Oecologia (2014) 174:1117–1126
DOI 10.1007/s00442-013-2851-0

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Drought enhances symbiotic dinitrogen fixation and competitive ability of a temperate forest tree

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Received: 31 July 2013 / Accepted: 26 November 2013 / Published online: 12 December 2013

Abstract General circulation models project more intense and frequent droughts over the next century, but many questions remain about how terrestrial ecosystems will respond. Of particular importance, is to understand how drought will alter the species composition of regenerating temperate forests wherein symbiotic dinitrogen (N_2)-fixing plants play a critical role. In experimental mesocosms we manipulated soil moisture to study the effect of drought on the physiology, growth and competitive interactions of four co-occurring North American tree species, one of which (*Robinia pseudoacacia*) is a symbiotic N_2-fixer. We hypothesized that drought would reduce growth by decreasing stomatal conductance, hydraulic conductance and increasing the water use efficiency of species with larger diameter xylem vessel elements (*Quercus rubra*, *R. pseudoacacia*) relative to those with smaller elements (*Acer rubrum* and *Liriodendron tulipifera*). We further hypothesized that N_2 fixation by *R. pseudoacacia* would decline with drought, reducing its competitive ability. Under drought, growth declined across all species; but, growth and physiological responses did not correspond to species' hydraulic architecture. Drought triggered an 80 % increase in nodule biomass and N accrual for *R. pseudoacacia*, improving its growth relative to other species. These results suggest that drought intensified soil N deficiency and that *R. pseudoacacia*'s ability to fix N_2 facilitated competition with non-fixing species when both water and N were limiting. Under scenarios of moderate drought, N_2 fixation may alleviate the N constraints resulting from low soil moisture and improve competitive ability of N_2-fixing species, and as a result, supply more new N to the ecosystem.

Keywords Climate change · Biogeochemistry · Hydraulic conductance · Plant physiology · Stomatal conductance

Introduction

Climate change during the twenty-first century is predicted to include novel climates—combinations of seasonal temperature and precipitation regimes that have no historical or modern counterpart (Williams and Jackson 2007; Williams et al. 2007). For example, individual drought events are projected to increase in duration (Carnicer et al. 2011) and spatial extent (Burke et al. 2006). How terrestrial ecosystems will respond to the increased frequency and duration of drought is unknown, creating uncertainties about the consequences for human well-being (e.g., ecosystem services) and changes to the global C balance.

Supporting the predictions of general circulation models, temperate forests of the southeastern US are experiencing an increased frequency of dry periods during the growing season (e.g., Ford et al. 2011). In these mesic forests, much like forests worldwide, previous drought events have triggered mortality of some tree species more so than others (Allen et al. 2010), suggesting that a chronic reduction

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Communicated by Allan T. G. Green.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-013-2851-0) contains supplementary material, which is available to authorized users.

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in precipitation could lead to a substantial shift in forest composition and ecosystem function. The fact that ecosystems recovering from major disturbances are particularly vulnerable to climatic stressors (Lindenmayer et al. 2011) is an additional consideration for the region. A significant portion of southeastern forests will be in various stages of early succession in the future as a result of new and continuing pressures for timber, biofuels and wood products (e.g., Hinchee et al. 2010; USDA Forest Service 2012). How the structure and function of regenerating forests are altered by drought will depend upon a suite of responses that range across spatial scales: from the physiological responses of individual trees (McDowell et al. 2011), to competitive interactions among tree species for water (Clark et al. 2011), and how these responses modulate broader cycles of C, N and water over the course of forest development (Jeffers et al. 2011).

In forest assemblages, xylem architecture may be a critical plant trait for determining which tree species are capable of persisting through drought events and which are likely to decline in growth or experience mortality. Drought can impair basic physiological functioning of trees (Hubbard et al. 2001) by reducing hydraulic conductivity and inducing cavitation (Tyree and Sperry 1989), but these physiological responses are often complex and depend on a number of factors. For example, there is often a trade off in constructing safe versus efficient conduits: plant hydraulic conductance is typically lower in low soil moisture environments reflecting higher C investment in conduits; while vulnerability to cavitation is typically higher in plants in high soil moisture environments (Maherali et al. 2004, 2006; Pockman and Sperry 2000). Architecturally, species with larger xylem lumen diameters (e.g., ring-porous species) are more predisposed to cavitate under drought than are species with smaller xylem lumen diameters (e.g., diffuse-porous species) (Cai and Tyree 2010; Sperry 2011; Taneda and Sperry 2008; Wheeler et al. 2005). While some plants can recover from hydraulic dysfunction by repairing embolized xylem conduits (Hacke and Sperry 2003), major losses of xylem conductance often lead to mortality (Tyree et al. 2003).

The response of forests to altered precipitation regimes also requires a consideration of species composition during stages of forest development. The composition of mature forests may hinge disproportionately upon the early establishment of a few key species: those that continue to persist through stages of succession and those that decline over time but facilitate the introduction of late successional species. For example, in eastern deciduous forests, *Liriodendron tulipifera* L., *Acer rubrum* L. and *Quercus rubra* L. remain common throughout phases of forest development. In recently harvested stands, *Q. rubra, L. tulipifera*, and *A. rubrum* establishment can be facilitated by stump sprouting as well as seed dispersal. *Robinia pseudoacacia* L. has the additional ability to root sprout and tends to accrue biomass rapidly during the early stage of reforestation. In contrast to other tree species, it declines in abundance over time and is relatively absent from mature stands (Boring and Swank 1984b; Elliott and Swank 2008).

