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SPECIAL TOPIC: NITROGEN DEPOSITION REASSESSED

Nitrogen deposition potentially contributes to oak regeneration failure in the Midwestern temperate forests of the USA

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Abstract We conducted a 7-year field study at two oak-dominated forest sites which differ in their atmospheric N deposition to test the hypothesis that red oak regeneration failure in the upper Midwestern US forests, at least in part, results from increased N load. The sites are located in Swallow Cliffs (SC) in Cook County, Illinois, and Indiana Dunes National Lakeshore (IDNL) in Porter County, Indiana. Annual wet NO_3^- deposition for the 22 years immediately prior to the experiments was significantly higher in IDNL than in the SC site. Results from common garden experiments showed that oak seedling biomass was 60 % lower at IDNL compared with SC, but there was little site effect on growth of maple seedlings. Experimental N addition also resulted in a 45 % decrease in the total biomass of the oak seedlings at SC, but had no significant effect on the biomass at IDNL. Maple seedlings responded little to experimental fertilization. The growth rate of mature oak trees was also lower at IDNL but to a much smaller extent than that of seedlings. Maple trees did not significantly differ between sites. We conclude that: (1) chronic N load adversely affects seedling performance of red oak, but not sugar maple, in these temperate forests; and (2) the seedling establishment phase rather than the adult tree is the likely target stage for this adverse effect of N loading. The exact mechanisms for the differential effects of N on these co-occurring species are not clear, but different plasticity in fractional biomass and N allocation to the leaves might be involved.

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Introduction

During the past few decades, numerous studies have focused on the scale and the consequences of chronic deposition of reactive N in ecological communities (Aber et al. 1989, 1998; Schulze et al. 1989; Galloway et al. 2003, 2004; Holland et al. 2005). These studies generally recognize N deposition as the main component of global change with disproportionately large consequences for ecosystem processes ranging from biogeochemistry to net primary productivity (NPP) (Magill et al. 1997; Vitousek et al. 1997; Norby 1998; Lovett and Rueth 1999; Matson et al. 2002; Clark and Tilman 2008; Ferretti et al. 2014). Earlier efforts in this field provided accumulating evidence that chronic N deposition affects NPP via the N-saturation mechanism (Aber et al. 1989), but emerging research underscores that atmospheric N deposition is also a major driver of change in species composition and loss of biodiversity (Sala et al. 2000; Stevens et al. 2004, 2006, 2011; Suding et al. 2005; Gilliam 2006; Bobbink et al. 2010; Isbell et al. 2013; Dirnböck et al. 2014).

Much of the evidence indicating a potential effect of N deposition on species composition comes from greenhouse trails or relatively short-term seedling transplant studies in the field, often showing dramatic species-specific responses to N addition (McNulty et al. 1996; Catovsky and Bazzaz 2002b; Aber et al. 2003; Lovett and Mitchell 2004). However, the extrapolation of these observations to canopy level and community structure is uncertain, and there is currently considerable debate about the exact mechanisms by which interspecific differences to N deposition can be explained. Oak decline in this region during the last few decades is well documented (Clark 1993; Lorimer 1993; Abrams 2003). There is, however, no consensus regarding the exact cause(s) of this decline though a complex set of interactions involving fire suppression, herbivory, light competition, logging, fragmentation, climate change, and soil moisture (Lorimer 1993; Canham et al. 1996; Abrams 2003; Dickie et al. 2007; Fei and Steiner 2008; Alexander et al. 2008, 2009) have been invoked as major culprits. The work presented here is an attempt to assess whether N deposition has been a contributing factor in the regeneration failure of oak in many temperate forest communities of the upper Midwestern regions of the US.

In northeast Illinois and northwest Indiana, the relative abundance of a number of oak species, especially red oak (*Quercus rubra*), has been precipitously declining in the last several decades with sugar maple (*Acer saccharum)* often replacing the oak (Bowles et al. 2003, 2008; Aldrich et al. 2005). Even though the problem of oak regeneration predates those of N deposition, much of its decline in recent decades coincides with the onset of N deposition problems. Since the early 1960s, forests in this region have been experiencing a relatively high N deposition rate. Atmospheric N deposition in this region is predominantly composed of NO_3^-, and it exhibits a relatively strong gradient: increasing N deposition from northwest to southeast [National Atmospheric Deposition Program (NADP)]. These documented cases of oak decline during the recent decades are consistent with both spatial and temporal patterns of increases in N deposition (Bowles et al. 2005; Borden 2007). This relationship, albeit circumstantial, raises the possibility that increased N availability may have, at least in part, contributed to the oak decline in this region. The likelihood that oak performance is negatively impacted by increased N availability is also supported by other studies (Dickie et al. 2007; Wallace et al. 2007; Cha et al. 2010), even though the results often underscore that the interaction with herbivory and other resources such as light and water availability condition N responses. The major objective of this study is to examine if red oak and sugar maple respond differently to a gradient of ambient N deposition as well as to manipulative N addition experiments. The inclusion of maple in this study was motivated by the fact that maple is a major subdominant species that has been progressively replacing oak in these systems (Bowles et al. 2005). Therefore, we tested the hypothesis that increased N availability will reduce growth of red oak more than sugar maple.

