NITROGEN AVAILABILITY INFLUENCES REGENERATION OF TEMperate TREE SPECIES IN THE UNDERSTORY SEEDLING BANK

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Abstract. While we have a good mechanistic understanding of effects of light availability on tree seedling regeneration in forests, we know little of the processes controlling seedling responses to changes in nutrient availability in the understory (low light). To examine how nitrogen availability may influence patterns of seedling regeneration in mixed temperate forests in eastern North America, we investigated nitrogen impacts on the dynamics of both coniferous and broad-leaved tree species in the understory. In addition, we compared these regeneration responses across coniferous (eastern hemlock) and broad-leaved (red oak) stand types. We applied nitrogen (0, 2.5, or 7.5 g m$^{-2}$ yr$^{-1}$) to replicated understory plots in three hemlock and three red oak dominated stands, and examined seedling survival and growth for three coniferous and three broad-leaved species over two years. Nitrogen addition influenced different demographic stages in contrasting ways, leading to a complex series of seedling responses to increased nitrogen availability in the understory. Hemlock was the only species to show a positive survival response to nitrogen addition, and these effects only became apparent after the first growing season. In contrast, a suite of midsuccessional species (red maple, white pine, and red spruce) underwent nitrogen-induced declines in survival, particularly at early stages of regeneration. Both successional position and species’ habitat preferences emerged as better determinants of species’ responses to nitrogen deposition in the understory than did leaf habit (coniferous vs. broad-leaved). Effects were often, but not always, more marked in hemlock stands (characterized by very low light availability), suggesting nitrogen-induced declines in seedling abundance were likely due to an imbalance between above- and belowground resources. In the present study, we have clearly demonstrated that nitrogen availability can be an important determinant of understory seedling bank dynamics in mixed temperate forests in eastern North America. Nitrogen-induced changes in seedling performance in the understory will ultimately influence the spatial and temporal patterns of regeneration of these mixed forests.

Key words: contrasting stand types; forest understory; mixed conifer–broad-leaved forests; nitrogen availability; seedling bank; seedling regeneration; temperate forest community dynamics.

INTRODUCTION

Light is typically regarded as the main resource limiting seedling regeneration in forests (Pacala et al. 1996), and has been the focus of a large body of ecological research (reviewed in Bazzaz and Wayne 1994). However, recent studies in forests suggest that plant responses to light are often contingent on soil nutrient availability (Coomes and Grubb 2000). Disturbance events coupled with individual tree effects on the environment generate substantial spatial and temporal heterogeneity in both aboveground (light) and belowground (nutrients, water) resources (Carlton and Bazzaz 1998, Battaglia et al. 2000). While we have considerable mechanistic information on how variation in light availability affects seedling regeneration dynamics (Pacala et al. 1994, Kobe et al. 1995), we know little of the specific processes controlling seedling responses to changes in nutrient availability under forest canopies (low light). Variation in nutrient availability, even under low light conditions, could be an important influence on performance at the very early stages of a seedling’s life. These effects may in turn influence forest composition, because an important component of the regeneration strategy of many species is the development of a seedling bank in closed-canopy forest (Marks and Gardescu 1998). Nutrient effects on understory seedling regeneration will be complex, as seedling responses to soil nutrient availability will be contingent both on species’ life history traits (Reich et al. 1998b) and the surrounding resource environment (Chapin et al. 1987).

Determining seedling responses to variation in soil nutrient levels will not only improve our understanding of how natural variation in nutrient availability affects forest dynamics, but may also help to better establish impacts of the widespread human-induced fertilization of natural ecosystems (Tilman et al. 2001). Agricultural intensification and fossil fuel burning are increasing...
nutrient loading in waterways, rainfall, and atmospheric particulate matter (Galloway et al. 1995), a great proportion of which finds its way into natural ecosystems (Lovett 1994). Ammonium and nitrate inputs into temperate forests are now of particular concern, because nitrogen deposition is concentrated in highly populated areas of the world: Europe, North America, and now parts of East Asia (Holland et al. 1999). Natural ecosystems in all these temperate regions are commonly nitrogen limited (Vitousek and Howarth 1991), and nitrogen deposition might exert profound effects on the structure and function of these systems (Aber et al. 1998). A better mechanistic understanding of seedling responses to nitrogen may significantly enhance our ability to predict the impacts of increased nitrogen inputs in temperate forests.

In the present study, we investigated the influence of nitrogen availability on patterns of seedling regeneration in mixed temperate forests in eastern North America, and contrast responses in stands of differing light availability. Soil nitrogen might be a particularly important factor controlling seedling regeneration in these mixed forests, because such forests contain a mixture of evergreen coniferous and deciduous broad-leaved trees, which differ greatly in many aspects of their basic biology (Walters and Reich 1999). As evergreen species tend to exhibit more conservative patterns of nutrient use than deciduous species (Chapin 1980, Aerts 1995), and as coniferous species are typically more slow-growing initially than broad-leaved species (Bond 1989, Reich et al. 1998a), we might expect these two major groups of trees to respond differently to variation in soil nutrient availability.