R. pseudoacacia is of particular functional importance because it associates with symbiotic N_2-fixing bacteria, which supply their host with the N required to sustain rapid growth; and as a result, this species introduces a substantial amount of new N to the ecosystem (Boring and Swank 1984a, b). For this and many other leguminous species, drought stress tends to reduce rates of N_2 fixation (Boring and Swank 1984b), due to the physiological constraints of the plant [e.g., high water demands (Du et al. 2011; Schulze et al. 1991)] or constraints on the symbiosis itself (Marino et al. 2007; Zahran 1999). In support of these ideas, a drought event of the 1980s caused substantial mortality of *R. pseudoacacia,* (as well as another ring-porous species, *Q. rubra*) relative to *L. tulipifera* and *A. rubrum* (diffuse-porous species) (Clinton et al. 1993; Elliott and Swank 1994b) and it reduced the amount of nodule biomass supported by *R. pseudoacacia* (Boring and Swank 1984b). Persistent drought may, therefore, not only negatively impact the abundance of *R. pseudoacacia* more so than other tree species, but it may also reduce the amount of new N entering the ecosystem, impairing a natural mechanism for recovery. Given the constraint of N on biomass accrual in regenerating forests (Rastetter et al. 2001), and the potential for this constraint to intensify under CO_2 fertilization (Norby et al. 2010), a reduction in *R. pseudoacacia* growth and N_2 fixation in the absence of other suitable N_2-fixing species, may reduce rates of forest biomass accretion and have consequences on species composition over ecosystem development.

Here, we conducted a manipulative experiment to determine how a reduction in soil moisture (hereafter referred to as “drought”) influences the physiology, growth and competitive interactions of four early successional tree species common in eastern deciduous forests. We hypothesized that species with larger xylem lumen diameters (ring-porous species such as *Q. rubra* and *R. pseudoacacia*) would be more susceptible to drought than species with smaller xylem lumen diameters (diffuse-porous species such as *L. tulipifera* and *A. rubrum*), and that drought-caused reductions in hydraulic conductivity would lead to reductions in growth and stomatal conductance. Because declines in stomatal conductance limit water loss, but allow subsequent C gain to continue (albeit by greater fixation of the heavier isotope of CO_2 that is generally discriminated against), we also expected increases in water use efficiency (WUE) to follow declines in hydraulic and stomatal conductances. Although *R. pseudoacacia* grows rapidly relative to other early successional species, previous drought events have led to significant reductions in growth and fecundity (Clark et al. 2012), high rates of mortality (Clark et al. 2012; Elliott and Swank 1994a), and a reduction in its ability to support nodule biomass (Boring and Swank 1984b). Therefore, we hypothesized that drought would negatively affect the ability of *R. pseudoacacia* to support the N_2-fixing symbiosis, and expected this to manifest as a reduction of nodule biomass and total N gained over the course of the experiment. In the context of competition among species, we hypothesized that the drought-induced decline of *R. pseudoacacia* and *Q. rubra* (because of hydraulic architecture and/or limitation of the N_2-fixing symbiosis) would result in the improved competitive abilities and growth of *L. tulipifera* and *A. rubrum*.

Materials and methods

Experimental design

We conducted a two-factor (species and drought) greenhouse experiment at the Coweeta Hydrologic Laboratory in western North Carolina, USA. Four species—*Robinia pseudoacacia*, *Liriodendron tulipifera*, *Acer rubrum* and *Quercus rubra*—were selected based on their prevalence in early successional forests and their xylem anatomy. *R. pseudoacacia* and *Q. rubra* are species with ring-porous xylem and have long vessels. Maximum vessel lengths in adult stem segments averaged 96 (±16 cm SD) in forests at the study site (C. F. Miniat, unpublished data). *L. tulipifera* and *A. rubrum* are species with diffuse-porous xylem and have shorter vessels. Maximum vessel lengths in adult stem segments averaged 5 (±0.7) and 12 (±0.9) cm, respectively, in forests at the study site (C. F. Miniat, unpublished data). We grew *R. pseudoacacia* from a Kentucky seed source for 16 weeks prior to the experiment, due to the lack of current-year seedlings in local forests; however, current-year seedlings of all other species were collected from local forests.

Our experimental mesocosms were 3.8 l in volume and contained a homogeneous, nutrient-poor soil media (1:1 ratio of sand and peat by volume) to ensure uniform soil porosity and hence, moisture availability. In June, four seedlings (one of each species) were transplanted into each of 40 mesocosms along with material to inoculate the roots with mycorrhizal fungi and other root-associating microbes. This material consisted of 125 ml of native, homogenized soil collected under adult forest trees of *Q. rubra*, *L. tulipifera* and *A. rubrum*, and 10 ml of rhizobial slurry consisting of pulverized nodules and roots in deionized water collected from *R. pseudoacacia* trees. Greenhouse temperature was maintained at 27 °C by a three-stage cooling system and a 12-h photoperiod was maintained using high-pressure sodium lamps triggered by a solar radiation sensor mounted inside the greenhouse.

