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Non-random species loss in a forest herbaceous layer following nitrogen addition

Christopher A. Walter,[1,5] Mary Beth Adams,[2] Frank S. Gilliam,[3] and William T. Peterjohn[4]

[1]*Department of Ecology, Evolution, and Behavior, University of Minnesota, 100 Ecology Building, 1987 Upper Buford Circle, Saint Paul, Minnesota 55108 USA*
[2]*Northern Research Station, USDA Forest Service, 180 Canfield Street, Morgantown, West Virginia 26505 USA*
[3]*Department of Biological Sciences, Marshall University, One John Marshall Drive, Huntington, West Virginia 25755 USA*
[4]*Department of Biology, West Virginia University, 53 Campus Drive, Morgantown, West Virginia 26506 USA*

Abstract. Nitrogen (N) additions have decreased species richness (*S*) in hardwood forest herbaceous layers, yet the functional mechanisms for these decreases have not been explicitly evaluated. We tested two hypothesized mechanisms, random species loss (RSL) and non-random species loss (NRSL), in the hardwood forest herbaceous layer of a long-term, plot-scale, fertilization experiment in the central Appalachian Mountains, USA. Using a random thinning algorithm, we simulated changes in species densities under RSL and compared the simulated densities to the observed densities among N-fertilized (+N), N-fertilized and limed (+N+L), and reference (REF) plots in regenerating forest stands. We found a lower *S* in the +N treatment across all survey years and determined that the reduction in *S* was a function of NRSL. Furthermore, non-random effects were observed in certain species, as they occurred at densities that were either higher or lower than expected due to RSL. Differential advantages were also observed among species between +N and +N+L treatments, suggesting that species responded to either the fertilization or acidification effects of N, though no consistent pattern emerged. Species nitrophily status was not a useful trait for predicting specific species losses, but was a significant factor when averaged across all treatments and sampling years. Our results provide strong evidence that declines in *S* in the forest herbaceous layer under N fertilization are due largely to NRSL and not simply a function of species rarity.

Key words: competitive exclusion; fertilization; nitrogen deposition; simulation; species diversity; temperate forest; understory.

Introduction

A negative relationship between nitrogen (N) inputs and plant species richness has been reported in many ecosystems (De Schrijver et al. 2011). This relationship has been widely observed in grasslands (Stevens et al. 2004, Dupre et al. 2010), heathlands (Phoenix et al. 2012, Southon et al. 2013), and, to a lesser extent, the herbaceous layer of forest ecosystems (Gilliam 2006, Hurteau and North 2008). Fewer studies, however, have investigated the mechanisms responsible for N-mediated declines in the species richness of plant communities. Since excess N addition is a global threat to ecosystems (Sala et al. 2000), likely contributing to an unprecedented level of species extinction (Tilman et al. 2001), understanding how N decreases species richness is critical for developing strategies to preserve biodiversity (Suding et al. 2005).

Globally, N availability constrains primary productivity (Vitousek and Howarth 1991), and N additions typically increase plant productivity by alleviating N limitation (LeBauer and Treseder 2008). The relationship between productivity and species richness is often unimodal, where richness is highest at an intermediate level of productivity (i.e., the "hump-backed model"; Grime 1973, but see Adler et al. 2011). There are two primary mechanistic hypotheses that explain why species are lost under N fertilization at the highest levels of productivity. The non-random species loss hypothesis (NRSL) states that species that are superior in nutrient acquisition, growth rate, and other growth strategies will displace species with inferior ability (Newman 1973, Tilman 1984, Wilson and Tilman 1993). With increased soil fertility, the superior species indirectly suppresses the growth of the subordinate species and different mortality rates between the two emerge. In contrast, the random species loss hypothesis (RSL) contends that mortality is equal among all species, and that the change in species composition under increased fertility is an effect of enhanced density-dependent mortality, where uncommon species are lost by chance (Goldberg and Miller 1990, Oksanen 1996, Stevens and Carson 1999). Neither NRSL nor RSL are necessarily mutually exclusive and the degree to which either mechanism alters community composition varies across systems (Suding et al. 2005), scales (Gross et al. 2000), and sites (Gough et al. 2000, Clark et al. 2007).

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[5] E-mail: walte732@umn.edu

Beyond increasing productivity, N additions also have the potential to acidify soil, which, in turn, can decrease the availability of base cations in the soil and increase the mobility of toxic metals (Vitousek et al. 1997). Thus, nitrophilic species may be able to either utilize excess N to outcompete non-nitrophilic species (Hautier et al. 2009), withstand the secondary effects of soil acidification (Schuster and Diekmann 2003, Peppler-Lisbach and Kleyer 2009), or both simultaneously. Distinguishing a biotic response between fertilization vs. acidification effects of N additions is difficult because multiple, interacting soil factors may be changed with N additions that can confound expected plant responses (Schaffers and Sykora 2000), altering the degree to which NRSL and RSL mechanisms may affect species richness.

Most research testing NRSL and RSL mechanisms on species richness has been done in grassland and old-field communities (Thomas et al. 1999): herb-dominated communities with relatively low species richness at broad scales. Whereas these studies have helped spur changes in plant community theory (Fraser et al. 2014), their results may not be generally applicable to forested systems. In contrast to grasslands and old fields, herbaceous layers of hardwood forests are species-rich communities of mostly perennial herbs, canes, graminoids, woody shrubs, and tree seedlings. Additionally, competition within this community for light, water, and nutrients occurs both within the herbaceous layer and between herbaceous layer plants and overstory trees (Gilliam and Roberts 2014, Neufeld and Young 2014). Community changes in the herbaceous layer of forests are of critical importance for forest managers interested in protecting biodiversity, because this forest stratum is responsible for more than 80% of plant species richness in hardwood forests (Gilliam 2007). Yet, to our knowledge, no tests of NRSL vs. RSL hypotheses have explicitly been carried out in a hardwood forest herbaceous layer.

