Research paper

Effects of nitrogen addition and increased precipitation on xylem growth of *Quercus acutissima* Caruth. in central China

Biyun Yu [iD] [1,2,3,4], Sergio Rossi[1,5], Hanxue Liang[6], Xiali Guo [iD] [7], Qianqian Ma[1,2,3], Shaokang Zhang [iD] [1,2,3], Jian Kang[4], Ping Zhao[1,2,3,4], Wei Zhang[1,2], Yuxi Ju[8] and Jian-Guo Huang[1,2,3,4,9]

[1]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China; [2]Core Botanical Gardens, Chinese Academy of Sciences, Guangzhou 510650, China; [3]Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China; [4]University of Chinese Academy of Sciences, Beijing 100049, China; [5]Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC G7H 2B1, Canada; [6]Institute of Loess Plateau, Shanxi University, Taiyuan 030006, China; [7]College of Forestry, Guangxi University, Nanning 530004, China; [8]Jigongshan National Natural Reserve, Xinyang 464000, China; [9]Corresponding author (huangjg@scbg.ac.cn)

Atmospheric nitrogen (N) deposition and increasing precipitation affect carbon sequestration in terrestrial ecosystems, but how these two concurrent global change variables affect xylem growth in trees (i.e., independently or interactively) remains unclear. We conducted novel experiments in central China to monitor the xylem growth in a dominant species (*Quercus acutissima* Caruth.) in response to N addition (CN), supplemental precipitation (CW) or both treatments (CNW), compared with untreated controls (C). Measurements were made at weekly intervals during 2014–15. We found that supplemental precipitation significantly enhanced xylem growth in the dry spring of 2015, indicating a time-varying effect of increased precipitation on intra-annual xylem growth. Elevated N had no significant effect on xylem increment, xylem growth rate, and lumen diameters and potential hydraulic conductivity (K_s) of earlywood vessels, but K_s with elevated N was significantly negatively related to xylem increment. The combination of additional N and supplemental precipitation suppressed the positive effect of supplemental precipitation on xylem increment in the dry spring of 2015. These findings indicated that xylem width was more responsive to supplemental precipitation than to increasing N in a dry early growing season; the positive effect of supplemental precipitation on xylem growth could be offset by elevated N resources. The negative interactive effect of N addition and supplemental precipitation also suggested that increasing N deposition and precipitation in the future might potentially affect carbon sequestration of *Q. acutissima* during the early growing season in central China. The effects of N addition and supplemental precipitation on tree growth are complex and might vary depending on the growth period and local climatic conditions. Therefore, future models of tree growth need to consider multiple-time scales and local climatic conditions when simulating and projecting global change.

Keywords: interactive effect, nitrogen deposition, seasonal response, supplemental precipitation, xylem growth.

Introduction

Nitrogen (N) is an essential macro element for plant growth (Ohyama 2010), mainly because N is a vital component of the thylakoids and enzymes in leaf chloroplasts that drive leaf photosynthetic carbon fixation (Evans 1989). Nitrogen also participates in nutrient transportation (Millard et al. 2006) and in building cellular structures (Cooke et al. 2003). Therefore,

N is of critical importance in the ecophysiological functions of plants and is particularly important for biomass production (Schulte-Uebbing and de Vries 2018). Previous studies have indicated that plants can obtain N by absorbing N through root, leaf stomata or cuticles following wet deposition from the atmosphere (Rennenberg and Gessler 1999, Sparks 2009, Nair et al. 2016). Therefore, alteration in the atmospheric N content

has great potential to affect the ecophysiological functions and biomass accumulation of plants (Schulte-Uebbing and de Vries 2018, Liang et al. 2020).

Precipitation is a vital climate factor for plant growth (Sarris et al. 2007, Die et al. 2012, Deslauriers et al. 2016) because it predominantly determines the amount of water available to plants (Neufeld et al. 1999, Deslauriers et al. 2016). Water from rain events is required for plant cell division and extension, transpiration and photosynthesis (Zweifel et al. 2001, Steppe et al. 2015), and affects N availability in plants (Kazanski et al. 2021); thus, water availability can modulate plant physiology, affect plant morphogenesis (Turcotte et al. 2009, Steppe et al. 2015), and ultimately affect plant growth and productivity (Zeppel et al. 2014, Fang et al. 2015). Therefore, the fluctuation in precipitation has great potential to alter plant growth.

In the context of global change, anthropogenic activities have enhanced atmospheric N deposition in the northern hemisphere and also shifted precipitation regimes (Galloway et al. 2004, Galloway et al. 2008, Chou and Lan 2012, IPCC 2013). The amount and the distribution of N deposition and precipitation are not uniform in the northern hemisphere. Asia (China, India), Western Europe and North America experience the highest rates of atmospheric N deposition (Dentener et al. 2006, Reay et al. 2008). Nitrogen deposition can increase N input to terrestrial ecosystems, thus altering the N availability of terrestrial ecosystems, whereas excessive N deposition can cause soil acidification (Lu et al. 2014) and change the soil N cycle and nutrient balance, thus affecting the carbon and N cycles of terrestrial ecosystems (Galloway et al. 2008). Simultaneously, global change has resulted not only in reduced precipitation and water shortages in mid-latitudes of the northern hemisphere (30°N–50°N) (Routson et al. 2019), but also in increased precipitation in other regions (Zhai et al. 2005). For example, compared with southern and north China with decreased precipitation, very heavy precipitation events have increased over the Yangtze River valley and the southeastern region of China due to the weakening East Asian summer monsoon during 1957–2014 (Zhai et al. 2005, Ma et al. 2015). Increases in precipitation can also bring a significant impact on terrestrial ecosystem (e.g., changing soil water availability or affecting soil nutrients, etc.) (Kong et al. 2013a, 2013b, Zhang et al. 2015); however, studies on the influence of increased precipitation on terrestrial ecosystems are still less reported, especially in forest ecosystems. Since widespread N deposition and increased precipitation have the potential to affect carbon uptake by altering N availability and soil water, respectively (Huxman et al. 2004, Santiago et al. 2004, Galloway et al. 2008, Niu et al. 2009, Janssens et al. 2010, Wang et al. 2019, Liang et al. 2020), both of them can affect plant growth.

Although these two aspects of global change are known to affect plant growth and carbon sequestration (Gentilesca et al. 2013, Lie et al. 2018), their effects on plant growth do not always occur in isolation, as these factors interact with each other (Yang et al. 2011, Shen et al. 2016, Han et al. 2017). Previous studies have shown that soil microbes responsible for nitrification are more active under high soil water availability (Chapin et al. 2002, Wang et al. 2006, Liu et al. 2009). On one hand, elevated water availability due to the increased precipitation can enhance net mineralization, increase soil N availability and mobility of inorganic N, and promote plant N uptake (Hooper and Johnson 1999, Wang et al. 2006). On the other hand, plant growth might be more limited by nutrient rather than water availability under increasing water availability, and thus might be more sensitive to N than to water addition (Hooper and Johnson 1999, Xiao et al. 2007, Xia and Wan 2008). Therefore, precipitation increase can regulate the effects of N on plant communities through its impact on N availability (McCulley et al. 2009, Nielsen and Ball 2015, Dijkstra et al. 2018), especially in arid systems where plants are water-limited (Zhang et al. 2015). However, excess precipitation can cause N loss through leaching (Chapin et al. 2002, Chen et al. 2019), which might reduce N- use efficiency if surplus water fails to elevate photosynthetic efficiency at a given N supply rate (Patterson et al. 1997, Zhang and Cao 2009). In the case of increased precipitation, N enrichment could offset water stress alleviation by promoting the growth of understory herbaceous species to reduce topsoil moisture (She et al. 2019). In addition, once N inputs exceed biotic demands, it might result in soil acidification, contributing to cation depletion and even causing nutrient (e.g., magnesium and manganese) imbalances of plants (Rennenberg and Gessler 1999, Nakaji et al. 2001, Lu et al. 2014). In this case, moderate precipitation increase can alleviate the negative effects brought by excessive N input on plants (Zhang et al. 2015), whereas excessive precipitation inputs are likely to exacerbate the loss of soil N (Kong et al. 2013a); consequently, it might restrict plant growth. Therefore, the interactive effect of N deposition and increased precipitation consequently has a profound influence on terrestrial carbon sequestration.

