Drought sensitivity of an N_2-fixing tree may slow temperate deciduous forest recovery from disturbance

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Abstract. Increased drought intensity and frequency due to climate change may reduce the abundance and activity of nitrogen (N_2)-fixing plants, which supply new N to terrestrial ecosystems. As a result, drought may indirectly reduce ecosystem productivity through its effect on the N cycle. Here, we manipulated growing season net rainfall across a series of plots in an early successional mesic deciduous forest to understand how drought affects the aboveground productivity of the N_2-fixing tree *Robinia pseudoacacia* and three co-occurring nonfixing tree species. We found that lower soil moisture was associated with reduced productivity of *R. pseudoacacia* but not of nonfixing trees. As a result, the relative biomass and density of *R. pseudoacacia* declined in drier soils over time. Greater aboveground biomass of *R. pseudoacacia* was also associated with greater total soil N, extractable inorganic N, N mineralization rates, and productivity of nonfixing trees. These soil N effects may reflect current N_2 fixation by *R. pseudoacacia* saplings, or the legacy effect of former trees in the same location. Our results suggest that *R. pseudoacacia* promotes the growth of nonfixing trees in early succession through its effect on the N cycle. However, the sensitivity of *R. pseudoacacia* to dry soils may reduce N_2 fixation under scenarios of increasing drought intensity and frequency, demonstrating a mechanism by which drought may indirectly diminish potential forest productivity and recovery rate from disturbance.

Key words: climate change; drought; nitrogen cycle; Robinia pseudoacacia *(black locust); symbiotic nitrogen fixation; throughfall reduction.*

Introduction

Global climate change is likely to reduce mean soil moisture in many terrestrial ecosystems and increase drought frequency globally (Intergovernmental Panel on Climate Change 2013, Wehner et al. 2017), which may impose water stress on trees and reduce forest productivity (Allen et al. 2010, Vose et al. 2016). Drought may also indirectly affect forest productivity through the nitrogen (N) cycle, by reducing the supply of available N to trees. Early successional temperate forests may be particularly sensitive to such indirect effects, because productivity is highly N limited (Vitousek and Howarth 1991, Wilson and Shure 1993). In these systems, symbiotic N_2 fixation (SNF) is often the main source of new N (Boring and Swank 1984*a, b*, Vitousek et al. 2002),

and provides a recovery mechanism from prior disturbance. However, reductions in soil moisture may diminish the SNF activity of N_2-fixing plants (hereafter N_2-fixers) or cause the abundance of N_2-fixers to decline.

N_2-fixers may be more sensitive to drought than nonfixers because of their unique N acquisition strategy. N_2-fixers allocate carbon (C) to symbiotic bacteria for access to the effectively limitless atmospheric N pool. However, SNF is energetically expensive and its benefits are potentially offset by greater C cost per unit N gained (Gutschick 1981, Vitousek and Field 1999). Thus, SNF should only be favored over soil N uptake when the marginal benefit—the difference between the C gained from an additional unit of N and the cost of fixing that N—is positive. Because light is plentiful in the early stages of forest succession, water may be the primary constraint to photosynthesis and therefore a key limiting factor for plant C supply. If water stress reduces leaf C uptake and assimilation, the relative value of C will increase and the benefit of having additional N to invest in photosynthesis may be limited, thereby reducing the marginal benefit of SNF over soil N uptake (Rastetter et al. 2001). Though many N_2-fixers regulate investment in SNF vs. soil N uptake based on soil N and water supply

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(Wurzburger and Miniat 2014, Menge et al. 2015), and light (Barron et al. 2011, Minucci et al. 2017, Taylor and Menge 2018), there are C costs to maintain this plasticity (Menge et al. 2009a, 2011). As a result, N_2-fixers may be poor competitors with nonfixing plants when water constraints make SNF unfavorable.

At the ecosystem level, the abundance of N_2-fixers represents the ultimate control on SNF, making it necessary to understand how soil moisture affects competition between N_2-fixers and nonfixers. If the marginal benefit of SNF vs. soil N uptake is diminished, N_2-fixers may not compete with nonfixers as well and experience reduced abundance in early successional forests. Relationships between N_2-fixers and soil moisture appear to vary across ecosystems and species, obscuring any global pattern. In arid ecosystems N_2-fixer abundance (both absolute and relative to nonfixers) and SNF activity increase with soil moisture (Schulze et al. 1991, Aranibar et al. 2004, Soper et al. 2015); yet N_2-fixers are more abundant in dry areas of the neotropics (Gei et al. 2018) and North America (Liao et al. 2017). Thus, it remains unclear how climate change–induced variation in soil moisture will affect the distribution and abundance of N_2-fixers, particularly those in mesic temperate ecosystems where species may be ill-adapted to survive drought. Limited evidence from a greenhouse study showed that *R. pseudoacacia* outcompeted nonfixers under drought conditions (Wurzburger and Miniat 2014). However, it is unclear how this greenhouse study with seedlings grown in small nutrient-poor mesocosms and controlled temperature and moisture conditions translates to field conditions with saplings growing in heterogeneous soils and variable climate. The marginal benefit of SNF may depend on the N cycling and temperature conditions that accompany drought and may require several growing seasons to manifest in changes in biomass and competitive interactions between N_2 fixers and nonfixers.

Forest productivity and resilience could be reduced if drought disproportionately affects N_2-fixer abundance. Disturbance events in forests are often accompanied by large losses of N, such as the removal of tree biomass during harvesting and leaching of dissolved inorganic N due to reduced uptake by trees (Knoepp and Swank 1997, Menge et al. 2009b, Boring et al. 2014, McLauchlan et al. 2014). Symbiotic N_2 fixation represents the largest natural source of new N to replace losses in these recovering systems (Boring and Swank 1984a, b). This process results in soils with locally elevated N mineralization and nitrification rates (Montagnini et al. 1986) and inorganic N availability (Goergen and Chambers 2009), which may facilitate the growth of neighboring nonfixers (DeBell et al. 1997). N_2-fixer biomass declines in late succession (Vitousek et al. 2002), and thus N limitation is often characteristic of later-successional forests without considerable anthropogenic inputs (Vitousek and Howarth 1991, Vitousek et al. 2013). The amount of N fixed in the early years of stand regeneration may thus determine the

severity of future N limitation, and the trajectory of forest growth decades after N_2-fixers are excluded (von Holle et al. 2013). As a result, SNF rates in early succession may determine the long-term ability of forests to recover from disturbance and store C.

Here we examined the direct and indirect effects of drought on forest productivity via a throughfall reduction experiment in an early-successional southern Appalachian forest. In these mesic deciduous forests, a single tree species, *Robinia pseudoacacia*, is the dominant N_2-fixer and it can significantly elevate soil N mineralization rates and N availability in dense stands (Boring and Swank 1984b, Montagnini et al. 1986). Over a 3-yr field experiment, we determined the effects of growing season soil moisture and soil N availability on the growth and competitive response of *R. pseudoacacia* and three co-occurring nonfixing trees. We hypothesized that (1) growth and SNF rate of *R. pseudoacacia* would be greatest at high soil moisture, and growth of nonfixing trees would be greatest under high N availability; (2) as a result of Hypothesis 1, the relative biomass and density of *R. pseudoacacia* would decline over time under low soil moisture; and (3) greater *R. pseudoacacia* biomass would be associated with elevated soil N availability and higher productivity of neighboring nonfixing trees.