Soil moisture treatments

At the beginning of the experiment we estimated seedling biomass using allometric equations (Table S1). Total biomass in each mesocosm was ranked into quartiles. Within each quartile, we randomly assigned treatments to mesocosms: half of the mesocosms received a drought treatment ($n = 20$), and half served as controls ($n = 20$). During the experiment we monitored volumetric soil moisture content (θ) at 0–15 cm depth [time domain reflectometry (TDR); CS616; Campbell Scientific], and recorded 15-min values on a subset of eight mesocosms in each treatment ($n = 16$). The period output from each TDR probe (μs) was converted to soil volumetric content using a calibration equation developed on sandy soils in the laboratory. Watering events were implemented treatment-wide when one of the monitored mesocosms reached a minimum soil moisture value. Each mesocosm received 2 l of water when the mean volumetric water content reached or was near 17 or 5 % for the control or the drought treatments, respectively. Because of the nutrient-poor nature of the potting medium, we fertilized with a highly diluted (20 % strength) plant fertilizer (15-30-15 NPK plus micronutrients; Miracle Grow) every 2 weeks throughout the experiment. Treatments were maintained for 22 weeks.

Control mesocosms had roughly twice the soil moisture values as those measured in the drought treatment, 20.3 (±4.8 % SD) compared to 9.9 (±3.2 % SD), respectively (Fig. S1). As a result, drought treatment soils were significantly drier than control soils ($t_{15,0.1} = 9.06$, $P < 0.001$).

Biomass measurements

After 22 weeks of treatment, seedlings were destructively harvested to determine: biomass growth; nodule biomass; foliar C, N and stable C isotope ratio (δ^{13}C); and stem hydraulic conductivity (described below). Seedlings were harvested by clipping stems at the soil level. Aboveground biomass was separated into stems and leaves. Root systems were disentangled with gentle agitation under water. Live nodule biomass was removed from *R. pseudoacacia* root systems with forceps. All biomass was dried at 65 °C in a forced-draft oven to a constant weight, and weighed to the nearest 0.01 g, except for nodules which were weighed to the nearest 0.1 mg. Growth was determined as the change in biomass over the course of the experiment to account for pre-treatment biomass differences.

N content was determined on all tissues at the end of the experiment. Dried biomass was ground to a fine powder in a ball mill grinder. A 13-mg sample of ground tissue was weighed to the nearest 0.01 mg, packed into a tin capsule and combusted according to the Dumas method on an elemental analyzer (Flash EA 1112 NC analyzer; Elantech, Lakewood, NJ).

Stomatal and hydraulic conductance

Leaf gas exchange measurements were made on each species three times during the experiment to determine the treatment effect on stomatal conductance to water vapor (mmol H_2O m^{-2} leaf area s^{-1}). Stomatal conductance was measured with a null balance porometer (LI-1600; LI-COR, Lincoln, NE) under ambient light conditions. One *A. rubrum* replicate and one replicate each of *A. rubrum* and *L. tulipifera* during the first and second measurement dates, respectively, had leaves that were not suitable for measurement due to herbivory. On the third measurement date, 11 replicates (four *L. tulipifera* and seven *R. pseudoacacia*) had no leaves suitable for measurement. Measurements were averaged across all dates for each replicate.

Upon harvesting, we measured stem hydraulic conductance, calculated as the mass flow rate of water through the stem divided by the pressure gradient across the stem segment (k_h; mmol H_2O s^{-1} MPa^{-1}) (Domec and Gartner 2001) using a pressure flow device (Xyl'em; Bronkhorst, Montigny-Les-Cormeilles, France). During harvest, stem xylem segments were re-cut under water, preserving the maximum length possible. Water flow (degassed, deionized 0.22-μm filtered water) through the stem segment was initiated and native k was measured at 40 mbar. We also measured k_h after rehydrating the stem samples with one end in water and a vacuum applied to the atmosphere for 24 h (for diffuse-porous species) or immediately under high pressure for 30 min (ring-porous species) to refill potentially embolized vessels. Percent loss was estimated as native minus rehydrated k_h divided by rehydrated k_h. The temperature of the solution (to correct flow rate for the change in viscosity associated with change in temperature), and the length of each sample were recorded before and after measurements. k_h values were normalized to a water temperature of 20 °C.

WUE estimates

To estimate integrated WUE, we measured the stable C isotope ratio ($\delta^{13}C$) of the leaf material of all plants at the end of the experiment (Farquhar et al. 1989). A 2-mg subsample of dried, ground tissue was analyzed on a continuous flow–combustion–isotope ratio mass spectrometer (Finnigan Delta C; Bremen, Germany) interfaced with an elemental analyser (NA1500; Carlo Erba Instruments, Milan) at the Analytical Chemistry Laboratory of the Odum School of Ecology, University of Georgia, to determine the $\delta^{13}C$.