Accordingly, the objectives of this research were to (1) determine the extent of N-mediated changes in plant density and species richness, diversity, and evenness; (2) explicitly test whether the NRSL or RSL mechanisms were responsible for the changes in those community metrics; (3) separate the effects of N fertilization from those of acidification; and (4) understand the effect of nitrophilic species on community composition under experimental N fertilization in a hardwood forest herbaceous layer. To meet these objectives, we analyzed long-term data collected from a plot-scale fertilization experiment located in the central Appalachian Mountains, USA.

Materials and Methods

Study area and sampling

This research was carried out at the Fernow Experimental Forest (FEF) in West Virginia, USA, in the long-term soil productivity experiment (LTSP; 39.0563° N, 79.6979° W). The FEF is a 1,902-ha research area that primarily contains Appalachian mixed mesophytic forest (Kochenderfer 2006). The LTSP is a 4 plot × 4 block randomized design that includes three experimental treatments and one uncut area in each block (Appendix S1: Fig. S1). For the purpose of this research, the uncut area was not examined. The three experimental treatments were whole-tree harvested (removal of all aboveground biomass) in the winter of 1996–1997 (Adams 2004). Since 1997, four plots have been fertilized at a rate of 35 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ with ammonium sulfate, applied by hand (+N), another four plots have been fertilized with ammonium sulfate at the same rate and limed at a rate of 22.5 kg $Ca^{++}{\cdot}ha^{-1}{\cdot}yr^{-1}$ with dolomitic lime (+N+L), and four plots have been allowed to regrow naturally with no experimental additions and are used as the reference in this experiment (REF). Each plot is ~ 0.37 ha and contains a 0.2 ha area in which measurements are made (a 7.6-m treated buffer surrounds each plot). The only recorded disturbance over the duration of this study was a microburst in December of 2009 that damaged the tree canopy. Qualitative measurements of canopy damage from the microburst showed that effect of the storm was to create canopy openness between 25% and 45% when averaged across the LTSP treatments (Peterjohn 2016*a*). No canopy measurements were made prior to the microburst, however, we assume that the canopy was closed.

The N-fertilization rate in LTSP was chosen to match the adjacent whole-watershed fertilization experiment (Watershed 3) at FEF, which began in 1989, and the long-term watershed fertilization experiment in the Bear Brook watershed in Maine. The fertilization rate is also approximately equal to three times the ambient rate of deposition in 1989. The lime addition rate in LTSP was chosen to match the loss of Ca++ in Watershed 3 in response to N fertilization. The mean wet deposition rate for total inorganic N over the duration of this research was 5.4 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ (National Atmospheric Deposition Program 2017), and total cumulative wet-deposited N at FEF is estimated to be 300 kg/ha since 1900 (W. T. Peterjohn, *unpublished data*). Tension lysimeter measurements taken over the duration of the LTSP experiment have revealed that soil water nitrate concentrations in +N and +N+L treatments are about three times greater when compared to the REF treatment (Peterjohn 2016*b*). Measurements of soil carbon (C) to N ratio in REF are 14.2, 16 in +N, and 15.1 in +N+L (Fowler 2015). Higher C:N values in N-fertilized treatments are likely a result of changes in soil microbial communities, lower organic matter decomposition rates (k) overall, or a function of the buildup of more recalcitrant carbon from a lower k at later stages of decomposition. Results from potential net N mineralization from a lab incubation of organic-horizon soil in 2015 reported mean N mineralization rates of 3.97 and 5.58 $\mu g\ N{\cdot}g\text{-}soil^{-1}{\cdot}d^{-1}$ for REF and +N, respectively (rate for +N+L was unavailable; J. E. Carrara, *unpublished data*).

Herbaceous layer sampling in 1996 and 1997 was done at 16 equally distant reference points within each plot. Within each subplot, each plant <1 m in height was identified to species and counted; individuals were defined as stems growing from the soil. For the years 2001, 2006, and 2011, sampling occurred within five 1-m circular subplots selected randomly from the 16 reference points. A total of 20 1-m^2 subplots (5 subplots × 4 plots) were sampled in each treatment during those years. Sampling was done between the months of June and July in 1996 (prior to treatment), and during the same months in 1997, 2001, 2006, and 2011.

Community metrics

Total plant density and species richness, diversity, and evenness were calculated in each subplot for each sampling year. Total plant density (D) was calculated as the sum of all individuals per 1 m^2, species richness (S) was defined as the total number of species per 1 m^2, diversity (H') was calculated using the Shannon-Weaver index, and evenness was calculated using Pielou's evenness metric (J; Hill 1973). To examine differences in D, S, H', and J, a two-way analysis of variance (ANOVA; with model effects of treatment, year, and treatment × year) was used at the subplot level, testing each metric for the effect of both treatment and year, and the differential effect between the two factors. Although testing the community metrics at the subplot level had the drawback of pseudo-replicating within plots and ignoring block effects, it had the advantage of increasing the initial sample size required for a robust bootstrap procedure (see *Non-random vs. random species loss*). Tukey's HSD test (THSD) was used to determine pairwise differences in means among years, among treatments, and among both factors simultaneously. Since the 1996 sampling period occurred prior to treatment, the ANOVA and THSD tested the sampling years 1997, 2001, 2006, and 2011. To test for any pre-treatment differences in D, S, H', and J among the treatments in 1996, a one-way ANOVA (with the model effect of treatment) and THSD were used to analyze the 1996 sampling year. Assumptions of normality and homoscedasticity were tested in ANOVA residuals and transformations were applied when necessary. The community metrics were all calculated using R package vegan (Oksanen et al. 2015); ANOVA analyses were performed using SAS JMP (SAS Institute 2015).