MATERIALS AND METHODS

Study site

Our study was conducted in a low-elevation (704–760 m above sea level [asl]), west-facing, 30-ha watershed located in the Cowee area near Franklin, North Carolina, within the Nantahala National Forest (35°16′43″ N, 83°21′43″ W). Prior to harvesting in 2010, the forest was characteristic of the mature southern Appalachian deciduous forests in the region. During harvest, approximately 75% of tree biomass was removed, with the remaining trees left to provide a seed source for regeneration. Following harvest, herbicide was applied to targeted harvested stumps and single stems of undesirable species in the understory (e.g., red maple [*Acer rubrum* L.], black gum [*Nyssa sylvatica* Marshall], silverbell [*Halesia* spp.], dogwood [*Cornus florida* L.], sourwood [*Oxydendrum arboreum* (L.) DC.] and striped maple [*Acer pensylvanicum* L.]). The intent was for the treatment to promote stand regeneration from seed sources rather than from stumps or from suppressed single stems. Soils were in the Cowee-Evard complex—a fine–loamy, parasesquic, mesic, Typic Hapludult—and the Saunook series—a fine–loamy, mixed mesic Humic Hapludult. Mean annual temperature and precipitation are approximately 14.0°C and 1,800 mm, respectively (Laseter et al. 2012). Total inorganic N deposition in the region is approximately 4 kg N ha^{-1} yr^{-1}, about 50% lower than average for the southeastern United States, and has been steady since the 1990s (Li et al. 2016).

Experimental design

We established eighteen 9-m^2 plots in spring of 2012. We haphazardly distributed three pairs of plots within each of three landscape positions: the north-facing side slope, the south-facing side slope, and the toe-slope lacking significant aspect. Paired plots were located within 10 m of each other. Plots were hydrologically isolated from the surrounding soil with 45-mil- (1.1 mm) thick ethylene propylene diene monomer (EPDE) plastic liner buried around them to a depth of 0.6 m. After the 2012 growing season, we tagged and measured the stem diameter and height of all individuals of four focal species: the N$_2$-fixing tree *R. pseudoacacia,* and three non-fixing species common in early succession, *Liriodendron tulipifera* L., *A. rubrum* and *Quercus velutina* Lam. The abundance of these four species in the plots was, on average, 17.0%, 31.4%, 29.2%, and 22.2% of total plot biomass, respectively (Appendix S1: Table S3).

Robinia pseudoacacia is the dominant N$_2$-fixing tree across its range in the Appalachian Mountain and Ozark regions (Boring and Swank 1984*a, b,* Liao et al. 2017). It is a member of the legume subfamily Papilionoideae, a group whose trees are rarely found outside of hot tropical and subtropical biomes (Legume Phylogeny Working Group 2017). Both *R. pseudoacacia* and *L. tulipifera* are fast-growing, shade-intolerant trees, whereas *A. rubrum* and *Q. velutina* are slower growing and shade tolerant. All four species can regenerate from the seed bank and sprout readily from stumps or roots following disturbance (Shure et al. 2006, Cofer et al. 2018). These species are known to compete for common resources (e.g., N, water, light) in southern Appalachian forests (Apsley 1987, Clark et al. 2010). Although the three nonfixing species often persist into late succession, *R. pseudoacacia* declines in abundance following canopy closure and therefore was likely to be rare in our study plots prior to cutting in 2010 (Boring and Swank 1984*b*, Boring et al. 2014, Liao and Menge 2016).

In July of 2013, we installed panels that removed either 0%, 20%, or 40% of incoming throughfall incident on the forest floor, to augment the natural level of soil moisture variability in our study site (Appendix S2). Almost all precipitation ends up as throughfall in these young forests, as canopy interception is low (Brantley et al. 2019). Panels remained in place during the 2013, 2014, and 2015 growing seasons (mid-May to mid-October), and were removed each winter. The 2013 growing season had 26% higher precipitation than the long-term average, while 2014 and 2015 were near average (7% and 0% higher, respectively; Miniat et al. 2017). Stand age was 3 yr at the start of the treatment period and 5 yr at the end of the experiment, which corresponded to mean tree heights in our plots of 0.32 m and 1.30 m, respectively. In the final year, some larger trees grew above the treatment panels, but changes to the treatment effect should have been minimal, because canopy interception

for trees of this age is low (Brantley et al. 2019). Soil moisture was estimated every 15 min across 0–15-cm mineral soil depth at the center of each plot, and hourly means were logged (CS-655, CR1000 Campbell Scientific, Logan, Utah) for the duration of the study. Soil moisture measurements were assumed to represent water availability to saplings, as the majority of roots for mature individuals of these species are distributed <50 cm deep (Hales et al. 2009, Ji et al. 2012, Hales and Miniat 2017) and our soil moisture measurements at 0–15 cm correlated with predawn and midday leaf water potentials, verifying this assumption (see below).

Tree biomass and growth rates

To quantify tree growth rates, we estimated tree aboveground biomass at the end of each growing season. For each tagged tree we measured the height of the apical meristem and the stem diameter at 5-cm height, and converted these measurements to total aboveground biomass using species-specific allometric equations (Boring and Swank 1984*b*, Elliott et al. 2002, Wurzburger and Miniat 2014, Minucci et al. 2017). We also tagged and measured newly recruited seedlings and noted cases of mortality. Relative aboveground productivity (g g^{-1} yr^{-1}) was calculated at the plot level for each species according to the equation:

$$\text{Rel. aboveground productivity} = \frac{\ln\left(\frac{X_2}{X_1}\right)}{t},$$

where X_2 = final biomass (g), X_1 = initial biomass (g), and t = time (yr).

Soil N cycling and availability

To assess how plant-available N varied across plots, we estimated several measures of soil N availability during the 2015 growing season. We quantified extractable ammonium and nitrate (i.e., inorganic N) at 0–10-cm depth three times during the summer with 2 mol/L KCl and carried out in 28-d in situ N mineralization assays by burying homogenized root-free soil in gas permeable polyethylene bags (Appendix S3). We also quantified the potential activity of leucine aminopeptidase (LAP) and β-N-acetylglucosaminidase (NAG), two enzymes involved in the release of N from organic material, using a modified fluorometrically labeled substrate method (Saiya-Cork et al. 2002, Appendix S3).

We determined total C and N stocks in our soils during the 2015 growing season, as well as the stable N isotope ratio (δ^{15}N) of the total soil N pool. In 2015, we subsampled approximately 2 g of soil from the same 0–10-cm depth soil samples used for extractions above. Soils were dried at 70°C to constant weight, ground in a ball mill, and a 15-mg subsample was combusted and analyzed (NA1500 CHN Analyzer, Carlo Erba, Milan,

Italy; IRMS, Delta V, Thermo-Finnigan, Bremen, Germany; Robinson 2001).