Considerable evidence now supports an interactive effect between N deposition and increased precipitation on plant growth (Zhao et al. 2015, Shen et al. 2016, Han et al. 2017). For example, an experiment conducted in southern California grassland demonstrated that increases in precipitation and N availability improved the dominance of *Lolium multiflorum* Lamk. and *Avena fatua* L. and their late-season carbon fluxes (Harpole et al. 2007). The findings demonstrated a strongly interdependent relationship between N deposition and future precipitation patterns on the grassland ecosystem. Similarly, a field manipulation experiment showed a positive combination effect of N deposition and increased precipitation on the growth of *Potentilla tanacetifolia* Willd. ex Schlecht. seedlings in a temperate steppe in China (Li et al. 2011). However, another study showed that N addition under elevated precipitation

reduced the N resorption efficiency in leaves of *Leymus chinensis* (Trin.) Tzvel. in a semiarid grassland in China (Shen et al. 2018). Most studies that have examined combinations of these two environmental factors have been conducted on herbaceous plants (Harpole et al. 2007, Shen et al. 2018) or seedlings (Wang et al. 2012, Yan et al. 2013), and mainly in desert and steppe ecosystems (Zhao et al. 2015, Shen et al. 2016, Han et al. 2017). In sum, the interactive effects of N addition and precipitation increase on plants in ecosystems are complex and not uniform, but vary with different environmental conditions. Until recently, few studies have considered the combined effects of these two environmental changes on tree growth in forest ecosystems (Yin et al. 2009, Lupi et al. 2012, Zhang et al. 2017, 2018).

Studies of the interactive effects of N deposition and increased precipitation on xylem growth are particularly lacking, although xylem growth is well recognized as a vital process for long-term carbon sequestration in forest ecosystems (Rossi et al. 2016). The dynamics of xylem growth reflect carbon sequestration by trees, as well as potential forest productivity in response to climate change, on a fine time-scale (e.g., weekly or seasonal) (Castagneri et al. 2017, Huang et al. 2018), and these dynamics could be influenced by multiple environmental factors (Sarris et al. 2007, Fonti et al. 2010). However, little is known about whether or how N deposition and increased precipitation interact to affect the xylem growth of forest trees.

In the present study, we addressed the issue mentioned above using a micro-sampling approach for weekly monitoring of the xylem growth of a dominant species (*Quercus acutissima* Caruth.) in a warm temperate forest of central China. The trees were treated with canopy N addition and precipitation manipulation to investigate whether and how increases in N deposition and precipitation might affect xylem growth. These forests are considered N-limited (Hedin 2004), but they receive abundant annual precipitation that might be enhancing the leaching and loss of N (Yang et al. 2009). Under higher levels of water availability, plant growth may shift from water- to nutrient-limited (Xiao et al. 2007, Xia and Wan 2008), thus plant growth and productivity may be more responsive to N addition rather than water increase (Hooper and Johnson 1999, Xia and Wan 2008). Therefore, we tested the hypotheses that (i) xylem growth is more sensitive to N addition than to increases in precipitation and (ii) the effect of N addition on xylem growth is mediated by the supplemental precipitation.

Materials and methods

Study area

This study was conducted in the Jigongshan National Nature Reserve (31°46′–31°52 N, 114°01′–114°06 E), in the Henan province of China, located in a climate transitional zone from the subtropical to the warm temperate regions. The stand consists of a mixed deciduous forest with 50-year-old trees. The mean values of the last 60-year meteorological data (1951–2010) indicate an average annual temperature of 15.2 °C (Zhang et al. 2015), and the mean annual precipitation of 1119 mm, with 80% of the total precipitation falling from April to October (Zhang et al. 2015). The rate of wet N deposition is 19.6 kg N ha^{-1} year^{-1} in the study area (Zhang et al. 2015). The soil texture is sandy loam (Shi et al. 2016). Vegetation at the study area belongs to a temperate deciduous broadleaf forest which is dominated by *Q. acutissima*, *Quercus variabilis* and *Liquidambar formosana* (Ouyang et al. 2017). *Q. uercus acutissima* is one of the dominant tree species on the study site and its important value calculated by summing the relative abundance, relative frequency and relative dominance index was 0.42 (Ouyang et al. 2017).

Experimental design

Circular plots with an area of 907 m^2 were set up in 2012 (Zhang et al. 2015). Four plots were selected for the present study, including (i) C: a control (no artificial N or water addition); (ii) CN: canopy N addition of 25 kg N ha^{-1} year^{-1}; (iii) CW: additional canopy precipitation of 30% of the mean annual precipitation (from 1951–2010); and (iv) CNW: addition of both canopy N and precipitation addition (with canopy N addition of 25 kg N ha^{-1} year^{-1} and precipitation increase of 30% of the mean annual precipitation). Nitrogen solution was sprayed to the canopy once per month, avoiding windy or rainy days. The total solution per year (∼ 20 mm) used in the CN treatment accounted for < 2% of the total annual precipitation (Table 1); therefore, its confounding effect caused by increased precipitation is marginal (Zhang et al. 2015). Supplemental precipitation was provided at weekly intervals (once per week) to avoid surface runoff (Shi et al. 2018). The chemical composition of the added water was approximated to natural rainfall by collecting rainfall in tanks near the study plots or from the nearby lakes when rainfall was scarce (Shi et al. 2018). Before the N solution was prepared each time, the content of N from added water was tested. These manipulations have been conducted during the growing season (April–October) since 2013.

Sampling, sample preparation and cellular measurements

In each treatment, three to four healthy adult trees with upright stem were monitored during 2014–15. The diameter at breast height of sampling trees under C, CN, CW and CNW is 35.15 ± 7.09, 37.78 ± 3.87, 36.70 ± 10.80 and 30.17 ± 6.19 cm, respectively. No significant difference in diameter at breast height of sampling trees was detected among different treatments. The wood microcores (2 mm diameter) were extracted from the stem at breast height with a Trephor (Rossi et al. 2006) at weekly intervals from March to December. Samples were extracted following a semi-helical pattern along the stem, and at least 2 cm from previous samplings points to

Table 1. The total precipitation (including ambient precipitation from Xinyang weather station and artificial precipitation (mm/m²) of the four treatments (C, CN, CW and CNW) in 2014 and 2015. The numbers outside and within the brackets indicate the amount of total and supplemental precipitation, respectively. C: control; CN: canopy nitrogen addition; CW: canopy precipitation supplementation; CNW: canopy nitrogen and precipitation additions in combination

Year	Treatment	Before monsoon season (April–May)	Monsoon season (June–August)	After monsoon season (September–November)
2014	C	235.9 (0)	355.4 (0)	228.3 (0)
	CN	241.5 (5.6)	363.8 (8.4)	233.9 (5.6)
	CW	316.5 (80.6)	539.2 (183.8)	294.0 (65.7)
	CNW	322.1 (86.2)	547.6 (192.2)	299.6 (71.3)
2015	C	133.1 (0)	581.9 (0)	197.4 (0)
	CN	138.7 (5.6)	590.3 (8.4)	203.0 (5.6)
	CW	213.7 (80.6)	765.7 (183.8)	263.1 (65.7)
	CNW	219.3 (86.2)	774.1 (192.2)	268.7 (71.3)

avoid anatomical malformation or disturbance by any previous sampling (Gruber et al. 2009). Bark was removed from trees with thick bark until the phloem was exposed. All samples were stored at 4 °C in 50% ethanol solution in Eppendorf microtubes.

For observations, the microcores were dehydrated in a graded series of ethanol and D-limonene and then embedded in paraffin (Rossi et al. 2006). Transverse sections of 8–10 μm thick were prepared, mounted on slides, dried and then deparaffinized by immersing the slides in D-limonene and ethanol. The sections were then stained with cresyl violet acetate and observed under an optical microscope. According to previous studies, the initiation of xylem growth was defined as when 50% of the observed radial files showed at least one first enlarging cell (which radial diameter at least twice the radial diameter of the cambial cells) (Rathgeber et al. 2011, Zhang et al. 2017). Unlike coniferous species, in which xylem is mainly composed of tracheids, the xylem structure of broad-leaved species generally includes wood fibers, axial parenchyma cells, vessel elements and vessel associated cells (Gričar 2010, Čufar et al. 2011). Because of the diversity and complexity of xylem cells in broad-leaved species, the radial width of the current xylem growth rings is generally applied to characterize xylem growth (Gričar 2010, Čufar et al. 2011). Therefore, three radial rows per sample were selected for measurement of the weekly xylem increments (μm) (Yu et al. 2019). Intra-annual xylem width data were log-transformed to reduce heteroscedasticity. The impact of the inherent growth trend of trees was minimized by computing intra-annual relative xylem growth (i.e., the ratio of the weekly xylem width to the average tree-ring width) of each tree. Each percentage represents the relative xylem growth in the study week. Additionally, the finial xylem increment (i.e., annual tree-ring width) during 2014–15 was determined based on a minimum of six microcores per tree extracted in 2016.

