

# Nitrogen nutrition addition mitigated drought stress by improving carbon exchange and reserves among two temperate trees

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## ABSTRACT

Climate change-driven increases in drought and atmospheric nitrogen (N) deposition frequency and severity across the world, and these changes have profound impacts on forest dynamics by affecting tree carbon balance. However, important knowledge gaps persist concerning the interactions between drought and N enrichment on carbon supply and reserve dynamics. We investigated gas exchange and carbohydrate reserve shifts and associated with leaf chemical composition across two temperate tree saplings coping with different levels of drought stress, N nutrition addition and their interactions. Our results showed that drought stress decreased net photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ), while  $A$  and  $g_s$  were increased by N addition, combined with increased  $g_s$  under the interactive effects of drought and N addition, indicating that N nutrient availability had suppressing effect on drought stress and improved the drought-induced negative conditions by altering carbon exchange traits. In addition, we found that N addition reduced the concentrations of nonstructural carbohydrate (NSC) and its components in relation to foliar carbon and nitrogen changes and the fast growth of saplings. Under the interaction of drought and N nutrition addition, no variation in carbohydrate concentrations demonstrate that carbon reserves play a critical role in regulating carbon exchange and growth. Our observations provide evidence of N nutrient availability mitigated drought stress by improving carbon exchange and reserves, which is expected to contribute to the predictions of future vegetation dynamics.

## 1. Introduction

Climate change has driven widespread shifts in global ecosystems and biogeochemical processes (Bahn et al., 2014; IPCC 2013; McDowell et al., 2020; Reichstein et al., 2013), and climate extremes, such as severe drought, are projected to be an important driver of terrestrial ecosystem shifts (Park Williams et al., 2012). Indeed, drought-induced forest mortality has risen with increasing frequency, duration and intensity of droughts across the world (Allen et al., 2010; Anderegg et al., 2019; McDowell et al., 2011). Severe droughts could compromise forest ecosystem functioning and the terrestrial carbon sink (Anderegg et al., 2020; Hartmann et al., 2013). While these impacts of single drought events have been widely studied, understanding combined impacts of climate change on tree growth, survival and mortality remains largely unknown and is crucial for predicting future vegetation dynamics. In particular, an increase in atmospheric N deposition may benefit forest

ecosystems through enhancing tree growth and productivity, which may in turn have impacts on drought-induced negative effects (Fowler et al., 2013; Reay et al., 2008; Villar-Salvador et al., 2013). Global atmospheric N deposition has increased by three-to five-fold over the past century due to increased fossil fuel combustion and artificial fertilizer application (Davidson, 2009; Galloway et al., 2008), and is predicted to increase by a factor of 2.5 by the end of the century (Fowler et al., 2013). Yet there is no clear consensus on the magnitude of how increasing N deposition affect drought-induced negative effects. Thus, studies on the combined effects of drought and nitrogen availability are of paramount importance.

Drought has many negative effects on forest ecosystems, such as constraining tree growth and gas exchange (Novick et al., 2016) and limiting carbon-water feedbacks (Mitchell et al., 2014; Skelton et al., 2017). Plants largely rely on both newly assimilated carbon and stored reserves of carbohydrates for growth, respiration and defense function

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(Hartmann and Trumbore, 2016). Carbon assimilation is affected via stomatal closure during water deficit, which can lead to a change of carbohydrates accumulation (McDowell, 2008). Non-structural carbohydrates (NSC) supplied from photosynthesis in leaves can be transported to shoots/roots in response to demand to support growth and respiration and as reserves for later use (Rennie and Turgeon, 2009), and typically in the form of soluble sugars and starch, are major substrates for all plant functional processes of primary and secondary metabolism (Hartmann and Trumbore, 2016). NSC reserves reflect the balance of carbon supply and utilization, which is strongly related to the ability of meet osmotic, metabolic and defensive carbon requirements, thus NSC have been implicated in mediating drought-induced tree mortality (Adams et al., 2017; Signori-Muller et al., 2021). Stored NSC can function as buffer to carbon demands when carbon supply is limited during drought stress (Adams et al., 2013; Galiano et al., 2011). Current researches support that carbon starvation, via imbalance between carbohydrate demand and supply, is an important physiological mechanism of drought-induced tree mortality (McDowell, 2011).

However, the pattern of drought-induced tree mortality might be modified with increasing atmospheric N deposition under changing climate. Generally, positive effects of N deposition on plant growth and ecosystem production due to higher N availability are much more common in the literature (Li et al., 2018; Zhang et al., 2021). Plant growth with increasing N availability is affected by changing leaf economics, carbon budget and water relations (Goldstein et al., 2013; Hacke et al., 2010; Zhang et al., 2018). A number of studies demonstrated that N nutrition addition could alter carbon reserves in trees via impacts on carbon source (gas exchange) and sink (growth and respiration) activities (Li et al., 2019; Quinn Thomas et al., 2009). Higher N availability can increase leaf N content, thus improving the photosynthetic enzymes and pigments, thereby stimulating photosynthetic capacity and water use efficiency (Evans, 1989; Zhang et al., 2018). Generally enhanced carbon gain under high N availability did not increase stored NSC were found in previous synthesis studies of woody plants (Du et al., 2020; Li et al., 2018). Because high growth rate due to N fertilization might have a cost in terms of reduced carbohydrates (Iivonen et al., 2001). These changes in functional traits of woody plants may influence plant survival and resistance under environmental stresses, particularly during drought. However, it is uncertain that whether the combined effects of drought and N addition on NSC are additive or not. To date, a previous meta-analysis conducted the interactive effects of multiple environmental change factors on NSC and its components, but the number of observations on the effects of the interactions between drought and N addition on woody plants were quite limited (only  $n = 2$ ).