Tree foliar sampling

As another estimate of soil N availability to trees, we determined foliar N concentration in August 2015. We also determined foliar stable C isotope ratio (δ^{13}C) to estimate integrated water-use efficiency (WUE) and foliar δ^{15}N to examine whether trees acquired N from different sources (Farquhar et al. 1989, Robinson 2001). Three fully extended leaves in full sun were sampled from three individuals of each species in each plot. A 1.5-mg subsample of leaf tissue was processed and analyzed as described above for soils. We attempted to calculate SNF rate by *R. pseudoacacia* with the δ^{15}N natural abundance method and a two end-member mixing model using *A. rubrum* as a reference for the δ^{15}N signature of soil N uptake (Robinson 2001). However, the δ^{15}N signature of *A. rubrum* (and nonfixers in general) varied as a function of *R. pseudoacacia* biomass nearby, thus violating the assumptions of the method.

Light availability

We used canopy light transmittance as a proxy for leaf area to estimate the level of light competition in our plots. At each plot we sampled photosynthetic photon flux density (PPFD; μmol m^{-2} s^{-1}; LI-250A with LI-193 sensor, Li-Cor, Lincoln, Nebraska) above the canopy (Q_o) and at three random points below the canopy at 75-cm height (Q_i). All readings were taken between 10:30 and 13:30 on the same day in July 2015. We calculated canopy transmittance as Q_i/Q_o.

Tree ecophysiological response to soil moisture

In July and September of year two (2014), we measured predawn and midday leaf water potential, transpiration, and stomatal conductance to determine whether tree species had physiological responses to variation in soil moisture. Predawn and midday leaf water potentials were measured for one individual per species per plot (Ψ_{pd} and Ψ_{md}, MPa; PMS Instruments, Albany, Oregon). Transpiration and stomatal conductance were measured under ambient light, humidity, and CO_2 conditions for two individuals per species per plot (E: μg H_2O cm^{-2} s^{-1}, g_s: mmol H_2O m^{-2} s^{-1}; LI-1600, LI-COR Inc., Lincoln, Nebraska). All samples were made on fully extended, full-sun leaves.

Statistical analysis

Although we originally intended to use throughfall reduction level as a categorical treatment effect, we found that landscape position explained more variation in percentage of soil moisture (%SM; 44% of variance) than did throughfall reduction treatment (22% of variance);

hence, we used %SM as a continuous variable in our analyses to capture both natural variation and the effect of throughfall exclusion (Appendix S1: Table S4). For example, for plots with 40%, 20%, and 0% throughfall reduction, mean growing season %SM ($\pm$SD) was 21.6% ($\pm$3.1), 25.3% ($\pm$3.3), and 23.1% ($\pm$1.6), respectively. The 3-yr mean growing season soil moisture (%SM) varied widely across plots ranging from 18.3 to 28.9% (Appendix S1: Table S2). Throughfall displacement treatments had no effect on the coefficient of variation of soil moisture ($\chi^2_1 = 1.43$, $P = 0.49$), and there was no correlation between the coefficient of variation and mean soil moisture (Pearson's correlation: $r = 0.10$, $P = 0.70$). To test for a lag in the treatment effect due to the panels being installed in early May of each year, we reanalyzed our growing season moisture data with May and June removed and found no difference in the significance of the treatment, landscape position or tree biomass effects compared to the full season results. Further, there was a high degree of correlation among yearly mean soil moisture measurements within plots, indicating that relative differences in moisture were consistent throughout the study, (Pearson's correlation: $0.72 < r < 0.94$). As a result, for all analyses beyond the assessment of the treatment effect, we used %SM as a continuous predictor that integrated both the pre-existing variability in moisture and the effect of our throughfall manipulation.

To determine species' sensitivity to soil moisture, N availability, and light, we modeled cumulative 3-yr relative aboveground productivity for each species and performed stepwise model selection with the Bayesian information criterion (BIC; Neath and Cavanaugh 2012). We used productivity (as opposed to total plot biomass) because these responses occurred during the study period where we monitored light and soil moisture. We then constructed linear mixed effects models (R, *nlme* package; Pinheiro et al. 2018) with the following potential fixed effects: 3-yr mean growing season soil moisture (%SM), canopy transmittance, N mineralization rate (N_{min}), extractable inorganic N, and all possible two-way interactions. The fixed effects of N_{min} and extractable inorganic N in the *R. pseudoacacia* model were square-root transformed to achieve normally distributed model error. Each model included landscape position and plot pair number as categorical random effects to account for spatial autocorrelation and variation in moisture and soil properties associated with topographic position within the watershed. The landscape random blocking effect isolated the effect of mean soil moisture from other factors that potentially differed among slope positions and aspects.

To determine the relationship between *R. pseudoacacia* biomass and both N availability and nonfixer productivity, we used the absolute abundance (total plot biomass) of *R. pseudoacacia* as the predictor variable, as these effects are likely density dependent. To analyze how trees responded physiologically to variation in soil moisture, we modeled stomatal conductance, transpiration,

predawn and midday leaf water potential, and leaf $\delta^{13}C$ as factors of mean moisture on the day or year ($\delta^{13}C$) that these measurements were taken. For stomatal conductance and transpiration, we also included time of day and ambient relative humidity as covariates. These analyses were carried out with linear mixed-effects models with landscape position and plot pair number as categorical random effects. Response variables measured at the tree-level (e.g., foliar N concentration) were averaged to produce one mean value per plot. Biomass of *R. pseudoacacia* was square-root transformed to achieve normally distributed residual model error. To determine whether variables were significant predictors in each model, we used likelihood ratio tests comparing the full model and a model with the term of interest dropped. All statistical analyses were performed with R 3.5.2 (R Development Core Team 2018).

RESULTS

Tree physiological response to soil moisture variation

Low daily mean soil moisture consistently reduced predawn and mid-day leaf water potentials for all species, indicating that our soil moisture measurements reflected differences in water availability to trees (Appendix S1: Fig. S1; Ψ_{pd}: $\chi^2_1 = 10.5$, $P = 0.001$; Ψ_{md}:

$\chi^2_1 = 5.7$, $P = 0.02$). A 1% decrease in daily mean soil moisture was correlated with a 0.06-MPa drop in both Ψ_{pd} and Ψ_{md} across all four species. Transpiration of *R. pseudoacacia* and *L. tulipifera* was approximately 73% greater than *A. rubrum* and 48% greater than *Q. velutina*. *Liriodendron tulipifera* was the only species to reduce stomatal conductance with declining soil moisture conditions (Appendix S1: Fig. S2; species by %SM interaction: $\chi^2_3 = 11.9$, $P = 0.01$); however, transpiration did not vary across soil moisture for any species (Appendix S1: Fig. S2; $\chi^2_1 = 2.5$, $P = 0.12$).

Low soil moisture increased integrated water-use efficiency for some, but not all, species (species by %SM interaction: $\chi^2_3 = 7.7$, $P = 0.05$). Water-use efficiency increased for *Robinia pseudoacacia* and *Q. velutina* with increasing soil dryness (Fig. 5a), but not for *L. tulipifera* or *A. rubrum*, or the average of all three nonfixers ($\chi^2_1 = 0.50$, $P = 0.48$).