In considering the possible causes of tree species' decline, it is important to assess if life stages matter. A number of studies have suggested that species-specific response during the seedling stage is the key mechanism that determines the regeneration of canopy trees (Catovsky and Bazzaz 2002a; Catovsky et al. 2002; Patterson et al. 2012). The critical role of the seedling phase is also consistent with reports that many of the oak regeneration problems in the US (Loftis and McGee 1993) and in Europe (Thomas et al. 2002) are linked to seedling survival. Therefore, another objective of this study was to assess if the responses of red oak and sugar maple to increased N availability varied according to demographic stages of the plants. We hypothesize that N deposition affects oak growth more prominently in seedlings than mature trees with no demographic consequences in maple.

Materials and methods

Site descriptions

We chose two oak/maple-dominated sites along an ambient gradient of atmospheric N deposition around the Chicagoland area (Avis et al. 2008). The sites are: Swallow cliffs (SC) Forest Preserve in Cook County, Illinois (41°40′N, 87°52′W) and Indiana Dunes National Lakeshore (IDNL) in Porter County, Indiana (41°38′N, 87°05′W). Each site is located within a 2.5-km radius of a NADP collection site that records the wet deposition of N. The wet deposition data during a 22-year period prior to the onset of this study show a relatively high rate of N deposition at both sites, but the deposition rates are on average 12 % higher at IDNL than SC (Table 1). The composition of the inorganic N in the wet deposition at both sites is similar; roughly a 4:1 NO_3^-:NH_4^+ ratio (Fig. 1; Table 1). A one-way ANOVA comparison of data between 1981 and 2002 (Fig. 1) indicated that NO_3^- deposition rates were significantly higher ($df = 21$, $F = 2.35$, $P = 0.026$) at IDNL than SC, but there was no significant difference in NH_4^+ deposition between the two sites. Neither site has been logged for at least 100 years, and both sites have very similar precipitation and temperature regimes as well as number of frost-free days per year (Borden 2007). The two sites also have very similar vegetation type and soil characteristics (Table 1). Oak, primarily white (*Quercus alba*), and red oak, dominates the experimental stands at both sides. Sugar maple also occurs but the relative abundance of this species is significantly higher at the IDNL than at the SC site (Table 1). The soil classification is identical at both sites: Morley silt loam, a fine illitic mesic Typic Hapludalf (formed on dolomitic parent material), and other important soil traits such as total N and pH are almost identical for both sites (Table 1). The two sites also have similar soil concentrations of P (17 ± 2 μg g^{-1} soil), Ca^{2+} (1,436 ± 130 μg g^{-1} soil), and Mg^{2+} (219 ± 16 μg g^{-1} soil). Even though the soil total N is very similar at both sites, available soil inorganic N (especially NO_3^-) and nitrification rates are significantly higher at IDNL than SC (Borden 2007). At both SC and IDNL, soil NO_3^- (and NH_4^+) levels are dynamic throughout the growing season, with the highest

Table 1 Location, soil and vegetation characteristics of the two forest sites used in this study

Sites (county, state)	SC (Cook, IL)	IDNL (Porter, IN)
Soil		
Series and classification	Morley silt loam	Morley silt loam
% N (A horizon)	0.26	0.27
pH	6.0	5.6
Vegetation		
Number of *Quercus* + *Acer* trees >10 cm DBH ha^{-1}	150	233
Relative dominance of *Quercus* in the stand	92 %	85.0 %
Relative dominance of *Acer* in the stand	0.5 %	10.3 %
Basal area (BA) by tree species (m^2 ha^{-1})		
Quercus alba	12.77	17.60
Quercus rubra	16.40	15.07
Acer rubrum	0.0	1.58
Acer saccharum	0.35	0.70
BA ratio of *Acer*:*Quercus* (%)	0.31	6.9
Wet deposition		
Total wet deposition, NO_3^- + NH_4^+ (kg h^{-1})	370	415
Ratio of NO_3^-:NH_4^+	3.8	4.2

The sites are oak dominated and represent an ambient gradient of atmospheric N deposition from relatively low (Swallow Cliffs; *SC*) to high (Indiana Dunes National Lakeshore; *IDNL*) deposition rates. Total wet depositions represent cumulative NH_4^+ + NO_3^- between 1981 and 2002