Previous studies have reported synergy (Damatta et al., 2002; Trubate et al., 2011), antagonism (Walters and Reich, 1989; Zhu et al., 2001) or no interaction (Kleiner et al., 1992) between drought stress and N nutrition addition on the stress tolerance of plants. Zhang et al. (2014) found that N fertilization has a positive effect on growth and water-use efficiency under low soil moisture conditions, whereas Dzedek et al. (2016) indicated that N deposition increased the drought sensitivity by decreasing biomass production, and Villar-Salvador et al. (2013) found that N addition and drought hardening exert opposite effects on the stress tolerance through quantifying water relations and gas exchange capacity. Zhang et al. (2021) recently observed that the effects of N addition on drought depended on species, organs and soil water status. These impacts of drought stress and N availability on trees have primarily been conducted on growth or water relations, the details of interactions between drought and N addition and how they affect tree carbon supply and reserves remain unclear.

Here, we investigated the responses of gas exchange, NSC concentrations and foliar C and N content to drought stress, N addition and their interactions among two temperate deciduous broad-leaved tree saplings. Our specific goal was to understand the pattern of how N nutrition addition affect drought tolerance associated with carbon

supply and reserve processes. We hypothesize that (1) drought stress reduces carbon supply and reserves by constraining gas exchange; (2) N nutrient availability affect carbon assimilation and NSC reserves by altering leaf C and N content; (3) N nutrient availability mitigates drought stress by changing carbon exchange and reserves.

## 2. Materials and methods

### 2.1. Site information

This study was conducted at the Research Station of Changbai Mountain Forest Ecosystems of Chinese Academy of Sciences, located in Jilin province, NE China (128°28'E, 42°24'N; 736 m altitude). The climate of this site is strongly influenced by the monsoon, and the region has temperate continental climate with cold winters and relatively short cool summers. The mean annual temperature and mean annual precipitation were 4.1 °C and 650.8 mm, respectively, across nearly 36 years (Meteorological data from the Research Station of Changbai Mountain Forest Ecosystems of the Chinese Academy of Sciences; described in (Zhang et al., 2021). In this region, the vegetation type is well-known for its high species richness among temperate forests, which is composed of broad-leaved deciduous tree species mixed with *Pinus koraiensis*. Two dominant deciduous broad-leaved tree species (*Quercus mongolica* and *Fraxinus mandshurica*) were selected for the present study. Among the two species, *Q. mongolica* is a simple-leaved tree species and *F. mandshurica* is a compound-leaved species.

### 2.2. Experimental design and treatments

In April 2017, five-year-old pot saplings with similar size (~1.5 m) were selected for each species ( $n = 36$ ). Six replicate saplings were selected for each treatment. The experimental design was the continuation and deepening of our previous researches and some details described in Zhang et al (2020a; 2021). Briefly, the potted saplings of each species were divided into three groups: well-watered (maintained at field capacity, which corresponds to the soil moisture measurements maintained between 0.38 and 0.40), moderate drought (40-50% field capacity) and severe drought (20-30% field capacity). We used capacitance probes (Stevens Hydraprobe, Stevens Water Monitoring Systems, Inc., USA) to measure soil moisture in each pot at 30 min intervals. According to our previous results of N addition (Zhang et al., 2020a, 2021), these tree species had maximum photosynthetic and hydraulic capacity under medium nitrogen addition (46 kg N ha<sup>-1</sup> year<sup>-1</sup>), which was based on natural atmospheric nitrogen deposition (23 kg N ha<sup>-1</sup> yr<sup>-1</sup>) at the study area (Guan et al., 2006; Sun et al., 2018). Thus, without nitrogen addition and medium nitrogen addition treatment subjected to each drought stress group. N addition treatments were carried out in 2017 and 2018 and drought stress was conducted from May 2018. Urea solutions with N addition concentrations (0.056 mol l<sup>-1</sup>) were sprayed into the pots once every month (45 ml per pot) from May to October in 2017 and 2018.

### 2.3. Gas exchange and leaf mass per area (LMA)

Gas exchange parameters including net photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were measured using a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) with a 6 cm<sup>2</sup> combined light source leaf chamber on sunny days from 09:00 to 11:00 in August 2018. For each individual measurement, sun-exposed portions and fully expanded leaves were selected for measuring. We set chamber CO<sub>2</sub> concentration to 400 ppm and the photosynthetically active radiation to 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. A high flow rate (500 μmol s<sup>-1</sup>) was set to minimize the time take for  $A$  and  $g_s$  to stabilize. The chamber conditions were kept close to ambient by matching air temperature and relative humidity. The intrinsic water-use efficiency (WUE<sub>i</sub>) was determined by dividing  $A$  by  $g_s$  for each measurement. We

used ImageJ software to measure leaf area. Leaf mass per area (LMA) was determined by leaf dry mass and leaf area.