Factors affecting aboveground productivity of Robinia pseudoacacia and nonfixers

The factors that best predicted relative net aboveground productivity differed between the N_2-fixer *R. pseudoacacia* and nonfixing trees (Appendix S1: Table S1). *Robinia pseudoacacia* was most productive with high soil moisture and low N_{min} (Fig. 1). In
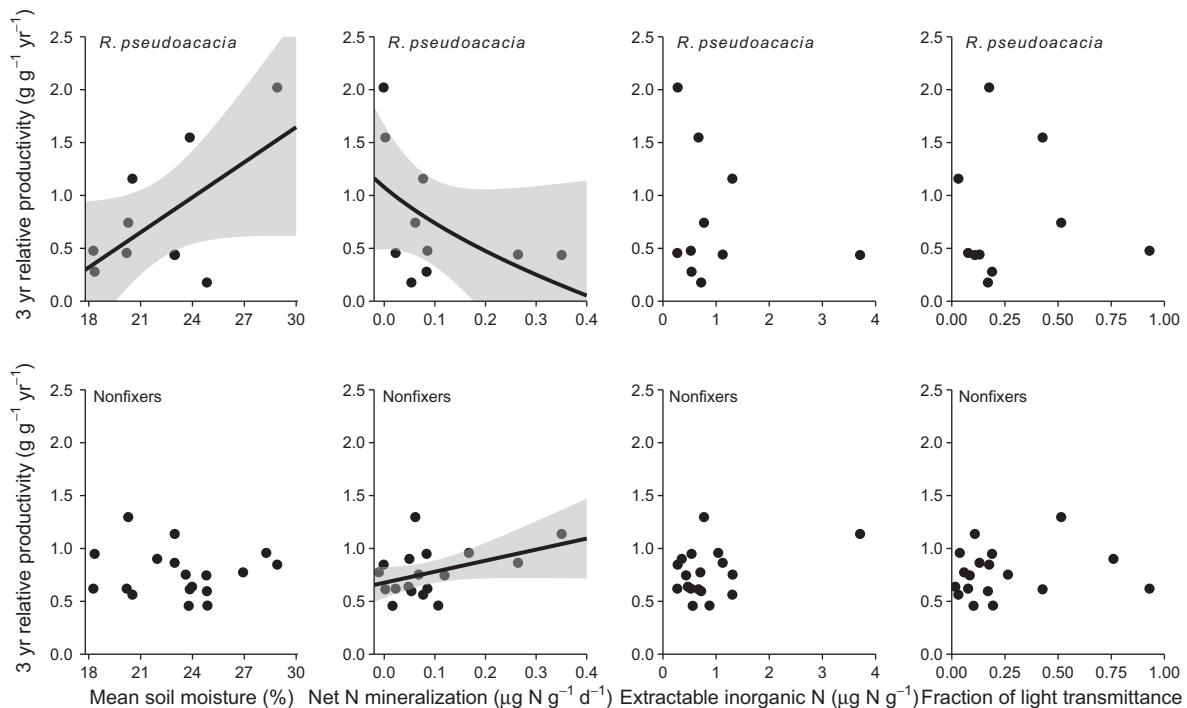

FIG. 1. Aboveground productivity of (top row) *Robinia pseudoacacia* and (bottom row) nonfixing tree species related to mean growing season soil moisture, net N mineralization, extractable inorganic N and light availability. Aboveground productivity and soil moisture are 3-yr means. Net N mineralization and extractable inorganic N were measured three times during the 2015 growing season using six randomly located 10-cm-deep cores homogenized to one sample per plot. Regression lines with 95% confidence intervals are included when the effect was present in the best model (as selected by BIC).

contrast, nonfixers as a group were most productive at high N_{min} (Fig. 1), regardless of moisture and light conditions. Non-fixers in plots that were in the top third of N_{min} rates were 25% more productive than those in plots in the bottom third. Individually, *L. tulipifera* was most productive with high soil moisture and light (positive interaction of %SM and transmittance; Appendix S1: Table S1). However, productivity was also greater with increasing N_{min} across all conditions (positive main effect of N_{min}). *Acer rubrum* was most productive with high N_{min}, regardless of moisture and light conditions (positive main effect of N_{min}; Appendix S1: Table S1). In contrast, *Q. velutina* was most productive under shadier conditions (negative main effect of canopy transmittance; Appendix S1: Table S1).

Change in relative biomass and density of Robinia pseudoacacia

Mean relative biomass of *R. pseudoacacia* (the percent of total tree biomass) increased slightly in year 1 (from 17.1 to 19.0%), but then declined in years 2 and 3 to a final relative biomass of 13.7%. The decline in years 2 and 3 was greater in plots with low %SM over this period (Fig. 2; $\chi^2_1 = 5.7$, $P = 0.02$). In plots where the relative biomass of *R. pseudoacacia* decreased in years 2 and 3, mortality explained 26% of the decline, rather than differences in growth. Relative density of stems similarly declined during this period (from 13.4 to 7.2%), with the greatest decrease again occurring in plots with low %SM (Appendix S1: Fig. S3; $\chi^2_1 = 4.9$, $P = 0.03$). In contrast, the relative biomass of *L. tulipifera* increased the most in plots where %SM was low between years 2 and 3 (Fig. 2; $\chi^2_1 = 4.0$, $P = 0.04$), while there were no significant changes in the abundance of *Q. velutina* or *A. rubrum*, and no changes in relative density of stems for any single nonfixing species.

Effect of Robinia pseudoacacia biomass on forest N cycle and nonfixer productivity

Higher biomass of *R. pseudoacacia* was associated with higher soil N_{min} (Fig. 3a; $\chi^2_1 = 13.9$, $P < 0.001$) and extractable inorganic N (Fig. 3b; $\chi^2_1 = 20.2$, $P < 0.001$), but not associated with differences in potential activity of N-acquiring enzymes NAG ($\chi^2_1 = 1.4$, $P = 0.23$) and LAP ($\chi^2_1 = 0.2$, $P = 0.64$). *Robinia pseudoacacia* biomass was also correlated with higher rates of nonfixing trees' productivity (Fig. 4; $\chi^2_1 = 7.9$, $P = 0.005$). When modeled by individual species, *A. rubrum* and *L. tulipifera* productivity increased with greater *R. pseudoacacia* biomass, but *Q. velutina* did not. There was no relationship between *R. pseudoacacia* biomass and its own productivity (Appendix S1: Fig. S6; $\chi^2_1 = 0.6$, $P = 0.43$). Foliar N concentration (Fig. 3c; $\chi^2_1 = 8.82$, $P = 0.02$) and $\delta^{15}N$ of nonfixers (Fig. 3d; $\chi^2_1 = 17.3$, $P < 0.001$) also increased with greater *R. pseudoacacia* biomass, suggesting that nonfixers assimilated atmospherically derived N. Foliar N
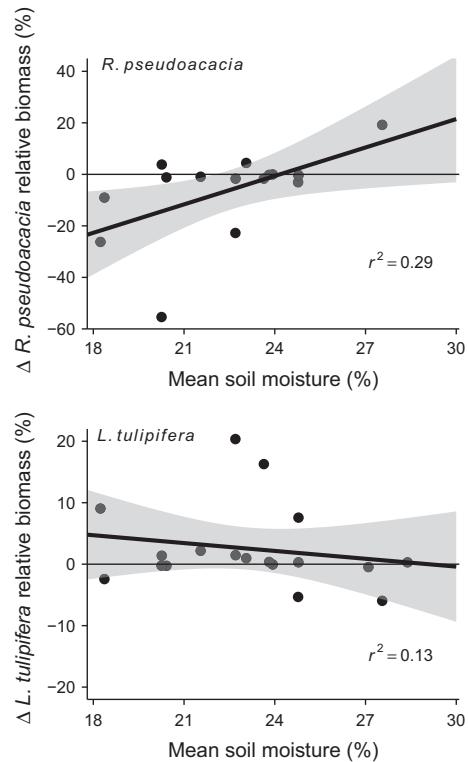

