have been found to decline under simulated N deposition (Treseder 2004; Van Diepen *et al.* 2007, 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 in our experiment (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 or a subsequent decrease in pH following  $\text{NH}_4^+$  uptake could be causes (Van Diepen *et al.* 2010). However, these mechanisms have not been observed in our study sites (Table 2); therefore, focus should now be directed towards a lack of AM fungal abundance under simulated N deposition. It is reasonable that the association between AM fungi and sugar maple could limit sugar maple seedling vigour in very early stages of establishment, thereby decreasing its survival under simulated N deposition.

Another possible mechanism leading to greater seedling mortality under simulated N deposition is the higher amounts of phenolic dissolved organic carbon (DOC) produced in forest floor exposed to simulated N deposition (Pregitzer *et al.* 2004), which is known to decrease the leaf area, biomass and development of sugar maple seedlings (Hane *et al.* 2003); for example, phenolic DOC production and leaching losses have increased remarkably under simulated N deposition in our experiment (Pregitzer *et al.* 2004), 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 (Hane *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.

#### ECOLOGICAL IMPLICATIONS

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 USA. Under simulated N deposition, trees in these forests have higher rates of NPP, and the biomass of dead mature trees also is 29% greater than under ambient N deposition (Pregitzer *et al.* 2008). Such a response suggests that simulated N deposition has increased the rate of forest stand development. Moreover, if trees are dying at earlier ages under simulated N deposition, in order for stand dynamics to remain unchanged, an established seedling must be recruited to the understorey and subsequently replace

each dead individual in the overstorey. We are unaware of a critical population of seedlings that must be established in order for recruitment of sugar maple trees to the overstorey 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 mass. This mechanism will be useful to include in simulations of ecosystem dynamics, specifically when sugar maple life-history traits are included to estimate regeneration, as in the SORTIE ecosystem model (Pacala *et al.* 1996). Analyses of this nature could help to identify demographic ‘bottlenecks’ that may alter the structure of sugar maple forests and subsequently the ecological services these forests provide to society. 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 USA, but further work is necessary to identify the causal agent of mortality in established seedlings. As atmospheric N deposition increases globally, it becomes necessary to understand and predict the mechanisms that can lead to population change of ecologically important tree species. Here, we have provided evidence that the slowing of decay and the accumulation of forest floor under chronic N deposition decrease seedling establishment in sugar maple-dominated northern hardwood forests across the entire Great Lakes region of the USA.