To further examine changes in the herbaceous layer community among treatments and across years, nonmetric multidimensional scaling (NMDS) was used. Distance matrices were calculated among treatments for each sampling year using Bray-Curtis dissimilarity calculations and iteratively plotted in an algorithm to maximize the correlation between calculated and plotted distances. Once the algorithm reached a solution, the NMDS axis scores for each treatment were averaged for each year and change vectors were calculated and plotted based on the Cartesian direction between years. To better visualize the effect of N fertilization and liming on plant community changes, the mean NMDS coordinate scores in REF for each year were also subtracted from the mean coordinate scores of the +N and +N+L treatments and the difference between the scores was plotted on NMDS axes. The stress value (an overall measure of fit; see Appendix S1: Fig. S2 for Shepard diagrams) was 0.19 in 1997, 0.2 in 2001, and 0.17 in 2011. The NMDS analysis was done using R package vegan (Oksanen et al. 2015).

Non-random vs. random species loss

To test the non-random (NRSL) vs. random (RSL) species loss hypotheses, a simulation of random thinning replicated what would occur if RSL was the primary mechanism determining composition in the herbaceous layer. More specifically, an algorithm was used to randomly thin all plants from each plot from their density in 1997 to their density in a later sampling year (2001, 2006, or 2011). The thinning simulation was then repeated multiple times to obtain a bootstrap distribution of the RSL density of each species (simulated distribution; D_S). Then, D_S in each plot was compared to the observed species density within the plot (a single value, the observed density, D_O). The difference between D_O and D_S is a distribution of differences between observed and simulated species losses and was denoted as δ. The δ value could then be determined for each species and if the mean δ was positive for a species, then there was evidence that the species was conferred some advantage by the treatment. Likewise, a negative δ indicated a disadvantage for a species. Differences in mean δ should be expected in the herbaceous layer of an early successional forest, as many factors could confer advantages or disadvantages among species (e.g., light and water). To understand how additions of N specifically affected these advantages and whether losses were due to random or non-random processes, differences between mean δ among treatments were tested for each species. The exact simulation approach we used was modified from Stevens and Carson (1999), and consisted of the following steps:

1). Twenty subplots were selected randomly, with replacement.
2). Within each subplot, individual plants were randomly selected from the total community of plants within the subplot in 1997, without replacement. The number of randomly selected plants was determined by the D_O of that same plot in 2001 (hence, simulated thinning of the subplot from the observed density in 1997 to the observed density in 2001). The plants that were randomly selected represented the remaining community after RSL. In cases where D_O in 1997 was less than D_O in 2001, individuals were randomly added to the subplot in the simulation, based proportionally on the community that was present in 1997.

3). The density of each remaining species was calculated for each subplot.
4). The simulation was repeated 15,000 times to create a distribution for each treatment of the mean density of each species (simulated random distribution; D_S).
5). Since differences between D_O and D_S are expected during early succession, the differences among the treatments between D_O (single value) and D_S (distribution with 15,000 values) were calculated:

$$\delta_{tmt1} = [D_{O_{tmt1}} - D_{S_{tmt1_i}}] \text{ resulting in 15,000 values of } \delta_{tmt1}$$

$$\delta_{tmt2} = [D_{O_{tmt2}} - D_{S_{tmt2_i}}] \text{ resulting in 15,000 values of } \delta_{tmt2}.$$

And then the mean differences were compared using a probability test

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{tmt1_i}}{n} < \frac{\sum_{i=1}^{n} \delta_{tmt2_i}}{n} \text{ then } p = 1 - \frac{\sum_{i=1}^{n} [\delta_{tmt1_i} < \delta_{tmt2_i}]}{n}$$

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{tmt1_i}}{n} > \frac{\sum_{i=1}^{n} \delta_{tmt2_i}}{n} \text{ then } p = 1 - \frac{\sum_{i=1}^{n} [\delta_{tmt1_i} > \delta_{tmt2_i}]}{n}$$

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{tmt1_i}}{n} = \frac{\sum_{i=1}^{n} \delta_{tmt2_i}}{n} \text{ then } p = 1$$

where tmt_1 and tmt_2 are two treatments for comparison and i denotes iteration number, n is the number of iterations, and p is the probability that the means are different.

6). The entire process was repeated for 2006 and 2011, using the initial density in 1997 for each year.
7). To control for the potential of type I errors in multiple comparisons, we used a sequential Bonferroni test on the P values from step six across all species, treatments, and years (Holm 1979, Rice 1988).

The thinning simulation was performed using R (R Core Team 2015) and R package vegan (Oksanen et al. 2015).

To test for differences between RSL and NRSL after the simulation, Stevens and Carson (1999) compared simulated mean S to the observed mean S. To compare these means, Bonferroni-corrected confidence intervals of simulated S were calculated, and means of observed S that fell outside of the confidence intervals were considered to have a significant portion of species loss due to non-random effects (Stevens and Carson 1999; Appendix S1: Fig. S3). However, since their comparison occurred over one year in an old-field, they did not encounter any species additions. In contrast, our experiment spanned 15 years and included multiple species additions during that time period. Therefore, a new method of examining the effects of RSL and NRSL that accounted for species additions was warranted.