FIG. 2. Change in relative biomass (percent of total tree biomass) of (top) *Robinia pseudoacacia* and (bottom) *Liriodendron tulipifera* during years 2 and 3 of the experiment related to mean soil moisture during that period. Lines represent regression fits with 95% confidence intervals and marginal r^2 values (variance explained by mean soil moisture alone).

concentration was significantly higher for *R. pseudoacacia* than for nonfixers (2.9% ±0.33 vs. 1.7% ±0.27; Welch's *t*-test: $t = 10.4$, $P < 0.001$).

Robinia pseudoacacia biomass was associated with higher total soil N ($\chi^2_1 = 3.97$, $P = 0.05$), but not C ($\chi^2_1 = 3.37$, $P = 0.07$) or C:N ratio ($\chi^2_1 = 0.08$, $P = 0.78$). And although the soil N pool was larger with more *R. pseudoacacia* biomass, we found no relationship between *R. pseudoacacia* biomass and the $\delta^{15}N$ of the total N pool (Appendix S1: Fig. S4; $\chi^2_1 = 0.02$, $P = 0.88$), which was 3.0‰, on average (Appendix S1: Table S2).

The association of *R. pseudoacacia* biomass with higher soil N cycling rates and nonfixer productivity was strongly influenced by one plot with very high biomass. The relationships between *R. pseudoacacia* biomass and *A. rubrum*, *L. tulipifera*, and total nonfixer productivity remained statistically significant if this plot was excluded from analysis. However, the relationships with N_{min}, extractable inorganic N, nonfixer foliar N content and $\delta^{15}N$, and total soil N were nonsignificant with this plot excluded. We concluded that this plot represented an important data point and was not an outlier, because multiple, independently measured, estimates of soil N availability were simultaneously elevated and were also
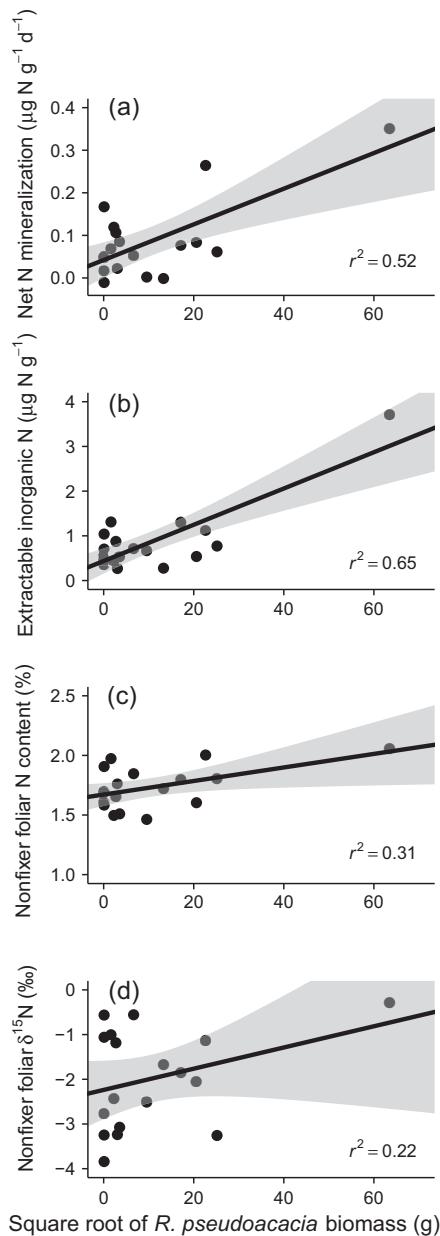
Fig. 3. *Robinia pseudoacacia* total plot biomass is positively related to (a) net N mineralization rate, (b) extractable inorganic N, (c) foliar N concentration of nonfixing trees, and (d) foliar $\delta^{15}N$ of nonfixing trees. Values for nonfixing trees represent the mean of the three individual species' mean responses. Biomass of *R. pseudoacacia* refers to total plot biomass in year 2, the midpoint of the study. Regression fits with 95% confidence intervals and marginal r^2 values (variance explained by *R. pseudoacacia* biomass alone) are included for significant relationships. All relationships become nonsignificant if the highest biomass plot is removed.
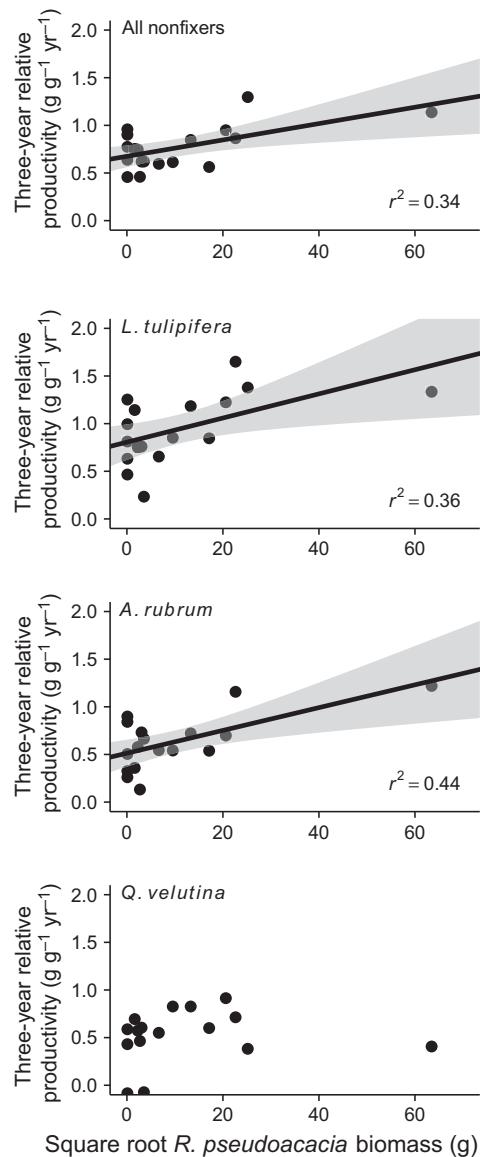

Fig. 4. *Robinia pseudoacacia* total plot biomass is positively related to relative net aboveground productivity of nonfixing trees (top). When nonfixers were modeled individually, there was a significant relationship for *Liriodendron tulipifera* and *Acer rubrum*, but not *Quercus velutina*. Regression fits with 95% confidence intervals and marginal r^2 values (variance explained by *R. pseudoacacia* biomass alone) are included for significant relationships. All relationships remain significant if the highest biomass plot is removed.

shown to increase soil N cycling in nearby forests (Boring and Swank 1984*b*, Montagnini et al. 1986).