As well as comparing the responses of coniferous and broad-leaved tree seedlings to changing nitrogen availability, the present study also contrasts their responses under coniferous and broad-leaved canopies. Mixed conifer–broad-leaved forests in eastern North America are commonly composed of a mosaic of either coniferous or broad-leaved dominated stands (Pastor and Mladenoff 1992). The prevalent conifer in these forests (eastern hemlock, Tsuga canadensis (L.) Carr.) frequently forms single-species stands within a matrix of broad-leaved tree species (Pastor and Broschart 1990). Light availability is significantly lower in hemlock stands than in red oak stands (dominant broad-leaved species in southern New England) (Catovsky 2000), which could in turn constrain species’ responses to increased nitrogen availability in the understory (e.g., Latham 1992, Crabtree and Bazzaz 1993, Canham et al. 1996, Finzi and Canham 2000).

Uncoupling the relative roles of light vs. nutrient availability in forest regeneration dynamics is difficult, given the experimental challenges posed by resource manipulations in forest systems. Previous studies have addressed the importance of resource interactions in forests mostly through examination of seedling responses to: (1) natural variation in resource availability (Kobe 1996, Walters and Reich 1997), (2) trenching treatments (Coomes and Grubb 1998, Lewis and Tanner 2000), and (3) different resource combinations in a common garden/greenhouse (Canham et al. 1996, Walters and Reich 2000). In the present study, we experimentally applied nitrogen in the field to manipulate nitrogen availability, a method that remarkably few contemporary studies have used (Grubb 1994, but see Fahey et al. 1998). We chose nitrogen levels that would act as a substantial perturbation to the natural nitrogen cycle. Thus, although our treatments were not designed to directly simulate the human-induced fertilization of temperate forests, the results of the experiment do provide valuable information on the mechanisms of seedling response to increasing nitrogen availability.

The current study tests the hypothesis that coniferous and broad-leaved tree seedlings show contrasting responses to nitrogen addition in the understory of mixed temperate forests, and that these responses are contingent on stand type. We used three coniferous and three broad-leaved tree species whose natural distributions overlap in southern New England (our study location). As we did not wish to confound effects of leaf habit with those related to successional position, we chose species within each group that ranged from mid- to late successional (Table 1, with data from Baker [1949]). We predicted that coniferous and late successional species would show smaller increases in growth and survival in response to nitrogen than would broad-leaved and early successional species, because their evergreen

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Leaf habit</th>
<th>Successional position</th>
<th>Seed mass (mg)</th>
<th>Seed collection years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsuga canadensis</td>
<td>eastern hemlock</td>
<td>evergreen coniferous</td>
<td>5</td>
<td>2.5</td>
<td>1996, 1998</td>
</tr>
<tr>
<td>Picea rubens</td>
<td>red spruce</td>
<td>evergreen coniferous</td>
<td>4</td>
<td>3.5</td>
<td>1996</td>
</tr>
<tr>
<td>Pinus strobus</td>
<td>white pine</td>
<td>evergreen coniferous</td>
<td>3</td>
<td>20</td>
<td>1996</td>
</tr>
<tr>
<td>Acer saccharum</td>
<td>sugar maple</td>
<td>deciduous broad-leaved</td>
<td>5</td>
<td>75</td>
<td>1996, 1998</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>red maple</td>
<td>deciduous broad-leaved</td>
<td>4</td>
<td>15</td>
<td>1997, 1998</td>
</tr>
<tr>
<td>Betula alleghaniensis</td>
<td>yellow birch</td>
<td>deciduous broad-leaved</td>
<td>3</td>
<td>0.5</td>
<td>1996, 1997</td>
</tr>
</tbody>
</table>

† Based on Baker’s (1949) table, where 1 is earliest and 5 is latest successional.
‡ Based on empirical measurements of seed samples.
§ Red maple seeds collected in spring.
habit and slow early growth rates would correlate with less flexible patterns of nutrient uptake (Bazzaz 1996, Aerts and Chapin 2000) and more conservative patterns of nutrient use (Reich et al. 1995, 1998b). We also predicted that seedling responses would be greater in the understory of broad-leaved tree stands than in hemlock stands, as the higher resource availability in broad-leaved stands should allow seedlings to take advantage of additional nitrogen to a greater extent (Latham 1992, Canham et al. 1996, Walters and Reich 2000).