To determine the effect of RSL and NRSL on species richness (S), we modified the species equilibrium theory equation (MacArthur and Wilson 1967) to divide species losses within a treatment into losses from RSL, and losses from NRSL

$$\Delta S_{t_0-t_x} = G_{t_0-t_x} - \left(L_{\mathrm{RSL}\,t_0-t_x} + L_{\mathrm{NRSL}_{t_0-t_x}}\right)$$

where $\Delta S_{t_0-t_x}$ is the change in S from 1997 to time x (2001, 2006, or 2011), $G_{t_0-t_x}$ is the input of new species from 1997 to time x, $L_{\mathrm{RSL}\,t_0-t_x}$ is the mean difference between observed S in 1997 and the expected S under RSL at time x (loss of species due to RSL), and $L_{\mathrm{NRSL}_{t_0-t_x}}$ is the mean number of species lost due to NRSL from 1997 to time x. Using this estimate, and noting that a net reduction in S is a negative quantity, the equation can be modified to solve for the number of species lost due to NRSL

$$L_{\mathrm{NRSL}_{t_0-t_x}} = G_{t_0-t_x} - L_{\mathrm{RSL}\,t_0-t_x} - \Delta S_{t_0-t_x}$$

This equation makes two assumptions: (1) that the error in detecting a species is equal to the error in not detecting a species in a treatment, and (2) that populations are undergoing RSL and NRSL simultaneously (see Appendix S1: Fig. S4 for an example iteration). To test if the contribution of NRSL to species losses varied among treatments, $L_{\mathrm{NRSL}_{t_0-t_x}}$ was compared using confidence intervals from the bootstrapped distribution. Specifically, $L_{\mathrm{NRSL}_{t_0-t_x}}$ was calculated using the modified equilibrium equation for each simulation iteration (n = 15,000) in each treatment; thus, $L_{\mathrm{NRSL}_{t_0-t_x}}$ is the product of a bootstrap distribution. In each sampling year, mean $L_{\mathrm{NRSL}_{t_0-t_x}}$ values in each treatment were compared to 95% confidence intervals of other treatments in a pairwise manner to determine significant differences. Family-wise error correction was avoided with such a small number of tests because of the inflation of the type-II error rate increases the likelihood of falsely reporting that mean species loss due to non-random effects was equal among treatments (Saville 1990).

Nitrophilic species

Since a plant trait database of nitrophily does not exist for the United States, in order to examine the presence and performance of nitrophilic species, we used published information to assign a nitrophily status to each species we found in LTSP (Appendix S2: Table S1). Where possible, we used species-specific experimental or observational results from the eastern North American hardwood forest region. If regional results were not available, species specific results from other regions were used. In many cases, we used the nitrophilic classification scheme for European plants: the Ellenberg index (Hill et al. 1999). The Ellenberg index assigns species to a number from 1 to 9 based on their affinity for N (9 being the highest level of nitrophily). Some of the species in the LTSP were listed in the Ellenberg index and, in those cases, we used the

published Ellenberg value. Some species were not in the Ellenberg index, but their congeners were. In those cases, the median Ellenberg nitrophily score of all congeneric species was assigned to an LTSP species. Since we relied on the Ellenberg index frequently, species whose nitrophily status was determined from studies other than Hill et al. (1999) were subjectively assigned an Ellenberg nitrophily scores based on their published response to N.

To test whether nitrophily status was a useful predictor of RSL or NRSL, the nitrophily index values were treated as a binary nominal variable. Species with index values >5 were categorized as nitrophilic, and species with index values ≤5 were categorized as non-nitrophilic. This step of assigning nitrophily into two categories was undertaken to help overcome the lack of species-specific nitrophily information (i.e., using congeners in nitrophily status assignment) and the subjective classification of non-Ellenberg listed species that were found in the published studies. A three-way Sheirer-Ray-Hare extension of the Kruskal-Wallis test (SRH; with model effects of nitrophily (NI) treatment (TMT), year (Y), NI × TMT, NI × Y, TMT × Y, and NI × TMT × Y) was used to compare differences in mean δ values between nitrophilic and non-nitrophilic species, among the treatments, and among the years 2001, 2006, and 2011. This test essentially functions as an ANOVA for ranked data and needed to be applied because of the severe positive kurtosis in the distribution of mean δ values. A THSD of the final model was also used to test for differences in mean δ between nitrophilic and non-nitrophilic species among treatments and years. We were unable to determine the nitrophily status for three species, *Zanthoxylum americanum*, *Podophyllum peltatum*, and *Streptopus lanceolatus*, and these species were excluded from the SRH and THSD tests.

Results

Community metrics

Prior to the beginning of treatment (1996 sampling), there were no differences among treatments in density (*D*), richness (*S*), diversity (*H*′), or evenness (*J*) among treatments. For the years following the beginning of experimental treatments, the effect of the various treatments on *D* did not depend on year. However, the experimental treatments did have an effect on *D* ($F_{2, 228} = 4.78$, $P = 0.0093$), as did year ($F_{3, 228} = 26.44$, $P < 0.0001$). Across all years, *D* was 25.9% lower in the fertilized (+N) treatment than in the reference (REF) treatment ($t = 3.64$, $P = 0.0131$), and 22.1% lower in the fertilized and limed (+N+L) treatment when compared to the REF treatment ($t = 2.46$, $P = 0.0392$). When averaged across all treatments, *D* decreased 49.9% between 1997 and 2001 ($t = 6.75$, $P < 0.0001$), 55.7% between 1997 and 2006 ($t = 7.52$, $P < 0.0001$), and 55.1% between 1997 and 2011 ($t = 7.44$, $P < 0.0001$; Fig. 1).

The effect of LTSP treatment on richness (*S*) did not depend on sampling year. However, treatment alone had an effect on *S* ($F_{2, 228} = 4.24$, $P = 0.0155$), as did year ($F_{3, 228} = 5.28$, $P = 0.0015$). When averaged across years, *S* declined by 13.6% in +N when compared to REF ($t = 2.91$, $P = 0.0109$; Fig. 1). When averaged across treatments, *S* declined by 13.3% from 1997 to 2001 ($t = 2.62$, $P = 0.0463$), by 13.5% from 1997 to 2006 ($t = 2.65$. $P = 0.0430$), and by 19.8% from 1997 to 2011 ($t = 3.85$, $P = 0.0009$).