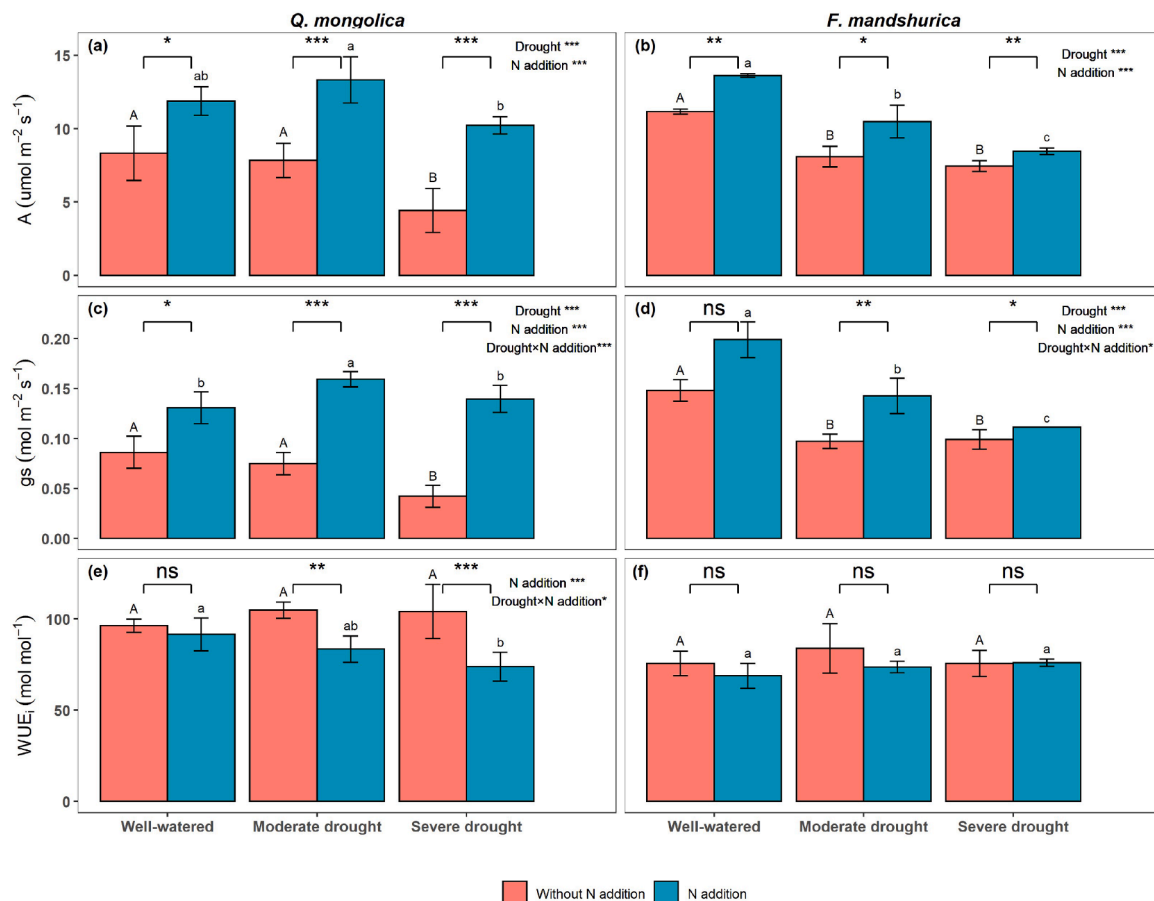
#### 2.4. Nonstructural carbohydrates (NSC) and foliar C and N content

Leaf samples for carbohydrate analysis were collected from the branches that were used for gas exchange measurements and dried in an oven at 105 °C for 30 min immediately after collection, and then dried at 65 °C for 72 h. The samples were ground into powders of 0.15 mm particle size with a ball mill (MM400, Retsch, Germany). Nonstructural carbohydrates extraction and quantification of samples were carried out following the modified anthrone method in Hansen and Moller (1975) and Mitchell et al. (2013), recently described by Zhang et al. (2021). Briefly, leaf sample was extracted with aqueous ethanol and the mixture was boiled in a water bath and centrifuged. The soluble sugar concentrations were determined by the supernatants using a predetermined standard curve by measuring the amount of glucose from UV-Vis spectrophotometer (Lambda 25, PerkinElmer, USA) of absorbance at 620 nm. The ethanol-insoluble residual separated after extraction was used for starch digestion after ethanol evaporation. Starch was extracted with distilled water and boiled in the water bath, then added 9.2 mol L<sup>-1</sup>HClO<sub>4</sub> and centrifuged. Removed supernatants and the procedure was repeated in the after replacing 9.2 mol L<sup>-1</sup>HClO<sub>4</sub> with 4.6 mol L<sup>-1</sup>HClO<sub>4</sub>. Starch concentration was also determined by UV-Vis spectrophotometer of absorbance at 620 nm. Starch concentration was calculated by multiplying the glucose hydrolysate concentration by a conversion factor of 0.9. The total NSC was calculated as the sum of soluble sugar and starch concentrations. We used elemental analyzer

(Vario EL, Elementar, Germany) to measure total foliar C and N content and C/N ratio were analyzed. Data is reported as mg/g dry weight or % content in this study.

#### 2.5. Statistics

All statistical analysis was performed using R statistical software (version 4.0.5; R Core Team, 2018). We have analyzed data for normality and homogeneity of variance before statistical analyses. We used two-way analysis of variance (ANOVA) to evaluate the main effects of drought, N addition, and their interactions on gas exchange ( $A$ ,  $g_s$  and  $WUE_i$ ), carbohydrates (soluble sugar, starch and NSC) and leaf chemical composition (C and N content, C/N ratio and LMA). The differences between without N addition and N addition at different levels of drought stress for each species were tested by Student's  $t$ -test. Multiple comparisons among values within each drought stress levels applying LSD post hoc test. A three-way ANOVA were used to determine the interactive effects of drought stress, N addition and tree species on each of carbon supply and reserve traits ( $A$ ,  $g_s$ ,  $WUE_i$ , soluble sugar, starch and NSC). We used linear mixed-effects models (LME) to evaluate the relationships among  $A$ , foliar N, LMA, C/N ratio, soluble sugar, foliar C and NSC. Correlation matrix was developed to evaluate relationships among all plant functional traits using corrplot package in the program R. The statistically significant correlations throughout the analyses was set at  $p < 0.05$ .



**Fig. 1.** The effects of N addition, drought stress and their interaction on gas exchange. Gas exchange parameters including net photosynthetic rate ( $A$ ; a,b), stomatal conductance ( $g_s$ ; c,d) and intrinsic water-use efficiency ( $WUE_i$ ; e,f) of *Q. mongolica* and *F. mandshurica*. The error bar represents one standard error of all measurements for each individual tree species ( $n = 3$ ). Different letters indicate significant difference within each watering treatment for each species (multiple comparisons). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , and 'ns' represents not significant.

