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Nitrogen fertilization interacts with light to increase *Rubus* spp. cover in a temperate forest

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Abstract Nitrogen additions have caused species composition changes in many ecosystems by facilitating the growth of nitrophilic species. After 24 years of nitrogen fertilization in a 40 year-old stand at the Fernow Experimental Forest (FEF) in Central Appalachia, USA, the cover of *Rubus* spp. has increased from 1 to 19 % of total herbaceous-layer cover. While *Rubus* spp. are generally associated with high-light conditions that are created after a disturbance event, some species are also known to be nitrophilic. We investigated whether the increase in cover in *Rubus* spp. was due to either nitrogen, light, or an interaction between these two factors. To test for the effect of nitrogen and light on *Rubus* spp. cover, we compared the relative cover of *Rubus* spp. among fertilized and unfertilized watersheds and among fertilized and unfertilized experimental plots, using estimates of canopy openness as a covariate. *Rubus* spp. plants were also grown ex situ in a field experiment using a 2-way factorial design, measuring leaf area, and using two levels of nitrogen and three levels of light. The effect of nitrogen fertilization on relative *Rubus* spp. cover depended on canopy openness in the watersheds ($F = 17.57$, $p = 0.0002$) and experimental plots ($F = 25.04$, $p = 0.0047$). A similar effect for leaf area was also observed among plants grown in the field experiment ($F = 4.12$, $p = 0.0247$). Our results confirm that, although *Rubus* spp. at FEF are nitrophilic, they require sufficient light to increase their cover. Furthermore, the dominance of *Rubus* spp. in the herbaceous layer likely contributes to the observed decline in species diversity.

Keywords Nitrogen deposition · Herbaceous layer · Fertilization · Forest understory · *Rubus* · Bramble

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Introduction

Plant community changes in response to nitrogen (N) amendments have been widely observed in grasslands and heathlands (Phoenix et al. 2012; Southon et al. 2013), but less commonly in forest herbs (Gilliam 2006). However, the herbaceous layer (defined as vascular plants <1 m above the ground) comprises, on average, more than 80 % of the total plant species richness in forests (Gilliam 2007). Existing studies on forest herbaceous-layer communities in response to increased N deposition have documented a general decline in the cover of many species and an increase in the cover of nitrophilic species (Dirnböck et al. 2014; Suding et al. 2005). Furthermore, a negative relationship between species richness and N availability has been reported in many ecosystems (De Schrijver et al. 2011; Field et al. 2014). Nitrogen additions can change the herbaceous-layer community by increasing the likelihood of mortality in all species and simultaneously select for survival and growth of nitrophilic species (Abrams et al. 1995; Grime 1979; Rajaniemi 2002).

In the Central Appalachian Mountains at the Fernow Experimental Forest (FEF), chronic N fertilization has changed the species composition of the forest herbaceous layer in favor of one particular genus. In a fertilized watershed within the forest, the relative cover of *Rubus* spp. (the percent of total herbaceous-layer cover that is *Rubus* spp.) has significantly increased concomitantly with a substantial decrease in species diversity (Gilliam et al. in press). Increases in *Rubus* spp. at other sites (hereafter referred to as *Rubus*) are mainly attributed to increases in light (Landhausser et al. 1997) and this genus is often dominant in recently disturbed areas (Hughes and Fahey 1991; Peterson and Pickett 1995; Peterson and Carson 1996). However, many species of *Rubus* are classified as nitrophilic (Hill et al. 1999) and forest disturbances that enhance light availability to the forest floor typically increase N availability (Vitousek and Melillo 1979). Vegetation surveys at other sites have also documented an increase in *Rubus* cover in response to N additions (Brunet et al. 1998; Falkengrengrerup 1993; Kellner 1993), and N fertilization in large quantities has indirectly increased the amount of light received by the herbaceous layer through tree leaf and branch mortality (Magill et al. 2004). Therefore, increased N availability could both directly and indirectly affect the dominance of *Rubus*, and it seems equally likely that increases in the cover of *Rubus* could be primarily the result of more light, more available N, or an interactive effect between these two factors.