There were neither differential nor main effects of treatment or year on species diversity (*H*′; Fig. 1). With respect to species evenness (*J*), there was marginal

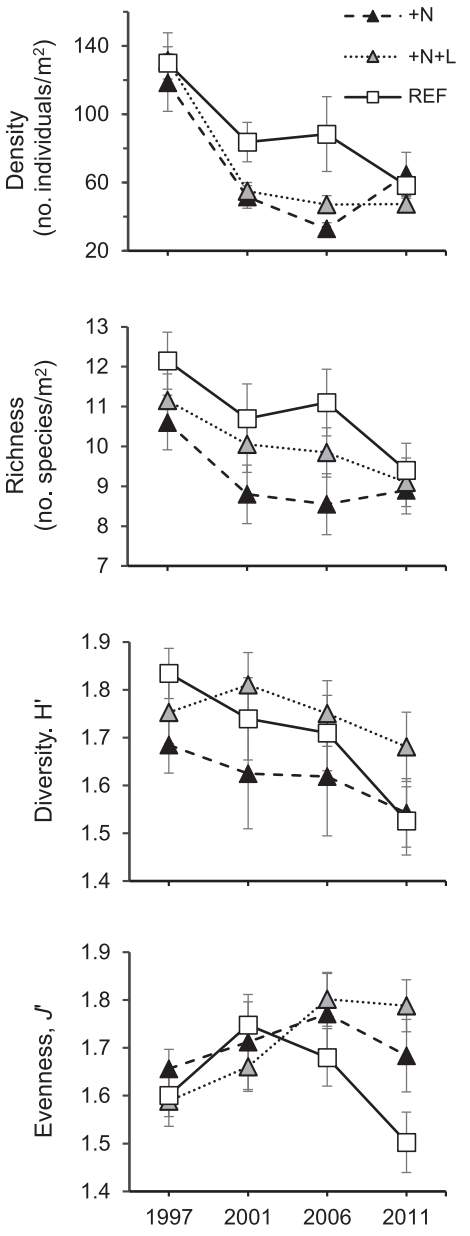

Fig. 1. Species metrics in the reference (REF), N-fertilized (+N), and N-fertilized and limed treatments (+N+L). Values are mean ± SE.

evidence overall that the effect of treatment depended upon the year ($F_{6,228} = 1.92$, $P = 0.0783$). When averaged across years, there was also marginal evidence that treatment had an effect on J ($F_{2,228} = 2.83$, $P = 0.0614$). However, there was an effect of year when averaged across treatments ($F_{3,228} = 3.86$, $P = 0.0102$). Specifically, J was 9.1% higher in all treatments in 2006 when compared to 1997 ($t = 3.23$, $P = 0.0076$).

Among treatments, plant communities in +N, +N+L, and REF treatments appeared to follow similar patterns of variation across years (Fig. 2a). Removing the expected community variation from successional dynamics and other potential environmental factors revealed that the +N and +N+L treatments had similar impacts on the community composition of the herbaceous layer in the first three sampling periods. However, a divergence in communities appeared in 2011 between +N and +N+L treatments (Fig. 2b).

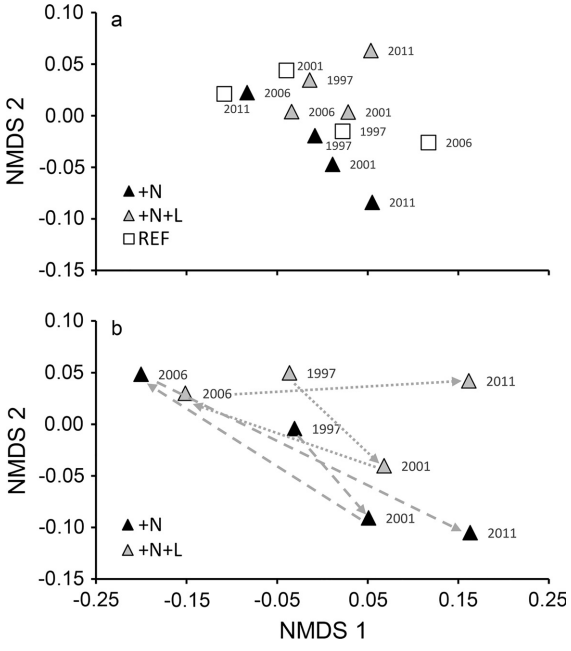

FIG. 2. Nonmetric multidimensional scaling (NMDS) of (a) plots of plant species communities in reference (REF), N-fertilized (+N), and N-fertilized and limed (+N+L) treatments and (b) the effect of the +N and N+L treatments after accounting for the variation in the REF treatment.

Non-random vs. random species loss

Species losses across all years and treatments ranged from 0.8 to 2.5 and, in all cases, the loss of species was due primarily to NRSL (Table 1). All treatments experienced species loss in any one sampling year, but there were differences among treatments in the percentage of species lost due to NRSL (Fig. 3). In 2001, the percent loss due to NRSL was lowest in the +N+L treatment: 29.9% lower than +N and 18.5% lower than REF. In 2006, the percent loss due to NRSL in +N+L was 14.6% lower than +N, but there were no differences between +N+L and REF. By 2011, the pattern had shifted among treatments, and there were no longer differences between +N and +N+L treatments. Instead, REF had a higher percent of species loss due to NRSL than both +N (12.3% lower) and +N+L (16.9% lower).