### 3. Results

#### 3.1. Effects on gas exchange

The effects of drought and N addition on gas exchange parameters were dependent on tree species. Drought stress significantly affected the net photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ) of *Q. mongolica* and *F. mandshurica* saplings ( $p < 0.001$ ; Fig. 1), but did not change the intrinsic water-use efficiency ( $WUE_i$ ). N addition did not change  $WUE_i$  for the two species, but increased both  $A$  and  $g_s$ , although the change in  $g_s$  of *F. mandshurica* was not statistically significant (Fig. 1). N addition significantly increased  $A$  and  $g_s$ , and significant decreased  $WUE_i$  of *Q. mongolica* under both moderate and severe drought stress. For *F. mandshurica*, N addition significantly increased  $A$  and  $g_s$  under both moderate and severe drought stress, but had no significant effects on  $WUE_i$  (Fig. 1). The interactions between drought stress and N addition were significant on  $g_s$  ( $p < 0.01$ ), but not for  $A$  and  $WUE_i$  (Table 1).

#### 3.2. Effects on carbohydrates

The soluble sugar, starch and total NSC concentrations of *F. mandshurica* were significantly decreased under drought stress conditions ( $p < 0.001$ , 0.01, 0.001 for soluble sugar, starch and total NSC, respectively; Fig. 2), but not for *Q. mongolica*. Under well-watered conditions, N addition decreased soluble sugar, starch and NSC concentrations for the two species although the change in starch of *Q. mongolica* was not statistically significant (Fig. 2). In addition, the effects of drought stress and N addition on NSC concentrations and its components were more pronounced in *F. mandshurica* than in *Q. mongolica*. Overall, the interactive effects of drought and N addition on soluble sugar and starch were significant for the two species ( $p < 0.05$ ; Table 1).

#### 3.3. Effects on foliar chemical composition

Drought, N addition and their interactive effects on foliar C and N content, C/N ratio and LMA varied with treatments and species (Fig. 3). Under well-watered conditions, N addition significantly increased foliar

C and N content, and significantly decreased C/N ratio for both species (Fig. 3a–f). N addition did not change LMA in *Q. mongolica* but significantly decreased LMA in *F. mandshurica* (Fig. 3g,h). Foliar N content significantly decreased under severe drought stress for *Q. mongolica* (Fig. 3c), but this decrease occurred under moderate drought stress for *F. mandshurica* (Fig. 3d). Overall, foliar C/N ratio significantly increased under drought stress for both *Q. mongolica* ( $p < 0.05$ ; Fig. 3e) and *F. mandshurica* ( $p < 0.001$ ; Fig. 3f). N addition significantly increased foliar N content ( $p < 0.05$ ) and decreased C/N ratio ( $p < 0.05$ ) under severe drought stress for *Q. mongolica*, but similar pattern occurred under moderate drought for *F. mandshurica*. The interactions between drought stress and N addition were significant on LMA ( $p < 0.05$ ) for *Q. mongolica* while not for *F. mandshurica* (Fig. 3).

#### 3.4. Relations across traits

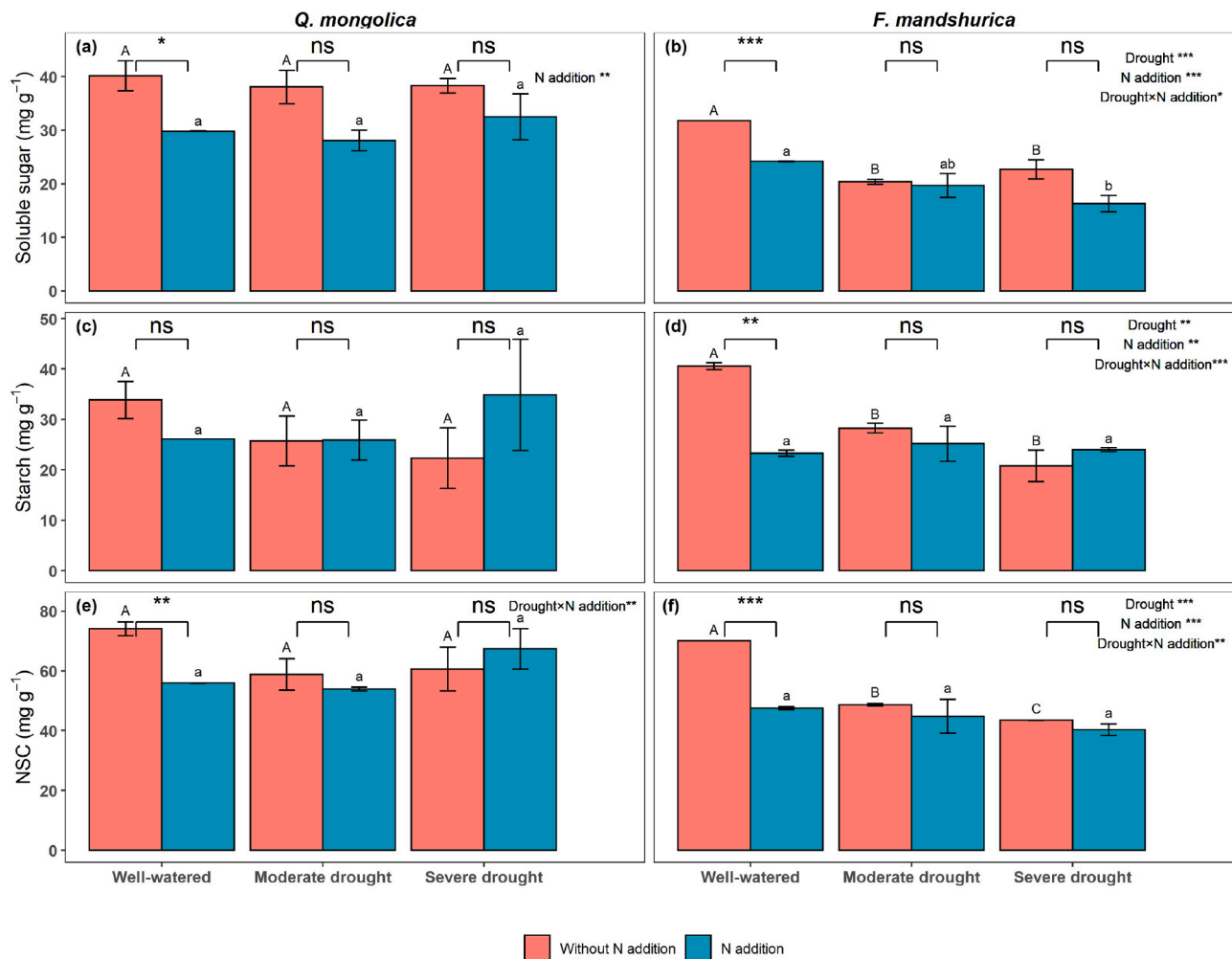
Significant linear correlations were found among  $A$ , foliar N, LMA, C/N ratio, soluble sugar, foliar C and NSC under the interaction of drought stress and N addition (Fig. 4). Specifically,  $A$  increased significantly with increasing foliar N content ( $p = 0.01$ ; Fig. 4a), LMA increased significantly with increasing  $A$  ( $p = 0.03$ ; Fig. 4b), and C/N ratio decreased significantly with decreasing foliar N content ( $p < 0.001$ ; Fig. 4c). Soluble sugar concentration were significantly correlated with foliar C content ( $p = 0.005$ ; Fig. 4d), the NSC concentration was strongly correlated with soluble sugar concentration ( $p < 0.001$ ; Fig. 4e), and LMA was positively correlated with starch concentration ( $p = 0.02$ ; Fig. 4f). Meanwhile, NSC concentration increased significantly with increasing starch concentration ( $p < 0.01$ ; Fig. S1). In addition,  $WUE_i$  significantly decreased with increasing  $g_s$  but increased with increasing soluble sugar contents (correlation coefficients are  $-0.77$  and  $0.72$ , respectively; Fig. S1).