Vessels are the vital functional structure for water transport in the xylem of broad-leaved trees (González-González et al. 2014, Zhang et al. 2019). Considering the high sensitivity of earlywood vessels to environmental signals (García-González and Fonti 2006) and the higher contribution of large vessel to hydraulic conductivity (González-González et al. 2014), each cross-section was then examined for the size (radial and tangential diameters, in um), number, and area of earlywood vessels (V_{area}) and total xylem area (um²). The earlywood vessels were defined as the area >7500 um² (González-González et al. 2014, Zhang et al. 2019). Each vessel area was computed as follow:

$$A = \pi (a \times b)^2 /4, \tag{1}$$

where a and b are major and minor perpendicular lumen diameters of each vessel. The vessel area percentage of the entire xylem cross-section (V_{ratio}) was calculated by dividing cumulative area of earlywood vessels by the entire xylem cross-section. Vessel density ($V_{density}$) was computed by dividing the number of vessels by the entire xylem cross-section. The important vessel hydraulic parameters: physical diameter (D_i), hydraulic diameter (D_h) and potential hydraulic conductivity (K_s) of earlywood vessels (Tyree and Ewers 1991, Tyree and Zimmermann 2002) were computed as:

$$D_i = 2(A/\pi)^{1/2} \tag{2}$$

$$D_h = \left(\left(\sum D_i{}^4 \right) /N \right)^{1/4} \tag{3}$$

$$K_s = \left(\pi \cdot \rho \cdot \sum D_i{}^4 \right) /128 \cdot \eta \cdot A_m, \tag{4}$$

where N is the number of vessels measured, and A_m is the xylem area analyzed. ρ and η are the density (998.2 kg m⁻³) and dynamic viscosity (1.002×10⁻⁹ MPa) of water at 20 °C.

Meteorological data collection

Daily climate data were obtained from the Xinyang weather station (32°08′N, 114°03′E) nearest to the sampling site. These data were available from the National Meteorological Information Center (http://data.cma.cn/), including daily maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperature, as well as precipitation.
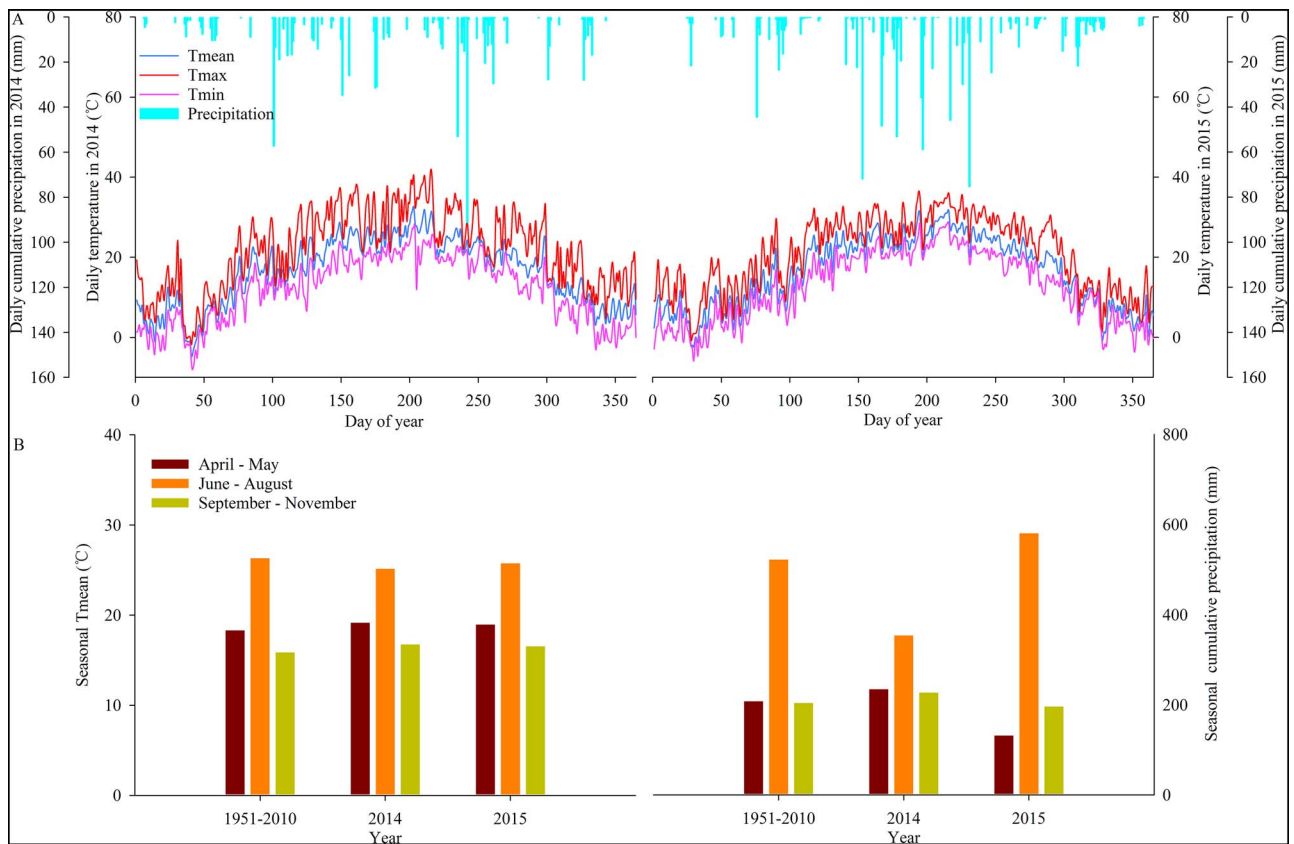
Figure 1. Daily (A) and seasonal (B) temperature and cumulative precipitation from the Xinyang weather station. Tmean, Tmax and Tmin represent the mean, maximum and minimum air temperature, respectively.

Statistical analysis

The climatic characteristics of the region were used to divide the growing season for intra-annual xylem formation into three seasons, April–May, June–August and September–November, corresponding to the periods before, during and after the monsoon season, respectively. Mixed-effects models could effectively estimate the fixed effects and deal with autocorrelation error in the repeated measurement over time (Lindstrom and Bates 1990, Davidian and Giltinan 1995). In order to investigate: (i) the effects of elevated N, increased precipitation and season on relative xylem growth, and (ii) the effects of elevated N and increased precipitation on earlywood vessel indexes (D_i, D_h, K_s, V_{area}, V_{ratio} and $V_{density}$), repeated measures analysis of variance (ANOVA) was performed using linear mixed models incorporated into the 'nlme' package in the R computing environment (Lindstrom and Bates 1990, Davidian and Giltinan 1995, Pinheiro and Bates 2000, Jose et al. 2017), with tree as a random factor.

The monthly xylem growth rate was also calculated using the mean xylem width minus that measured in the previous month. Three-way ANOVA was used to examine the effects of N, precipitation and the factor time (year or season) on the seasonal xylem growth rate. Post hoc analysis after the significant interactive effect was performed using the 'pairs' function in the 'lsmeans' package in the R computing environment (Lenth 2016), where the least-squares means (adjust means) for factor combinations in the fitted linear models was computed using the 'lsmeans' function in 'lsmeans' package (Searle et al. 1980, Lenth 2016) and the 'cld' function in 'multcomp' package (Piepho 2004).