Foliar $\delta^{15}N$ and N concentration

Nonfixing trees' foliar $\delta^{15}N$ (calculated as the mean for all nonfixing species) increased with greater abundance of *R. pseudoacacia* (i.e., it approached zero; Fig. 3d), suggesting that SNF affected the $\delta^{15}N$ of the soil available N

associated with high nonfixer productivity. Although we could not determine if SNF by *R. pseudoacacia* or some other confounding factor caused the high N availability in this specific plot, SNF by *R. pseudoacacia* has been

pool, which prevented our ability to distinguish between N derived from soil vs. atmosphere in high *R. pseudoacacia* biomass plots. Although we could not reliably calculate N derived from the atmosphere, foliar N concentration decreased with declining soil moisture for *R. pseudoacacia* (Fig. 5b; $\chi^2_1 = 6.14$, $P = 0.01$), but not for nonfixers (Fig. 5b; $\chi^2_1 = 0.48$, $P = 0.49$).

Relationship between N-acquiring soil enzymes and tree aboveground productivity

There was no relationship between potential activity of the N-acquiring soil extracellular enzymes leucine aminopeptidase (LAP) and β-N-acetylglucosaminidase (NAG) and relative aboveground productivity of *R. pseudoacacia*, *L. tulipifera*, or *A. rubrum*. However, productivity of *Q. velutina* was negatively associated with potential activity of NAG, but not LAP (Appendix S1: Fig. S5; $\chi^2_1 = 8.4$, $P = 0.004$).

DISCUSSION

Our field study demonstrates that biomass of an N_2-fixing tree species was positively associated with available soil N and the growth of nonfixing trees, suggesting its role as a facilitator of recovery in an early successional mesic temperate forest. However, we found that low soil moisture can reduce the relative abundance of this N_2-fixer. Thus, increasing drought frequency and intensity due to climate change may shift competition between this N_2-fixing species and nonfixers, and as a result diminish the beneficial role of N_2-fixers in forest recovery. Our findings point to the marginal benefit of SNF vs. soil N uptake as a potential mechanism driving the greater sensitivity of N_2-fixers to low moisture compared to nonfixers.

Divergent constraints on productivity of the N_2-fixer Robinia pseudoacacia vs. nonfixers

Robinia pseudoacacia was most productive under high soil moisture but low N mineralization, whereas nonfixers as a group were unresponsive to our measured range of soil moisture and most productive under high N mineralization, supporting our first hypothesis. Although our study did not directly test competitive interactions, these species compete for common resources that limit their growth and fecundity (Apsley 1987, Clark et al. 2010), and our findings suggest that water and N availability shape interactions among these species. Under high soil moisture but low N mineralization, *R. pseudoacacia* was more productive than its nonfixing counterparts (Fig. 1), which suggests that SNF is more beneficial when the C value of additional N is large. However, this competitive advantage appeared to decline at low soil moisture, supporting the idea that the C benefits of additional N are reduced when C acquisition via photosynthesis is limited by water. The contrasting relationships of *R. pseudoacacia* and nonfixer productivity with soil N availability further suggest that nonfixers outcompete *R. pseudoacacia* when the benefit of additional N is low. Even if *R. pseudoacacia* downregulated SNF when soil N availability was high, it still may be a poor competitor because of the high C costs of maintaining N uptake plasticity (Menge et al. 2009a, 2011). Such energetic costs may include the maintenance of cellular infrastructure for detecting low N supply and the repeated construction and excision of root nodules (Menge et al. 2011), which may be exacerbated by frequent dry periods (Minucci et al. 2017).

Although productivity of the nonfixer community as a whole was correlated only with N mineralization, the best predictors of productivity varied by species (Fig. 6,
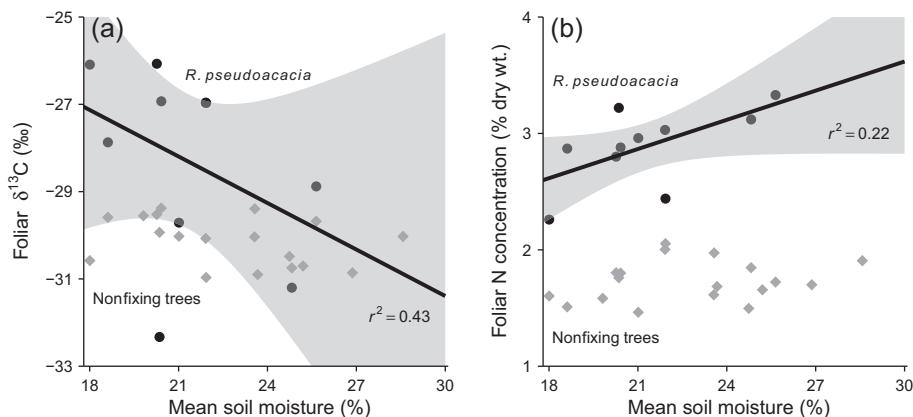