Statistical analyses

To test the effectiveness of our treatment on volumetric soil moisture content, we used a *t*-test ($\alpha = 0.05$, one-tailed, $n = 8$) on the average θ during the experiment. To test the effects of reduced soil moisture and species on growth, stomatal conductance, native hydraulic conductance, loss of hydraulic conductance, total plant N content, tissue N concentration and WUE ($\delta^{13}C$ of leaf tissue) during the experiment, we used a split-plot design with treatment and species as the whole-plot (one-tailed, $\alpha = 0.05$) and split-plot factors (two-tailed, $\alpha = 0.05$), respectively. We used PROC MIXED (SAS v9.3, SAS Institute, Cary, NC) with the whole-plot error modeled as a random effect. To meet normality assumptions, growth, hydraulic conductivity, and total plant N content data were natural log transformed, and tissue N concentration data were arcsin transformed.

To test the effect of reduced soil moisture on *R. pseudoacacia* nodule biomass, we used a one-way ANOVA ($\alpha = 0.05$, two-tailed) with growth as a covariate (PROC GLM). Data were square root transformed to meet normality assumptions.

We conducted two linear regression analyses to evaluate competitive interactions. In the first analysis, we related *R. pseudoacacia* growth to the total mesocosm biomass in a simple linear regression with no intercept (PROC GLM). In the second analysis, we used an analysis of covariance to relate growth of each species with the growth of *R. pseudoacacia*, both expressed as a fraction of total mesocosm biomass. In the overall model, growth of each species was the continuous dependent variable, *R. pseudoacacia* growth was the continuous independent variable, and species (two-tailed, $\alpha = 0.05$) and treatment (one-tailed, $\alpha = 0.05$) were categorical variables (PROC GLM). If species, treatment, or the interaction were significant, separate regressions were done for each species and treatment.

Results

Effects of drought on seedling physiology and growth

Contrary to our first hypothesis, hydraulic architecture did not consistently influence the physiological and growth responses of seedlings to drought. On average, drought reduced seedling biomass across species by 40 % ($F_{1,38} = 7.23$, $P = 0.01$; Fig. 1). While species differed in growth during the experiment ($F_{3,111} = 19.58$, $P < 0.001$), drought affected each species similarly (no drought by species interaction, $F_{3,111} = 1.65$, $P = 0.18$). *R. pseudoacacia* had the greatest growth among all species, even under drought. Root biomass followed the same pattern as total seedling growth: among species root biomass differed, but

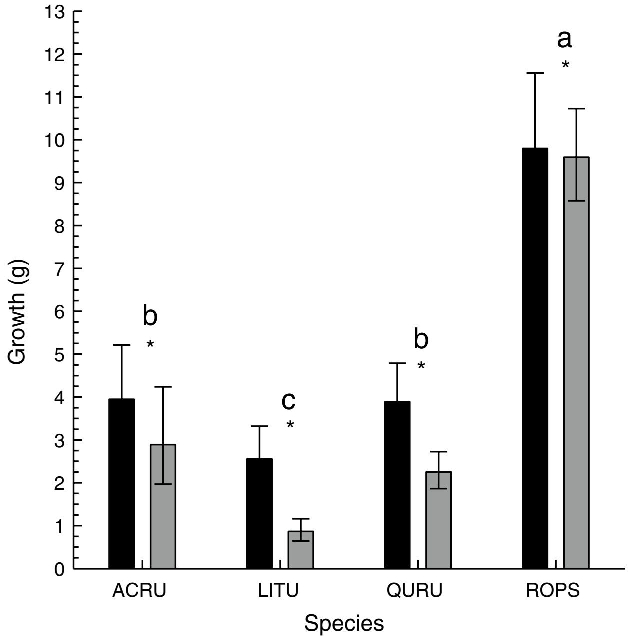

Fig. 1 Plant biomass growth in control (*black bars*) and drought (*grey bars*) experimental mesocosms ($n = 20$). The drought treatment was significant for all species (denoted with an *asterisk*). *Different letters* indicate significant differences among species. Values are untransformed mean and SE. *ACRU Acer rubrum*, *LITU Liriodendron tulipifera*, *QURU Quercus rubra*, *ROPS Robinia pseudoacacia*

drought affected all species similarly, with reductions of 32 % ($F_{1,38} = 7.37$, $P = 0.01$).

Across all species and measurement dates, stomatal conductance declined, on average, by ~10 % under drought (Fig. 2a). While stomatal conductance varied significantly among species (species effect $F_{3,114} = 34.36$, $P < 0.001$), drought affected the stomatal conductance of all species similarly (drought effect $F_{1,38} = 2.76$, $P = 0.10$; no species by treatment interaction). Stomatal and hydraulic conductances were significantly correlated ($R = 0.77$, $P = 0.02$, $n = 8$).