Results

Meteorological differences between years

The climatic variables of the study site differed between 2014 and 2015 (Figure 1). The mean Tmax of the three seasons (April–May, June–August and September–November) was 25.72, 31.40 and 22.41 in 2014, 5.27, 4.78 and 5.49% higher than that in 2015, respectively. During June–August in 2014, a reduction in the accumulative precipitation, corresponding to 32.10% of that occurring during the last 60 years (1951–2010) and 38.93% of that in 2015, resulting in a drought during July–August in 2014. A drier early growing season (April–May, prior to the monsoon season) occurred in 2015, resulting in a cumulative precipitation of 36.39% less than that during 1951–2010 and 43.58% less than in 2014.
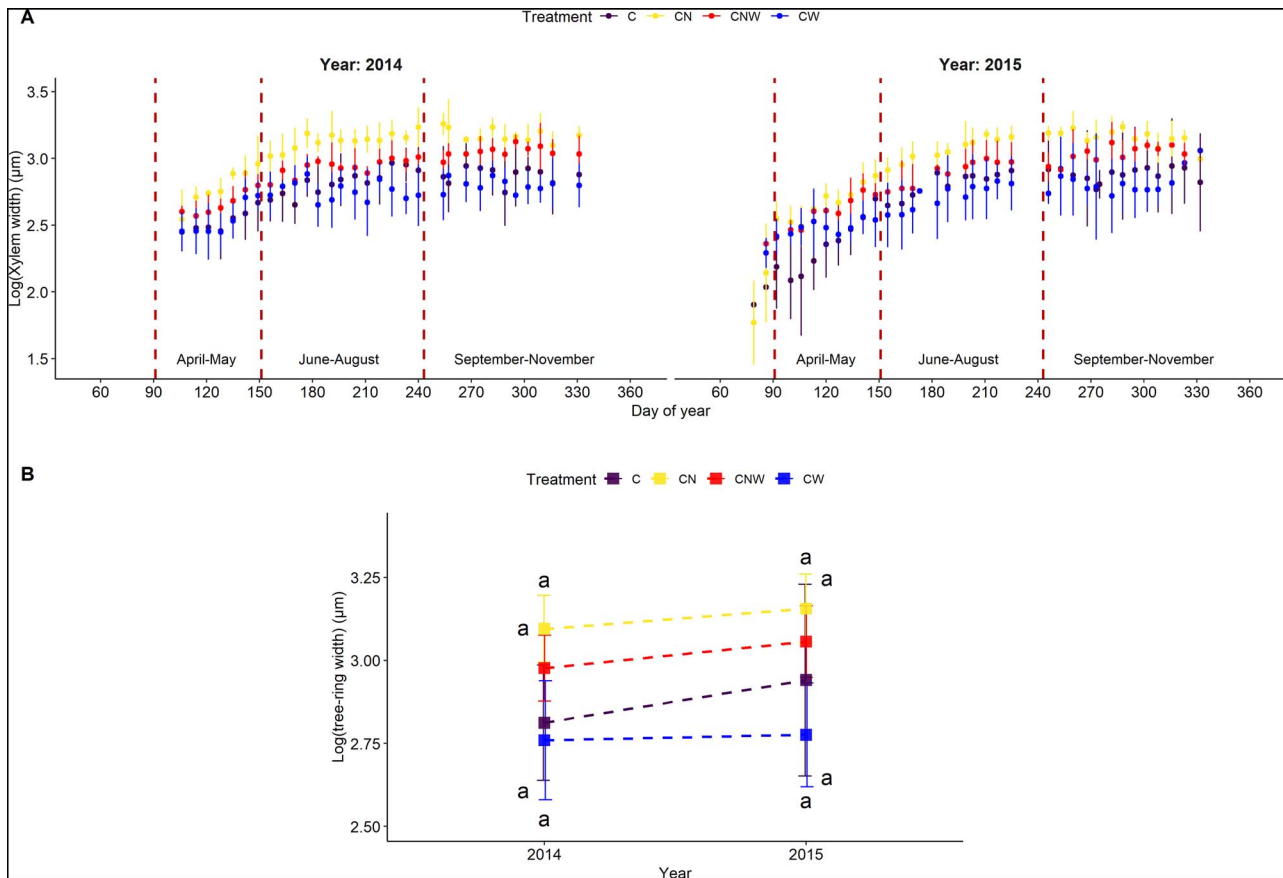
Figure 2. Log-transformed weekly xylem width (A) and tree-ring width (B) in 2014 and 2015. C: control; CN: canopy nitrogen addition; CW: canopy precipitation addition; CNW: canopy nitrogen and precipitation addition in combination. Values are presented as mean ± SD. Different letters denote significant differences in the width among treatments for each year at $P < 0.05$ by Tukey test.

Xylem growth dynamics

Overall, xylem formation started before the first monitored date in 2014 (day of the year (DOY) 106) and 2015 (DOY 79) (Figure 2A). The weekly xylem width before DOY 130 (mid-May) in 2015 was higher under CW than under C conditions. In contrast, the xylem growth dynamic during this phase in 2014 was similar between CW and C. The xylem growth under all treatments in 2014 slowed down after July (DOY 181), whereas it continued with an upward trend during July–August in 2015. In 2014 and 2015, the weekly xylem width was wider under CN than under other treatments (C, CW and CNW) and the weekly xylem width was lower under CW than under CNW, but no significant difference was detected between CN and C, CN and CW, CN and CNW, or between CW and CNW in both monitored years. The cumulative xylem width during April–May accounted for >75% of the mean total xylem width for each treatment in both monitored years (Figure 3). At the inter-annual scale, no significant difference in final xylem width was detected between any two treatments during the monitored years (2014 and 2015) (Figure 2B). Sample trees in C conditions produced significantly wider final xylem width in 2015 than in 2014; however, no significant difference in the

final xylem width between the two monitored years was found for sample trees in CN, CW or CNW (Figure 2B).

Relative xylem growth and xylem growth rate

Large differences were observed in relative xylem growth among treatments before the monsoon season (April and May) in both monitored years, but especially in 2015 (Figure 3). The interactive effects of N addition and supplemental precipitation on relative xylem growth varied with years and seasons (Table 2). A significant effect of season was observed on relative xylem formation in 2014 and 2015. No significant effect was detected for either N addition or supplemental precipitation regarding the relative xylem growth in the 2 study years. The interaction variables of supplemental precipitation × season and elevated N × supplemental precipitation × season on relative xylem growth were only significant before the monsoon season (April–May) in 2015.

The relative xylem width under CW showed a higher mean value than that under C, CN and CNW during April–May in 2015, but only the difference in relative xylem width between CW and C reached statistical significance ($P < 0.05$) in 2015 (Figure 3). The mean relative xylem width under CNW before (April and
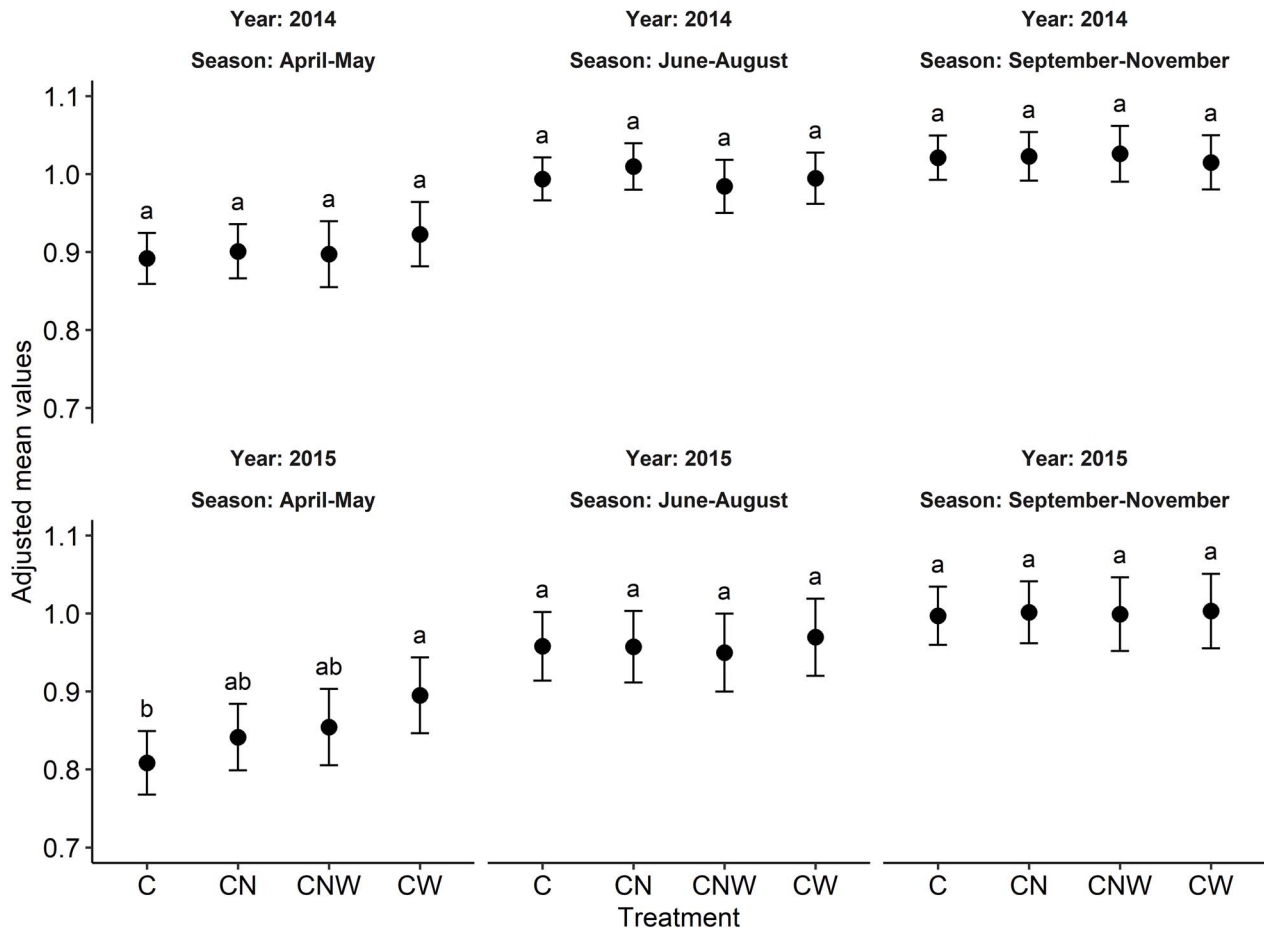
Figure 3. Seasonal relative xylem width under different treatments (C, CN, CW and CNW) in 2014 and 2015. C: control; CN: canopy nitrogen addition; CW: canopy precipitation addition; CNW: canopy nitrogen and precipitation addition in combination. The adjust mean values represent the least-squares means computed from the mixed effects model using the R package 'lsmean'. The error bars represent the 95% confidence interval. Different lowercase letters stand for significant variation in the relative xylem width among different treatments during each season in each year.