FIG. 5. Changes in $\delta^{13}C$ and foliar N concentration with soil moisture. (a) Foliar $\delta^{13}C$ of *Robinia pseudoacacia*, a measurement of water-use efficiency, increases with declining soil moisture, but there is no effect for nonfixing trees. (b) Foliar N increases with increasing moisture for *R. pseudoacacia* (circles and solid line), but not nonfixing trees (diamonds). Values for nonfixing trees represent the mean of the three individual species' mean responses. Soil moisture, foliar N and $\delta^{13}C$ measurements are from the 2015 growing season. Regression fits with 95% confidence intervals and marginal r^2 values (variance explained by mean soil moisture alone) are included for significant relationships.
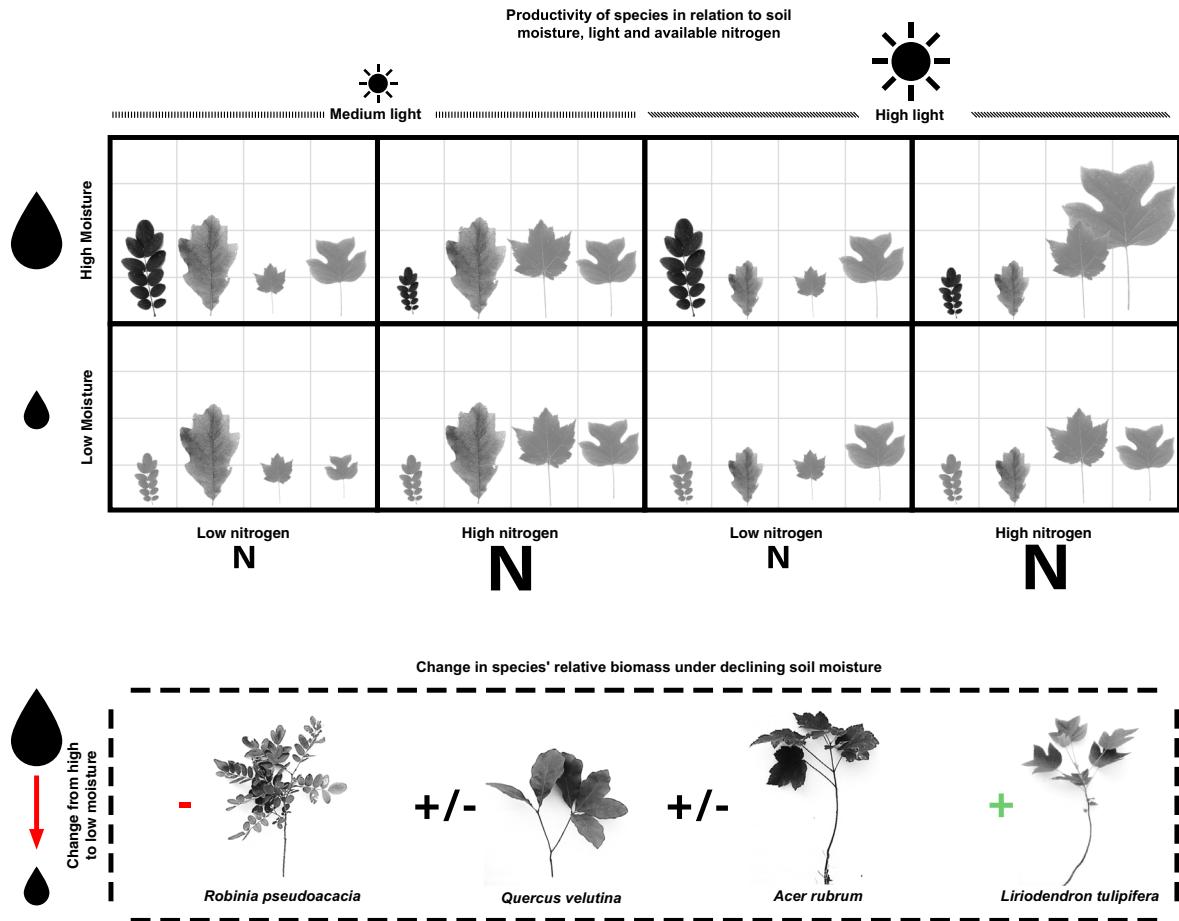
FIG. 6. Synthesis of individual and community-level responses to declining soil moisture. Top panel depicts aboveground productivity and leaf N concentrations for each species in relation to gradients of soil moisture, light, and available nitrogen, where leaf size denotes productivity, and leaf darkness denotes nitrogen concentration. In each box, leaves are ordered from left to right (*Robinia pseudoacacia*, *Quercus velutina*, *Acer rubrum*, and *Liriodendron tulipifera*). Bottom panel depicts change in relative biomass among these four species under declining soil moisture, where + denotes increase, +/− denotes no change, and − denotes decrease.

top). Productivity of *L. tulipifera* was strongly correlated with a combination of water and light, with growth increasing when both were in high supply. However, N mineralization was positively associated with *L. tulipifera* productivity across all water and light conditions, suggesting that soil N availability may be the primary limiting factor for this species. In contrast, productivity of *Q. velutina* was not explained by our measures of soil N availability, and light was the only significant predictor. The negative relationship between light and *Q. velutina* growth may reflect the poor ability of oaks to compete with faster-growing shade-intolerant species (such as *L. tulipifera*) under high light (Kaelke et al. 2001, Loftis et al. 2011). In addition, *Q. velutina* was the only nonfixing species that did not increase productivity, foliar N concentration, or δ^{15}N with greater *R. pseudoacacia* biomass. One potential explanation for this lack of sensitivity is that *Q. velutina* associates with ectomycorrhizal fungi, which can mine N from stable organic matter via the excretion of extracellular enzymes (Read and

Perez-Moreno 2003, Phillips et al. 2013), allowing this species access to soil N, independent of inorganic N supply. Interestingly, *Q. velutina* productivity was greater where potential activity of NAG, an enzyme that degrades chitin, was low, suggesting that fungal investment in this N-liberating enzyme is highest when N is most limiting the productivity of *Q. velutina* (Sinsabaugh and Moorhead 1994). In contrast to *Q. velutina*, *A. rubrum*, and *L. tulipifera* associate with arbuscular mycorrhizal fungi that scavenge inorganic N, and thus may be more sensitive to differences in inorganic N availability.

Soil moisture shapes competition between Robinia pseudoacacia *and nonfixers*

We found that relative abundance of the N_2-fixer *R. pseudoacacia*, in terms of both biomass and stem number, declined over time under low soil moisture (Fig. 6, bottom), supporting our hypothesis that *R. pseudoacacia*

would be outcompeted by nonfixers at low moisture due to the low marginal benefit of SNF. Concurrent with this decline, we found an increase in relative biomass of *L. tulipifera* (Fig. 6, bottom). The high relative aboveground productivity of *L. tulipifera* (approximately 60% greater than the other nonfixers), along with high water use and foliar N content, makes this species the most likely candidate to outcompete *R. pseudoacacia* for light or other resources over our 3-yr study period (Apsley 1987). Changes in the marginal benefit of SNF vs. soil N uptake may be responsible for this shift in competition under low soil moisture. If stomatal limitation due to soil moisture deficit reduces the potential C gain from photosynthesis, the primary use of N, then there is less benefit from acquiring additional N. Although we did not observe significant changes in instantaneous measures of stomatal conductance for any species, we found that long-term water-use efficiency ($\delta^{13}C$) increased for *R. pseudoacacia* and *Q. velutina,* but not *L. tulipifera* or *A. rubrum*, with declining soil moisture. This trend is consistent with prior evidence that *R. pseudoacacia* reduces stomatal conductance and increases its water-use efficiency under dry conditions (Wurzburger and Miniat 2014, Mantovani et al. 2015, Minucci et al. 2017). Additionally, we found that low moisture was associated with a decline in foliar N concentration of *R. pseudoacacia,* but not nonfixing species, supporting the idea that reductions in SNF (due to its lower marginal benefit) may explain the reduced competitive ability of *R. pseudoacacia*.

Robinia pseudoacacia *may drive soil N availability and nonfixer productivity*

We found that the presence of *R. pseudoacacia* was associated with greater soil N availability and productivity of nonfixing trees, supporting our third hypothesis. We also found that nonfixing trees had elevated foliar $\delta^{15}N$ and N concentrations in plots where *R. pseudoacacia* was most abundant, suggesting that new N inputs to soils, via SNF, enhanced soil N availability and facilitated the growth of nonfixing trees. Although we could not reliably calculate SNF from foliar $\delta^{15}N$, it is likely that *R. pseudoacacia* stems were actively fixing N_2 during our study period based on findings of previous studies in recently disturbed forests (Boring and Swank 1984a, b) and nodule sampling and acetylene reduction assays at the same study site (Wurzburger, *unpublished data*). Because we did not manipulate *R. pseudoacacia* abundance or soil N directly, we cannot rule out the possibility that other differences among plots led to these relationships. Specifically, these findings could have arisen if all four of the tree species were most productive in the same plots; but two of our results counter that idea. First, *R. pseudoacacia* and nonfixers were most productive in opposite conditions: *R. pseudoacacia* in plots with low N mineralization and high soil moisture, and nonfixers in plots with high N mineralization

(Appendix S1: Table S1). Second, over the course of the experiment, we observed a decrease in *R. pseudoacacia* relative biomass with declining soil moisture, suggesting that moisture disproportionately affected *R. pseudoacacia* relative to other species, reducing its competitive dominance. Although woody N_2-fixers can increase productivity of nonfixing trees in mixed-species plantations (DeBell et al. 1997) and in natural forests (Apsley 1987), they can also suppress nonfixer growth through competitive interactions (Taylor et al. 2017). The net effect of N_2-fixers (i.e., facilitation vs. competition) likely depends on the rate of SNF, and the extent to which nonfixers are limited by N vs. light, water, or other nutrients, which may vary through succession and across ecosystems.

