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Impacts of elevated nitrogen inputs on oak reproductive and seed ecology

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Abstract

The effects of increased anthropogenic inputs of reactive nitrogen (N) have been studied at the Harvard Forest Chronic N Experiment, where NH_4NO_3 has been applied experimentally since 1988 to increase atmospheric deposition rates ~6- and ~18-fold above ambient. This paper asks whether conditions favorable to primary production also resulted in plastic increases to flower, fruit, and seedling traits, and focuses primarily on the oaks that dominate the hardwood stands of the Harvard Forest experiment. Litterfall samples collected between 1996 and 2001 revealed that flowers and fruits were significantly more abundant in N-treated plots, and an analysis of oak tree abundance found significant variation both among and within plots. Acorn samples collected during 2003 and 2004 (a mast and a postmast year) were therefore analyzed using ANCOVA models that included an estimate of oak tree abundance. This tree abundance estimate was the only significant driver of increased acorn production during the mast year, and in both years it was a significant factor on plots receiving the highest levels of N. In the postmast year, acorn production was increased in direct response to N-related factors other than tree abundance. Our comparisons of control and N-treated plots for acorn quality traits (e.g. rates of acorn damage, germination percentage, seedling growth) revealed negligible or only transient differences. Shifts in overall acorn abundance – particularly disproportionate N-mediated increases during nonmast years – could have a wide range of ecological consequences beyond the more frequently examined impacts of N deposition on primary production and carbon sequestration.

Keywords: acorn density, acorn weight, *Curculio*, elevated CO_2, masting, N deposition, perennials, *Quercus*, reproductive allocation, seed predation, soil nutrients

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Introduction

As human activities are elevating atmospheric CO_2 and altering climate, equally drastic changes are occurring in the cycling of biologically active nitrogen (N) compounds from the atmosphere into both aquatic and terrestrial ecosystems (Matson *et al.*, 2002; Galloway *et al.*, 2003). Concerns about the long-term effects of increased rates of N deposition on natural forest ecosystems in the northeastern United States have motivated several large-scale studies as part of a larger international network of research (e.g. Gilliam *et al.*, 2001; Aber *et al.*, 2003). The Chronic N Amendment Study at Harvard Forest (Petersham, MA, USA) involves comparisons between control plots (which have been N-limited historically) and experimental plots that have received >15 years of artificial N amendments (representing six- and 18-fold increases over ambient inputs) (Aber *et al.*, 1998; Magill *et al.*, 2004).

The Chronic N study primarily addresses how a major ecosystem manipulation – artificially elevated N deposition – influences ecosystem function, including rates of net primary production through changes in incremental vegetative growth. The experiment has monitored the N-mediated plasticity of traits such as aboveground woody biomass, N content of fine roots, and the biomass and N content of foliage (Magill *et al.*, 2000, 2004). Data about vegetative plasticity alone, however, is unlikely to yield significant insights into the future of forest ecosystems and the communities and populations within them. Predicting the response

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of forests in the middle- to long-term will require similarly detailed data about indirect effects of increased N deposition, such as shifts in herbivory (e.g. Throop & Lerdau, 2004) and, as addressed in this paper, the plasticity of plant reproductive and offspring traits (Ackerly & Bazzaz, 1995).

Our study complements a handful of studies addressing the response of woody plants to CO_2 enrichment, another ecosystem manipulation that generally enhances vegetative growth. Results to date indicate that several dominant woody species disproportionately increase reproductive output in response to CO_2-induced enhancement of vegetative growth, and that there are sometimes positive correlations between increased aboveground growth and offspring traits (e.g. seed size, germination rates, and seedling size) (Hussain *et al*., 2001; LaDeau & Clark, 2001, 2006; Stiling *et al*., 2004; Darbah *et al*., 2007). There is a more extensive literature on how dominant grassland species respond to CO_2 enrichment. Positive, negative, and near-zero correlations have been found between species-specific vegetative biomass responses and reproductive or offspring traits (e.g. Huxman *et al*., 1998, 1999; Edwards *et al*., 2001; Jablonski *et al*., 2002; Thurig *et al*., 2003).