DBH Diameter at breast height

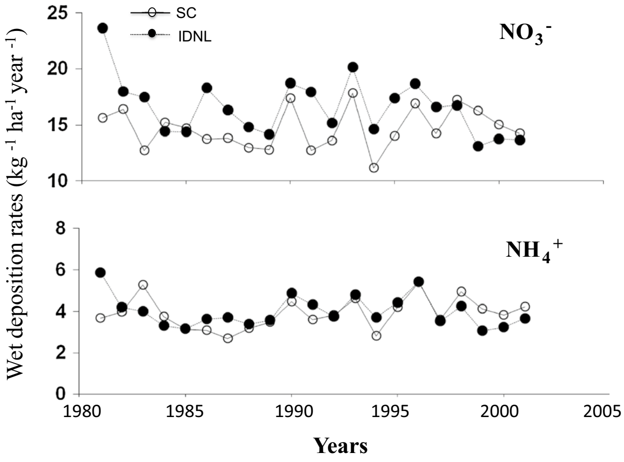

Fig. 1 Annual wet deposition rates for NO_3^- (*top*) and NH_4^+ (*bottom*) at two National Atmospheric Deposition Program (NADP) sites (http://nadp.sws.uiuc.edu/). The NADP collection sites are within 2.5 km of their corresponding experimental sites; Swallow Cliff (SC), Illinois; and Indiana Dunes National Lakeshore (IDNL), Indiana

soil NO_3^- levels occurring in July (detailed in Borden 2007). Under ambient N deposition conditions, soil NO_3^- in July is significantly higher at IDNL than SC (39 vs. 25 μg g^{-1} soil, respectively). For the same sampling period, soil NH_4^+ was 4.0 μg g^{-1} at IDNL vs. 3.2 μg g^{-1} at SC (Borden 2007).

Seedling propagation, field transplant, and experimental N additions

In 2002, locally collected acorns of red oak and seeds of sugar maple were stratified in a freezer for 3 months prior to germination. Roughly 200 evenly sized germinated seedlings were then transferred into 1-l pots containing a 1:1 by volume river-washed sand and potting mix (Miracle Gro; Scotts Miracle-Gro, Marysville, OH). Seedlings were grown in greenhouses conditions under natural photoperiod and thermal regimes during the entire growing season in 2002, and transplanted into the field in May 2003. At each site, we cleared eight 1 × 1-m plots of surface vegetation. Plots were at least 2 m apart. To the extent possible, plots were created in areas with similar topographies, under- and overstory plants and light conditions. Species and N treatment were randomly assigned to each plot. We transplanted 25 vigorous, but equal-size seedlings of either oak or maple in each plot using a soil core (10-cm diameter to a depth of 20 cm). We fenced each plot on all sides including the top (height 1 m) with chicken wire to protect seedlings from large animal herbivory. Plots were regularly weeded to keep competition from all other potential neighbors to a minimum. For each species, two of the plots were designated as control and two were designated as N fertilized. The N-fertilized plots received N addition at a rate of three times the ambient wet N deposition rate. This application rate is intermediate relative to many other N addition studies (Gilliam et al. 2001). Our experimental N addition method was relatively unique both in terms of total amount of N added as well as the monthly application intervals. More specifically, N addition was applied monthly (12 applications $year^{-1}$) using a 3-G capacity lawn and garden sprayer. At each site the application rate for each month was determined from the average monthly wet deposition rates of NO_3^- and NH_4^+ from the previous 5 years (NADP data). For example, in April 2004, we applied three times the average deposition for April 1999–2003. To further mimic the exact nature of the ambient deposition, we applied NO_3^- to NH_4^+ in a ratio identical to the 5-year monthly ratios reported by the corresponding NADP stations. Inorganic N sources came from a combination of KNO_3 and $(NH_4)_2SO_4$, generally in a 4:1 ratio. The fertilized plots received N at an average rate of roughly 18.8 and 20.2 kg h^{-1} $year^{-1}$ of NH_4^+ plus NO_3^- (or 7.1 and 7.6 kg N h^{-1} $year^{-1}$) at SC and IDNL, respectively. At SC, NO_3^--N and NH_4^+-N application rates averaged roughly 4.2 and 2.9 kg N h^{-1} $year^{-1}$, respectively. At IDNL, the application rates of NO_3^--N and NH_4^+-N were 4.6 and 3.0 kg N h^{-1} $year^{-1}$, respectively. Salts of NO_3^- and NH_4^+ were dissolved in water, and applied at a rate of 0.6 l per plot. Only 20 % of the total N came from $(NH_4)_2SO_4$, and, given the naturally high levels of cations in these dolomitic parent materials as well as counterbalancing effects of K in KNO_3^-, it is unlikely that this NH_4^+ salt would cause a significant effect on soil pH. Control plots received an equal volume of deionized water.