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## References

- Aber, J.D., Nadelhoffer, K.J., Steudler, P. & Melillo, J.M. (1989) Nitrogen saturation in northern forest ecosystems. *BioScience*, **39**, 378–386.
- Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.L., Magill, A.H., Martin, M.E., Hallett, R.A. & Stoddard, J.L. (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? *BioScience*, **53**, 375–389.
- Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**, 1–67.
- Bailey, S.W., Horsley, S.B. & Long, R.P. (2005) Thirty years of change in forest soils of the Allegheny Plateau, Pennsylvania. *Soil Science Society of America Journal*, **69**, 681–690.
- Barrett, L.I. (1931) Influence of forest litter on the germination and early survival of chestnut oak, *Quercus montana*, Willd. *Ecology*, **12**, 476–484.
- Burton, A.J., Ramm, C.W., Pregitzer, K.S. & Reed, D.D. (1991) Use of multivariate methods in forest research site selection. *Canadian Journal of Forest Research*, **21**, 1573–1580.
- Catovsky, S. & Bazzaz, F.A. (2002) Nitrogen availability influences regeneration of temperate tree species in the understory seedling bank. *Ecological Applications*, **12**, 1056–1070.
- Chen, S.L., Li, J.K., Wang, S.S., Fritz, E., Huttermann, A. & Altman, A. (2003) Effects of NaCl on shoot growth, transpiration, ion compartmentation, and transport in regenerated plants of *Populus euphratica* and *Populus tomentosa*. *Canadian Journal of Forest Research*, **33**, 967–975.
- Clark, C.M. & Tilman, D. (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, **451**, 712–715.
- Duchesne, L., Ouimet, R. & Houle, D. (2002) Basal area growth of sugar maple in relation to acid deposition, stand health, and soil nutrients. *Journal of Environmental Quality*, **31**, 1676–1683.
- Dzwonko, Z. & Gawronski, S. (2002) Influence of litter and weather on seedling recruitment in a mixed oak-pine woodland. *Annals of Botany*, **90**, 245–251.
- Ellsworth, D.S. & Liu, X. (1994) Photosynthesis and canopy nutrition of 4 sugar maple forests on acid soils in northern Vermont. *Canadian Journal of Forest Research*, **24**, 2118–2127.
- Facelli, J.M. & Pickett, S.T.A. (1991) Plants litter—its dynamics and effects on plant community structure. *Botanical Review*, **57**, 1–32.
- Gabriel, W.J. (1978) *Genetic Variation in Seed and Fruit Characters in Sugar Maple*, Vol. 404. USDA Forest Service Resources Papers of the North East, Washington, DC.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. & Vorosmarty, C.J. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.
- Godman, R.M., Yawney, H.W. & Tubbs, C.H. (1990) *Acer saccharum* Marsh. Sugar maple. *Silvics of North America. Volume 2. Hardwoods* (eds R.M. Burns & B.H. Honkala), pp. 78–91. Agriculture Handbook Number 250. U.S. Department of Agriculture, Forest Service, Washington, DC, USA.
- Grubb, P.J. (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Review of the Cambridge Philosophical Society*, **52**, 102–145.
- Haase, J., Brandl, R., Scheu, S. & Schädler, M. (2008) Above- and below-ground interactions are mediated by nutrient availability. *Ecology*, **89**, 3072–3081.
- Hallett, R.A., Bailey, S.W., Horsley, S.B. & Long, R.P. (2006) Influence of nutrition and stress on sugar maple at a regional scale. *Canadian Journal of Forest Research*, **36**, 2235–2246.
- Hamrick, J.L. & Lee, J.M. (1987) Effect of soil surface-topography and litter cover on the germination, survival, and growth of musk thistle (*Carduus nutans*). *American Journal of Botany*, **74**, 451–457.
- Hane, E.N., Hamburg, S.P., Barber, A.L. & Plaut, J.A. (2003) Phytotoxicity of American beech leaf leachate to sugar maple seedlings in a greenhouse experiment. *Canadian Journal of Forest Research*, **33**, 814–821.
- Holland, E.A., Braswell, B.H., Sulzman, J. & Lamarque, J.F. (2005) Nitrogen deposition onto the United States and western Europe: synthesis of observations and models. *Ecological Applications*, **15**, 38–57.
- Horsley, S.B., Long, R.P., Bailey, S.W., Hallett, R.A. & Wargo, P.M. (2002) Health of eastern North American sugar maple forests and factors affecting decline. *Northern Journal of Applied Forestry*, **19**, 34–44.
- Houle, G. & Payette, S. (1991) Seed dynamics of *Abies balsamea* and *Acer saccharum* in a deciduous forest of northeastern North America. *American Journal of Botany*, **78**, 895–905.
- Houle, D., Duchesne, L., Moore, J.D., Lafeche, M.R. & Ouimet, R. (2002) Soil and tree-ring chemistry response to liming in a sugar maple stand. *Journal of Environmental Quality*, **31**, 1993–2000.
- Ibanez, I. & Schupp, E.W. (2002) Effects of litter, soil surface conditions, and microhabitat on *Cercocarpus ledifolius* Nutt. seedling emergence and establishment. *Journal of Arid Environments*, **52**, 209–221.
- Johnson, N.M., Likens, G.E., Bormann, F.H. & Pierce, R.S. (1968) Rate of chemical weathering of silicate minerals in New Hampshire. *Geochimica et Cosmochimica Acta*, **32**, 531–540.
- Jones, M.E., Paine, T.D. & Fenn, M.E. (2008) The effect of nitrogen additions on oak foliage and herbivore communities at sites with high and low atmospheric pollution. *Environmental Pollution*, **151**, 434–442.
- Juice, S.M., Fahey, T.J., Siccama, T.G., Driscoll, C.T., Denny, E.G., Eagar, C., Cleavitt, N.L., Minocha, R. & Richardson, A.D. (2006) Response of sugar maple to calcium addition to Northern Hardwood Forest. *Ecology*, **87**, 1267–1280.
- Kolb, T.E. & McCormick, L.H. (1993) Etiology of sugar maple decline in four Pennsylvania stands. *Canadian Journal of Forest Research*, **23**, 2395–2402.
- Lal, R. & Shukla, M.K. (2004) *Principles of Soil Physics*. Marcel Dekker, New York, NY.
- Long, R.P. & Horsley, S.B. (1997) Sugar maple health and mortality in relation to slope position and glaciation on the Allegheny Plateau. *Bulletin of the Ecological Society of America*, **78**, 134.
- Long, R.P., Horsley, S.B. & Lilja, P.R. (1997) Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Canadian Journal of Forest Research*, **27**, 1560–1573.
- Lovett, G.M. & Mitchell, M.J. (2004) Sugar maple and nitrogen cycling in the forests of eastern North America. *Frontiers in Ecology and the Environment*, **2**, 81–88.