**Materials and Methods**

**Nitrogen Addition Treatments**

We set up experimental plots in three hemlock- and three red oak-dominated forest stands at Harvard Forest in central Massachusetts, USA (42°32’ N, 72°11’ W, elevation 340 m). The sites were established in the Tom Swamp tract, and were chosen so that hemlock and red oak contributed over 50% of the basal area in each of three stands. Details of the stands are clearly described in an earlier paper (Catovsky 2000). At each site, we set up nine 12-m³ plots (2 × 6 m) within a 20 × 20 m square. Each experimental plot received one of three nitrogen addition treatments (0, 2.5, or 7.5 g N m⁻² yr⁻¹), with the result that each treatment was replicated three times per site. Plots were arranged in a 3 × 3 grid, and nitrogen treatments were applied in a Latin square design (each row and column received one of each treatment level). In both 1998 and 1999, plots were given nitrogen eight times per year at approximately three-weekly intervals beginning mid-April and ending mid-September. At each addition, nitrogen was applied as prilled ammonium nitrate pellets (J. T. Baker, Phillipsburg, New Jersey, USA) using a hand-held seed spreader to ensure adequate and even broadcast. After addition, each plot was given 5 L of water to partially dissolve the pellets. We chose to apply nitrogen as pellets, as preliminary observations suggested that this method substantially reduced leaf burn (pellets were too heavy to settle on leaves), and allowed for a more gradual release of nitrogen into the soil between additions. Our treatments were designed to provide a substantial perturbation to the natural nitrogen cycle, representing nitrogen treatments that added considerably to background nitrogen mineralization rates (4–8 g N m⁻² yr⁻¹; Catovsky 2000) and current levels of nitrogen deposition (0.6 g N m⁻² yr⁻¹; Munger et al. 1998) at Harvard Forest.

**Assessment of Seedling Regeneration Patterns**

Each plot was divided into three 4-m² subplots (2 × 2 m), in order to examine nitrogen addition impacts on a different demographic component of seedling bank regeneration dynamics. The soil was not scarified in any of the seedling treatments, and any establishment from seed occurred as a result of natural patterns of germination and emergence through the upper litter layers.

**Planted Seedling Plots: Growth and Survival of Established Seedlings**

Seeds of each study species were collected from multiple trees at Harvard Forest in the autumn of 1996, 1997, and 1998 (Table 1). Any seeds that were not to be used the following year were air-dried and stored at 4°C. In late autumn 1997 and 1998, seeds were placed in cloth bags and buried in trays of wet, coarse sand. These trays were placed outside throughout the winter to stratify the seeds, and were collected the following spring. In 10-cm deep germination flats, seeds were spread out evenly over a peat-based potting mix with added perlite, and then covered with a thin layer of vermiculite. These flats were then placed in shade-houses (neutral shade-cloth filtering 95% incoming PAR) in an experimental garden at Harvard University (Cambridge, Massachusetts, USA) in mid-April, and the seeds were left to germinate. The flats were monitored once every three days and watered when necessary. Seedlings began to germinate in early to mid-May. In mid-June, the seedling flats were transported to Harvard Forest. At this point, most seedlings had between two and four true leaves. In both 1998 and 1999, five seedlings of each species were transplanted into each “seedling” plot. Plots were watered immediately afterwards to prevent early transplant-related mortality. Two weeks later, any dead seedlings were replaced with new transplants. Typically, one to two seedlings of each species died per plot.

**Seed Addition Plots: Seedling Establishment from Seed in the Absence of Dispersal Limitation**

Seeds were collected in the same way as those used for the planted seedling plots. At the end of the autumn in 1997 and 1998, seeds were broadcast over each subplot using a hand-held seed spreader to ensure even spread. The number of seeds added per plot varied according to species (Table 2), and depended on seed quantity collected, probable seed rain inputs, and seed viability estimated from germination trials. Although seed addition plots were an attempt to remove effects of spatial variation in seed rain, in some cases, the background seed fall was much higher than the number of seeds added experimentally, e.g., hemlock and birch seeds in hemlock stands in 1999 (Table 2, a vs. b). In those cases, dispersal limitation may still underlie emergence patterns in contrasting stand types.

**Natural Regeneration Plots: Patterns of Regeneration from the Soil Seed Bank**

In the third of each subplot, no seeds or seedlings were added. Instead, the natural dynamics of seedlings emerging from the soil seed bank were monitored.

From June 1998 until September 1999, we took monthly censuses of seedling emergence and survival in all study plots during the growing season. Seedlings were individually tagged once they were planted or once they emerged from the seed bank, and their survival was subsequently monitored monthly during the main growing seasons of 1998 and 1999, and once after the 1998–1999 winter.
TABLE 2. Details of seed input and seedling emergence patterns for both (a) seed addition and (b) natural regeneration plots.