Growth, biomass, and N mass fractions in tree seedlings

After seven full seasons of field treatments, all seedlings were harvested by first cutting the shoots at the junction with the roots and placing them into individual paper bags. Roots were then excavated to a depth of 30 cm using a corer (10-cm diameter, 14.5 cm deep) and placed in individual plastic bags before transferring them to the lab. Obviously, a fraction of the roots system is lost due to the limited diameter of the core. We do not have an estimate of this fraction, but it is reasonable to assume that lateral roots farther away from the tap root are likely to represent a higher order and lighter weight fraction. Future work in this area of research, especially that which requires an exact knowledge of biomass allocation, should determine the recovery rate of the root harvest protocol. Roots were processed by first thoroughly washing them clean of soil while making sure to minimize fine root losses. Shoots and roots were further separated into individual tissue type, e.g., leaves, stems, fine and coarse roots. Light-colored roots smaller than 1 mm in diameter were designated as “fine roots.” All tissues were oven dried at 65 °C to a constant mass. Dry tissues were measured for biomass and biomass allocation. These tissues were subsequently ground finely in a rotary mill (Foss Tecator Cyclotec sample mill, model 1093; Hoganas, Sweden) and then analyzed for %N using an elemental analyzer (Fisson NA 2500; Elantech, NJ). We calculated N mass for each tissue type from the product of the biomass and N concentration. For both biomass and N mass, the data are presented as fractional (percent) allocation to each tissue type.

Measurement of mature tree growth

During the 7 years of the study period, we also examined responses of mature oak and maple trees at both forest sites. Five pairs of mature oak and maple trees were selected at each site in the control and fertilized plots. Each oak and maple pair was similar in size [diameter at breast height (DBH) 55–75 cm] and growing in close proximity to each other to avoid large differences in soil and other environmental conditions on plant growth. Each tree was fitted with a dendrometer band in 2003, and the increase in circumference was converted to basal area increment (BAI) according to: $\mathrm{BAI} = \pi(R_{t0}^2 - R_{t1}^2)$ where R_{t0} and R_{t1} represent radius at the start and the end of the study, respectively (Mendivelso et al. 2013). Even though we had selected trees of relatively equal size, DBH varied by as much as 30 % among target trees. Therefore, we normalized for this initial size difference by expressing the BAI of each individual as a function of its initial basal area to obtain relative BAI (RBAI) (Wallace et al. 2007). The resulting RBAI is in units of centimeter squared per centimeter squared per year, or simply in per year.

Statistical analyses

In seedlings, differences in biomass, biomass allocation and N mass allocation were analyzed using three-way ANOVA (species × site × fertilization) followed by a Student’s *t*-test for mean comparisons. In mature trees, differences in relative grow rate were compared between species and sites using two-way ANOVA (species × site). Data were transformed before analyses whenever necessary. We used either R or Minitab (version 17; Minitab) software to conduct these statistical analyses.

Results

Growth responses in seedlings and mature trees

The growth responses to forest sites and experimental N fertilization were distinct between seedlings and trees, and between red oak and sugar maple. For example, growth of oak seedlings was reduced by roughly 60 % in IDNL compared to SC, but growth of the mature trees was only 30 % lower at the IDNL relative to SC site (Table 1; Figs. 2 and 3). Growth of trees or seedlings of maple did not differ significantly between the two sites (Figs. 2 and 3). The results of experimental N addition on seedling growth also depended on the site (Table 2). For example, supplemental N reduced the growth of red oak seedlings by about 45 % at the site with the lower N deposition rate (SC), but seedling growth was not significantly affected by N fertilization

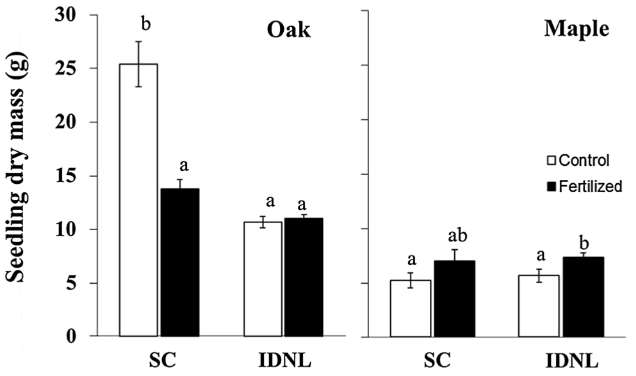

Fig. 2 Effects of the ambient N deposition at the two forest sites (SC and IDNL) and experimental N addition (*control* and *fertilized*) on seedling biomass of red oak and sugar maple. Data are mean ± SD, $n = 15$–30. In *each panel*, means significantly different from each other are designated with *different letters*. For abbreviations, see Fig. 1

at the IDNL site. Maple seedlings generally responded positively to experimental N addition, but the effect was significant only at IDNL (Fig. 2).