Table 2. Results of repeated measures ANOVA on the effects of elevated nitrogen ($N_{addition}$), increased precipitation ($P_{increased}$) and season (April–May, June–August and September–November) on relative xylem growth in 2014 and 2015

Year	Source of variation	F-value	P-value
2014	$N_{addition}$	0.056	0.818
	$P_{increased}$	0.001	0.983
	Season	248.135	<0.001
	$N_{addition} \times P_{increased}$	1.142	0.310
	$N_{addition} \times Season$	0.605	0.546
	$P_{increased} \times Season$	2.854	0.059
	$N_{addition} \times P_{increased} \times Season$	2.556	0.079
2015	$N_{addition}$	0.054	0.822
	$P_{increased}$	1.638	0.230
	Season	360.885	<0.001
	$N_{addition} \times P_{increased}$	2.642	0.135
	$N_{addition} \times Season$	0.207	0.813
	$P_{increased} \times Season$	9.974	<0.001
	$N_{addition} \times P_{increased} \times Season$	4.186	0.016

May) and during June–August fell between the values under CN and CW, but no significant difference was detected in the mean relative xylem width among these three treatments. No significant difference was observed in the mean relative xylem growth among treatments during September–November (after the monsoon season) in both years. In addition, during the two growing season, a relatively larger fluctuation of xylem growth rate was found in April–May compared with the other two seasons (Figure 4). However, no significant difference was detected among the treatments at this season during both monitored years.

The results from post hoc analysis for the interaction showed a significantly higher relative xylem growth with supplemental precipitation (P_1) than without supplemental precipitation (P_0) ($P < 0.001$) when no artificial N was given (N_0) during April–May in 2015 (Figure 5 and Table 3). However, no significant difference was found between the relative xylem growth under P_1 and P_0 when N was added (N_1) (Table 3). In addition, no
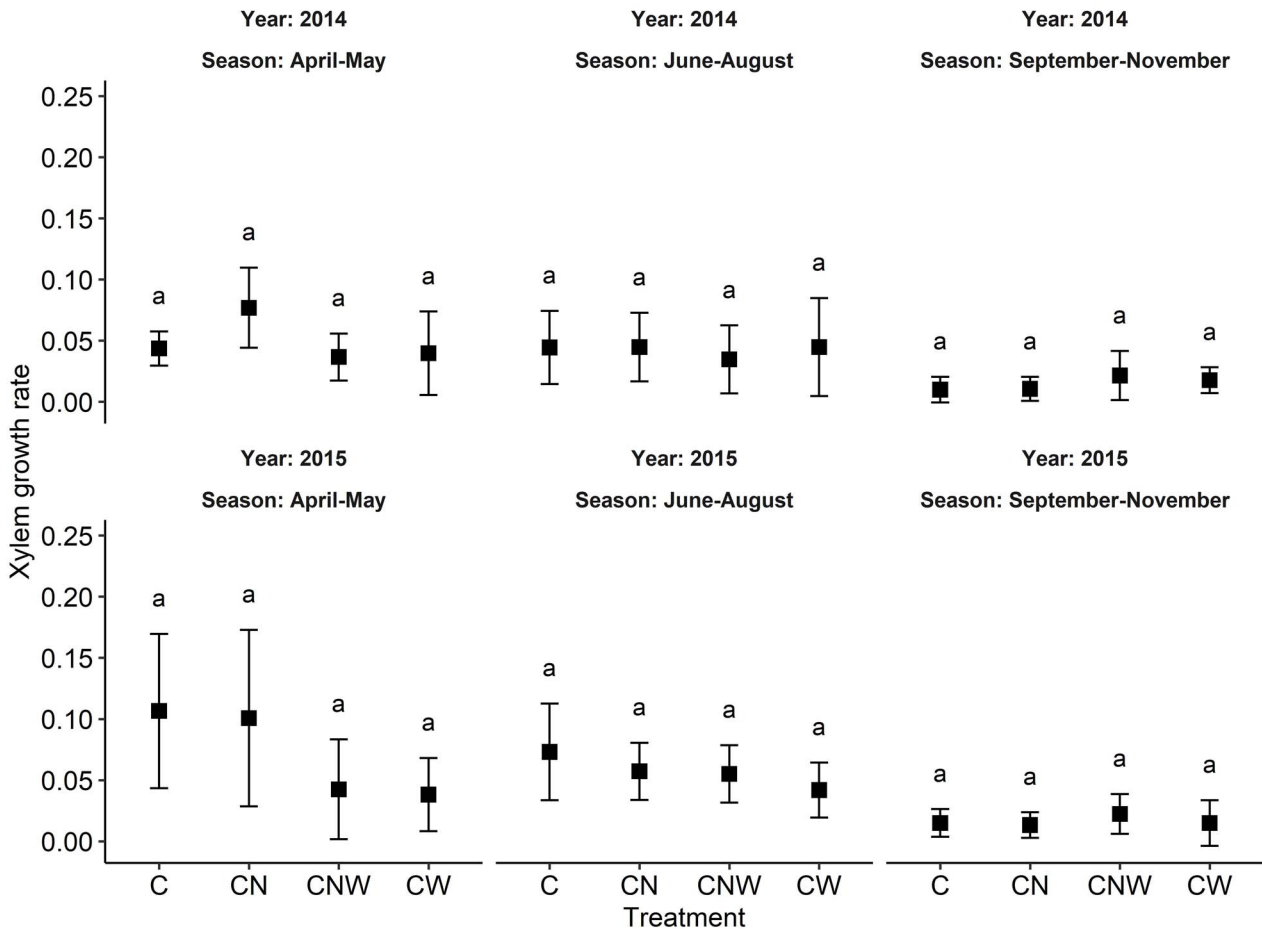
Figure 4. Seasonal xylem growth rate under different treatments (C, CN, CW and CNW) in 2014 and 2015. C: control; CN: canopy nitrogen addition; CW: canopy precipitation addition; CNW: canopy nitrogen and precipitation addition in combination. Values are presented as mean ± SD. Different lowercase letters stand for significant variation in the xylem growth rate among different treatments during each season in each year at $P < 0.05$ by Tukey test.

significant difference was observed in the relative xylem growth between the groups with (N_1) and without N addition (N_0) during this season when precipitation increase was set as the fixed factor.

Earlywood vessel indexes and its relationship with xylem increment

On a whole, the mean vessel size (physical and hydraulic diameter and vessel area) of earlywood under CN was relatively higher than that under other treatments (C, CNW and CW) (Figure 6). Multiple comparison results showed significant differences in physical and hydraulic diameter and vessel area between CN and CW during 2015. In contrast to vessel size, the vessel density under CN was significantly lower than under CW during both years. However, no remarkable differences in all vessel indexes, including physical (D_i) and hydraulic diameter (D_h), mean vessel area (V_{area_mean}), percentage of total vessel area (V_{ratio}), potential hydraulic conductivity (K_s)

and vessel density ($V_{density}$), were detected between C and CN, C and CNW, or C and CW during both years. Interaction analysis for N addition, precipitation increase, and year showed that, the main effect of year had significant effects on all earlywood vessel indexes (Table 4). Significant main effect of N addition was only detected on $V_{density}$, in which $V_{density}$ with N addition was significantly lower than that without N applied (estimate = −84.7, $t = −2.543$, $P = 0.029$). No significant main effect of increased precipitation and interactive effect of N addition and precipitation increase was found on V_{ratio}, D_i, D_h, K_s and V_{area_mean}.