No significant three-way interactive effects of drought stress, N addition and tree species were found for  $A$  and starch, ( $p > 0.05$ ), while significant interactions were found for  $g_s$  and  $WUE_i$ , soluble sugar and NSC ( $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.001$ ,  $p < 0.05$ , respectively; Table 1). Furthermore, the interactive effects between N addition and tree species were significant for all carbon supply and reserve traits (Table 1).

**Table 1**

Results ( $F$  and  $P$  value) of three-way ANOVA on the effects of drought stress, N addition and tree species on carbon supply and reserve traits. Significance at  $P < 0.05$  is presented in bold.  $A$ , net photosynthetic rate;  $g_s$ , stomatal conductance;  $WUE_i$ , intrinsic water-use efficiency; Sugar, soluble sugar; NSC, total nonstructural carbohydrates.

Dependent variable		Drought	N addition	Tree species	Drought $\times$ N addition	Drought $\times$ tree species	N addition $\times$ tree species	Drought $\times$ N addition $\times$ tree species
$A$	$F$ -value	28.306	157.707	16.217	2.504	2.130	28.281	2.365
	$P$ -value	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.01</math></b>	0.094	0.156	<b><math>p &lt; 0.001</math></b>	0.107
$g_s$	$F$ -value	21.744	311.672	19.206	5.451	4.588	52.193	6.976
	$P$ -value	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.01</math></b>	<b><math>p &lt; 0.05</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.01</math></b>
$WUE_i$	$F$ -value	1.506	42.339	36.717	2.291	1.025	15.664	3.247
	$P$ -value	0.234	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	0.114	0.368	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.05</math></b>
Sugar	$F$ -value	16.306	16.748	272.885	2.823	12.466	7.810	4.023
	$P$ -value	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.05</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.001</math></b>
Starch	$F$ -value	1.098	1.743	1.441	3.102	3.896	2.512	1.432
	$P$ -value	0.363	0.190	0.250	<b><math>p &lt; 0.05</math></b>	<b><math>p &lt; 0.05</math></b>	<b><math>p &lt; 0.05</math></b>	0.198
NSC	$F$ -value	7.297	3.736	62.585	3.418	7.752	3.971	2.314
	$P$ -value	<b><math>p &lt; 0.01</math></b>	<b><math>p &lt; 0.05</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.01</math></b>	<b><math>p &lt; 0.001</math></b>	<b><math>p &lt; 0.01</math></b>	<b><math>p &lt; 0.05</math></b>