We observed that plots containing *R. pseudoacacia* had greater soil N stocks, which suggests that *R. pseudoacacia* SNF can enrich the total soil N pool within 5 yr of forest harvest. This finding suggests a more rapid effect of SNF on ecosystem N stocks than previously reported (Boring and Swank 1984b). However, because *R. pseudoacacia* can both sprout from viable root stock (Shure et al. 2006) and recruit from a persistent seed bank (Hille Ris Lambers et al. 2005, Cofer et al. 2018), it is possible that elevated total soil N in our study was, at least in part, a legacy of SNF from prior forest structure and disturbance events. In support of this idea, soil N availability can remain elevated for a decade following local losses of *R. pseudoacacia* (von Holle et al. 2013). Interestingly, we did not find a relationship between total soil $\delta^{15}N$ and *R. pseudoacacia* biomass, despite the pattern of increasing foliar $\delta^{15}N$ of nonfixers. The lack of correspondence of $\delta^{15}N$ between the total soil N and nonfixer foliage may be explained by the small size of the plant-available inorganic N pool relative to the total soil N pool (8–11 orders of magnitude smaller in our study; Knoepp and Vose 2007) and differences in isotopic composition between these N pools. Although we did not analyze the $\delta^{15}N$ signature of the available N pool, it is possible that it more closely reflects the signature of *R. pseudoacacia* litter, which may be preferentially mineralized by microbes because of its high N content (Melillo et al. 1982).

Our results suggest that drought may reduce ecosystem-level SNF by decreasing the abundance of N_2-fixers; however, drought may also reduce the SNF rate of individual trees. Although we did not measure SNF rate directly, we found that foliar N concentration of the N_2-fixer *R. pseudoacacia* declined as mean soil moisture decreased, and there was no such decline for nonfixers (Fig. 5b). Although it is possible that this reduction in foliar N concentration was due to decreased mass flow or diffusion of N in dry soils, foliar N concentration did not decline for nonfixers along the same gradient, suggesting that a reduction in SNF rate is a more likely explanation. In fact, SNF rate can be correlated with foliar N concentration for *R. pseudoacacia* seedlings (Minucci et al. 2017) and Mediterranean shrub species (Dovrat and Sheffer 2018). Our previous work

demonstrates that drought effects on SNF by *R. pseudoacacia* can be dynamic at the scale of weeks and months, and can depend on drought intensity, duration, and frequency (Wurzburger and Miniat 2014, Minucci et al. 2017). Therefore, critical and unanswered questions remain: how did the temporal dynamics of SNF mediate the responses of saplings to drier conditions in our 3-yr study, and how will SNF dynamics mediate the responses of trees over decades of forest recovery?

Global change factors and the marginal benefit of SNF

Our results suggest that the marginal benefit of SNF over soil N uptake may be reduced under low soil moisture or high soil N. Tolerance to prolonged droughts is a key trait for trees in some ecosystems, and N_2-fixers can increase water-use efficiency and fix more C under drought by investing fixed N in photosynthetic enzymes (Tobita et al. 2010, Adams et al. 2016, Minucci et al. 2017). Such a physiological response may explain how *R. pseudoacacia* invested more in SNF under drought and outgrew nonfixers when directly competing for soil resources in small, low-nutrient greenhouse mesocosms (Wurzburger and Miniat 2014). We also observed higher water-use efficiency under dry conditions in our field study; however, it was not accompanied by increased foliar N concentration and productivity, suggesting that *R. pseudoacacia* was not fixing or acquiring enough N to invest in photosynthetic enzymes. The inability of *R. pseudoacacia* to increase foliar N concentrations under dry conditions in this study may reflect the transient nature of dry periods in the field; the longest consecutive dry period with soil moisture in the bottom quartile was 13 d, and the mean duration was 5 d. In a greenhouse study on drought frequency and duration, *R. pseudoacacia* increased foliar N concentration and SNF under 8-week droughts, but not during 2- or 4-week droughts interspersed with wet conditions (Minucci et al. 2017). Thus, the number of consecutive dry days and the frequency of small rainfall events appear to play an important role in determining the physiological response of *R. pseudoacacia* to drought. Interestingly, the number of rain days is decreasing in the southern Appalachian Mountains during the growing season (Burt et al. 2018), a pattern which has been linked to decreased annual growth rate for trees (Elliott et al. 2015). Therefore, how the system responds to drought as a whole may be dictated by both reductions in mean soil moisture and a lower frequency of rain events.

Global change–induced drought will coincide with other abiotic and biotic changes, such as elevated temperature and CO_2, and increased pest and pathogen outbreaks, which could also alter the marginal benefit of SNF over soil N uptake. Warmer climates and longer growing seasons may result in increased annual growth potential, and therefore greater potential C gain per unit N fixed. However, warmer temperatures may also result in increased N mineralization rates in soils with high organic content (Brookshire et al. 2011), which would reduce the benefit of fixed N. Increased temperature also raises respiratory costs for trees (Oishi et al. 2018), and these costs may be even more severe for N_2-fixers that have higher N concentrations in leaf tissues (Mitchell et al. 1999). Atmospheric CO_2 enrichment appears to benefit N_2-fixers over nonfixers (Cernusak et al. 2011), and this effect may be driven by higher growth rates, which lead to greater C return on investment in SNF (Terrer et al. 2018). In addition, there may be variety of other mechanisms by which SNF indirectly provides C gains to N_2-fixers, including investment in herbivore defense compounds (Thamer et al. 2011) or extracellular enzymes to liberate other limiting nutrients such as phosphorus (Houlton et al. 2008). Thus, predicting how global change will influence the marginal benefit of SNF will require understanding the net effect of several, co-occurring physiological and biogeochemical responses, and may be dependent on the functional traits of N_2-fixers as well as their ecosystem context.

CONCLUSIONS

Our study demonstrates the potential for drought to indirectly decrease forest productivity through its reduction of SNF. We found that low soil moisture was associated with reduced aboveground productivity of the N_2-fixing tree, *Robinia pseudoacacia*, and a decline in the relative biomass and density of this species over time. Reductions in the abundance of this species could negatively affect forest productivity, as the presence of *R. pseudoacacia* was correlated with greater soil N cycling rates, soil N stocks, and nonfixer productivity. However, it remains unclear over what timescales the indirect effects of drought will manifest, as both the elevated mineralization rates of fixed N from *R. pseudoacacia* detritus, and the legacy effects of SNF from prior disturbance events, may persist for decades. Perhaps most importantly, our findings raise the possibility that the indirect effects of drought, through interactions with symbiotic N_2-fixing plants, can shape how ecosystems respond to global change.

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