The relationships between earlywood vessel indexes and total xylem increment varied at different years and treatments. Both D_i and D_h under C had significant positive relationship with total xylem increment in 2014, but no significant positive relationship between K_s and total xylem increment was observed in both years. K_s under CN was remarkably negatively related to total xylem increment in both monitored years (Table 5).
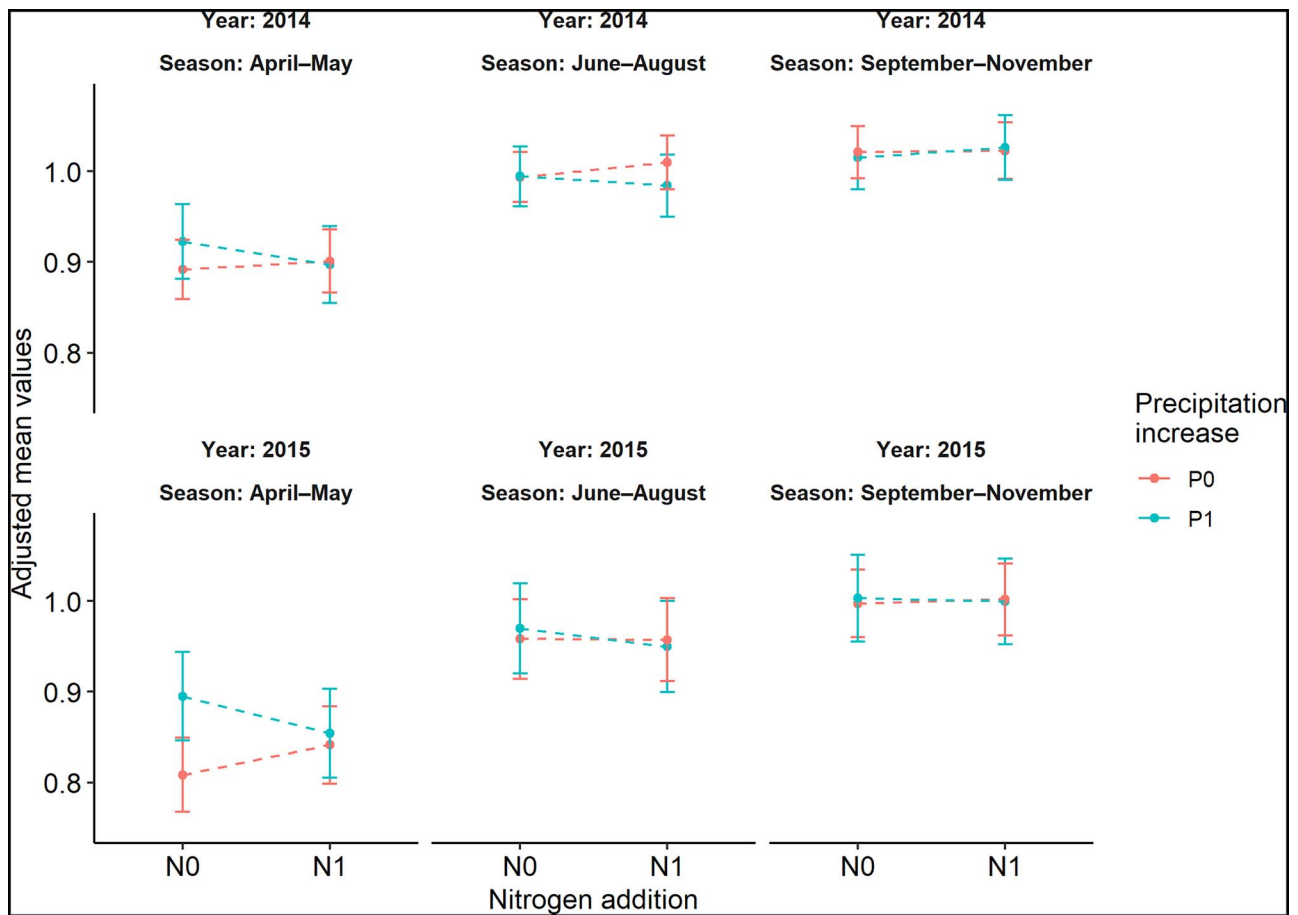
Figure 5. Seasonal interaction plots between nitrogen (N) addition and precipitation (P) increase for xylem formation in 2014 and 2015. The adjust mean values represent the least-squares means computed from the mixed effects model using the R package 'lsmean'. The error bars represent the 95% confidence interval. N_0: without N addition; N_1: with N addition; P_0: without increased precipitation; P_1: with increased precipitation.

Table 3. Post hoc analysis after the significant interactive effect of nitrogen addition (N_0 and N_1), precipitation increase (P_0 and P_1) and season (S_1, S_2 and S_3) on relative xylem growth in 2015 made using the 'pairs' function in R package 'lsmeans'

Contrast	Estimate	SE	t-value	P-value
$N_1\,P_0\,S_1 – N_0\,P_0\,S_1$	0.033	0.017	1.992	0.692
$N_1\,P_1\,S_1 — N_0\,P_1\,S_1$	−0.041	0.019	−2.168	0.597
$N_0\,P_1\,S_1 – N_0\,P_0\,S_1$	0.086	0.018	4.888	0.017
$N_1\,P_1\,S_1 – N_1\,P_0\,S_1$	0.013	0.018	0.729	1.000
$N_1\,P_0\,S_2 – N_0\,P_0\,S_2$	−0.001	0.018	−0.038	1.000
$N_1\,P_1\,S_2 – N_0\,P_1\,S_2$	−0.019	0.019	−1.036	0.992
$N_0\,P_1\,S_2 – N_0\,P_0\,S_2$	0.012	0.019	0.626	1.000
$N_1\,P_1\,S_2 – N_1\,P_0\,S_2$	−0.008	0.018	−0.409	1.000
$N_1\,P_0\,S_3 – N_0\,P_0\,S_3$	0.004	0.015	0.287	1.000
$N_1\,P_1\,S_3 – N_0\,P_1\,S_3$	−0.004	0.018	−0.220	1.000
$N_0\,P_1\,S_3 – N_0\,P_0\,S_3$	0.006	0.017	0.361	1.000
$N_1\,P_1\,S_3 – N_1\,P_0\,S_3$	−0.002	0.017	−0.138	1.000

N_0: without N addition; N_1: with N addition; P_0: without increased precipitation; P_1: with increased precipitation; S_1: April–May; S_2: June–August; S_3: September–November.

Discussion

Elucidating how concurrent atmospheric N deposition and increased precipitation affect xylem formation of forest trees would help us improve the understanding of tree growth and forest productivity in the context of global change. Through a 2-year (2014 and 2015) investigation of the effects of N addition and supplemental precipitation on xylem growth of *Q. acutissima*, this study found that no significant main effect
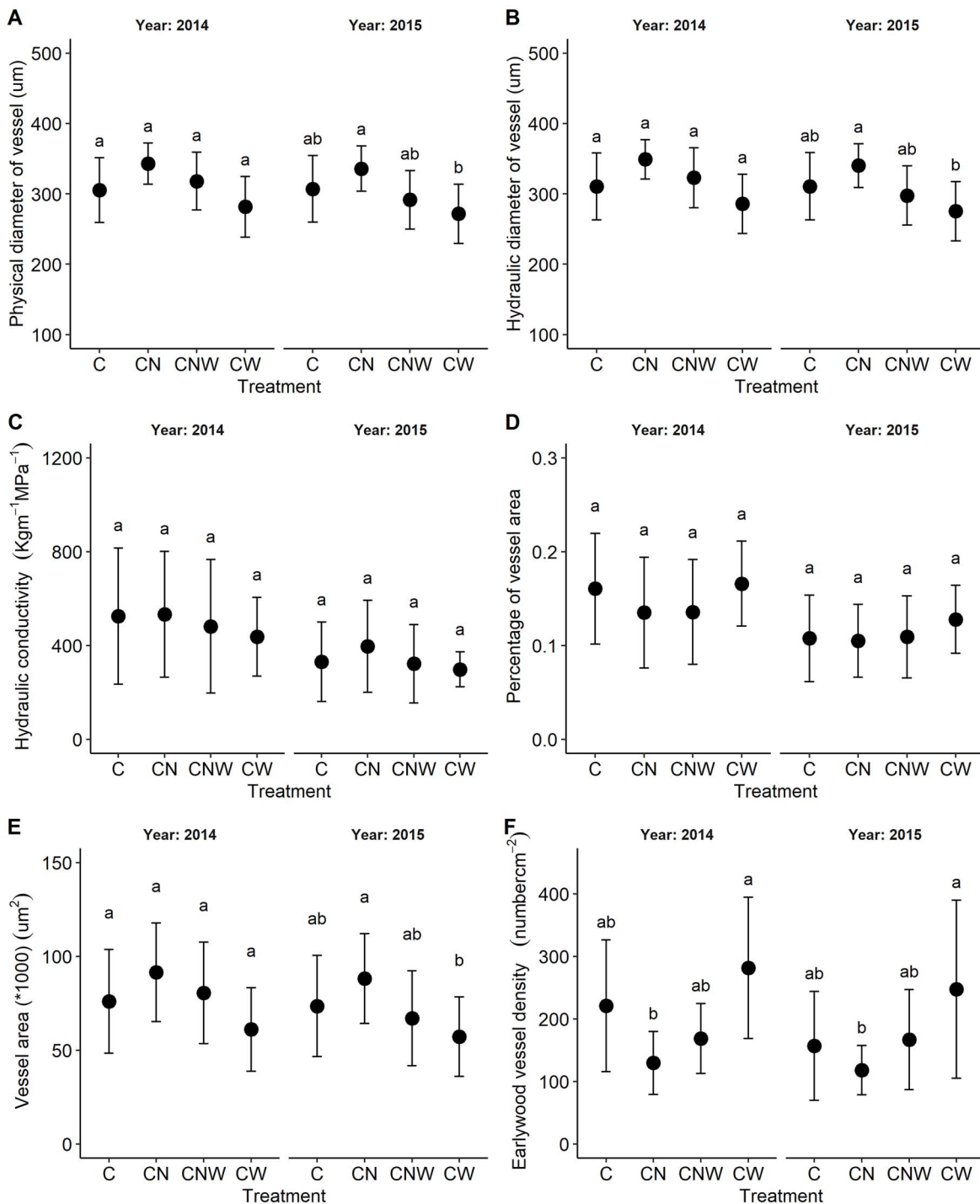
Figure 6. The earlywood vessel indexes (physical diameter (μm), hydraulic diameter (μm) and potential hydraulic conductivity (kg m^{-1} MPa^{-1})), vessel area (μm^2), percentage of vessel area and vessel density (number cm^{-2}) of *Q. acutissima* under different treatments (C, CN, CNW and CW) in 2014 and 2015. Values are presented as mean $\pm$ SD. C: control; CN: canopy nitrogen addition; CW: canopy precipitation supplementation; CNW: canopy nitrogen and precipitation additions in combination.