Similar studies are clearly needed to investigate whether and how biomass responses to increasing N inputs correlate with reproductive and offspring trait responses. We have conducted a set of studies on the Harvard Forest Chronic N plots. Our studies address two specific questions and concentrate on the two closely related oak species that are dominants in the hardwood forests in the northeastern United States, *Quercus rubra* and *Q. velutina* (red oak and black oak). First, we ask whether oaks plastically increase reproductive output in response to conditions that are, in the short-term, favorable to rates of primary productivity. Because these trees have experienced past N-limited (and therefore suboptimal) conditions, plasticity is expected to be especially strong in this forest stand (Aber *et al*., 1998; Sultan, 2003). Second, since offspring are locally dispersed with respect to the scale of variation in N deposition rates (Aber *et al*., 2003), we examine whether increased N inputs in the maternal environment elicits plasticity in individual offspring traits (Roach & Wulff, 1987; Donohue & Schmitt, 1998). Changes in the reproductive or seed ecology of oaks are particularly interesting given their masting habit, which involves very high reproductive output in certain years. Mast years are followed by 1 or more years when reproduction output is quite limited, most likely because trees fail to surpass a minimal resource threshold (Isagi *et al*., 1997; Kelly & Sork, 2002).

We investigated whether N amendments increased reproductive tissue production by the oak species dominating these hardwood stands by sorting and comparing litterfall samples from control and experimental plots, collected between 1997 and 2001. We also sampled acorns that fell into these experimental and control plots during a mast and a postmast year (2003 and 2004). These data were used to determine whether mature acorn production – both the number of mature acorns and total acorn biomass – increased with N amendments. Finally, to examine the possibility that phenotypic plasticity in offspring traits is triggered by variation in the maternal environment, we compared the quality of acorns derived from control and experimental plots using four metrics: (1) mean acorn size, (2) frequency of damage by insects, paying particular attention to weevil species that infest mature acorns (*Curculio* spp.), (3) germination rates, and (4) differences in the size of greenhouse-grown seedlings.

Methods

Experimental plots and treatments

The mixed hardwood stand chosen for the Chronic Nitrogen Amendment experiment at Harvard Forest in Central Massachusetts is dominated by black and red oaks (*Q. velutina* Lam. and *Q. borealis* Michx.), with additional representation by black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), American beech (*Fagus grandifolia* Ehrh.), and black cherry (*Prunus serotina* Ehrh.). As detailed in Aber *et al*. (1993), applications of N as a concentrated aqueous solution of NH_4NO_3 have occurred monthly since 1989 during the growing season (May–September). Rates of 150 and $50\,kg\,ha^{-1}\,yr^{-1}$ on the 'high' and 'low' plots, respectively, represent approximately 18- and six-fold increases over control plots receiving ambient levels (Ollinger *et al*., 1993). Treatments have been applied on four $30\,m \times 30\,m$ plots divided into thirty-six $5\,m \times 5\,m$ subplots; only the interior, nonedge 16 subplots were used for sample collection. Throughout this paper, we refer to the high plots as 'N-150' and the two types of low plots as 'N-50' and 'NS-50.' The NS-50 plot initially received both low N and sulfur (S) treatments ($74\,kg\,ha^{-1}\,yr^{-1}$ Na_2SO_4), but S applications were discontinued in 1998 because no significant differences were observed between the NS-50 and N-50 plots (Magill *et al*., 2000).

Litterfall sampling and analysis

Using nine plastic baskets ($0.2345\,m^2$) located within nine of the 16 interior subplots, cumulative litterfall was collected three times per year on or near June 1 (spring), September 1 (summer) and November 15 (fall). Only leaf tissue biomass has been reported previously (Aber *et al.*, 1993; Magill *et al.*, 2000, 2004). Here, we report on nonfoliage tissues collected between 1997 and 2002.

After these nonfoliage samples were weighed, tissues identifiable as oak flowers and acorns (i.e. fruits) were sorted out and reweighed. Data for red and black oaks in this study have been pooled (Magill *et al.*, 2000, 2004) because it can be difficult to distinguish between species and these two taxa hybridize (Sander, 1990). The experimental unit used in statistical analyses of these data was the sum of all three samples from a subplot, and results of statistical tests should be interpreted conservatively since the spatial contiguity of subplots potentially violates the assumption that they are independent replicates (Hurlbert, 1984).