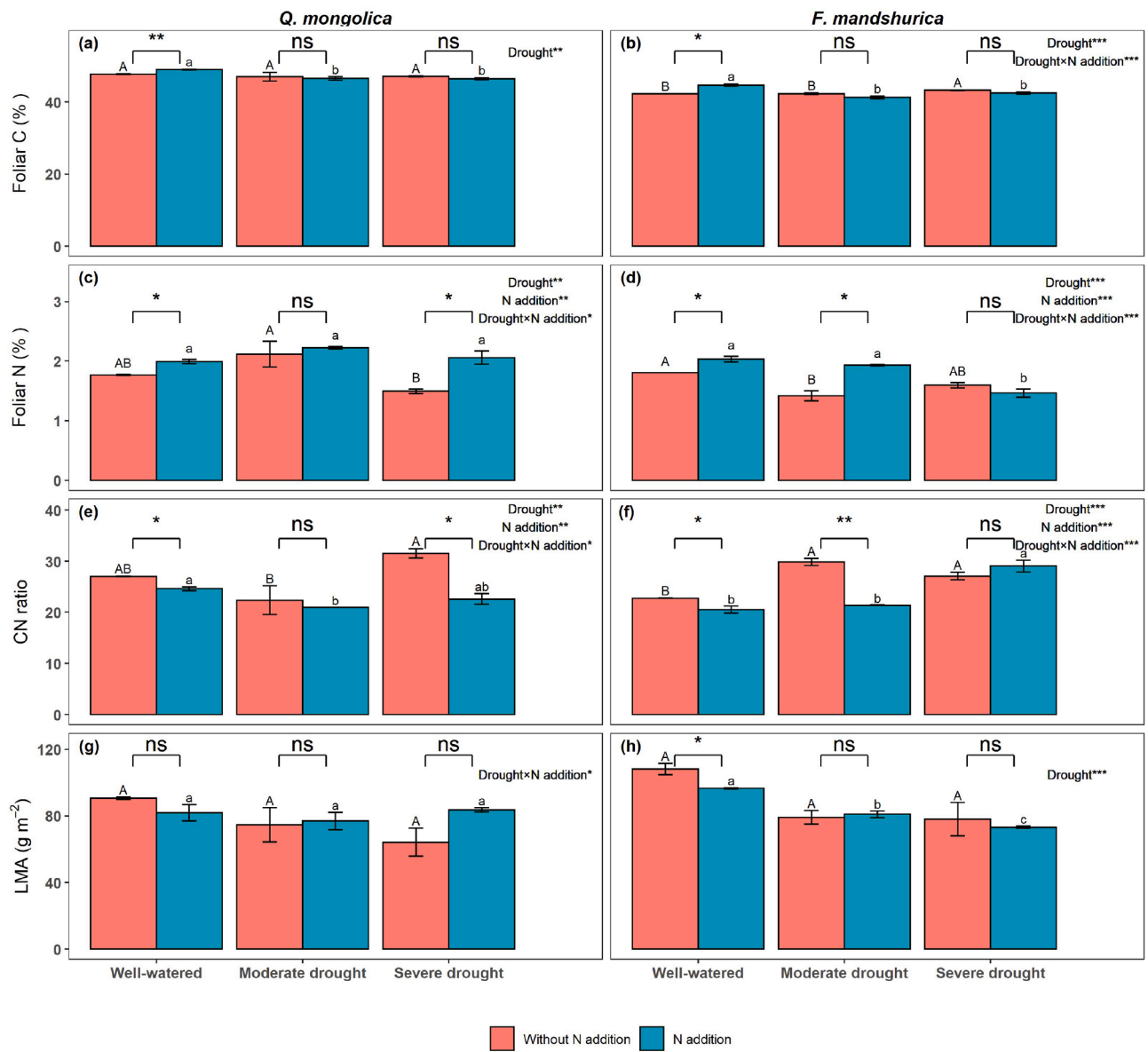
**Fig. 2.** The effects of N addition, drought stress and their interaction on carbohydrate concentrations. Carbohydrates including soluble sugar (a,b), starch (c,d) and total nonstructural carbohydrates (NSC; e,f) of *Q. mongolica* and *F. mandshurica*. The error bar represents one standard error of all measurements for each individual tree species ( $n = 3$ ). Different letters indicate significant difference within each watering treatment for each species (multiple comparisons). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , and 'ns' represents not significant.

## 4. Discussion

We examined the effects of drought stress, N nutrition addition and their interactions on gas exchange ( $A$ ,  $g_s$  and  $WUE_i$ ), carbohydrates (soluble sugar, starch and total NSC) and leaf chemical composition (C and N content, C/N ratio and LMA). This study provides us with an assessment of how N nutrition addition affects drought tolerance related to carbon supply and reserves. The results supported our first hypothesis, that  $A$  and  $g_s$  of the two tree species declining under different levels of drought stress and carbohydrates declined in *F. mandshurica*, suggesting that gas exchange and carbon reserves were constrained under drought stress. Consistent with our second hypothesis, we found that N nutrition addition stimulated carbon assimilation rate by increasing foliar N content. Meanwhile, N addition decreased carbohydrate concentrations among the two species. According to our last hypothesis, we found that gas exchange parameters were improved under the interactive effects of drought and N nutrition addition. Additionally, both drought stress and N addition reduced carbohydrate reserves, but carbohydrate concentrations did not change in the interaction between drought and N nutrition addition. These results indicate that N nutrient availability had suppressing effects on drought stress and could improve drought stress conditions by changing carbon supply and reserves.

### 4.1. N nutrient availability alters tree carbon exchange with different watering regimes

Overall, drought has major impacts on net photosynthetic rate and stomatal conductance of the two temperate tree saplings, especially in severe drought (Fig. 1). This is consistent with many previous findings that stomatal closure typically occurs to maintain water use and osmotic pressure under drought conditions (Brodribb et al., 2003; Pedrero et al., 2014; Yang et al., 2021), which leading to a strong reduction in photosynthesis (Burchett et al., 1984; Suárez and Medina, 2006). However, net photosynthetic rate and stomatal conductance were enhanced by N nutrition addition under both well-watered and drought stress conditions in our study, and this increase in gas exchange was more pronounced under limiting water conditions for most cases (Fig. 1). Thus N nutrition had opposite effects on drought stress and can improve the negative effects of drought stress by affecting gas exchange of the tree saplings. Higher nutrient uptake could trigger plant responses to drought by stimulating the production of photosynthetic proteins (e.g., Rubisco), thereby enhancing carbon assimilation (Evans, 1989), as reflected in our foliar N content results (Figs. 3 and 4a). The increased net photosynthetic rate was positively correlated with foliar N content, which is consistent with previous results that leaf N content play critical roles in photosynthetic capacity (Quinn Thomas et al., 2009; Sparks, 2009). Changes in leaf compounds may strengthen the carbon sink



**Fig. 3.** The effects of N addition, drought stress and their interaction on foliar chemical traits. Foliar chemical traits including foliar C content (a,b), foliar N content (c,d), C/N ratio (e,f) and leaf mass per area (LMA; g,h) of *Q. mongolica* and *F. mandshurica*. The error bar represents one standard error of all measurements for each individual tree species ( $n = 3$ ). Different letters indicate significant difference within each watering treatment for each species (multiple comparisons). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , and 'ns' represents not significant.