of N addition was detected at the xylem increment, xylem growth rate, mean vessel area, and hydraulic diameters and conductivity of earlywood vessels during the two monitored years. Compared with N addition, supplemental precipitation had

a significant positive effect on xylem increment of *Q. acutissima*, but only during a relatively dry early growing season in 2015 and without artificial N addition (Figure 5 and Table 3). The study suggested that xylem width of *Q. acutissima* was more

Table 4. Results of repeated measures ANOVA on the effects of elevated nitrogen ($N_{addition}$), increased precipitation ($P_{increased}$) and year on earlywood vessel indexes (vessel area percentage (V_{ratio}), physical diameter (D_i), hydraulic diameter (D_h), potential hydraulic conductivity (K_s), mean vessel area (V_{area_mean}) and vessel density ($V_{density}$))

Parameters	Source of variation	F-value	P-value
V_{ratio}	$N_{addition}$	2.205	0.168
	$P_{increased}$	0.514	0.490
	Year	20.286	<0.001
	$N_{addition} \times P_{increased}$	0.149	0.707
	$N_{addition} \times$ Year	0.902	0.344
	$P_{increased} \times$ Year	0.250	0.618
	$N_{addition} \times P_{increased} \times$ Year	0.144	0.705
D_i	$N_{addition}$	3.156	0.106
	$P_{increased}$	3.104	0.109
	Year	3.949	0.049
	$N_{addition} \times P_{increased}$	0.011	0.920
	$N_{addition} \times$ Year	0.774	0.380
	$P_{increased} \times$ Year	3.364	0.069
	$N_{addition} \times P_{increased} \times$ Year	0.016	0.901
D_h	$N_{addition}$	3.076	0.110
	$P_{increased}$	2.868	0.121
	Year	5.283	0.023
	$N_{addition} \times P_{increased}$	0.006	0.941
	$N_{addition} \times$ Year	0.652	0.421
	$P_{increased} \times$ Year	2.988	0.086
	$N_{addition} \times P_{increased} \times$ Year	0.001	0.970
K_s	$N_{addition}$	0.196	0.667
	$P_{increased}$	0.584	0.462
	Year	20.344	<0.001
	$N_{addition} \times P_{increased}$	0.000	0.984
	$N_{addition} \times$ Year	0.222	0.638
	$P_{increased} \times$ Year	0.008	0.930
	$N_{addition} \times P_{increased} \times$ Year	0.372	0.543
V_{area_mean}	$N_{addition}$	3.762	0.081
	$P_{increased}$	3.430	0.094
	Year	5.061	0.025
	$N_{addition} \times P_{increased}$	0.001	0.974
	$N_{addition} \times$ Year	0.721	0.396
	$P_{increased} \times$ Year	2.209	0.138
	$N_{addition} \times P_{increased} \times$ Year	0.392	0.532
$V_{density}$	$N_{addition}$	6.324	0.031
	$P_{increased}$	3.821	0.079
	Year	3.973	0.048
	$N_{addition} \times P_{increased}$	0.297	0.598
	$N_{addition} \times$ Year	1.385	0.241
	$P_{increased} \times$ Year	0.826	0.365
	$N_{addition} \times P_{increased} \times$ Year	0.309	0.579

sensitive to precipitation increase than N addition in a short term, but the effect of supplemental precipitation on xylem growth was mediated by the N resource condition and varied with time, which did not support our hypotheses. Last but not least, xylogenesis of *Q. acutissima* had already begun before we sampled, which indicated a need of earlier monitoring for future studies in order to obtain the complete process of xylem formation during the year.

Effects of N addition on xylem growth

Trees canopy can uptake N from wet deposition through leaf stomata or cuticle (Rennenberg and Gessler 1999, Sievering et al. 2007, Tomaszewski and Sievering 2007, Sparks 2009, Wortman et al. 2012, Nair et al. 2016). The absorbed N can be used to form chloroplast thylakoids and enzymes (i.e., Rubisco) which are vital for leaf photosynthesis (Evans 1989, Nakaji et al. 2001) and carbon sequestration of plant (Wortman et al. 2012,

Table 5. The relationship between the earlywood vessel hydraulic indexes (mean vessel area (V_{area_mean}), physical diameter (D_i), hydraulic diameter (D_h) and potential hydraulic conductivity (K_s)) and radial xylem increment (D_r)) of *Q. acutissima* under different treatments (C, CN, CNW and CW). C: control; CN: canopy nitrogen addition; CW: canopy precipitation supplementation; CNW: canopy nitrogen and precipitation additions in combination

Year	Treatment	Fixed effect	Estimated value	SE	*t*-value	*P*-value
2014	C	V_{area_mean}	0.001	0.000	1.587	0.117
		D_i	1.908	0.734	2.600	0.016
		D_h	2.213	0.767	2.884	0.008
		K_s	0.063	0.107	0.592	0.559
	CN	V_{area_mean}	0.000	0.000	−0.073	0.942
		D_i	0.371	1.506	0.246	0.809
		D_h	0.763	1.586	0.481	0.637
		K_s	−0.417	0.158	−2.644	0.018
	CNW	V_{area_mean}	−0.001	0.001	−0.901	0.373
		D_i	−1.240	2.191	−0.566	0.583
		D_h	−0.998	2.140	−0.466	0.650
		K_s	−0.252	0.237	−1.065	0.310
	CW	V_{area_mean}	0.000	0.000	0.136	0.892
		D_i	1.037	0.600	1.730	0.101
		D_h	1.077	0.638	1.688	0.109
		K_s	−0.037	0.138	−0.266	0.794
2015	C	V_{area_mean}	0.001	0.001	0.526	0.601
		D_i	0.201	1.509	0.133	0.895
		D_h	0.837	1.610	0.520	0.609
		K_s	−0.642	0.344	−1.867	0.075
	CN	V_{area_mean}	0.001	0.001	0.831	0.410
		D_i	−0.525	2.180	−0.241	0.813
		D_h	−0.490	2.271	−0.216	0.832
		K_s	−0.790	0.307	−2.577	0.021
	CNW	V_{area_mean}	−0.001	0.001	−0.989	0.328
		D_i	−0.412	2.233	−0.184	0.857
		D_h	−0.651	2.290	−0.285	0.781
		K_s	−0.834	0.413	−2.018	0.069
	CW	V_{area_mean}	−0.000	0.001	−0.232	0.818
		D_i	−0.767	1.060	−0.724	0.483
		D_h	−0.535	1.074	−0.498	0.628
		K_s	−0.609	0.323	−1.882	0.084

Nair et al. 2016). Therefore, canopy photosynthesis relies on N supply conditions and leaf N content (Nakaji et al. 2001, Chiwa et al. 2004). Xylem cell development depends heavily on the nonstructural carbon (NSC, i.e., sucrose) produced by leaf photosynthesis (Krabel and Roloff 1999), thus it might be affected by N addition. However, the effect of N addition on xylem growth (a long-term process of carbon sink in terrestrial ecosystems) varies with tree species and the amount of N addition (Zhang et al. 2018, Yu et al. 2019). Previous studies at the same study site found that the same amount of canopy N addition (50 kg N ha^{-1} year^{-1}) played a different role in xylem growth of different tree species, in which elevated N could significantly promote xylem growth of *Q. acutissima* (Yu et al. 2019), whereas no significant effect of elevated N on xylem growth was detected for *Q. variabilis* (Yu et al. 2019) or *L. formosana* (Zhang et al. 2018), indicating a species-specific N effect. This might be related to the different N requirements of different tree species. For example, a study on

leaf N and phosphorus concentrations and their stoichiometry of *Quercus* species across China revealed that, compared with *Q. variabilis*, *Q. acutissima* is slightly N-limited, whereas *Q. variabilis* is phosphorus-limited (Wu et al. 2012), thus *Q. acutissima* is more responsive to N addition than *Q. variabilis*. In addition, the degree to which trees respond to elevated N also depends on the amount of N supplied. In contrast, no significant effect on the xylem growth of *Q. acutissima* was noted for canopy N addition of 25 kg N ha^{-1} year^{-1} (half of that in the previous study) in the present study, suggesting that the N supplied in this study might be not enough to cause a significant increase in the xylem width of *Q. acutissima*.