We compared nonfoliage litterfall biomass among plots and across years using SAS PROC MIXED to estimate a repeated-measures ANOVA model in which treatment was the among-subject effect and year the within-subject effect. In the REPEATED procedure, we chose a first-order autoregressive covariance structure. This choice followed a comparison of several covariance structures using the Akaike information criterion (AIC) goodness-of-fit statistic, and was logically consistent with data collected in consecutive years (Littell *et al.*, 1996; SAS Institute Inc., 2002, 1998, Cary, NC, USA). After fitting this model, comparisons among plots employed a Scheffe's multiple comparison procedure.

Tree abundance estimates

Experimental N applications have resulted in significant net primary productivity increases on experimental plots (Magill *et al.*, 2004). Changes in reproductive traits, especially increases in acorn abundance, may reflect both the direct impact of N applications and the indirect impact of increases in aboveground woody tissue biomass, particularly larger canopy volumes. To statistically disentangle these direct and indirect contributions to acorn abundance, we needed an estimate of oak abundance on each subplot. After obtaining a database of individual tree diameters at breast height (d, in cm) (A. Magill, 2002, unpublished data), these estimates were converted to basal area ($g = \pi d^2/40\,000$, in m^2). We summed total red and black oak basal area on a focal subplot and on the eight surrounding subplots. The average basal area of these nine subplots was converted to stand basal area [$G = g/(subplot\ area)$, in $\mathrm{m}^2\,\mathrm{ha}^{-1}$]. This served as our estimate of oak tree abundance for a given subplot.

Acorn abundance, biomass, and damage rates

We estimated acorn abundance on 12–16 of the interior 16 subplots. In both years, sampling was done on the same date (September 30), several weeks before trees begin shedding leaves. In an effort to sample only that year's acorns, we gathered only newly fallen acorns visible on the ground without disturbing the past season's leaf litter. When more than one individual participated in sampling, we minimized disparities in sampling effort and intensities by initially sampling in tandem. By working in adjacent subplots, it was also possible to frequently cross-check the consistency of sampling protocols throughout the day.

After estimating the total biomass of each sample, acorns were sorted into three categories and counted: immature, mature/intact, and mature/damaged. To estimate the mean individual acorn size within a subplot, the total biomass of mature/intact acorns was divided by the count.

For each subplot, we also estimated proportion of acorns damaged. In 2003, this was done by checking for exit hole damage associated with infestation by *Curculio* spp. weevils (Riccardi *et al.*, 2004; Miller & Schlarbaum, 2005). Because this failed to identify many infested acorns (see below), we used a float test in 2004 to identify damaged acorns, and then dissected damaged acorns to discern which were infested with *Curculio* spp. weevils. We angularly transformed proportion data to improve normality (Zar, 1999).

We analyzed these data using a multivariate linear model. The fixed effects in the model were N-treatment (categorical) and oak abundance (continuous). The model also included the interaction between N-treatment and oak abundance (SAS Institute Inc., 2002). Separate analyses were conducted for 2003 and 2004 data because we examined a slightly different variable to estimate acorn damage rates. With 2003 data, we examined among-plot variation in four response variables: total acorn biomass, acorn number, individual acorn size, and proportion damaged. In 2004, proportion damage by *Curculio* spp. was added as a fifth dependent variable. Also, sampling designs were not identical between years. Specifically, 2003 data had fewer samples and did not including samples from the NS-50 plot. When a multivariate model was significant, we then conducted univariate analyses for individual dependent variables.

Greenhouse study of germination and seedling growth

The abundance of acorns produced in 2003 enabled us to initiate a germination study to compare acorns derived from control, N-50, and N-150 plots. All available acorns from the control plot ($N_{\mathrm{Control}} = 233$) and randomly chosen acorns from the other two plots ($N_{\text{N-50}} = 240$, $N_{\text{N-150}} = 240$) were cold/dark stratified (4 °C) in moist, sterile vermiculite between October and March. All acorns were transferred to Barnard's Ross Greenhouse on April 9, 2004, and allowed to germinate under natural photoperiods and cool temperatures (~ 19–23 °C).