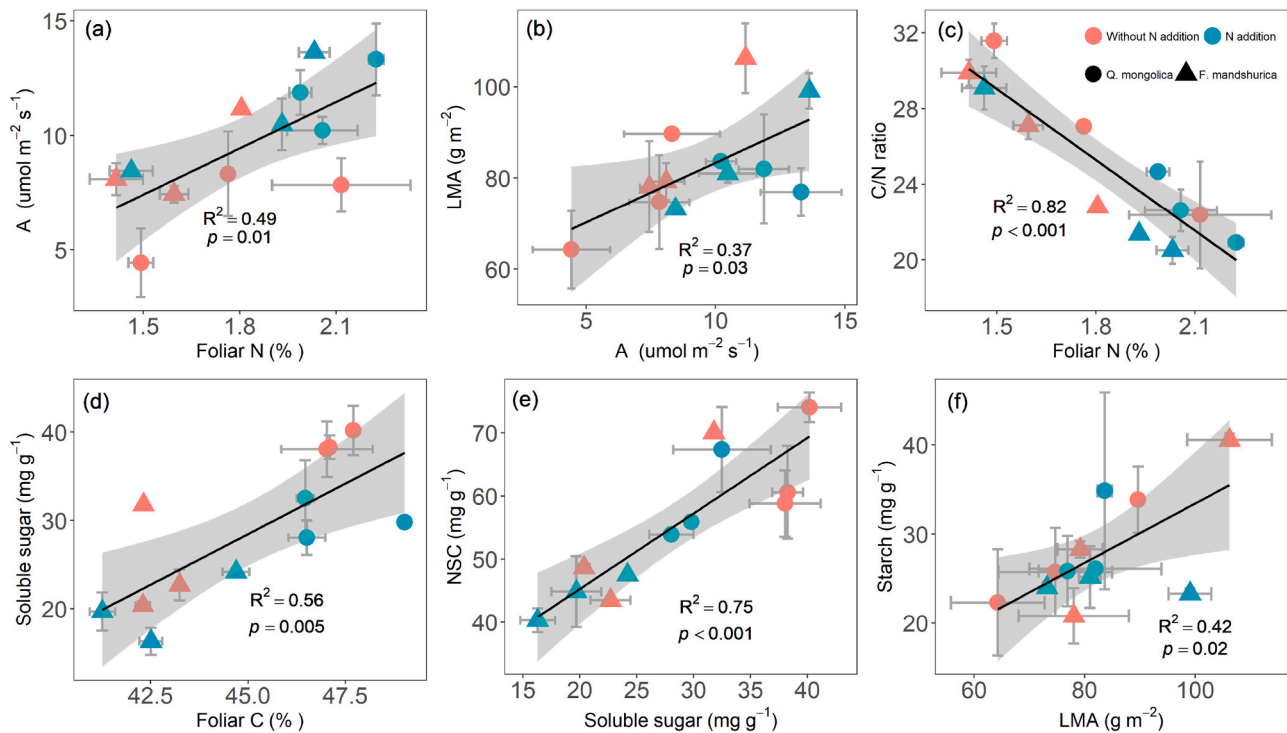
function and affect osmotic adjustment, which influence survival and resistance of woody plants under environmental stresses, particularly during drought (Galiano et al., 2017; Hoegberg et al., 2006; Villagra et al., 2013). Thus, N nutrition addition and drought hardening exert opposite effects on the carbon exchange of the two species. Similarly, a previous study reported that N nutrition addition and drought hardening exert opposite effects on the drought tolerance by observing gas exchange, water relations and growth (Villar-Salvador et al., 2013).

The interactive effects of N nutrition addition and drought stress were not significant for net photosynthetic rate (Fig. 1a and b), but were significant for stomatal conductance of the two species (Fig. 1c and d), suggesting that N nutrition addition can regulate stomatal closure to maintain water and carbon fluxes under drought stress conditions. Stomatal responses in relation to leaf N nutrient availability determine the dynamics of carbon exchange, which contributes to overall drought tolerance. Indeed, plants can drive stomatal responses in leaves and thereby control the whole-plant conductance with N addition (Domec et al., 2009; Plavcová et al., 2013). Furthermore, N nutrition

accumulation can not only improve carbon exchange (Chen et al., 1993; Shiratsuchi et al., 2006), but also enhance plant nutritional status to promote growth (Ågren, 2004; Montti et al., 2014). Our previous studies observed that medium N addition (the same N addition level in this study) increased gas exchange and water transport capacity of these temperate trees, but these effects diminished or disappeared at higher N addition levels (Zhang et al., 2020a; 2021), suggesting that the N addition level in this study did not reach the threshold of soil N availability and as a nutrient beneficial to plant growth. This is also consistent with our results that LMA increased significantly with increasing net photosynthetic rate (Fig. 4b).