Upon harvest, species varied significantly in hydraulic conductance (species effect $F_{3,93} = 18.69$, $P < 0.001$; Fig. 2b); but, this did not vary predictably by hydraulic architecture. The ring-porous *Q. rubra* had the lowest hydraulic conductance, followed by *L. tulipifera*; *R. pseudoacacia* and *A. rubrum* together had the highest hydraulic conductance. Drought only affected the stem hydraulic conductance of *L. tulipifera* (drought by species interaction $F_{3,93} = 2.52$, $P = 0.06$; Fig. 2b), which experienced a 34 % decline with drought. The loss of hydraulic conductance measured at harvest varied by species as well as by drought (treatment effect $F_{1,35} = 4.37$, $P = 0.04$; species effect

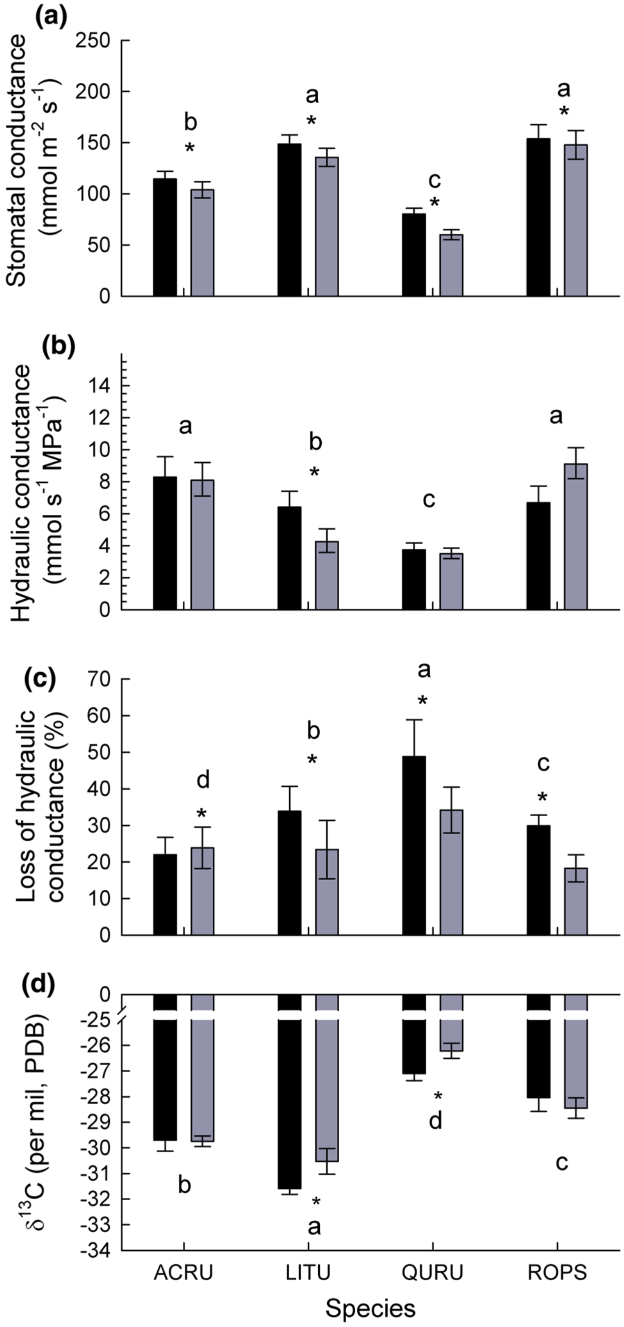

Fig. 2 Physiological responses of seedlings in control (*black bars*) and drought (*grey bars*) experimental mesocosms, for **a** stomatal conductance to water vapor (mmol H_2O m^{-2} leaf s^{-1}), **b** stem hydraulic conductance (mmol H_2O s^{-1} MPa^{-1}), **c** loss of hydraulic conductivity (%), and **d** stable C isotope ratio [$\delta^{13}C$; ‰ Pee Dee belemnite (*PDB*)] of leaf tissue. For stomatal conductance and loss of hydraulic conductivity, a drought response was significant for all species (*asterisks*). *Different letters* indicate significant differences among species. For stem hydraulic conductance and leaf δ^{13}C, a treatment by species interaction was present (denoted with an *asterisk*). Values are untransformed mean and SE. For abbreviations, see Fig. 1

$F_{3,44} = 4.15$, $P = 0.01$; Fig. 2c); but this did not vary predictably by hydraulic architecture. Across both treatments, the ring-porous *Q. rubra* had the highest amount of hydraulic dysfunction (41 %), followed by *L. tulipifera* (29 %), *R. pseudoacacia* (24 %), and *A. rubrum* (23 %) together. All species had a higher loss of hydraulic conductance in the control treatment compared to the drought treatment (33 vs. 25 %).

WUE increased for some species with drought (drought by species interaction $F_{3,93} = 2.56$, $P = 0.06$; Fig. 2d). Differences in WUE among species followed our expectations, where *L. tulipifera* had the lowest WUE, followed by *A. rubrum*, *R. pseudoacacia* and *Q. rubra* (species effect $F_{3,47} = 69.42$, $P < 0.001$). However, in response to drought, only *Q. rubra* and *L. tulipifera* displayed greater WUE (both increased by 3 %).

N_2 fixation and accumulation in plant biomass

Contrary to our second hypothesis, drought increased the biomass of N_2-fixing nodules of *R. pseudoacacia* by roughly 80 % relative to the control treatment (Fig. 3). Nodule biomass was positively correlated with total plant biomass ($R = 0.52$, $P < 0.001$); after adjusting for total plant biomass, we observed a significant drought effect on nodule biomass ($F_{1,37} = 4.43$, $P = 0.04$).