Fractional biomass and N mass allocation

Patterns of biomass fraction allocated to different plant parts and their responses to increased N availability were significantly different between the two species (Table 3). Overall, biomass fractions in oak seedlings were relatively conservative in response to fertilization with the exception of a roughly 42 % drop in fine roots at SC. The fraction of biomass allocated to coarse roots was on average roughly 27 % higher in IDNL than in SC, but the biomass fraction allocated to fine roots more than doubled in IDNL compared with SC (Table 3). In maple seedlings, fractional biomass allocation was much more plastic in response to experimental N addition than to different sites. The proportion of maple biomass allocated to coarse roots increased in response to fertilization by an average of 77 %, but there was little change in this compartment in response to differences in atmospheric N deposition among the two sites (Table 3). This increase in coarse root biomass in response to fertilization came largely at the cost of a decrease in biomass allocation to the stem. Across the two sites, biomass allocation to the stem tissue decreased by an average of 85 % when seedlings were grown with supplemental N (Table 3). Fractional N mass allocation generally did not follow biomass partitioning. For example, on average, oak seedlings allocated more than threefold N mass compared with the biomass to the leaf compartment. However, the pattern of N pool responses to various compartments responded similarly to increased N availability due either to fertilization or site differences.

Discussion

Oak decline and its regeneration failure has been a focus of numerous local, regional and international concerns, but there is still little consensus on the exact mechanisms that have undermined the success of this species in much of its native range during the last several decades. We suggest that the exact causes of oak replacement in these and many other forests around the world are far from being resolved in this study. Undoubtedly, no one study can comprehensively assess the role of a single factor unless the possible role of other factors has been explicitly tested. We acknowledge that in our forest sites, like many others, the apparent loss of oak species is complex and is most likely a function of multiple factors. Furthermore, the relative importance of each factor might change depending on the availability of other resources and conditions. However, the results from this work provide evidence that chronic atmospheric N deposition is a significant contributor to red oak regeneration failure in these Midwestern forest communities. Below, we consider the evidence for this contention followed by a discussion of the possible mechanisms.

Parallel responses of oak to atmospheric deposition or experimental fertilization

The total wet deposition at these sites averaged for the 22 years immediately preceding our experiments indicated that IDNL receives significantly ($P < 0.05$) more NO_3^- than SC (Fig. 1). Additionally, the net nitrification rate and available soil inorganic N (largely NO_3^-) at both sites exhibit a strong seasonal pattern, and are consistent with the deposition patterns, i.e., significantly higher in IDNL than SC site (Borden 2007). The fact that the two sites are almost identical with regard to parent material, soil type, soil pH, precipitation and temperature regimes, and land use history, leads to the conclusion that increased N availability in these stands may play a role in determining the negative growth responses in red oak. Furthermore, we found that the effect of the higher N deposition at the IDNL site on oak seedlings was largely reproduced by experimental N addition. At the SC site, N fertilization decreased seedling growth by 45 %; somewhat smaller than a 60 % decrease in response to the gradient, but still a marked decline. Taken together this study provides an explicit test that red oak, especially during the seedling stage, is highly sensitive to increased N availability. Growth of mature red oak trees also decreased in response to increased N availability along the gradient, but the relative adverse effect of N was considerably less pronounced than those reported for the seedlings (Fig. 2). We are not sure exactly what drives this asymmetric ontogenic/demographic response, but these observations highlight the possibility that seedling recruitment may be the bottleneck phase mediating

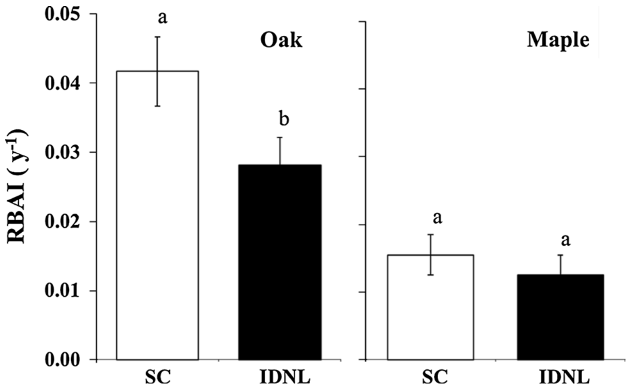

Fig. 3 Relative basal area increment (*RBAI*) responses of mature oak and maple trees at SC and IDNL forest sites. Data are means of four to nine replications ± SD. Means significantly different from each other are designated with *different letters*. For other abbreviations, see Fig. 1