There is experimental evidence that a combination of both N and light are important in *Rubus* germination and growth. Jobidon et al. (1989) observed that the application of mulch in a clearcut balsam fir-spruce (*Abies balsamea*, *Picea mariana*) forest—a practice designed to decrease soil-available N—decreased the cover, frequency, and leaf nitrogen content of *Rubus idaeus*. In a separate study, N fertilization without canopy disturbance in a mature balsam fir-spruce forest stimulated germination of dormant *Rubus idaeus* seeds (Jobidon 1993). However, because of very low light under the closed canopy, the *Rubus idaeus* seedlings that emerged survived less than one year after germination (Jobidon 1993). These results suggest the nitrophilic nature of *Rubus*, and underscore the importance of canopy openness to their survival.

Given previous observations on the effect of both N and light on the growth of *Rubus*, the purpose of this study was to determine if the effect of N on *Rubus* cover depends on the light level in (i) the forest herbaceous layer of both fertilized and unfertilized treatments, and (ii) among transplanted plants grown in a smaller scale experiment. These questions were examined at FEF in two long-term fertilization experiments—utilizing the natural variation in canopy openness—and among *Rubus* plants that were transplanted and grown ex situ at a farm site in both fertilized and unfertilized soils, and with different levels of artificial shading to experimentally control differences in both N fertilization and light.

Methods

Study sites and experimental design

The FEF is a 1902-ha research forest located in the Allegheny Mountain physiographic province of north-central West Virginia, near the town of Parsons (Kochenderfer 2006). Within FEF, two watersheds and a long-term replicated experiment were chosen to carry out this study. Watershed 3 (WS3; 34 ha) was clearcut between 1969 and 1972 and is currently the site of a whole-watershed fertilization study that was initiated in 1989. Since then, 35 kg N ha^{-1} $year^{-1}$ in the form of ammonium sulfate has been applied to the watershed annually by aircraft (Adams et al. 2006). Watershed 7 (WS7; 24 ha) was clearcut in two stages from 1963 to 1967 and maintained barren with herbicide until 1969. Since 1969, WS7 has been allowed to recover naturally and serves as the unfertilized reference for WS3 in this study. To control for differences in aspect between watersheds, areas within both WS3 and WS7 were classified based on three aspect strata: 1—“northeast,” 30–90°; 2—“south,” 150–210°; and 3—“northwest,” 270–330°. In each watershed, eighteen 10-m radius plots were randomly chosen from an existing network of study sites in order to establish six plots for each of the three aspect classifications. Within each plot, five 1-m^2 circular subplots were randomly selected based on polar coordinates to measure the herbaceous-layer cover and averaged together in the analysis to the plot-level. We defined the herbaceous layer as all vascular plants that were growing one meter or less above the soil surface or less (Gilliam and Roberts 2014).

The Long-Term Soil Productivity Experiment (LTSP) is a randomized block design that includes four plots of each fertilized and unfertilized treatments (Adams 2004). Each plot is ~0.37 ha and contains a 0.2 ha area in which measurements are made (7.6 m treated buffer around each plot). All aboveground biomass was removed (whole-tree harvesting) in both the unfertilized (WT) and the fertilized plots (WT + NS) in LTSP in 1996. Since then, WT + NS plots have been treated with 35 kg N ha^{-1} $year^{-1}$ as ammonium sulfate, applied by hand. In the LTSP, the four replicate plots of each treatment (WT, WT + NS) were used. Within each of these plots, four 1-m radius subplots were randomly located to measure *Rubus* cover. Since the entire LTSP experiment shares the same aspect, no stratification based on aspect was necessary. In both the watershed and LTSP experiments, variation in canopy cover was assumed to be caused by ordinary forest dynamics. We also assumed that differences in soil N availability were not directly affected by canopy openings (e.g., treefalls which increased soil N)—a potential factor-on-factor interaction.