After observing high germination rates, we incorporated germinants into a study of early seedling growth traits. Seedlings were transferred to 21 cm deep Super Cell cone-tainers (Ray Leach, Canby, OR, USA) containing standard growing mix (Fafard #2, Agawam, MA, USA) supplemented by 1/4 teaspoon (~ 1.2 g) of Osmocote pelletized N–P–K fertilizer (Ortho, Marysville, OH, USA) and watered three to four times per week.

At 10 weeks after germination, we estimated seedling size nondestructively by measuring the length of all leaves and the height of the seedling from the apex to the first internode; these lengths were summed to provide an estimate of seedling size. After these measurements were done at 10 weeks, half of the seedlings were harvested and separated into leaves, stems, and roots. After drying to constant temperature in an approximately 60 °C oven, tissues were weighed. At 20 weeks, remaining seedlings were similarly processed. We conducted a one-way ANOVA on both the 10- and 20-week nondestructive size estimates, after which comparison of seedlings derived from control and experimental plots were conducted using Scheffe's procedure.

Results

Litterfall from 1997 to 2002

There was significant year-to-year variation in the biomass of nonfoliage litterfall, with especially high litterfall in 2001. Across years, the mean biomass of these tissues sampled from N-150 plot was significantly greater than control plots. Means for the N-50 and NS-50 plots were intermediate and did not differ significantly from each other, nor did the mean of the NS-50 differ significantly from the control (Table 1, Fig. 1a). To confirm that the oaks dominating the stand were driving this pattern of interannual and among plot variation, we repeated the analysis using only oak tissue biomass data of the nonfoliage litterfall samples. Results were similar (Table 1, Fig. 1b). Detailed examination of these tissue samples (not shown) revealed positive correlation between total acorn tissue produced and male and female oak flower tissues produced. Also, acorns accounted for ~ 80–90% of the total reproductive tissue biomass produced in all plots and in both seasons.

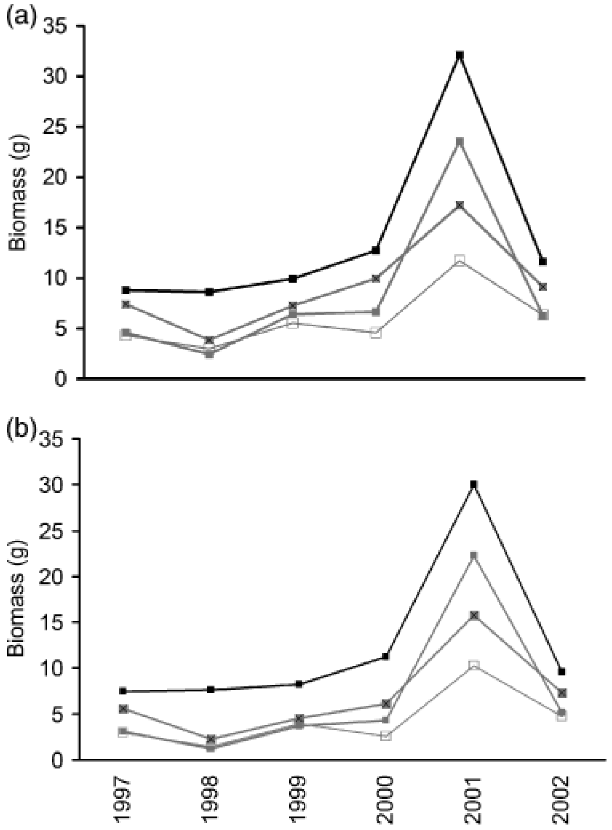

Fig. 1 Among-plot and across-year variation in the biomass of litterfall samples: (a) all nonfoliage litter; (b) oak nonfoliage litter. H-150 plots, black lines and ■ ; N-50 plots, gray line and ■; NS-50 plots, gray line and ⊠; control plots, thin gray line and □. See text and Table 1 for tests using a repeated measures mixed-model ANOVA and for comparison of each plot's mean biomass using a Scheffé's procedure.