Table 2 ANOVA for whole-seedling biomass (three-way) and relative growth rate of trunk diameter of the mature trees (two-way) following 7 years of field trial

	Source of variation	*F*	*P*
Seedlings	Species	45.24	<0.0001
	Site	186.29	<0.0001
	N fertilization	5.39	0.021
	Species × site	3.78	0.054
	Species × N fertilization	7.17	0.008
	Site × N fertilization	8.36	0.004
	Species × site × N fertilization	4.27	0.040
Mature trees	Species	14.44	0.001
	Site	4.87	0.039
	Species × site	5.26	0.032

Biomass and growth responses are assessed as a function of species (red oak and sugar maple), site (SC and INLS), and experimental N addition. For abbreviations, see Table 1

the regeneration failure of red oak in these forest stands. We found relatively low seedling mortality in response to either experimental N addition or forest sites; 85 and 90 % of oak seedlings survived in SC and IDNL, respectively. Therefore, we can further speculate that if oak seedling recruitment into these systems is adversely affected by increased N availability, seedling vigor rather than mortality might be the underlying mechanism.

Possible mechanism(s) of an apparent negative response of oak to N addition

A number of other field studies have also shown a negative effect of N addition on growth of oak seedlings, including red (Zaccherio and Finzi 2007) and pin oak species (Dickie et al. 2007), yet the commonality of this response and the potential mecanisms responsible for it are not understood. There is some evidence that under field conditions increased N availability may decrease oak growth due to increased susceptibility to insect herbivory (Cha et al. 2010). While we did not systematically analyze seedlings for insect damage, there were no obvious signs of differences in leaf herbivory between N treatments and/or sites. Another possible mechanism underlying the inhibitory effects of increased N availability on oak seedlings might involve loss of compatible ectomycorrhizal (EM) and/or increased predominance of arbuscular mycorrhizal fungal species. Evidence suggests that increased N loading decreases EM species richness in many coniferous forests (Karen et al. 1997; Jonsson et al. 2000; Dighton et al. 2004; Edwards et al. 2004; Cox et al. 2010), and similar trends have also been reported in other temperate forests (Baxter et al. 1999). During the early stages of this project, we conducted a survey of fungal communities using morphological identification and found that the total number of species was similar at the two sites, but the proportion of EM fungi to the total fungal species decreased dramatically from 40 % at SC to 20 % in IDNL. Later, Avis et al. (2008) used molecular and morphological approaches at the same sites and showed that N fertilization resulted in a 20 % decrease in EM fungal species richness.

The idea that increased N availability could shift the floristic composition from ecto- to endotrophic trees (here exemplified by red oak and sugar maple, respectively) is supported by other, albeit limited, studies (Francis and Read 1994; McNulty et al. 1996; Thomas et al. 2010). Although generalization of an N-induced shift in favor of endotrophic tree species is premature, the concept is consistent with Read's conceptual model (1991) of how mycorrhizal fungi affect the distribution/succession of ectotrophs and endotrophs. This idea is also consistent with recent conceptual models suggesting that plant adaptation to changes in resource availability is linked to mycorrhizal symbionts (van der Heijden et al. 1998, 2008a, b; Klironomos et al. 2011; Phillips et al. 2013) though the linkages can be highly context dependent (Jonsson et al. 2001; Johnson 2010).

Well-documented conceptual models of plant response to changes in resource availability (Tilman 1988; Bazzaz 1997) predict that plasticity in biomass and N mass fraction are important mechanisms that may determine the adaptation of species to changes in resource availability. In our study, fractional allocation of biomass N to different organs was significantly different among species, and was affected by fertilization and by site differences in N deposition (Table 3), but the interaction terms were not statistically different (Table 2). This indicates that although the two species show some level of plasticity in response to increased N availability, the response is not dependent on

Table 3 Fractional biomass and N mass allocation responses of seedlings of red oak and sugar maple to different forest sites (SC and IDNL), and experimental N addition [control (*C*), fertilized (*F*)] following 7 years of a field trial