- Lovett, G.M. & Rueth, H. (1999) Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient. *Ecological Applications*, **9**, 1330–1344.
- Lovett, G.M., Weathers, K.C., Arthur, M.A. & Schultz, J.C. (2004) Nitrogen cycling in a northern hardwood forest: do species matter? *Biogeochemistry*, **67**, 289–308.
- MacDonald, N.W., Burton, A.J., Liechty, H.O., Witter, J.A., Pregitzer, K.S., Mroz, G.D. & Richter, D.D. (1992) Ion leaching in forest ecosystems along a Great lakes air pollution gradient. *Journal of Environmental Quality*, **21**, 614–623.
- Marks, P. L. & Gardescu, S. (1998) A case study of sugar maple (*Acer saccharum*) as a forest seedling bank species. *Journal of the Torrey Botanical Society* **125**, 287–296.
- Metcalfe, D.J. & Grubb, P.J. (1997) The responses to shade of seedlings of very small-seeded tree and shrub species from tropical rain forest in Singapore. *Functional Ecology*, **11**, 215–221.
- Molofsky, J. & Augspurger, C.K. (1992) The effect of leaf litter on early seedling establishment in a tropical forest. *Ecology*, **73**, 68–77.
- Moore, J.D., Camire, C. & Ouimet, R. (2000) Effects of liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair Watershed, Quebec, Canada. *Canadian Journal of Forest Research*, **30**, 725–732.
- Pacala, S.W., Canham, C.D., Saponara, J., Silander, J.A., Kobe, R.K. & Ribbens, E. (1996) Forest models defined by field measurements: estimation, error analysis and dynamics. *Ecological Monographs*, **66**, 1–43.
- Pregitzer, K.S., Zak, D.R., Burton, A.J., Ashby, J.A. & MacDonald, N.W. (2004) Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry*, **68**, 179–197.
- Pregitzer, K.S., Burton, A.J., Zak, D.R. & Talhelm, A.F. (2008) Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. *Global Change Biology*, **14**, 142–153.
- Richards, L.A. (1954) *Diagnosis and Improvement of Saline and Alkali Soils*. US Department of Agriculture, Handbook 60, Washington, DC.
- Schaberg, P.G., Tilley, J.W., Hawley, G.J., DeHayes, D.H. & Bailey, S.W. (2006) Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. *Forest Ecology and Management*, **223**, 159–169.
- Schaetzl, R.J., Darden, J.T. & Brandt, D. (2009) *Michigan Geography and Geology*. Pearson Custom Publishers, Upper Saddle River, NJ. 672 pp.
- Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, **303**, 1876–1879.
- Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G. & Pennings, S. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4387–4392.
- Tao, D.L., Xu, Z.B. & Li, X. (1987) Effect of litter layer on natural regeneration of companion tree species in the Korean Pine Forest. *Environmental and Experimental Botany*, **27**, 53–65.
- Taylor, K.M. & Aarssen, L.W. (1989) Neighbor effects in mast year seedlings of *Acer saccharum*. *American Journal of Botany*, **76**, 546–554.
- Throop, H.L. & Lerdau, M.T. (2004) Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. *Ecosystems*, **7**, 109–133.
- Treseder, K.K. (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO<sub>2</sub> in field studies. *New Phytologist*, **164**, 347–355.
- Van Diepen, L.T.A., Lilleskov, E.A., Pregitzer, K.S. & Miller, R.M. (2007) Decline of arbuscular mycorrhizal fungi in northern hardwood forests exposed to chronic nitrogen additions. *New Phytologist*, **176**, 175–183.
- Van Diepen, L.T.A., Lilleskov, E.A., Pregitzer, K.S. & Miller, R.M. (2010) Simulated nitrogen deposition causes a decline of intra- and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in Northern Hardwood Forests. *Ecosystems*, **13**, 683–695.
- Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B. & Sprent, J.I. (2002) Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry*, **57**, 1–45.
- Wargo, P.M., Minocha, R., Wong, B.L., Long, R.P., Horsley, S.B. & Hall, T.J. (2002) Measuring changes in stress and vitality indicators in limed sugar maple on the Allegheny Plateau in north-central Pennsylvania. *Canadian Journal of Forest Research*, **32**, 629–641.
- Zaccherio, M.T. & Finzi, A.C. (2007) Atmospheric deposition may affect northern hardwood forest composition by altering soil nutrient supply. *Ecological Applications*, **17**, 1929–1941.
- Zak, D.R., Holmes, W.E., Burton, A.J., Pregitzer, K.S. & Talhelm, A.F. (2008) Simulated atmospheric NO<sub>3</sub>- deposition increases soil organic matter by slowing decomposition. *Ecological Applications*, **18**, 2016–2027.
- Zang, H.-X., Song, D. & Liu, Z.-X. (2008) Study on physiological characteristics and salt tolerance for seedlings of 11 tree species. *Forest Research*, **21**, 168–175.

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