Table 1 Effects of plot and year on the biomass of flower and acorn tissues by canopy trees, from a mixed-model repeated-measures ANOVA

		Total flower and acorn biomass		Oak flower and acorn biomass	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Nitrogen (N)-treatment	3, 43	9.99	< 0.0001	11.21	< 0.0001
Year	5, 122	21.69	< 0.0001	24.24	< 0.0001
Treatment × year	15, 122	1.34	0.19	1.41	0.15

See Fig. 1 and text for additional details.

Variation in acorn traits

Among-plot variation in acorn traits was attributed to different combinations of factors between the 2 years. In 2003, tests of the multivariate linear model indicated a significant main effect of N-treatment and a significant interaction between N-treatment and tree abundance.

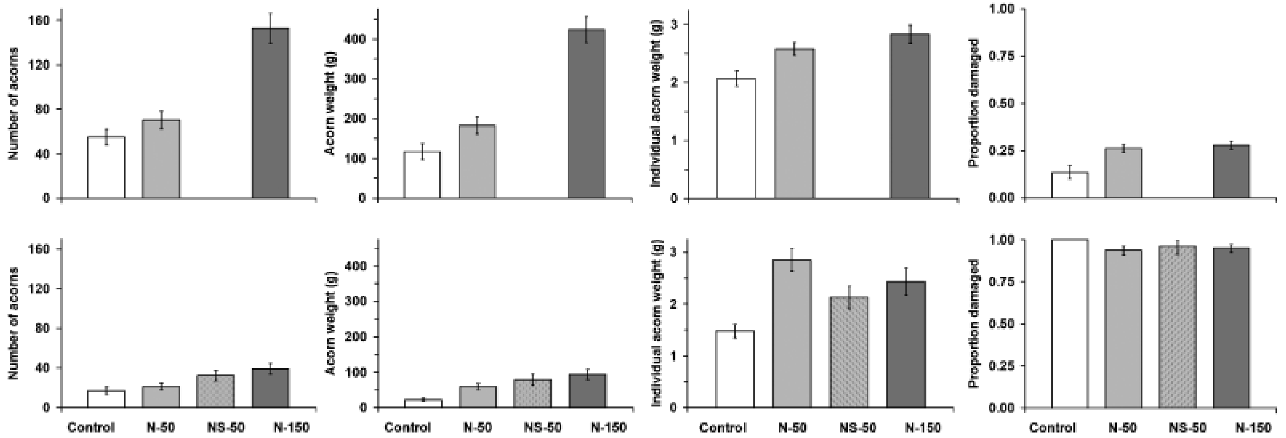

Fig. 2 Means (±1 SE) for acorn number, acorn biomass, individual acorn size, and proportion damaged. Top and bottom rows of figures are 2003 and 2004 data, respectively.

Table 2a Summary of multivariate models examining effects of N-treatment and oak abundance on acorn traits

Source of variation	df	Wilks' λ	*F*	*P*
2003				
Nitrogen (N)-treatment	8, 54	0.493	2.86	0.01
Oak abundance	4, 27	0.781	1.9	0.14
Treatment × abundance	8, 54	0.437	3.46	0.0028
2004				
N-treatment	15, 125	0.352	3.82	<0.0001
Oak abundance	5, 45	0.726	3.4	0.0109
Treatment × abundance	15, 125	0.340	3.97	<0.0001

Separate models were fit in 2003 and 2004.

The following year, when acorns were much less abundant and a larger number of samples were analyzed, tests of the multivariate model found that the interaction term and both main effects were significant sources of variation (Table 2a).

The two acorn abundance traits – number and biomass – were generally lowest in the control plot, intermediate in the N-50 plot, and highest in the N-150 plot in both years (Fig. 2). During 2003, a mast year, a major driver of variation in acorn abundance across all plots was the abundance of oak trees on and near a given subplot. Univariate models for that year indicate that only the main effect of tree abundance was significant; the interaction between tree abundance and N-treatment was either nonsignificant or only marginally significant (Fig. 3a and c; Table 2b). In 2004, a postmast year with much sparser acorn production, variation in these two acorn abundance traits depended on specific combinations of oak abundance and N-treatment. On all N-treated plots, we found greater acorn abundance relative to control plots. These increases covaried with tree abundance only on the N-150 plots (Fig. 3b and d; Table 2b).