There was also evidence that +N and +N+L treatments affected NRSL at the species level. By comparing the difference between simulated and observed species abundances (δ), we determined if species were advantaged by one treatment over another (significantly larger mean δ values), or disadvantaged (significantly smaller mean δ value). There were 32 species with significantly different mean δ values among treatments in at least one sampling year (Appendix S2: Tables S2–S4), and 12 of those species had mean δ values ≥ |1| among treatments in at least one sampling year (Fig. 4). For the species conferred the largest advantages or disadvantages (species with the highest δ values; δ > |1| for one or more years), the differences in δ among treatments did not follow clear patterns. The treatment effects on the difference

TABLE 1. Variables used in the calculation of species richness due to non-random species loss (NRSL) and random species loss (RSL) and the percent contribution of NRSL to species losses in reference (REF), fertilized and limed (+N+L), and fertilized plots (+N).

	2001			2006			2011		
Metric	REF	+N+L	+N	REF	+N+L	+N	REF	+N+L	+N
S_{1997}	11.9	11.2	11.0	11.9	11.2	11.0	11.9	11.2	11.0
S_{t1}	10.7	10.1	8.8	11.1	9.9	8.6	9.4	9.1	8.9
$\Delta S_{1997\text{-}t1}$	−1.2	−1.1	−2.2	−0.8	−1.4	−2.5	−2.5	−2.1	−2.1
No. species additions ($G_{1997\text{-}t1}$)	4.1	3.6	3.3	5.3	4.6	4.3	4.9	4.3	4.5
Expected species loss due to RSL ($L_{\text{RSL }1997\text{-}t1}$)	0.8	1.6	0.2	1.1	1.4	0.6	−0.2	0.9	0.6
Expected S due to RSL alone	9.9	8.5	8.6	10.0	8.5	8.0	9.6	8.2	8.3
Estimated species loss due to NRSL ($L_{\text{NRSL }1997\text{-}t1}$)	4.5	3.1	5.3	4.9	4.6	6.2	7.6	5.5	5.9
Species loss due to NRSL (%)	84.9^{A}	66.4^{A}	96.3^{B}	81.2^{AB}	77.1^{A}	91.7^{B}	102.7^{A}	85.8^{B}	90.4^{B}

Notes: S denotes species richness and t_1 = year indicated in column. Differing letters indicate significant differences in means within the same sampling year. The percentage greater than 100 occurred because the mean species richness predicted from the bootstrap simulations was greater than the observed species richness.

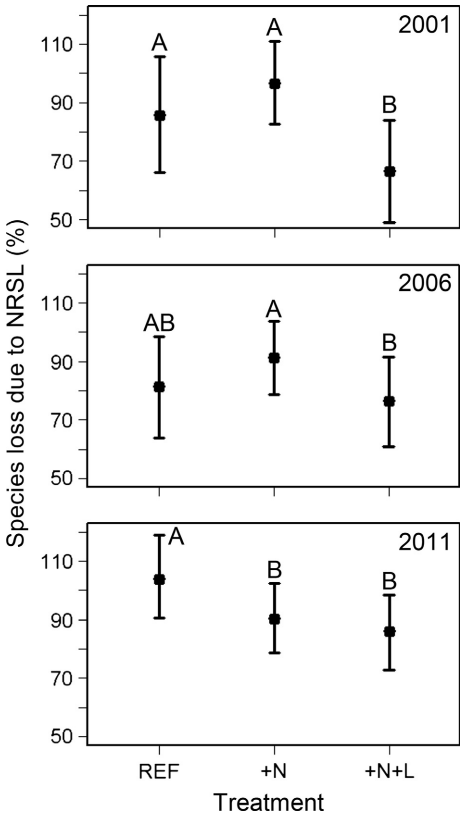

FIG. 3. Percentage of the total species loss (mean) that was due to non-random species loss (NRSL) among treatments and sampling years. Error bars indicate 95% confidence intervals, differing letters indicate significant differences in means within the same sampling year. Values greater than 100% occurred when the species richness predicted from the bootstrap simulations was greater than the observed species richness.

between simulated and observed density among *Thelypteris noveboracensis*, *Prunus pensylvanica*, and *Acer rubrum* diverged through time. Conversely, the δ values of *Betula alleghaniensis*, *Ageratina altissima*, and graminoids converged though time. Other species, like *Quercus rubra*, *Glycyrrhiza lepidota*, and *Galium* spp. tended to have large deviations in δ in 2006 relative to the values in 2001 and 2011 (Fig. 4). Overall, any effect of treatment on mean δ values was dependent on the species, with no obvious universal pattern of treatment effects among species.

Nitrophilic species

Differences in mean δ between nitrophilic and non-nitrophilic species were detected when averaged across all treatments and all years ($H = 5.92$, $P = 0.0149$). Nitrophilic species collectively had a density that was 0.14 individuals per m^2 higher than expected under the RSL simulation. Although non-nitrophilic species grew at a density of 0.33 individuals/m^2 less than expected under RSL, the differences between simulated and observed density of nitrophilic and non-nitrophilic species was neither dependent on treatment nor year.

DISCUSSION

Our results show that N additions to a hardwood forest herbaceous layer reduced both plant density (*D*) and species richness (*S*), and that non-random species loss (NRSL) was the mechanism for reductions in *S* (Table 1). In fact, the species loss equation revealed that the losses across all treatments were due to NRSL. Furthermore, among individual species, there were instances where the density of a particular species deviated significantly from the simulated density predicted by random species loss (RSL): evidence that certain treatments either favored or inhibited a species density. Such deviations from the predicted RSL are consistent with NRSL. Tests of the bootstrap simulation on individual species found that 41.8% of the species present in the sampling years from 2001 to 2011 were either more or less dense than predicted under RSL in at least one sampling year.