To test the relationship between N fertilization and canopy openness in a controlled setting and to mitigate any potential factor-on-factor interaction, *Rubus* plants were grown ex situ in a two-way factorial experiment with two levels of N fertilization and three levels of shade. *Rubus allegheniensis* rhizomes were collected on May 27, 2014 from an untreated area adjacent to the LTSP plots and grown in full sunlight at the West Virginia University Agronomy Farm (39.6606°N, 79.9046°W; Sadhu 1989). After the rhizomes were taken from FEF, they were shaken free of soil, trimmed of fine roots, and weighed. The rhizomes were then randomly assigned a treatment and planted in 12.7 cm wide × 18.4 cm tall circular, plastic pots. The potting soil was a 2:1 mixture of PRO-MIX BX (sphagnum moss, perlite, and vermiculite) and Turface MVP (clay soil conditioner). To prevent the pots from drying quickly, the entire pot was buried so that the top of the pot was level with the top of the soil in the farm field. The pots were planted randomly in a 4 × 15 grid with a 1.5 m space between each pot to prevent shading between the plants. Sixty plants were initially planted—ten receiving each treatment—but some rhizomes never sprouted canes and others died while sprouting at the beginning of the experiment. The final number of replicates for each treatment was five for low-shade/low-N, nine for low-shade/high-N, nine for medium-shade/low-N, six for medium-shade/high-N, six for high-shade/low-N, and seven for high-shade/high-N (Fig. 1). The rhizomes were planted on May 29 and the plants were harvested on July 30, 2014.

The shade levels in the field experiment were achieved by placing wire cages above the pots and covering all sides of the cages with shade cloth. The shade cloth levels were selected based on the nominal percentage of direct light that they block and were used to simulate the broad range of light levels from canopy openings that are found in both WS3 and WS7. The low-light level used 90 % shade cloth, medium-light

		Light level		
Nitrogen level		**Low** (90%)	**Medium** (60%)	**High** (30%)
	High (1244 μM N)	n = 9	n = 6	n = 7
	Low (100 μM N)	n = 5	n = 9	n = 6

Fig. 1 Experimental design, treatment groups, and sample sizes used in the two-way field experiment. The values within light treatment indicate the percentage of ambient light purportedly blocked by the shade cloth, and the values within nitrogen treatment indicate the amount of nitrogen delivered at each of the 10 fertilizer applications

level used 60 % shade cloth, and the high-light level used 30 % shade cloth. The actual light levels achieved by these treatments were measured using HOBO pendant light sensors, model UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). Sensors were placed randomly in two pots of each shade treatment and one sensor was placed in full light to measure ambient levels. The light intensity was measured in Lux over 25 days by each of the sensors and the mean intensity recorded for each light treatment level was compared to the mean intensity measured for ambient light. These measurements revealed that the low-light level received 5 % of ambient light, the medium-light level received 11.4 % of ambient light, and the high-light level was received 50 % of ambient light. The two N fertilization levels in the factorial experiment were designed to match the soil N availability in both the unfertilized WS7 (low N) and the fertilized WS3 (high N). Nitrogen was applied to the plants using a nutrient solution modified from Johnson et al. (1957; Appendix 1) and the low N level was half of the concentration of 200 µM N found in the soil water of a reference area at FEF (Edwards et al. 2006). The nutrient solution in the high N level was the same except that it included an additional 35 kg N ha^{-1} as ammonium sulfate over the duration of the experiment—the same amount of fertilizer that WS3 receives annually. The nutrient solution was delivered to the plants in ten separate 500-ml applications over the course of the experiment. Therefore, in each application, the low N level plants received 100 µM of N, and the high N level plants received 1244 µM of N (Fig. 1).