### a) Seed addition plots

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Stand type</th>
<th>Seeds added (seeds/m²)²</th>
<th>Emergence (seedlings/m²)³</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>1998</td>
<td>hemlock</td>
<td>100</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>100</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>250</td>
<td>18.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>250</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Picea rubens</em></td>
<td>1998</td>
<td>hemlock</td>
<td>100</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>100</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>200</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>200</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Pinus strobas</em></td>
<td>1998</td>
<td>hemlock</td>
<td>50</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>50</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>150</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>150</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Acer saccharum</em></td>
<td>1998</td>
<td>hemlock</td>
<td>50</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>50</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>75</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>75</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>1998</td>
<td>hemlock</td>
<td>100</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>100</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>250</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>250</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Betula alleghaniensis</em></td>
<td>1998</td>
<td>hemlock</td>
<td>500</td>
<td>26.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>500</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>250</td>
<td>36.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>250</td>
<td>1.33</td>
</tr>
</tbody>
</table>

### b) Natural regeneration plots

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Stand type</th>
<th>Seed rain (seeds/m²)‡§</th>
<th>Emergence (seedlings/m²)³</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>1998</td>
<td>hemlock</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>2500</td>
<td>15.40</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>1998</td>
<td>hemlock</td>
<td>75</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>45</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>12</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>10</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Betula spp.</em></td>
<td>1998</td>
<td>hemlock</td>
<td>500</td>
<td>15.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>100</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>hemlock</td>
<td>2000</td>
<td>28.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red oak</td>
<td>120</td>
<td>0.37</td>
</tr>
</tbody>
</table>

† As seed germination commonly takes place the year after seed fall, seed inputs refer to the previous year, i.e., 1997 and 1998. The temporal relationship between seed rain and emergence is less well coupled in *Acer rubrum*, as a proportion of seeds that fall in spring germinate that year.

‡ Values in boldface represent significant differences between stand types for each year–species combination (post hoc tests, \(P < 0.05\)).

§ Based on seed trap sampling. See Catovsky (2000) for full details.

Seedling biomass and foliar nitrogen measurements

In August 1999, in every planted seedling plot, leaf samples were taken from one surviving seedling of each species belonging to each seedling cohort (1998, 1999). For maple seedlings with relatively large leaves, three leaf discs (6-mm diameter) were punched. For all other seedlings, three to six leaves were removed, depending on seedling size. In these cases of unknown leaf area, samples were photocopied and leaf area subsequently calculated using NIH Image software v1.6 (NIH, Bethesda, Maryland, USA). To determine leaf chlorophyll concentrations, two to four of these leaf discs/samples were placed in 3 mL dimethyl-formamide and left for 72 h at 4°C in complete darkness (García and Nicolás 1998). Absorbance of the resulting solution at 664 and 647 nm was measured using a Spectronics 20D spectrophotometer (Spectronic Unicam, Rochester, New York, USA), and chlorophyll concentrations calculated from standard equations (Wellburn 1994). The remainder of each set of leaf samples was weighed after drying in an oven for 48 h at 70°C, and leaf mass per unit area calculated (LMA, g/m²). In this way, chlorophyll concentration could be expressed on both a leaf area and mass basis, and also at the whole-plant canopy level.

At the end of the second growing season (late Sep-
tember 1999), all remaining seedlings were harvested. All seedlings were gently extracted from the soil and washed to remove any soil. Seedlings from seed addition and natural regeneration plots were weighed whole after one week drying in an oven at 70°C. Planted seedling cohorts were additionally separated into leaves, stems, and roots.

Environmental measurements

To determine how nitrogen addition affected seedling resource environment, above- and belowground resource availability were measured for each plot during the 1999 growing season. We used methods and sampling protocols that would provide a good indication of "plant available" resources. Season-long integrated nitrogen availability was determined using ion exchange resin bags placed in each nitrogen plot from June until October 1999 (Binkley and Vitousek 1989). The bags were constructed with 1.5 tablespoons (22 mL) of mixed bed strong acid (cation) and strong base (anion) gel resins (Sybron Chemicals, Birmingham, New Jersey, USA) sealed in nylon mesh, and placed at a depth of 5 cm in the soil. After removal from the soil, 4 g of dried resin (at 70°C, overnight) was extracted with 100 mL of 2 N potassium chloride solution (25°C, 24 h), and then frozen immediately following suction-filtration. Ammonium and nitrate in all soil and resin extracts were measured using a LaChat continuous flow ion analyzer using methods 12-107-06-1-A and 12-407-04-1-B (LaChat Instruments, Milwaukee, Wisconsin, USA). Blanks were created from resin bags that had been sealed in polyethylene bags for the length of the growing season. These resins were extracted in the same way as the field bags to determine the lower threshold for detection.