Mean individual acorn biomass was lower in the control plots as compared with experimental plots in both years (Fig. 2). In 2003, the only significant factor contributing to variation in this trait was the treatment-by-abundance interaction term, and it was only marginally significant. In 2004, both the interaction term and the main effect of N-treatment were highly significant (Table 2b). That year, trees on the N-50 stand produced the largest acorns and, unexpectedly, there was an increase in per-acorn biomass with tree abundance (Fig. 3e and f).

In 2003, a year of high acorn abundance, infestation rates were estimated as ∼20–30% in all plots (Fig. 2). A subset of undamaged acorns from this sample was cold stratified for use in the greenhouse study. Numerous *Curculio* spp. weevil larvae were observed on the greenhouse bench and floor shortly after these seeds were placed in the warm greenhouse environment. We interpreted this as evidence that many seemingly intact acorns had been infested, possibly accounting for poor germination rates. Excavation and examination of ungerminated acorns revealed that these or other unidentified pests had destroyed many embryos. This motivated the change in protocol for assessing damage rates for the much smaller 2004 acorn samples, in which damage rates were uniformly high (>90%) (Fig. 2) and damage attributed to *Curculio* was similar in all plots (Fig. 4). Despite differences in methods for assessing damage rates, in both years among-plot variation was limited and univariate models failed to detect significant effects of the different plot types, indicating that damage rates were not sensitive to either the N-treatment applied or other among-plot differences (Table 2b, Fig. 4).

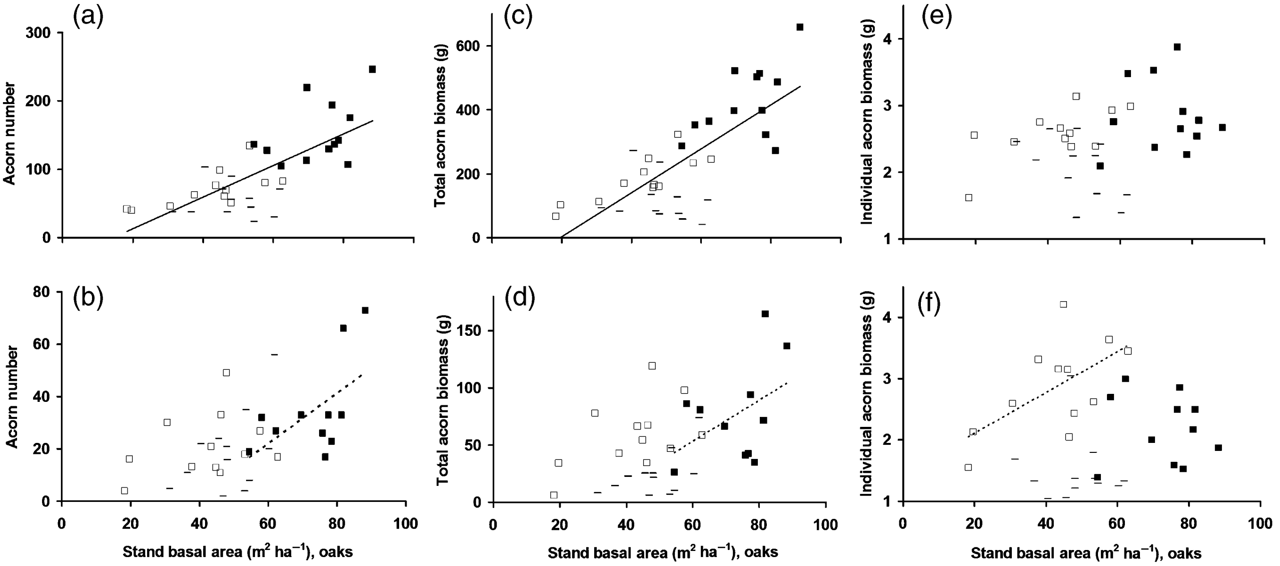

Fig. 3 Scatterplots relating oak tree abundance (as estimated by stand basal area) to 2003 and 2004 subplot means for (a, b) acorn number, (c, d) total acorn biomass, and (e, f) individual acorn biomass. Control plots are indicated by –, N-50 plots by □ and N-150 plots by ■. Solid trend lines are included when there was a significant main effect of abundance in ANCOVA models; dashed lines are included to indicate where there was a significant abundance by N-treatment interactions in the ANCOVA models, and show trends that were specific to a given N-treatment level. See Table 2b and text.