Forest experiment measurements

Plant cover was measured in each subplot by comparing the area of the plant with the area of an observer's hand. Observers estimated the cover of herbs by placing a hand, palm-down and fingers closed, directly above the foliar surface of a plant. The observer then determined the size of the leaf in relation to their hand. The units of measure were "hands" and observers worked in pairs to estimate cover separately, then averaged their estimates together to improve precision through active feedback (Wintle et al. 2013). This method proved to be very precise when hand-measured leaves were regressed against the same leaves measured using a leaf area meter (average $R^2 = 0.94$ for individual *Rubus* plants; Walter et al. 2015). Two categories of plant cover were measured in each subplot—the cover of *Rubus*, and the total cover of all herbaceous-layer plants. The vast majority of *Rubus* plants at FEF are *R. allegheniensis*, but *Rubus idaeus* has also been observed. Since *Rubus* species hybridize and can be difficult to identify in the field, *Rubus* cover was measured on plants identified at the genus level. The relative *Rubus* cover was calculated as the fraction of all herb cover in a plot that was *Rubus*. To determine the effect of canopy light on *Rubus* cover in both the watersheds and LTSP plots, we relied on the strong association between the amount of photosynthetically active radiation that reaches the forest floor and canopy openness (Becker et al. 1989; McCarthy and Robison 2003; Rich 1990). A spherical densiometer was used to measure the canopy openness inside each of the 1-m^2 subplots. One densiometer reading was taken in each of the cardinal directions in the subplot and averaged to estimate the mean canopy openness. The relative *Rubus* cover and canopy openness were measured in the watersheds and LTSP WT and WT + NS treatments in June 2012.

Field experiment measurements

To test for differences in plant cover, the leaf area of each potted *Rubus* plant was measured using a leaf area meter (LI-3100, LI-COR, Nebraska, USA). To determine if N fertilization led to an increase in leaf chlorophyll and/or leaf N, the relative leaf chlorophyll content was estimated using a SPAD meter (SPAD-502, Spectrum Technologies, Aurora, IL, USA). SPAD was measured on the terminal leaflet of five leaves on each plant and averaged to obtain a mean SPAD value for each plant. SPAD measurements are unit-less, and provided a non-destructive, relative measure for leaf chlorophyll and nitrogen content. To measure leaf N concentrations directly, the leaves of each plant were dried, ground, and analyzed for their N content using a Carlo Erba NCS elemental analyzer, model NA 1500. Total leaf N for each plant was then calculated by multiplying the concentration of leaf N (% N by mass) by the total leaf mass per plant.

Statistical analysis

To test if the effect of N fertilization on *Rubus* cover depended on canopy openness in the forest measurements, a two-way analysis of covariance (ANCOVA) was performed for the two watersheds (WS3 and WS7) and both LTSP treatments (WT and WT + NS). The order of the relationship (linear vs. polynomial) between canopy openness and relative *Rubus* cover in the ANCOVA models was determined by choosing models with the lowest corrected Akaike information criterion statistic. One-way analysis of variance tests (ANOVA) was used to compare the mean relative *Rubus* cover and mean canopy openness between the watersheds and LTSP treatments. To test if the effect of N fertilization on *Rubus* leaf area depended on the light level in the ex situ field experiment, a two-way ANCOVA was used to test for differences in leaf area, SPAD, percent leaf N, and total leaf N. Initial rhizome mass was used as a covariate in the ANCOVA models to correct for any contributions to growth from larger rhizomes. Student's *t* tests were used for post hoc pairwise comparisons of means because family-wise error correction in multiple comparison tests inflates the probability of Type II errors (Saville 1990) and because of the relatively small number of comparisons. All statistical analyses were performed using SAS JMP (SAS Institute 2013). Transformations to normalize residuals and independent samples ANOVA tests were applied when appropriate.