All species declined in total N content under drought ($F_{3,114} = 29.52$, $P < 0.001$; Fig. S2). *R. pseudoacacia* had the greatest accumulation of N (346 mg), nearly 3.5 times that of all the other species. And even though *R. pseudoacacia* experienced an increase in nodule biomass with drought, on average, we observed a 28 % reduction in total plant N across all species by the end of the experiment in response to drought ($F_{1,38} = 5.34$, $P = 0.03$; no drought by species interaction, $F_{3,114} = 2.09$, $P = 0.11$). The pattern observed with N content was not a result of changes in N concentration in response to drought ($F_{1,38} = 0.12$, $P = 0.73$; Figs. S2 and S3).

Interspecific competition

Contrary to our third hypothesis, *R. pseudoacacia* was the best competitor among the four species, even under drought. In general, *R. pseudoacacia* growth comprised a similar proportion of total growth in each mesocosm (42 %) in both treatments (no treatment effect, $F_{2,37} = 2.23$, $P = 0.12$; overall model, slope = 0.42, $F_{1,39} = 144.75$, $P < 0.001$, $R^2 = 0.14$). Growth by all other species was negatively correlated with that of *R. pseudoacacia* (slope = −0.33, $F_{1,113} = 33.44$, $P < 0.001$); but, the strength of the negative relationship depended on the treatment (no treatment effect; species effect $F_{2,113} = 7.08$, $P = 0.001$; species by treatment interaction $F_{2,113} = 2.52$, $P = 0.08$; Fig. 4). *A. rubrum* growth was negatively related to *R. pseudoacacia* growth; but, this relationship was only significant under drought (linear regression, $F_{1,18} = 20.56$, $P < 0.001$, $R^2 = 0.53$, slope = −0.65; Fig. 4a). For *L. tulipifera*, a negative relationship in growth was only significant under control conditions (linear regression, $F_{1,18} = 21.15$, $P < 0.001$, $R^2 = 0.54$, slope = −0.67; Fig. 4b). This pattern emerged because *L. tulipifera* grew the least among all species, with the largest individuals occurring in the control treatment. The proportional change in *Q. rubra* growth was negatively related to *R. pseudoacacia* growth; but, this relationship was only significant under drought (linear regression, $F_{1,18} = 7.40$, $P = 0.01$, $R^2 = 0.29$, slope = −0.24; Fig. 4c).

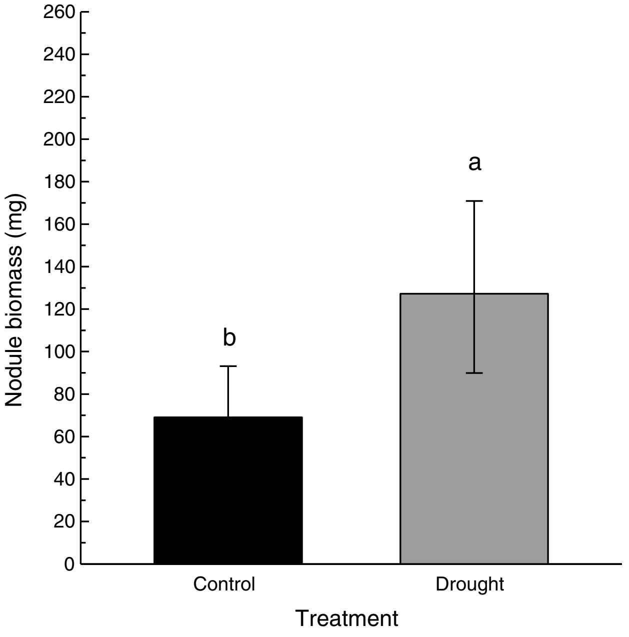

Fig. 3 Root nodule biomass of *R. pseudoacacia* in control and drought experimental mesocosms. *Different letters* indicate a significant treatment difference. Values are untransformed means and SE

Discussion

Our study demonstrates a fundamental relationship between water and N in regulating competitive interactions among four tree species in response to drought. In temperate forests responding to disturbance, N constrains primary productivity (Gerber et al. 2013; Vitousek and Howarth 1991), making the natural source of new N to ecosystems, symbiotic N_2 fixation, critical for alleviating N limitation and facilitating forest recovery (Boring et al. 1988). Therefore, understanding the effect of drought on ecosystem processes requires knowledge about the effects of water limitation on the N cycle in general and symbiotic N_2 fixation in particular.