In the life cycle of trees, vessel size and density are sensitive to ecological environmental changes, especially sensitive to climatic events and nutrient availability (Fonti et al. 2010). The hydraulic benefit of ring-porous trees is mainly affected by the size of the vessel. Previous N-addition experiments have revealed that high N resource can affect whole-plant hydraulic

conductance by altering vessel diameter and density (Plavcova and Hacke 2012, Goldstein et al. 2013); consequently, it might increase hydraulic conductivity (Bucci et al. 2006, Hacke et al. 2010, Goldstein et al. 2013). In addition to xylem hydraulic structure, hydraulic function of plants is also closely associated with stored NSC concentrations (McDowell 2011, Anderegg and Anderegg 2013, Zhang et al. 2021). As osmolytes, NSC (i.e., soluble sugar, one of main components of NSC) can adjust osmotic pressure, maintain tissue water potential, and participate in embolism repair process, thus it is vital to regulate hydraulic function (Clifford et al. 1998, O'Brien et al. 2014, Hartmann and Trumbore 2016). Nitrogen addition could significantly increase NSC consumption through promoting growth and respiration (Li et al. 2018, 2019). Decreased NSC storage in plants under N addition could subsequently impact hydraulic function of plants. Additionally, enhanced growth of xylem tissues under elevated N conditions could change the hydraulic traits of xylem (e.g., produce wider diameter); consequently, it might affect hydraulic efficiency and/or safety (Zhang et al. 2021). On one hand, N addition results in a greater hydraulic efficiency; on the other hand, it also decreases hydraulic safety, induces to higher risk of embolism and might be more vulnerable to hydraulic failure (Hacke et al. 2006). Consequently, it might lead to more negative water potential and reduce carbon assimilation (Faustino et al. 2013). In our study, although N addition had no significant effect on the vessel diameter (D_i and D_h) and area of the earlywood vessel, the mean value of these indexes was relatively higher under N addition treatment (CN) than under other treatments (Figure 6). Moreover, a significant negative correlation between the potential hydraulic conductivity (K_s) and xylem increment was found under N addition treatment (CN) during both years (Table 5), which indicates that N addition-induced larger vessels and higher hydraulic efficiency might decrease hydraulic safety. Our results were consistent with previous studies (Zhang et al. 2021).

Effect of increased precipitation on xylem growth

Water availability is crucial for the growth of xylem cells (Die et al. 2012, Deslauriers et al. 2016). In general, water supplied from precipitation would cause an increase in water transport in stem, potentially enhancing water transportation in the stem sap (Myburg and Sederoff 2001). Because xylem cell differentiation and cell enlargement are turgor-driven processes, thus differentiating xylem cells require sufficient water to generate an adequate wall-yielding turgor pressure to allow cell enlargement and growth (Turcotte et al. 2009, Steppe et al. 2015). The increased water availability in the stem sap could be transported to cambium via aquaporins; then, it increases the turgor required for the bending of cell wall and promotes the division and expansion of xylem cells during cambium division (Turcotte et al. 2009, Steppe et al. 2015). Since cell division and expansion determine the radial growth of the xylem (Myburg

and Sederoff 2001); consequently, increases in precipitation have the potential to facilitate secondary xylem growth. Similarly, water availability from precipitation also could affect cell division and expansion by influencing photosynthesis and the translocation of assimilates (Zweifel et al. 2001, Steppe et al. 2015). The development of wood cell is known to have a strong dependence on the availability of photoassimilates (Krabel and Roloff 1999, Deslauriers et al. 2009). Regardless of the way in which precipitation affects plants, the influence of precipitation on plants varies with time. Previous studies have indicated that both the size and the timing of rainfall events could strongly drive ecological processes (Zeppel et al. 2014). Seasonal changes in precipitation may have larger effects when they occur in dry seasons than wet seasons (Zeppel et al. 2014). In our study, the relative xylem growth was higher under CW during April–May (before the monsoon season, relatively dry) than under C during the drier spring in 2015 (Figure 3). The cumulative xylem width was also >75% of the mean total xylem width of each treatment during April–May in both monitored years (Figure 3), indicating a vigorous phase for xylem growth. With less precipitation and the increasing demand for water for rapid xylem growth during this period (April–May), xylem growth would benefit from increased precipitation because both cell division and cell enlargement requires enough water and photosynthate. This finding was consistent with the positive correlation found between tree radial growth and precipitation in the early growing season on the Tibetan Plateau, as most of the ring-width formation occurred prior to the arrival of the monsoon front (Fang et al. 2015). Studies have shown that the effect of N on plant growth depends on moisture conditions (Buljovcic and Engels 2001), mainly because soil microbes which N nitrification depends on are more active under high soil moisture conditions (Chapin et al. 2002, Wang et al. 2006, Liu et al. 2009). Under a dry condition, plants might be more water-limited rather than N-limited, especially during the fast-growing stage when a lot of water is required for plants growth. Therefore, when compared with the wet (June–August) or the slow growth period (September–November), xylem growth during April–May might prove to be more sensitive to increases in precipitation.

Interactive effects of N addition and supplemental precipitation on xylem growth

The effects of atmospheric N deposition and increased precipitation on plant growth do not occur in isolation (Jia et al. 2015, Wang et al. 2019); rather, these two factors often interact to affect plant growth (Harpole et al. 2007). The addition of N had no marked effect on xylem growth; however, we found significantly greater xylem growth during April–May (before the monsoon season) in 2015 with supplemental precipitation than with ambient precipitation. Interestingly, this increased growth did not occur when N was applied concomitantly with

the additional water. In other words, N addition reduced the positive effect of supplemental precipitation on xylem formation of *Q. acutissima* during the pre-monsoon season. Previous studies conducted in grasslands have demonstrated that elevated N had reduced soil moisture through stimulating ecosystem evapotranspiration (Niu et al. 2009). Additionally, broad-leaved trees respond to N by forming wider earlywood and larger vessels, leading to a greater hydraulic efficiency (Watanabe et al. 2008). However, in dry conditions, increased xylem hydraulic efficiency (high hydraulic conductance and rapidly transport water) might have an adverse effect on plant growth (Hacke et al. 2006). Wider vessels can simultaneously elevate the risk of cavitation and drought-induced embolism, which might decrease hydraulic safety, consequently impeding water transport and reducing plant assimilation (Tyree and Sperry 1989). In the study, we found that the mean diameter and mean area of earlywood vessels under CN was relatively higher than other treatmets, especially significantly higher than that under increased precipitation (CW) in 2015 (had a drier early growing season compared with 2014) (Figure 6). In addition, the xylem hydraulic index K_s under CN was significantly negatively correlated with xylem increment (Table 5). Our results suggested that elevated N-induced larger hydraulic conductance of earlywood vessels might be not conducive to water utilization by *Q. acutissima*. Since plant growth during the early growing season requires sufficient water, thus a decrease in water availability will probably reduce plant growth, especially under water-deficient conditions. These might be the reasons why significant positive effect of supplemental precipitation on xylem increment of *Q. acutissima* during the early growing season (April–May) in 2015 was disappeared under N addition condition.

Conclusions

The study of concurrent effects of N deposition and increased precipitation on xylem growth of trees is critical for obtaining a precise assessment of tree growth and for forecasting the growth of trees and forests in response to anthropogenic activities in the future. Here, we used manipulation experiments to investigate the effects of N addition and supplemental precipitation on the xylem growth of *Q. acutissima* in central China. We found that xylem growth was more sensitive to supplemental precipitation than to N addition at a drier early growing season (April–May in 2015), and the effect of supplemental precipitation on xylem growth could be mediated by N addition, but was time-dependent. Nitrogen addition could reduce the positive effect of supplemental precipitation on the xylem growth of *Q. acutissima* in the dry early growing season (April–May) in 2015, suggesting that the interactive effect of supplemental N and precipitation on xylem growth possibly depended on specific climatic conditions. Our findings emphasized that comprehensive prediction of the influence of global change on forest systems requires investigation of the impact of multiple simultaneously changing global variables on tree growth at a finer time scales, while also taking specific site conditions into account.

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