		Field sites				Sources of variation, three-way ANOVA						
		SC		IDNL		Site	N Treatment (N)	Species (Sp.)	Site × N	Site × Sp.	N × Sp.	Site × N × Sp.
		C	*F*	*C*	*F*							
Fractional biomass allocation (%)												
Oak	CR	51.10	57.63	70.39	68.77	***	***	***	†	*	***	NS
	FR	4.92	2.83	8.54	7.64	***		***	NS	**	***	NS
	LF	15.16	11.61	9.55	7.13	***	***	*	NS	**	NS	NS
	ST	28.83	27.93	11.53	16.47	***	***	†	NS	*	***	NS
Maple	CR	35.59	67.93	41.81	69.93							
	FR	7.97	17.00	7.37	14.55							
	LF	11.51	8.29	9.93	8.32							
	ST	44.93	6.78	40.89	7.50							
Fractional N mass allocation (%)												
Oak	CR	35.51	48.08	58.87	56.84	***	***	NS	**	†	***	†
	FR	5.32	3.72	8.95	9.27	***	***	***	NS	**	***	NS
	LF	41.25	31.6	24.29	21.45	***	***	***	*	**	NS	NS
	ST	17.92	16.6	7.89	12.44	***	***	**	†	NS	***	NS
Maple	CR	30.17	57.97	30.18	62.23							
	FR	9.68	20.5	9.44	17.78							
	LF	24.72	16.82	21.4	15.5							
	ST	35.44	4.71	38.98	4.49							

Biomass and N mass fractions are shown for coarse roots (*CR*), fine roots (*FR*), leaf (*LF*) and stem (*ST*). Data are means

* $P < 0.5$, ** $P < 0.01$, *** $P < 0.001$, † $P < 0.1$, *NS* not significant

species. The one exception to this conclusion is the fact that the fraction of whole-plant N mass partitioned to leaves decreased significantly in response to increased N availability both in response to N fertilization and increased site N deposition rate (Table 3). The plasticity in fractional N allocation to different plant organs does not always follow the pattern of biomass fractions (Abrahamson and Caswell 1982; Newingham et al. 2007) and this may have ecological and evolutionary consequences. We found, however, that biomass and N mass responses of oak seedlings to increased N availability were qualitatively similar (Table 3) indicating that tissue quality was unchanged. In fact, leaf N concentration of oak seedlings was independent of site and N fertilization (data not shown). Given the universal positive correlation between fractional N allocation (and/or N concentration) of the leaf and the photosynthetic rate (Field and Mooney 1986; Evans 1989), our data suggest that N deposition did not adversely affect the growth of oak seedlings via photosynthetic capacity. Rather, the lower biomass and N mass fractional allocation to the photosynthetic tissues must have reduced the whole-plant C gain of the oak seedlings when grown under higher N availability. It must also be noted that a lower leaf biomass fraction response to increased N availability is in contrast with the expectations of the functional equilibrium hypothesis (Gutschick and Kay 1995; Reynolds and D'Antonio 1996; Brouwer 1983; Poorter and Nagel 2000). We are not sure what caused this marked decrease in the relative production of the photosynthetic tissue, but future studies addressing this issue must consider the metabolic cost (as well as the predominant site of NO_3^- reduction, e.g., root vs. shoot) of assimilating NO_3^- vs. C. Compared with NH_4^+, uptake and assimilation of NO_3^- is associated with a relatively high energetic cost. The cost of NO_3^- uptake and reduction may compete with C assimilation, a condition that is more likely to occur under light-limited environments. Leaf status of other nutrients including P and a number of cations could have also been involved (e.g., changes in stoichiometry of $N{:}P{:}Ca^{2+}$) in mediating the negative growth response in red oak and lack of a negative response in sugar maple. In fact, when negative growth responses have been reported under chronic N deposition, the proposed mechanism involves loss of base cations from the soil followed by foliar deficiency of these essential elements (Aber et al.1989; McNulty et al. 1996; Zaccherio and Finzi 2007). The soils in our experimental sites are derived from dolomitic parent material that are rich in base cations including Ca^{2+} and Mg^{2+}, making this a less likely mechanism to mediate

N-induced contrasting responses in oak or maple seedlings. Increased N availability at IDNL compared to SC had little effect on fractional N allocation in leaves of maple seedlings leading to the conclusion that unlike oak, whole-plant C gain of maple seedlings was not affected.

The negative response of red oak to increased N availability may also represent this species' relative preference for the ratio of the two N forms rather than a direct effect of N availability per se. This assertion is consistent with a number of studies that have generally shown a clear preference for NH_4^+ over NO_3^- in oak species (Zerihun and BassiriRad 2001; Templer and Dawson 2004). The relative abundance of the inorganic N forms, rather than total inorganic N, is arguably a stronger selection pressure in plant ecology and biodiversity (Boudsocq et al. 2012; Britto and Kronzucker 2013), hence the gradual shift from oak to maple in these systems might represent a shift in the dominant form of available N. The composition of deposited N at these sites favors NO_3^- over NH_4^+ by a 4:1 ratio, and even though the ratio is not quite that large in soil available N, NO_3^- often dominates soil inorganic N (Borden 2007). A number of studies have shown that relative to other deciduous trees, oaks generally have a low nitrate reductase activity (Downs et al. 1993; Truax et al. 1994; Thomas and Hilker 2000), which means that their ability to assimilate NO_3^- is highly limited. Therefore, the inhibitory effects of the ambient gradient and/or the experimental N addition on red oak are likely to be coupled to the inability of this species to meet its N demand from increased NO_3^- availability.