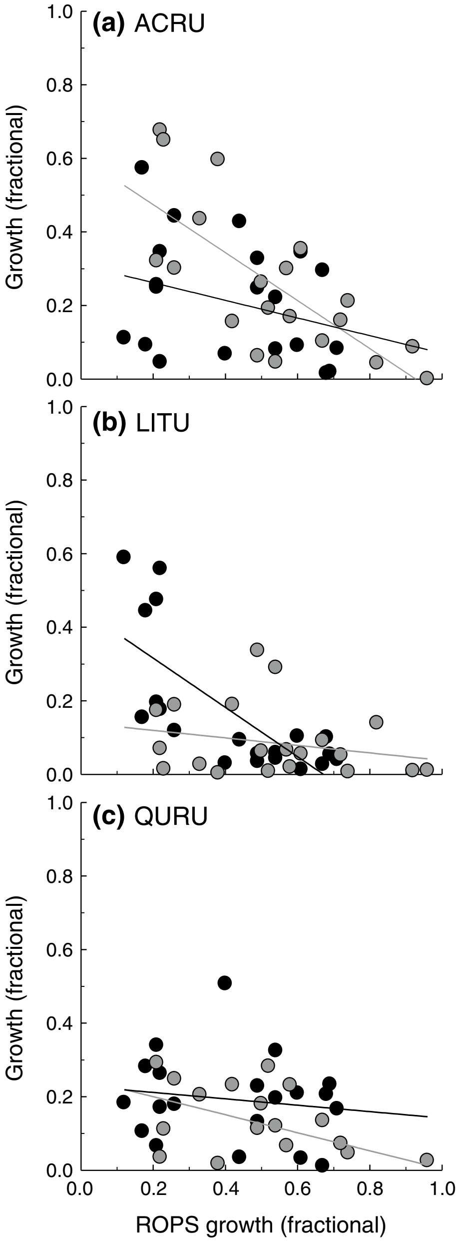

Fig. 4 Biomass growth for each species relative to that of ROPS in control (*black*) and drought (*grey*) experimental mesocosms for **a** ACRU, control $y = 0.31 - 0.24x$, $R^2 = 0.10$, $P = 0.18$; drought $y = 0.60 - 0.65x$, $R^2 = 0.53$, $P < 0.01$; **b** LITU, control $y = 0.45 - 0.67x$, $R^2 = 0.54$, $P < 0.01$; drought $y = 0.14 - 0.10x$, $R^2 = 0.05$, $P = 0.21$ and **c** QURU, control $y = 0.23 - 0.09x$, $R^2 = 0.02$, $P = 0.21$; drought $y = 0.25 - 0.25x$, $R^2 = 0.29$, $P = 0.01$. Growth is expressed as a fraction of total mesocosm growth. Competitive relationships differed for drought and control treatments depending on the species. For abbreviations, see Fig. 1

Our results provide evidence that drought can exacerbate N deficiency, induce nodulation by an N_2-fixer, and modulate competitive relationships among N_2-fixing and non-fixing tree species. Under drought, *R. pseudoacacia* increased its investment in nodulation in order to support its rapid growth, which negatively affected the growth of *A. rubrum* and *Q. rubra*. This competitive interaction had been absent under moist soil conditions. While *L. tulipifera* biomass was not negatively correlated with that of *R. pseudoacacia* under drought, it was the species that generally grew the least. This species maintained a relatively high stomatal conductance compared to the other species; yet, it had relatively few leaves, reflecting its drought-deciduous (i.e., drought avoidance) nature. In general, species that lacked the ability to fix N_2 experienced compounding effects of water and N limitation. In contrast, drought induced nodulation and improved the competitive ability of *R. pseudoacacia*, which was contrary to our expectation and to previous observations of *R. pseudoacacia* in response to drought in natural ecosystems.

The energetic cost of N_2 fixation (Rastetter et al. 2001) ensures that the trait is only favorable when the acquisition of N from soil becomes more costly for meeting plant N demands. In the case of facultative N_2 fixation, plant investment in bacterial symbionts is regulated by the cost of fixation relative to that of acquiring N from the soil environment (Menge et al. 2009). *R. pseudoacacia* demonstrates this type of regulation; the species increases N_2 fixation in response to declining availability of soil N (e.g., Johnsen and Bongarten 1991, 1992). Therefore, in our experiment, an increased investment in N_2-fixing nodules by *R. pseudoacacia* under drought suggests that the treatment resulted in a reduction in the available N pool, or a deficiency of soil moisture for facilitating the diffusion of N to plant roots.

Soil moisture presents a new layer of complexity in the consideration of resource acquisition trade-offs for symbiotic N_2-fixers. Reductions in soil moisture can trigger N deficiency in soils (Cramer et al. 2010) that may in turn favor N_2 fixation as a means to acquire N over the construction of additional root biomass. The intricacy of relationships among energetic resources, water and N in regulating N_2 fixation can be gleaned from the literature across diverse terrestrial ecosystems. N_2-fixing species become relatively more abundant than non-fixers with increasing aridity in N-poor shrublands (Monks et al. 2012) and N_2 fixation rates increase with declining precipitation across savanna ecosystems (Schulze et al. 1991). However, reductions in soil moisture can present an ultimate physiological constraint on N_2-fixing organisms (Du et al. 2011; Freiberg 1998; Schulze et al. 1991). Along a continuum of soil moisture conditions, N_2 fixation may increase in response to drought-induced N deficiency until the point at which water limitation exceeds the level of physiological tolerance for supporting symbiotic N_2 fixation. Tolerance thresholds may vary among N_2-fixing species and ecosystems, but this conceptual framework can resolve the seemingly contradictory responses of N_2 fixation to drought in the literature. For example, a drought threshold can be exceeded through mechanisms of water limitation on hydraulic processes (Du et al. 2011; Schulze et al. 1991) or on the physiology and activity of the bacterial symbionts (Marino et al. 2007; Zahran 1999). In field studies, while N_2 fixation tends to increase along gradients of aridity, it declines at the most arid of sites (Aranibar et al. 2004; Schulze et al. 1991). For *R. pseudoacacia* in temperate forests, severe summer droughts have reduced nodule biomass (Boring and Swank 1984a) and prolonged drought has led to substantial tree mortality (Elliott and Swank 1994b). In our study, despite the fact that our drought treatment was severe enough to constrain growth across all species, we speculate that it remained within the range of drought tolerance for *R. pseudoacacia* and that the influence of water on soil N had a stronger influence on N_2 fixation than did reduction of water alone.