Table 2b Summary of corresponding univariate models

		Acorn number		Total acorn biomass		Individual acorn biomass		Proportion of damaged acorns		Proportion damage by weevils	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
2003											
Nitrogen (N)-treatment	2, 30	0.78	0.47	1.86	0.17	2.44	0.10	1.00	0.38	na	na
Abundance	1, 30	4.00	0.055	4.99	0.03	0.13	0.73	2.36	0.14	na	na
N × abundance	2, 30	1.42	0.26	3.11	0.06	3.27	0.05	1.17	0.33	na	na
2004											
N-treatment	3, 49	3.44	0.02	9.82	<0.0001	4.27	0.0093	1.29	0.29	1.81	0.16
Abundance	1, 49	1.69	0.20	2.95	0.09	2.44	0.13	1.98	0.17	0.01	0.94
N × abundance	3, 19	3.03	0.04	9.52	<0.0001	4.83	0.0050	1.24	0.31	1.98	0.13

Greenhouse study

Germination rates were lower for acorns derived from control and N-50 plots (both about 19%) and slightly higher for those derived from N-150 plots (27%), but the N-treatment effect was nonsignificant ($F_{2,33} = 1.27$, $P = 0.295$). Seedling size data from the greenhouse study, which was conducted with seedlings derived from 2003 acorn samples, indicated that the effect of N-amendment level on seedling size was significant at the 10-week harvest ($F_{2,129} = 5.17$, $P < 0.01$). At that point, seedlings derived from the N-150 plots were about 30% larger than those from control plots; seedlings from N-50 and control plots did not differ significantly in size. At the 20-week harvest, there was no longer a significant effect of N-amendment treatment on seedling size (Fig. 5). We were not able to relate transient differences in seedling size with differences in initial acorn size (i.e. by using initial acorn size as a covariate) because individual acorns were not weighed at the start of the germination study. We do know that acorns from the control and N-50 subplots are, on average, smaller in size (Figs 2 and 3e).

Discussion

While N deposition and its potential relevance to soil function, primary production, and carbon sequestration

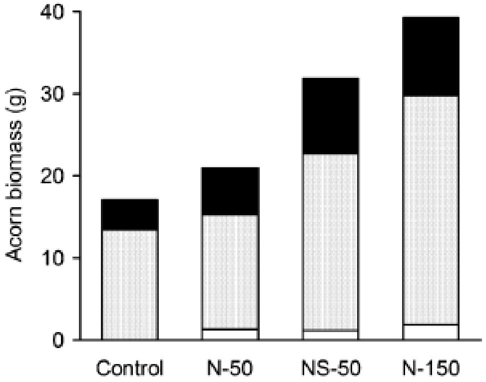

Fig. 4 From Fall 2004, biomass of undamaged and damaged acorns, with solid portion of bar indicating acorns damage by *Curculio* weevils, dotted cross portion indicating other types of damage, and open portion of bar indicating no damage.

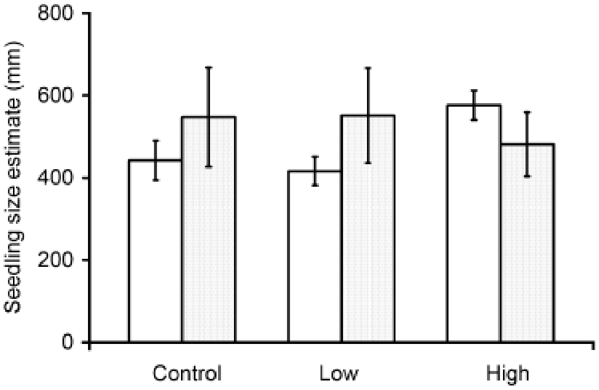

Fig. 5 Variation among control and experimental plots in mean (±1 SE) seedling size at 10 weeks (open bars) and 20 weeks (stippled bars). Greenhouse-grown seedlings were derived from acorns collected in Fall 2003.