Studies of the effects of N in grassland and old-field systems have discovered evidence for species loss due to both NRSL (Hautier et al. 2009) and RSL (Stevens and Carson 1999), and evidence for both occurring simultaneously (Suding et al. 2005). Research testing the two mechanisms in a fertilized coniferous forest understory determined that RSL was the mechanism responsible for declines in *S* (Thomas et al. 1999). Discrepancies in results among these studies, and ours, are likely due to the variety environmental factors that affect plant density and the collection of plant functional types that are present at each site (Suding et al. 2005). Published studies vary widely with respect to ecosystem type, N-fertilization amounts, land-use history, and cumulative N load: all factors that could affect NRSL and RSL mechanisms. Additionally, the secondary effects of N fertilization, like shifts in soil microbial communities (Johnson et al. 2003), increases in plant litter accretion (Foster and Gross 1998, Lamb 2008) and changes in herbivory, pathogenic infections, and earthworm activity (Gilliam 2006) can cause differential competitive advantages or disadvantages that contribute to NRSL.

One, or many N-induced, indirect environmental changes could explain the differences in advantages and disadvantages we observed at the species level in the hardwood forest herbaceous layer (Rajaniemi 2003). However, the dominant resource competed for under N additions is likely light (Hautier et al. 2009, DeMalach et al. 2016), as competition among species shifts from belowground nutrient acquisition to aboveground light acquisition (Newman 1973, Tilman 1987). Therefore, changes in density of species in response to N additions is due mainly to increased competition for light, and not to other indirect effects of N. Results from a field experiment on the nitrophilic *Rubus allegheniensis* at the Fernow Experimental Forest (FEF) support this idea. Walter et al. (2016) found that, at high light levels, N

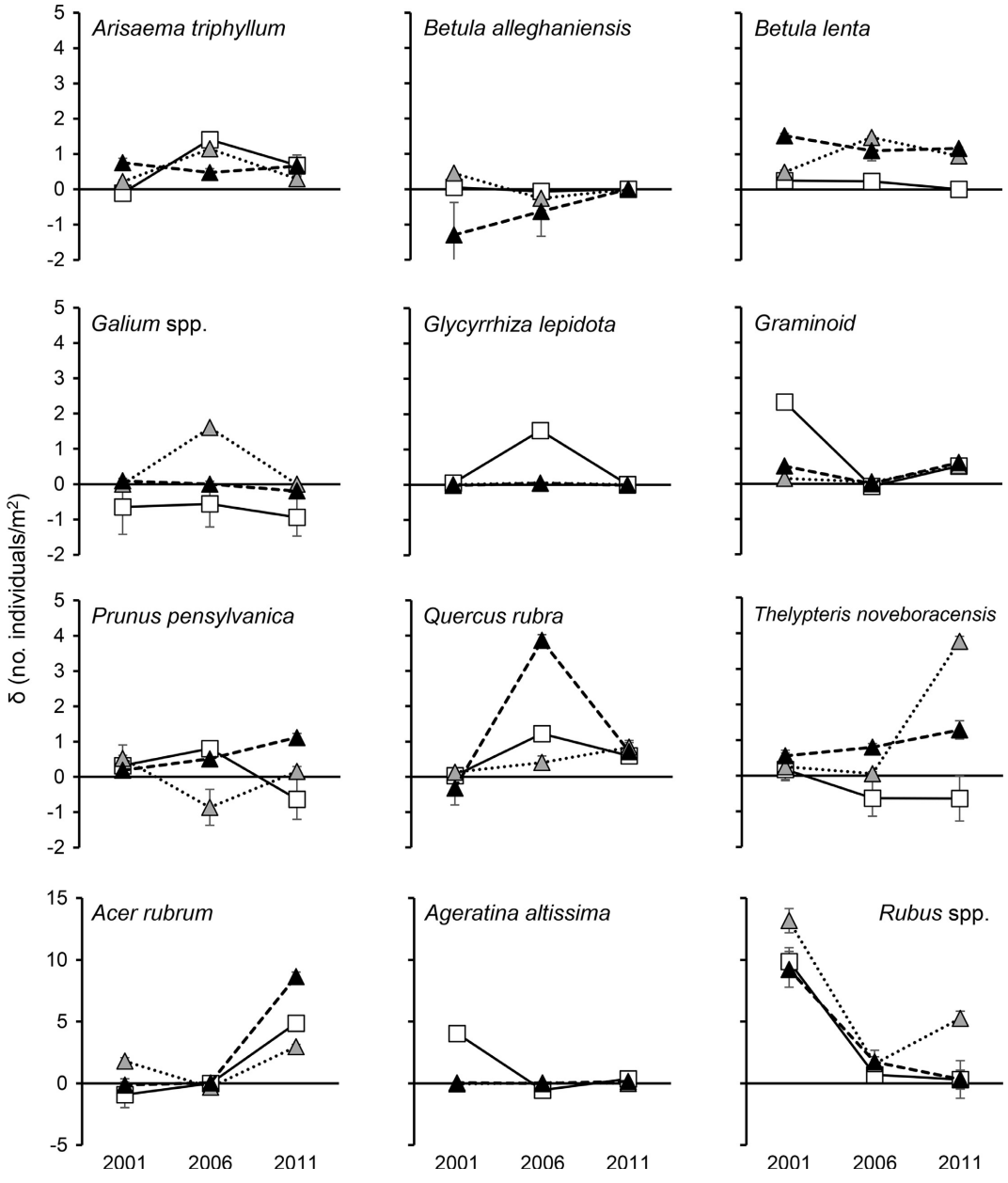

FIG. 4. Difference in simulated vs. observed density (δ) for the 12 species with δ values >1 in at least one sampling year among reference (REF), N-fertilized (+N), and N-fertilized and limed treatments (+N+L). Values are mean ± SE.

fertilization caused a substantial increase in the leaf area of *Rubus allegheniensis*, all without changes in herbivory, earthworm activity, or obvious pathogenic infections.