Light availability was measured using canopy hemispherical photographs (Canham 1988). On a uniformly overcast day, photos were taken at 5 cm and 1 m above the ground in the center of each plot using a fish-eye lens (180°) attached to a Canon camera body (with self-timer). The camera was leveled and oriented toward north before each photo. Two-hundred speed color slide film was used to capture the photos. The resulting images were scanned into the computer with a slide scanner (500 dpi resolution) and analyzed using GLI/C software (courtesy of C. D. Canham, Institute of Ecosystem Studies, Millbrook, New York, USA). Thresholds were set manually in both blue and green spectra on each image separately, and the software used to calculate the percentage of open sky, percentage of direct beam radiation, percentage of diffuse beam radiation, and the percentage of global radiation (direct plus diffuse).

Water availability was determined by taking a soil core (2-cm diameter, 20-cm depth) from the edge of each plot. Sieved soil (2-mm mesh) was dried at 105°C for 48 h and weighed to give gravimetric soil moisture. Further soil cores (10-cm diameter, 4–12 cm deep) were taken to determine soil pH in the organic horizon. The pH of sieved soil (5.6-mm mesh) was measured on a mixture of 2 g of air-dried soil in 20 mL distilled water (pH 5.5) using an Orion 250A pH meter (Orion Instruments, Boston, Massachusetts, USA).

**Statistical analysis**

Multifactor analyses of variance were used to investigate influence of nitrogen addition on understory seedling bank development. Linear models included nitrogen addition as a continuous factor, and different combinations (plus interactions) of a variety of fixed, discrete factors (stand type, species, year, month, seedling age). Models also included a nested series of random factors. Site was nested within stand, and plot was nested within site (stand), with the result that stand mean square was tested over site, and site was tested over plot mean square. Significant multifactor interactions involving nitrogen were investigated by examining the magnitude and significance of regression slopes (dependent variable vs. nitrogen addition) using standard errors. Post hoc comparisons of means were carried out using Scheffé multiple comparisons (Day and Quinn 1989), and comparisons between slope coefficients were carried out using T', GT2, and Tukey-Kramer methods (Sokal and Rohlf 1995).

Similar linear models were used to investigate changes in allocation between roots and shoots under different nitrogen treatments, with leaf mass ratio (LMR, leaf/total biomass) and root mass ratio (RMR, root/total biomass) calculated for individual seedlings. Then, to distinguish ontogenetic changes from true allocation responses, we examined changes in Model II linear regression slopes (geometric mean regression) for the natural logarithm of root or shoot biomass against natural logarithm of total biomass, according to the method described by McConnaughay and Coleman (1999). Allometric analyses were carried out for individual species, nitrogen treatments, and stand types.

When necessary, data were transformed to ensure that the assumptions of analysis of variance were met (normality of residuals, homoscedascity). Common transformations included natural logarithm (biomass), natural logarithm of square root (emergence), and the logit function (proportional survival) (Sokal and Rohlf 1995). For figures, the level to which data were pooled across treatments was determined by the highest order significant interaction in the models.

**Results**

**Environmental conditions**

Nitrogen addition significantly increased availability of both ammonium and nitrate (Fig. 1; $F_{1,46} = 21.5$ and 44.6, respectively, $P < 0.0001$), and these effects were consistent across stand types (no N x stand interactions, $F_{1,46} < 1.9$, $P < 0.17$). In contrast, nitrogen addition did not significantly affect availability of other
Effects of nitrogen addition (white = 0, light gray = 2.5, and dark gray = 7.5 g N m\(^{-2}\) yr\(^{-1}\)) on (a) ammonium and (b) nitrate availability in hemlock and red oak stands (mean ± 1 SE), determined from mixed anion–cation resin bags.

Nitrogen effects on seedling demography

In many treatments, there were significant species × stand (× year) interactions, which followed the basic seedling dynamics observed in a parallel natural demography experiment in the same stands (Catovsky 2000). For clarity, these basic patterns are not addressed here, but are described in more detail in this additional paper. Here we report predominantly on significant effects caused by the nitrogen treatment itself.

Emergence

In seed addition plots, all study species emerged from added seed except sugar maple, which suffered high seed predation (Table 2). In natural regeneration plots, the major species to emerge from the soil seed bank were birch, hemlock, and red maple (Table 2). At these early stages of the life cycle, birch species could not be distinguished beyond genus. Nitrogen addition altered some of these natural emergence patterns, but mainly in plots where seeds were added (significant N × species × stand interaction only in seed addition plots, \(F_{4,450} = 2.52, P = 0.041\); and not in natural regeneration plots, \(F_{4,450} = 0.17, P = 0.95\)). In seed addition plots, effects of nitrogen on seedling emergence were only significant in hemlock stands (Fig. 2), where nitrogen addition enhanced birch emergence by almost three-fold (from 16 to 46 seedlings/m\(^2\)), but decreased white pine emergence.