Contrary to our expectations, hydraulic architecture did not function as a general determinant of a species' growth response to drought. We expected growth of the two ring-porous species, *Q. rubra* and *R. pseudoacacia*, to be the most negatively affected by the drought treatment. While *Q. rubra* was the most severely affected by drought in all physiological parameters measured (it demonstrated the lowest values of stomatal and stem hydraulic conductance and increased its WUE), *R. pseudoacacia* was one of the least affected by drought. *R. pseudoacacia* had either high or intermediate levels of stomatal and stem hydraulic conductance and WUE relative to the other species, similar to previous studies (e.g., Du et al. 2011). In fact, hydraulic conductance moderately increased with drought for *R. pseudoacacia,* suggesting that, for this species, N_2 fixation may partially offset physiological constraints of water when both water and N are limiting.

We also expected that the two species with smaller xylem lumen diameters, *A. rubrum* and *L. tulipifera*, would be less affected by the drought treatment compared to the ring-porous species. Indeed *A. rubrum* was the least impaired by drought; although it was not the best competitor (*R. pseudoacacia* remained the dominant competitor). In contrast, *L. tulipifera* was severely affected by drought, both in mean changes in biomass growth and among physiological parameters. In terms of biomass growth, *L. tulipifera* had ~70 % reduction in biomass relative to the control, and was generally the least WUE species; it produced nearly 80 % less leaf biomass than it did in the control treatment (data not presented); thus, the water use by the plant was low. It is unclear why hydraulic architecture was a poor predictor of tree species' response to drought in our study. Vulnerability to cavitation can also be affected by wood density, decreasing as wood density increases (Lens et al. 2011), and vessel length. Species with shorter vessels are more resistant to cavitation compared to those with longer vessels, due in part to the greater number of interconduit pits in the latter compared to the former (Christman et al. 2012; Lens et al. 2011). A myriad of factors affect vulnerability to cavitation, and there are multiple hydric solutions for coping with water stress, such as positive root pressure and activation of plasma membrane intrinsic proteins (Leng et al. 2013); however, little is known about the point beyond which embolism repair is not possible (Meinzer and McCulloh 2013; Ogasa et al. 2013).

Our results demonstrate the complexity of factors that determine forest response to drought, including the regulation of water use at the leaf and xylem levels, to competition among species for water and N. Extending these results to field conditions requires several additional considerations. While our mesocosm approach provided a uniform platform for determining drought effects on tree species, the design is limited in its ability to replicate the full range of regeneration mechanisms that characterizes recently disturbed forests (e.g., seed dispersal, stump- and root-sprouting). Therefore, in field conditions, tree populations are likely to demonstrate greater variance in their access to belowground resources than was demonstrated in our study. However, we speculate that the competitive dominance we observed in *R. pseudoacacia* is likely to hold in field conditions, as this species vigorously sprouts from root networks and produces deep sinker roots (Apsley 1987 and references therein) unlike the other species investigated here. This root architecture is common among N_2-fixing savanna species (e.g., Cramer et al. 2010) allowing for greater access to water resources, and could further modulate the threshold of tolerance for *R. pseudoacacia* in scenarios of drought. Future work, including in situ soil moisture and N manipulations, would help provide more insight into the mechanisms driving tree species competition in response to drought.

Conclusion

Our results suggest that drought effects on regenerating temperate forests will, in part, be influenced by the means by which tree species acquire water and N. Our study demonstrated relationships among water, N and species competition that an alternative experimental approach (e.g., tests of single species response to drought) may have failed to detect. Based on the results of our study and observations of *R. pseudoacacia* in the field, drought effects on symbiotic N_2 fixation are likely to be complex and result from a variety of trade-offs among energetic resources, N and water availability and physiological traits of trees. If moderate drought favors *R. pseudoacacia* growth relative to other species because of declines in soil N availability, new questions emerge about how enhanced rates of N_2 fixation may change patterns in biomass accretion over ecosystem development (Rastetter et al. 2001). Furthermore, the implications of extreme drought are unclear. Severe-drought scenarios could lead to diminished inputs of N via N_2 fixation and intensified N constraints on primary production. Therefore, it is of critical importance to understand tolerance thresholds for symbiotic N_2 fixation and determine the consequences of drought on biogeochemical cycles over the course of forest regeneration.

Acknowledgments This study was supported by the United States Department of Agriculture Forest Service, Southern Research Station, and by cooperative agreement number 11-CA-11330140-095 to N. Wurzburger at the University of Georgia. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the authors and do not necessarily reflect the views of the USDA Forest Service or the University of Georgia. We thank Neal Muldoon, Shialoh Wilson, Sheena Zhang, Courtney Collins, Steven T. Brantley, and Jeff Minucci for their assistance with this research. We are grateful to Lindsay Boring, Ford Ballantyne and two anonymous reviewers for their constructive comments on the manuscript.

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