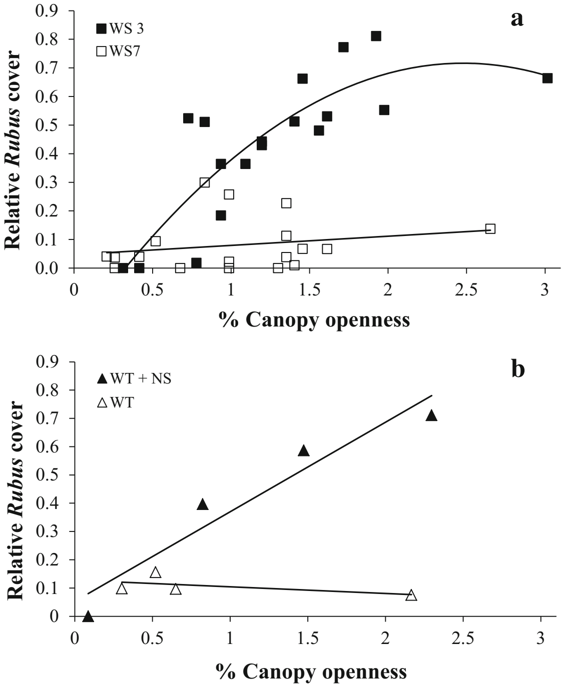

Fig. 2 The relative *Rubus* cover (the fraction of herbaceous layer cover in a plot that is *Rubus*) in a fertilized (WS3) vs. unfertilized (WS7) watershed (A) and in fertilized (WT + NS) and unfertilized (WT) treatments in LTSP (B) vs. canopy openness as measured by a densiometer

Results

Forest experiments

The effect of N fertilization on relative *Rubus* cover in the watersheds depended on canopy openness ($F = 17.57$, $p = 0.0002$). Specifically, the mean relative *Rubus* cover in WS3 was 84.2 % higher than in WS7 at the highest level of canopy openness, but equal at the lowest level of canopy openness (Fig. 2a). The best fit ANCOVA model included watershed (WS), canopy openness (C), WS × C, C^2, and WS × C^2 effects. The effect of canopy openness on relative *Rubus* cover in the LTSP treatments (WT compared to WT + NS) was also dependent on fertilization ($F = 25.04$, $p = 0.0047$). At the highest canopy openness, the relative *Rubus* cover was 85.7 % higher in WT + NS when compared to the WT, but equal at the lowest level of canopy openness (Fig. 2b). The best fit ANCOVA model for LTSP included the effects of treatment (T), C, and T × C. Overall, the mean relative *Rubus* cover was higher in both WS3 ($t = 5.71$, $p < 0.0001$) and the LTSP WT + NS treatment ($t = 2.03$, $p = 0.0444$) when compared to their unfertilized counterparts, WS 7 and LTSP WT. However, the average canopy openness did not differ between the fertilized and unfertilized watersheds nor between the LTSP treatments.

Field experiment

The effect of N fertilization on *Rubus* leaf area per plant depended on the light level ($F = 4.12$, $p = 0.0247$). The initial rhizome mass also had a significant positive effect on leaf area ($F = 5.46$, $p = 0.0253$). Post hoc comparisons using *t* tests determined that leaf area at the high-N/high-light treatment was significantly greater than the leaf area of the plants grown at low-N/high-light ($t = 2.13$, $p = 0.0401$). Specifically, the mean leaf area at the high-N/high-light treatment was 130.2 % greater than in the low-N/high-light treatment (Fig. 3). Additionally, the *t* tests revealed that mean leaf area at the high-N/high-light treatment was 83.3 % greater than at the high-N/low-light treatment ($t = 2.04$, $p = 0.0489$). The final ANCOVA model included light (L), nitrogen (N), initial root mass, and L × N as effects.

The effect of N fertilization on *Rubus* SPAD and percent leaf N did not depend on the light level and N fertilization alone did not have an effect. However, light did have a positive effect on both SPAD ($F = 4.85$, $p = 0.0138$) and percent leaf N ($F = 10.19$, $p = 0.0003$; Fig. 4). Post hoc *t* tests determined that SPAD was 16.9 % higher at the high-light level when compared to low-light ($p = 0.0231$) and percent leaf N was 35.9 % higher at the high-light level when compared to low-light ($t = 5.12$, $p < 0.0001$; Fig. 4). Percent leaf N was also found to be higher at the high-light level when compared to medium-light ($t = 3.00$, $p = 0.0047$) and higher at medium-light when compared to low-light ($t = 2.25$, $p = 0.0302$). The effect of N fertilization on *Rubus* total leaf N (g plant^{-1}) did not depend on the light level and there were no additive effects of light or N fertilization on the total leaf N. Yet, there was a significant positive effect of initial root mass on total leaf N ($F = 14.13$, $p = 0.0010$). Light (L), nitrogen (N), initial root mass, and L × N were included as effects in the final ANCOVA models for SPAD, percent leaf N, and total leaf N.