#### 4.2. N nutrient availability alters carbohydrate reserves with different watering regimes

Single effect of drought stress or single effect of N nutrition addition both decreased NSC reserves, although the change of *Q. mongolica* in drought was not statistically significant (Fig. 2). Decreased NSC under



**Fig. 4.** Relationships across tree key functional traits. Relationship between net photosynthetic rate ( $A$ ) and foliar N content (a); between  $A$  and leaf mass per area (LMA; b); between foliar N content and C/N ratio (c); between foliar C content and soluble sugar concentrations (d), between soluble sugar concentrations and total nonstructural carbohydrates (NSC; e), and between LMA and starch concentrations (f). The error bar represents one standard error of all measurements for each individual tree species ( $n = 3$ ). A linear model is fitted to the data. The confidence interval of the linear regression is shown in light grey color. The coefficients of determination and  $p$ -value are also shown.

drought stress supports that stored NSC can be mobilized and used to support metabolic activity for survival when carbon assimilation is insufficient to meet demand during drought stress (McDowell et al., 2008; McDowell, 2011). On the other hand, decreased NSC under N addition possibly indicates that stimulating growth was given priority over storing NSC. As mentioned earlier, N nutrient availability generally stimulated tree carbon assimilation rate and growth. Most of the additional carbon assimilated by photosynthesis was allocated to growth and respiration, but less to NSC reserves. The fast growth rate in saplings under optimal N fertilization seems to have a cost in carbohydrates consumption. The species in our study are both fast-growing species, and the newly assimilated carbon investment for growth and respiration are prioritized over NSC storage, which is confirmed in previous studies (Li et al., 2019; Minchin and Lacomte, 2005). However, no significant variation in NSC concentrations under N addition in the mature trees has been found in a previous study (Zhang et al., 2021). Thus the N nutrient availability alters carbohydrate reserves under drought stress conditions might be large difference between saplings and field mature trees, because mature trees established growth systems and N fertilization might have no effect on their fast growth. Although differences in carbohydrate dynamics of seedlings and mature trees have been proposed (Hartmann et al., 2018; Zhang et al., 2020b), clearly there is a need for a deeper understanding of how N nutrition addition affect carbohydrate reserves in mature trees, particularly in interaction with drought stress.

Notably, we observed no variation of NSC in the interactions between drought stress and N nutrition addition (Fig. 2). Combined with the decline of NSC both under drought and N addition, we speculate that N nutrient availability improved the negative effects of drought by altering carbohydrate reserves dynamics, that is, N addition had suppressing effects on drought stress. N addition has a major effect on C/N ratio in leaves and soluble sugar concentration were significantly correlated with foliar C content (Fig. 4c and d), and NSC concentration

was strongly depend on soluble sugar and starch concentration (Figs. 4e and S1). This suggested that N nutrition addition altered carbohydrate reserves by changing foliar C and N content, which could contribute to improve drought stress conditions. We also found that  $WUE_i$  significantly increased with increasing soluble sugar contents, and starch contents positively correlated with LMA (Figs. 4f and S1), which suggested that mitigated drought-induced effects under N nutrition addition was partly attributed from improving plant water use and leaf growth. A previous study also indicated that a positive feedback loop might exist where soil nutrient availability improves the metabolism and functioning of the roots and might restore drought-induced alterations in carbon and nitrogen allocation, by contributing to the maintenance of cellular functions, consequently strengthening carbon sinks (Schonbeck et al., 2021).

#### 4.3. Divergent responses of the interactions between drought and N nutrition addition among species

The three-way interactive effects of drought, N nutrition addition and tree species were significant for most carbon supply and reserve traits ( $g_s$ ,  $WUE_i$ , soluble sugar and NSC; three-way ANOVA), and the interaction between N nutrition addition and tree species were significant for all carbon supply and reserve traits (Table 1), indicating that the responses of carbon supply and reserves to drought and N addition are strongly depended on species. *F. mandshurica* showed higher mean  $g_s$  than *Q. mongolica* (Fig. 1), suggesting that *F. mandshurica* had a stronger gas exchange capacity. This is consistent with previous gas exchange results conducted on the same tree species (Liu et al., 2015; Zhang et al., 2021). In addition, we found that the interactive effects of drought and N addition on carbohydrate reserves were more remarkable in *F. mandshurica* than in *Q. mongolica* (Fig. 2). These differences between the two tree species may be related to the divergence of simple (*Q. mongolica*) and compound leaves (*F. mandshurica*).

Compound-leaved tree species are more likely to protect themselves from stress conditions via controlling stomata and shifting leaf traits (Gorai et al., 2015; Song et al., 2017). Consistently, we found that N addition significantly decreased intrinsic water-use efficiency (WUE<sub>i</sub>) of *Q. mongolica* under drought stress conditions, but not for *F. mandshurica*. This may be associated with high stomatal regulation capacity in compound leaves (Yang et al., 2019). Additionally, N nutrition addition has major impact on foliar N content and C/N ratio for *F. mandshurica* under severe drought stress, but this similar pattern occurred under moderate drought stress for *Q. mongolica*, which confirmed that the leaflets from compound-leaved species were responsive prior to simple leaves under unfavorable environmental conditions (Wang et al., 2016; Zhang et al., 2021). Note that the limited number of tree species observations in the present study limits the power of our assessment, thus a comprehensive evaluation of multiple tree species responses to the interaction of drought and N enrichment is urgently needed.

## 5. Conclusions

We investigated the shifts of carbon exchange (gas exchange) and reserves (NSC concentrations and its components) associated with leaf chemical composition across two tree species under drought, N nutrition addition and their interactions. N nutrient availability enhanced growth and gas exchange in juvenile tree species were related to foliar N content under drought stress. On the other hand, N nutrition addition reduced carbohydrate reserves due to fast growth in tree saplings. Meanwhile, N nutrient availability changed NSC reserve dynamics by altering foliar C and N contents under limiting water conditions, which most likely improved plant water use and leaf growth. Our results provide experimental evidence that N nutrition addition had suppressing effects on drought stress and mitigated drought-induced negative effects by improving carbon exchange and reserve traits. Additionally, the divergences in most carbon supply and reserve traits among species might related to leaf forms. Considering the expected increase in drought frequency and severity, and the magnitude of atmospheric N deposition across the world, further studies are needed on the variation in carbon and water balance of mature trees to better assess how multiple factors affect drought tolerance under future climate change.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

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