Survival

Increasing nitrogen availability led to overall changes in first-year seedling survival in both seed addition and natural regeneration plots, but did not affect survival in planted seedling plots (no significant N × species interactions, \(F_{5,2350} = 1.31, P = 0.26\)). In seed addition plots, the differential responses of species to increased nitrogen were contingent on both stand type and year (significant N × species × stand and N × species × year interactions, \(F_{4,1815} = 3.68\) and 3.92, \(P = 0.0054\) and 0.0036, respectively). Nitrogen addition decreased first-year survival for red maple, white pine, and red spruce seedlings, but only in hemlock stands (Fig. 3a) and not in red oak stands (Fig. 3b). For both red maple and white pine, increasing nitrogen exacerbated the lower overall seedling survival in hemlock stands vs. red oak stands. Nitrogen effects were stronger for red maple and red spruce in 1998 (Fig. 3c), and for white pine in 1999 (Fig. 3d). We also found sig-
significant declines in red maple survival with increasing nitrogen in natural regeneration plots (Fig. 3e), but the effects were not contingent on stand type or year (significant N × species interaction, $F_{2,891} = 3.68, P = 0.0025$; but no significant higher order interactions, $F_{1-6,891} < 1.52, P > 0.17$ for all).

Although we were unable to detect nitrogen effects on planted seedlings in the first year, influences of increasing nitrogen appeared after the first growing season. Nitrogen significantly increased hemlock survival over the seedlings’ first winter, and led to nonsignificant declines in red maple and sugar maple survival (Fig. 4a; significant N × species interaction, $F_{5,171} = 3.06, P = 0.011$). Hemlock survival in the second growing season was also enhanced by increasing nitrogen, but only in stands dominated by hemlock trees (Fig. 4b; significant N × species × stand interaction, $F_{3,100} = 2.72, P = 0.049$). Nitrogen addition also de-
Nitrogen availability in forest understory

Figure 4. Effects of nitrogen addition (white = 0, light gray = 2.5, and dark gray = 7.5 g N·m⁻²·yr⁻¹) on survival of planted seedlings after the first growing season (mean ± 1 se). Effects are shown pooled across stand types for overwinter survival (a), and separately for second-year survival (across full length of growing season) in hemlock (b) and red oak (c) stands. Due to low seedling numbers, second-year survival for birch and sugar maple seedlings (in hemlock stands) could not be calculated. Means and standard errors were calculated from logit transformed data and then back-transformed. Values above histogram bars for each species represent slope coefficients calculated using transformed data. Slopes significantly greater than zero (P < 0.05) are shown with an asterisk.

Increased red maple seedling survival in the second growing season, but in contrast to seed addition plots, this effect was only significant in red oak stands (Fig. 4c). In hemlock stands, there was a tendency for nitrogen to decrease survival in second-year planted seedling plots for the same species that showed nitrogen-induced declines in survival in seed addition plots in the first year, i.e., red maple, white pine, and red spruce (Fig. 4b). High overall seedling mortality in hemlock stands, however, reduced sizes of seedling populations at the start of the second growing season. Low sample sizes increased variability in nitrogen effects and reduced significance of these species-specific nitrogen-induced declines. In seed addition and natural regeneration plots, mortality both within the growing season and over winter was so high that sample sizes were inadequate to examine regeneration dynamics of any second-year seedlings.

Biomass, allocation patterns, and tissue chlorophyll

By the end of 1999, only planted seedling plots contained enough seedlings to harvest. Nitrogen addition did not, however, significantly influence final seedling biomass in these plots (no significant N interaction terms, F₁⁻⁴,₅₆₀ < 0.78, P > 0.38), and the result was no different whether we considered individual seedling biomass or total plot-level biomass (F₁⁻⁴,₂₁₀ < 1.33, P > 0.25).

Although final seedling biomass was not affected by increasing nitrogen availability, seedling allocation patterns did change significantly following nitrogen addition treatments. Basic linear models of leaf and root mass ratios produced no significant N terms (F₁⁻⁴,₆₁₀ < 0.55, P > 0.46), showing that nitrogen addition had no effect on either of these measures of allocation (Table 3). However, when we accounted for size-related shifts in allometry, we were able to detect some changes in seedling allocation patterns between leaves and roots (Figs. 5 and 6). For allocation to leaves, significant effects of nitrogen were only observed when pooling across stand types (equivalent to no significant N × species × stand interaction), while there were clear differences in nitrogen-induced changes in root allocation between contrasting stand types. Red maple,