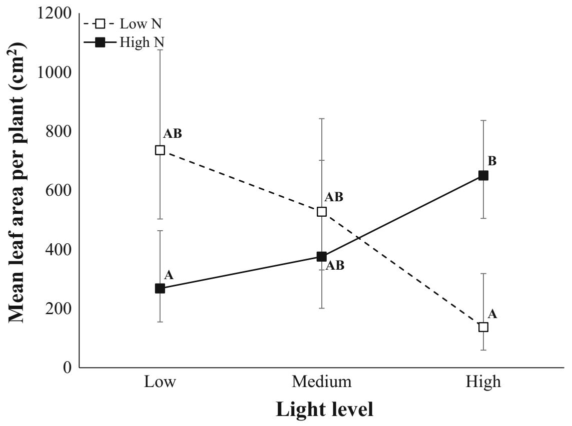

Fig. 3 Mean leaf area per *Rubus* plant grown at two nitrogen levels and three light levels, achieved by using three densities of shade cloth designed to block a percentage of ambient light—high light blocked 30 %, medium blocked 60 %, and low blocked 90 %. The means were back-transformed after analysis from log-transformed data and the *error bars* represent 95 % confidence limits. *Differing letters* indicate significant differences ($p < 0.05$) between means using Student's *t* test

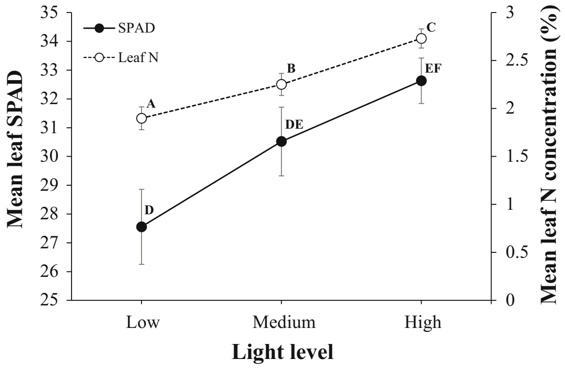

Fig. 4 Mean *Rubus* leaf SPAD and percent leaf nitrogen in plants grown across three light levels achieved by using three densities of shade cloth designed to block a percentage of ambient light—high light blocked 30 %, medium blocked 60 %, and low blocked 90 %. *Differing letters* indicate significant differences ($p < 0.05$) at each light level using Student's *t* test and *error bars* represent one standard error

Discussion

In this study, we investigated the effect of N and light on the cover of *Rubus* in forest and field experiments. Chronic N fertilization in the forest experiment led to a striking increase in the relative *Rubus* cover in the fertilized watershed and LTSP plots. Species of the *Rubus* genus are typically found in abundance after forest canopy disturbances, when light levels are high (Hughes and Fahey 1991; Peterson and Carson 1996; Peterson and Pickett 1995; Phillippe et al. 2010). However, in the absence of major forest disturbances or differences in canopy openness between fertilized and unfertilized treatments, the relative *Rubus* cover was considerably higher in the fertilized treatments (Fig. 2). Yet, light was indeed an important factor, as the effect of N on the cover of Rubus depended on canopy openness. Therefore, the increase in the relative *Rubus* cover in the fertilized treatments was only realized because of the increase in cover in areas with higher canopy openness. The differential effect of N and light was also observed among *Rubus* plants grown the field experiment. At the highest light level in the field experiment, *Rubus* leaf area was substantially higher in the plants grown at high N when compared to those grown at low N (Fig. 3). These results demonstrate that *Rubus* plants growing in fertilized areas were able to utilize the increased light from larger canopy openings to increase cover.

Interactions between light and nutrients have been documented in other herbaceous-layer plants (Baeten et al. 2010; Eickmeier and Schussler 1993; Rodriguez-Garcia and Bravo 2013) and in coniferous forest systems (Hedwall et al. 2010, 2013; Strengbom and Nordin 2012; Thomas et al. 1999), but less so in temperate deciduous forests (Gilliam 2007). While light is thought to be the most limiting resource in the forest herbaceous layer (Coomes and Grubb 2000; Neufeld and Young 2014), the effect of light has been observed to be dependent on the level of soil N (Walters and Reich 1997). However, light was the only factor affecting *Rubus* leaf N content when grown in our field experiment (Fig. 4). *Rubus* plants grown in the field experiment had higher leaf N concentrations in higher light regardless of their level of N fertilization. The lack of a differential effect between N and light on leaf N concentration is consistent with previous leaf research that has determined that light is the major factor controlling leaf N (Evans 1989).