are widely recognized (Aber *et al*., 1998, 2003; Magnani *et al*., 2007), previous studies have not addressed its potential to influence the reproductive ecology or seed ecology of forest trees. On the hardwood plots at the Harvard Forest Chronic N site, 15 + years of experimental N applications have increased the size of reproductively mature trees (Magill *et al*., 2004), and our analyses demonstrate that increases in the abundance and size of oak trees have resulted in increased acorn production, and in some instances increases in individual acorn size. During a nonmast year, experimental applications of N had a direct influence on trees that further increased acorn availability. Such N-mediated shifts in overall acorn abundance – particularly increases during nonmast years – could have a wide range of ecological consequences.

For example, such changes could interfere with the hypothesized function of masting as a predator-satiating strategy (Kelly & Sork, 2002). This hypothesis, if true, predicts a negative correlation between acorn abundance and rates of predation. Our short-term study found higher infestation rates in a postmast year, in agreement with this prediction, but this difference was confounded with a switch in how we assessed damage rates. Within a year, despite substantial among-plot variation in acorn availability, we found that acorn infestation rates were quite uniform. Longer-term data would be useful for more rigorously testing hypotheses about masting's function, and in general for investigating whether N-mediated changes in acorn availability influence the population dynamics of arthropods and vertebrates known to depend on this temporally variable resource (e.g. Jones *et al*., 1998; Ostfeld *et al*., 2006).

In recent years, many other research groups have exposed forest ecosystems to long-term experimental manipulations but only a handful of these researchers have investigated the reproductive and seed ecology of trees. LaDeau & Clark (2001, 2006) found that female cone production doubled on elevated CO_2 FACE plots initiated in an immature *Pinus taeda* plantation in North Carolina. This was mainly because of earlier onset of reproduction driven directly by the impact of CO_2 levels on tree development and indirectly by CO_2-induced differences in vegetative growth rates. Additionally, *P. taeda* seeds derived from these elevated CO_2 plots were 91% heavier and had greatly enhanced germination rates, and grew into seedlings with longer roots and more needles, but similar biomass (Hussain *et al*., 2001).

In a study of paper birch (*Betula papyrifera*) growing on FACE plots in Wisconsin, Darbah *et al*. (2007) found that both male and female flower production increased with increasing CO_2, and that elevated O_3 increased female flower production. Seedling germination was also affected, with elevated CO_2 increasing and O_3 decreasing germination rates. Seedling vigor and survivorship were also enhanced by elevated CO_2. As with the studies of *P. taeda*, these birch trees were growing in evenly aged, near-monoculture conditions rather than in a natural, mixed-age forest.

A more naturalistic study was conducted by Stiling *et al*. (2004), examining natural postfire regeneration of Florida scrub-oak forests under elevated CO_2 conditions. Among plots exposed to five seasons of either elevated CO_2 or a control treatment, they found that acorn abundance increased substantially in response to CO_2 in two dominant oak species but not in a third, subdominant species. For the two dominant species, enhanced CO_2 apparently increased reproduction directly by altering allocation patterns (acorns per twig) and indirectly by stimulating vegetative growth (number of twigs). The study also paralleled ours in finding that CO_2 had negligible or inconsistent impacts on individual acorn traits such as acorn biomass, germination rates, or rates of infestation by *Curculio* spp.

Both our results and those of Stiling *et al.* (2004) are consistent with the hypothesis that dominant oak species increase reproductive output but not offspring quality when exposed to ecosystem manipulations that enhance vegetative growth. This hypothesis requires testing in a range of forest ecosystems subject to CO_2 enhancements, N amendments, or combinations of the two, since so few studies have investigated the plasticity of reproductive and offspring traits for woody species in natural, intact ecosystems. Indeed, our study is apparently unique in examining how tree reproductive traits respond to long-term experimental increases in N deposition. Future research needs to improve our knowledge of how often relationships between growth, reproductive ecology, and seed ecology are shifted by ecosystem manipulations, and to probe the underlying basis and broader consequences of such shifts.

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