Table 3. Effects of nitrogen addition (0, 2.5, or 7.5 g·m⁻²·yr⁻¹) on seedling allocation to leaves (leaf mass ratio, g/g) and roots (root mass ratio, g/g), pooled across stand types and age classes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Nitrogen addition (g·m⁻²·yr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>a) Leaf mass ratio</td>
<td></td>
</tr>
<tr>
<td>Red maple</td>
<td>0.22</td>
</tr>
<tr>
<td>Sugar maple</td>
<td>0.24</td>
</tr>
<tr>
<td>White pine</td>
<td>0.57</td>
</tr>
<tr>
<td>Red spruce</td>
<td>0.53</td>
</tr>
<tr>
<td>Hemlock</td>
<td>0.47</td>
</tr>
<tr>
<td>b) Root mass ratio</td>
<td></td>
</tr>
<tr>
<td>Red maple</td>
<td>0.44</td>
</tr>
<tr>
<td>Sugar maple</td>
<td>0.47</td>
</tr>
<tr>
<td>White pine</td>
<td>0.22</td>
</tr>
<tr>
<td>Red spruce</td>
<td>0.22</td>
</tr>
<tr>
<td>Hemlock</td>
<td>0.26</td>
</tr>
</tbody>
</table>
white pine, and red spruce all showed significant increases in allocation to leaves (when accounting for size) in response to nitrogen addition (Fig. 5), and particularly significant decreases in allocation to roots, but only in hemlock stands (Fig. 6).

Species also demonstrated some flexibility in leaf composition in response to increasing nitrogen availability in the understory. Nitrogen addition altered species’ foliar chlorophyll concentrations (on both an area and mass basis), although the effects were contingent on stand type (significant N * species * stand interaction, \( F_{4, 397} = 3.01 \) for area and 3.48 for mass, \( P = 0.019 \) and 0.009, respectively). In red oak stands, increasing nitrogen availability led to significantly increased foliar chlorophyll for red maple, red spruce, and hemlock (Fig. 7b). In hemlock stands, only white
pine and hemlock showed significant increases in chlorophyll concentration, while red maple showed a significant decline (Fig. 7a). In contrast, whole-plant chlorophyll and the ratio of chlorophyll $a$ to $b$ were both unaffected by nitrogen treatments ($F_{1,4,222} < 1.33$, $P > 0.26$ for all N effects).

**DISCUSSION**

**Nitrogen effects on understory seedling bank dynamics**

Demographic impacts of nitrogen availability in the understory of mixed conifer–broad-leaved forests were
Effects of nitrogen addition on foliar chlorophyll concentrations (mean ± 1 SE, pooled across seedling ages), expressed on a leaf area basis, in both (a) hemlock and (b) red oak stands. Values above histogram bars for each species represent slope coefficients produced from linear models. Slopes significantly greater than zero \((P < 0.05)\) are shown with an asterisk.

**Influence of stand composition**

The contingency of species’ responses to nutrient availability might be particularly marked in the forest understory, as canopy trees each create a unique combination of resource conditions beneath them (Boettcher and Kalisz 1990, Vesterdal and Raulund-Rasmussen 1998). In the present study, seedling responses to nitrogen addition were often strongly dependent on canopy composition (hemlock vs. red oak stands). Environmental measurements in this current study and in an earlier study (Catovsky 2000) clearly demonstrated that both light availability and pH were significantly lower in hemlock stands than in red oak stands. In the current experiment, we found that often, but not always, these differences in resource environment increased species’ sensitivity to nitrogen addition in hemlock stands compared with red oak stands. Birch emergence was only enhanced by nitrogen addition in hemlock stands, while nitrogen-induced declines in survival of mid-successional species were more marked in general in hemlock stands. Thus, rather than increasing shade tolerance of other potential seedling bank species (e.g., Kobe 1996), such as red maple and white pine, nitrogen addition actually accentuates current seedling dynamics in hemlock stands in favor of a hemlock positive feedback.

**Mechanisms underlying seedling responses to nitrogen**

Contrary to our hypothesis, successional position emerged as a more important determinant of species’ responses to nitrogen than did leaf habit (coniferous...
FIG. 8. Summary of main seedling demographic responses to increased nitrogen addition under each experimental treatment, showing species-specific effects that were significantly positive, +; significantly negative, −; or marginally negative, (−) (determined from regression coefficients). All other nonsignificant changes are indicated by NS. If effects were contingent on stand type or year, they are shown separately. When data were not available, the box is shaded gray.

vs. broad-leaved). For example, red maple and red spruce (similar tolerance ranking, but contrasting leaf habit) both showed similar declines in survival following nitrogen addition. Hemlock was the only species whose survival was increased by nitrogen addition in the forest understory, and also represents the only study species that is able to persist for decades in the deeply shaded forest understory as a sapling (Kobe et al. 1995). However, our results do not give overriding support to the importance of successional position in determining nitrogen responsiveness, due to (1) shortage of data for the other late successional species in the study (shortage of sugar maple data was due to poor overall seed and seedling survival), and (2) the somewhat positive response of yellow birch to nitrogen (emergence only).