The substantial increase in the relative cover of *Rubus* under N fertilization suggests that *Rubus* species at FEF are indeed nitrophilic (Craine 2009; Dirnböck et al. 2014). Nitrophilic plants also often have thorns and a planophilic leaf angle distribution (Craine 2009), both notable traits of *Rubus* (Balandier et al. 2013). Under N fertilization, nitrophilic plants can cause shifts in herbaceous-layer species composition through increased competition for resources (Clark et al. 2007; Cleland et al. 2008; Suding et al. 2005). Thus, plants that respond to N fertilization by increasing cover can potentially out-compete neighboring plants for light (Newman 1973; Wilson and Tilman 1991). Specifically in *Rubus*, increases in cover at other sites have led to decreases tree seedling growth and survival by creating deep-shade (Balandier et al. 2013). Furthermore, the ability of *Rubus* to propagate vegetatively allows it to reproduce and spread quickly (Eilts et al. 2011)—which is likely the major factor causing the decline in diversity observed in after 25 years of experimental N fertilization in WS3 at FEF (Gilliam et al. in press).

While intraspecific competition changes help to explain the dominance of *Rubus* following N fertilization, other N-mediated processes could be shifting simultaneously in the forest herbaceous layer. Higher soil N can result in increased plant N uptake which, in turn, increases the quality of plant tissue for foraging herbivores (Throop and Lerdau 2004). Increased N availability can also lead to increases in pathogenic infections (Mitchell et al. 2003; Strengbom et al. 2002), decreased resistance to species invasion (Cassidy et al. 2004), and composition shifts in soil microbial communities (Brandrud and Timmermann 1998; Compton et al. 2004). However, the *Rubus* plants grown in the field experiment experienced neither competition, species invasion, nor obvious signs of herbivory or pathogens, and leaf area was considerably higher at the high-N treatment when compared to the low-N treatment at the highest level of light. Therefore, a shift in herbaceous-layer composition toward nitrophilic species in N-fertilized treatments at FEF is likely due primarily to a decline in the heterogeneity of soil nutrients under N fertilization (Beatty 2014; Eilts et al. 2011; Small and McCarthy 2003), and not due to other secondary N-mediated processes, consistent with the predictions of the N homogeneity hypothesis (Gilliam 2006).

Overall, our results underscore the effect of both N and light on *Rubus* in the forest herbaceous layer. These effects were observed over a large span of temporal and spatial scales—from a 1-year pot experiment, a 16-year early successional plot experiment, and a 23-year aggrading forest watershed experiment. At each level, the response of *Rubus* under N fertilization at FEF follows the pattern suggested by the soil nutrient homogeneity hypothesis, whereby a more homogeneous soil nutrient environment enhances the competitive ability of nitrophilic species and species richness can be reduced (Gilliam 2006). If our results are indicative of herbaceous layers in other temperate forest regions, then there is still potential for large losses of biodiversity under continued N deposition—at least, in part, driven by an increased dominance of nitrophilic species like *Rubus*.

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Appendix 1: The concentrations of chemical constituents used in the nutrient solution applied to *Rubus* plants grown in the field experiment, modified from Johnson et al. (1957)

Constituent	Concentration (μM)
KNO_3	50
$Ca(NO_3)_2\cdot4H_2O$	25
NH_4NO_3	50
KH_2PO_4	6.25
$MgSO_4\cdot7H_2O$	12.5
KCl	20
H_3BO_3	25
$MnSO_4\cdot H_2O$	2
$ZnSO_4\cdot7H_2O$	2
$CuSO_4\cdot5H_2O$	0.5
Na_2MoO_4	0.5
$CoCl_2\cdot6H_2O$	0.5
$C_{10}H_{12}N_2NaFeO_8$	20

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