Previous studies of forest herbaceous layers typically have found N-induced reductions in species diversity (Strengbom and Nordin 2008, Hedwall et al. 2011, Gilliam et al. 2016). In contrast, we found no evidence that species diversity decreased after 15 years of N fertilization. We did find marginal evidence that evenness (J) was higher in +N+L than REF treatments across all years, signifying that N fertilization and liming may maintain higher J. In an adjacent fertilized watershed at Fernow Experimental Forest, Gilliam et al. (2016) did not observe a decrease in H' in the forest herbaceous layer until ~25 years of fertilization at 35 kg $N \cdot ha^{-1} \cdot yr^{-1}$. This suggests that a similar decrease in H' in response to N fertilization in the Long Term Soil Productivity (LTSP) Experiment may not be realized until ca. 2021. However, differences in stand age between the fertilized watershed and LTSP may create differences in N uptake, which may have an effect on H' in the herbaceous layer.

Gilliam (2006) predicted in the nitrogen homogeneity hypothesis that the dominance of nitrophilic species should cause both J and S to decrease after chronic N additions, leading to a decrease in H'. One alternate explanation for why there was no difference in H' among treatments may be a drastic increase in light from damage to the forest canopy in LTSP during a microburst in December 2009. Since competition for light is a major factor influencing diversity under N additions (Hautier et al. 2009), it is likely that storm-induced increases in light at the forest floor stimulated enhanced competition for light across all LTSP treatments. Since the soil nutrients in +N were undergoing homogenization with N additions, the 2009 storm disturbance likely created a higher and more homogenous light environment, which could have led to the observed maintenance of species evenness in fertilized treatments in 2011 (Fig. 1), driving higher H'. There is also evidence that tree damage from the 2009 microburst was greater in N-fertilized plots (Walter et al., *in review*), which may have led to differences among treatments in post-storm effects on the herbaceous layer.

Regarding species loss, such losses were more random in +N+L relative to both +N and REF treatments in 2001, and again more random in 2006 when compared to +N (Fig. 3). It is possible that additions of lime equalized competition by increasing niche space (Silvertown 2004). When competition becomes increasingly equal among species, the proportion of species losses via random processes should increase (Silvertown 2004, Pearman et al. 2008). In 2011, species losses in both +N and +N+L were more random relative to REF. We suspect that the increase in NRSL in REF in 2011 was related to the relatively low J in REF during that year (Fig. 1), and possibly a result of the microburst (see *Materials and Methods*) that disturbed the canopy in December 2009. If belowground competition decreased in +N and +N+L treatments by the addition of N, community composition since 1996 was likely changing to favor species with superior aboveground competition (i.e., growth; Rajaniemi 2003). However, the community composition in REF would still be determined by both below- and aboveground competition. The microburst that opened the canopy likely also caused a flush of soil nutrient availability with the increase in disturbance-related litterfall (Vitousek 1985, Prescott 2002). As a consequence of sudden nutrient addition from the canopy disturbance, the herbaceous community in REF may have shifted abruptly from 2006 to 2011 to favor aboveground competitors, leading to NRSL and a decline in J. However, fertilization in +N and +N+L treatments may have already changed community composition by that time to favor aboveground competitors. Thus, the effect of the microburst-opened canopy may not have led to a shift in species composition or a decline in J in +N and +N+L, resulting in a lower portion of species loss due to NRSL in those treatments relative to REF.

Unlike most studies of effects of N on plant communities (Stevens and Carson 1999, Clark et al. 2007, Dupre et al. 2010, Southon et al. 2013), the design of the LTSP allows for separation of the acidification and fertilization effects of N additions. The addition of lime had no effect on plant density that was different than +N alone. However, liming did maintain S such that it was not different than REF. Surprisingly, there were no detectable trends across years and among species on how liming contributed to advantages or disadvantages with N addition (Fig. 4). Some species were advantaged by the addition of lime relative to N alone, while others were disadvantaged or exhibited no response. Effects were also not consistent across years within a species. However, the overall community composition of the +N and +N+L treatments appeared to diverge in 2011 (Fig. 2).

Nitrophily status as a functional trait was not useful for predicting specific species losses among treatments. Since the nitrophily index was created explicitly for this research from previous papers with a variety of N treatments and observation studies, misclassifications of species could have occurred. However, nitrophily status was a significant factor when averaged across both treatments and years, indicating that it may be useful for broad classification at the community level. After 15 years of experimental treatments, *Acer rubrum* and *Rubus* spp. were the herbaceous layer species with the largest differences between simulated and observed densities (Fig. 4). The advantage from N in *Acer rubrum* is somewhat surprising considering it was identified as a non-nitrophilic species. However, the advantage of N in *Acer rubrum* was realized in 2011, after storm disturbance increased light availability and at a time when *Acer rubrum* in all treatments appeared to benefit from the increased light. Additionally, the observed advantages from N additions in both *Acer rubrum* and *Rubus* spp. is consistent with observations from a nearby, fertilized watershed at FEF (Gilliam et al. 2016) and in response to N additions in boreal forests (Strengbom and Nordin 2008, Hedwall et al. 2011).

Overall, this research demonstrates the substantial role of non-random species loss under N additions in the forest herbaceous layer. As previously suggested by Gilliam (2006), N-induced changes in a hardwood forest herbaceous layer are a function of the advantages conferred to few nitrophilic species, a function that would lead to non-random species loss. Our results suggest that species losses are indeed governed by these non-random effects, wherein certain species are conferred advantages and disadvantages from N additions. Thus, in aggrading forests receiving similar rates of atmospheric N deposition, species loss is not simply a function of species rarity.

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