In these forests, leaf habit is not well correlated with species’ habitat preferences, perhaps explaining the lack of predictive power provided by this species’ trait for understanding responses to nitrogen availability. In fact, species’ site preferences emerged as an equally parsimonious explanation for our results than did their shade tolerance class. For example, two of the main species that showed negative nitrogen responses in our experiment (red maple and white pine) are typically found on poorer soils in New England forests than a number of our other study species (e.g., hemlock and yellow birch) (Whitney 1991). In addition, red maple
has lower foliar nitrogen concentrations than many co-occurring forest species, again supporting the notion that it has a preference for lower nutrient sites (Abrams 1998). Successional position and species’ nutrient requirements may interact with one another in complex ways to determine seedling responses to nitrogen in the understory (e.g., Mitchell and Chandler 1939, Spurr 1956). Clearly, establishing the underlying physiological mechanisms for these responses will provide the best method for untangling these effects.

In the present study, hemlock was the only species to respond positively to nitrogen addition under low light conditions, and was the only species to show consistent increases in leaf chlorophyll concentrations with nitrogen (although no species showed significant increases in whole-plant chlorophyll). Species differ in the degree to which low light availability may constrain their responsiveness to nutrients (Walters and Reich 1996, 1997, Catovsky and Bazzaz 2000), and late successional species might have a better capacity to respond to increased nutrient availability under low light scenarios than earlier successional species (Walters and Reich 1997, 2000, Finzi and Canham 2000). For less shade-tolerant species, manufacturing additional chlorophyll in low light environments may represent a metabolic cost that is not offset by the resulting small increase in carbon gain.

In contrast, the more marked nitrogen-induced declines in survival of a number of species in the understory of hemlock stands when compared with red oak stands strongly suggest that resource imbalance may be driving the negative effects of nitrogen on seedling regeneration. In hemlock stands, where light availability is particularly low, nitrogen addition is more likely to create an imbalance between above- and belowground resource availability. Nutrient amendment has been shown to reduce seedling survival at low light availability (Grubb et al. 1996), although the actual mechanism has still not been clearly established. In the present study, we found that the species whose survival significantly declined in nitrogen addition plots showed the greatest allocational flexibility in response to nitrogen. Red maple, red spruce, and white pine all allocated more biomass to leaves and less biomass to roots with increasing nitrogen availability.

From an optimality standpoint, this strategy appears beneficial (Bazzaz 1997). As nitrogen becomes less limiting, plants allocate less belowground, and increase their leaf area ratio to improve their capacity for carbon gain aboveground (Peace and Grubb 1982). The greater allocation flexibility of the earlier successional species in the present study agrees with ecological theory on succession (Bazzaz 1996), and has been previously found for seedlings of these specific species growing under varying combinations of light and soil resources (Canham et al. 1996). This flexibility in response is often advantageous for responding to rapid increases in resource availability, but may be detrimental in a forest understory where resources are severely limiting. The reasons for shifts in allocation leading to increased mortality are not clear in this case, but could be related to seedlings allocating carbon to improve growth potential rather than survival (Walters and Reich 2000). Kobe (1997) has shown that more shade-tolerant species had higher concentrations of nonstructural carbohydrates in their roots than less tolerant species, and suggested that a trade-off exists between growth and survival. An alternative hypothesis is that species that allocate less biomass to roots in response to nitrogen addition could be more sensitive to drought episodes (Canham et al. 1999).

Implications for forest dynamics

The structure and dynamics of temperate forests are currently threatened by a suite of novel human-induced perturbations, such as increased nutrient loading, increased frequency of biological invasions (e.g., hemlock woolly adelgid), changing human land-use patterns, and altered atmospheric chemical composition (Foster et al. 1997). Predicting the exact nature of any future impacts is challenging given the uncertainties in the magnitude and timing of these changes. A multiple resource perspective is useful in this regard, as many novel perturbations involve changes in seedlings’ resource environment (Field et al. 1992, Bazzaz and Catovsky 2001). If we can understand how species behave along a suite of resource axes, we should be able to predict patterns of forest dynamics under a range of future change scenarios.

We have clearly demonstrated that nitrogen availability can be an important determinant of understory seedling bank dynamics in mixed temperate forests in eastern North America. Seedling responses to soil nitrogen levels were a complex function of individual species’ traits and local resource environment (light availability, mostly). Differential survival responses to nitrogen availability increased the success of hemlock seedlings in the understory relative to other common seedling bank species, such as red maple and white pine, whose survival declined following nitrogen addition. These nitrogen-induced changes were often stronger when hemlock dominated the canopy, and thus suggest that resource imbalance may be driving effects on seedling regeneration. For many tree species, maintaining a seedling bank is a critical component of their regeneration strategy, and thus nitrogen-induced changes in seedling performance in the understory will ultimately influence the spatial and temporal patterns of regeneration of these mixed temperate forests.

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LITERATURE CITED