We found that higher N deposition rate at IDNL compared to SC did not significantly affect the growth of the mature maple trees although experimental fertilization had an overall slight positive effect on the total biomass production of the seedlings. This N-induced stable or positive response in seedlings of sugar maple has been observed by others (Stanturf et al. 1989; Elvir et al. 2003). In a field study, Elvir et al. (2003) examined responses of sugar maple and red spruce over a 10-year period of N fertilization at the Bear Brook Watershed in Maine. They reported that only sugar maple responded positively to this relatively long-term N addition. Later, Elvir et al. (2006) also showed that when multiple species were examined in this system, N addition improved leaf N status and photosynthetic rate only in sugar maple. However, it is not clear if this apparent stability and/or enhancement of sugar maple response to chronic N deposition is the direct effect of N per se, or due to the rapid demise of its major competitor species such as beech (Lovett and Mitchell 2004) and oak in our system. Furthermore, there is evidence that the effect of N deposition on sugar maple in the northeastern forests of the USA cannot be extrapolated to other regions. In fact, multi-year experimental N addition experiments in a mixed species forest of northeastern USA (Zaccherio and Finzi 2007) as well as an N gradient study in northern hardwood forests of the Upper Great Lake region (Patterson et al. 2012; Talhelm et al. 2013) show a significant decrease in abundance and growth of sugar maple seedlings in response to increased N availability.

Potential interactions with community-level processes and consequences on biodiversity

Clearly these contrasting observations highlight the site-specific nature of the N responses and the need for robust experimental protocols that could separate the effect of N from other environmental variables. Currently, the results of N deposition studies come from disparate bodies of literature that vary considerably in terms of experimental protocol including the length of data collection, N addition vs. ambient gradient, biotic factors, parent material, land use history etc. What remains ubiquitous, however, is the overwhelming evidence that regardless of site-specific characteristics and experimental approaches, chronic N addition differentially affects growth and survival of co-occurring species causing a significant change in diversity and floristic composition (Catovsky and Bazzaz 2002b; Zaccherio and Finzi 2007; Bedison and McNeil 2009; Bobbink et al. 2010; Clark et al. 2013; Southon et al. 2013; Talhelm et al. 2013). The observed pattern of oak replacement by maple species in the Midwestern region of the US (Abrams 2003) is undoubtedly driven by a complex set of drivers and multiple factors (Vitousek et al. 1997; Aber and Driscoll 1997; Lovett et al. 2000; McNeil et al. 2006). A number of key factors such as fire suppression, mesophication, animal herbivory, climate change and land use (Lorimer 1993; Canham et al. 1996; Abrams 2003; Dickie et al. 2007; Fei and Steiner 2008; Alexander et al. 2008, 2009) which have often been invoked as potential mechanisms cannot be ruled out from the results presented here. The study presented here suggests, however, that chronic N deposition is also a likely factor. Above, we considered some of the potential mechanisms by which N may directly affect the performance of oak compared to maple. The observation that oak in these forest sites responds directly to increased N availability does not preclude the possibility that other mediating but indirect mechanisms may also be involved. Gilliam (2006, 2007) has proposed that in many temperate forest ecosystems, changes in resources may affect the composition of the canopy species by shifting the competitive interactions of tree seedlings and the herbaceous species. This is a possibility that was not directly tested here, but deserves greater attention in future studies addressing the demise of oak in these Midwestern forests.

Conclusion

The forest sites examined in this study have been undergoing a shift in species composition in recent decades which has manifested in a gradual replacement of oak by maple. Much effort has gone into understanding the potential causes of oak's failure to regenerate and its progressive replacement by species such as sugar maple. The picture that emerges indicates a collage of factors ranging from light competition to fire to herbivory to mesophication as potential contributing factors (Abrams 2003). However, the role of chronic N deposition as a driving force shifting community composition of these Midwestern forests in favor of maple has not been considered previously. Our study shows in the absence of major differences in factors, such as large animal herbivory, edaphic and environmental characteristics, that chronic N deposition and experimental N addition caused a large decrease in the growth of red oak but not sugar maple. These findings have implication for understanding the community and population ecology of plants in these forests. Shift in species composition in favor of maple could affect biogeochemical processes (e.g., increase nitrification) further undermining the stability and the competitive balance of the dominant species. This work also underscores the need to fully integrate chronic atmospheric N deposition and perhaps other components of global change into management and conservation efforts designed to halt the loss of this flagship species.

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