

1 **Divergences in hydraulic conductance and anatomical traits of stems and**
2 **leaves in three temperate tree species coping with drought,**
3 **N addition and their interactions**

4
5 **Short running title: Drought and N addition affects tree hydraulics**
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7 **Hongxia Zhang**^{1,6}, **Nate G. McDowell**², **Henry D. Adams**³, **Anzhi Wang**¹, **Jiabing Wu**¹, **Changjie Jin**¹,
8 **Jinyuan Tian**^{1,6}, **Kai Zhu**^{1,6}, **Weibin Li**⁴, **Yushu Zhang**⁵, **Fenghui Yuan**^{1,*} and **Dexin Guan**^{1,*}

9 (¹ Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016,
10 China; ² Pacific Northwest National Laboratory, Richland, WA 99352, USA; ³ Department of Plant Biology, Ecology and Evolution,
11 Oklahoma State University, Stillwater, OK 74078-3013, USA; ⁴ State Key Laboratory of Grassland and Agro-ecosystems, Key
12 Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture
13 Science and Technology, Lanzhou University, Lanzhou 730020, China; ⁵ Institute of Atmospheric Environment, China Meteorological
14 Administration, Shenyang 110166, China; ⁶ University of Chinese Academy of Sciences, Beijing 100049, China)

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21 **Corresponding author:*

22 *E-mail address:* fh yuan@iae.ac.cn (Fenghui Yuan), Tel: 86-24-83970336;

23 *E-mail address:* dx guan@iae.ac.cn (Dexin Guan), Tel: 86-24-88087869.

24 Abstract

25 Drought and N addition have been shown to affect tree hydraulic traits, but few studies have been
26 made on their interactions across species with different wood types or leaf forms. We examined the
27 responses of hydraulic conductance and xylem anatomical traits of *Quercus mongolica* (ring-porous
28 with simple-leaves), *Fraxinus mandshurica* (ring-porous with compound-leaves), and *Tilia*
29 *amurensis* (diffuse-porous with simple-leaves) to drought, N addition and their interactions. Drought
30 stress decreased current-year xylem-specific conductivity in stems (K_{sx}) and leaf hydraulic
31 conductance (K_{leaf}), but N addition affected K_{sx} and K_{leaf} differently among species and watering
32 regimes. These divergent effects were associated with different responses of anatomical traits and
33 leaf forms. Higher mean vessel diameter in stems (D_{stem}) and lower vessel density in leaves (VD_{vein})
34 were observed with N addition. The three-way interactive effects of drought, N addition and tree
35 species were significant for most values of anatomical traits. These results were also reflected in
36 large differences in vessel diameter and density among species with different wood types or leaf
37 forms. The two-way interactive effects of drought and N addition were significant on K_{leaf} and
38 predawn water potential (Ψ_{pd}), but not K_{sx} , indicating that leaves were more sensitive than stems to a
39 combination of drought stress and N addition. Our results provide mechanistic insight into the
40 variable responses of xylem water transport to the interactions of drought and N availability.

41

42 1 Introduction

43 Extreme drought events and atmospheric nitrogen (N) deposition have become two major factors in
44 global environmental changes (Dore 2005; Reay et al. 2008; IPCC 2013), which could strongly
45 affect plant water relations and functional traits in terrestrial ecosystems (Manes et al. 2006; Duan et

46 al. 2015; Borghetti et al. 2017). Drought-induced forest mortality has risen with increasing frequency,
47 duration and intensity of droughts across the world (Allen et al. 2010; Anderegg et al. 2015, 2016;
48 Hartmann et al. 2018). An increase in atmospheric N deposition may benefit forest ecosystems
49 through enhancing tree growth and productivity, which in turn affect drought tolerance
50 (Villar-Salvador et al. 2013), yet there is no clear consensus on the magnitude of this effect.

51 Previous studies have proposed that hydraulic failure is an important physiological mechanism
52 involved in drought-induced tree mortality (McDowell et al. 2008; Sala et al. 2010; Hartmann et al.
53 2013; Sevanto et al. 2014; McDowell et al. 2015; Adams et al. 2017). Drought increases the risk of
54 embolism and hydraulic dysfunction and thus partially or completely hampers xylem function,
55 resulting in shoot dieback, and in severe cases, tree death (Brodribb and Cochard 2009; Hoffmann et
56 al. 2011; Choat 2013). Different wood types (e.g. ring-porous and diffuse-porous) reflect different
57 responses to a trade-off between vulnerability and conductivity (Taneda and Sperry 2008).
58 Ring-porous trees generally have wide vessels but may experience more embolism than
59 diffuse-porous trees (Christman et al. 2012). Xylem anatomical traits vary among species and plant
60 organs (Beikircher et al. 2008), which might contribute to divergent hydraulic functional or leaf
61 stomatal conductance responses to N deposition or drought among species (Fusaro et al. 2017;
62 Borghetti et al. 2017). Hydraulic architecture is somewhat plastic in its response to environmental
63 conditions (Plavcova and Hacke 2012), but the pattern of its responses to drought are unclear. Plant
64 resistance to water flow through leaves is higher than in stems (Sack et al. 2003; Wang et al. 2015),
65 hence the hydraulic conductance of leaves has a large influence over plant water transport (Brodribb
66 et al. 2005).

67 Tree hydraulic traits also vary with soil nitrogen (N) conditions (Ghashghaie and Saugier 1989;

68 Tan and Hogan 1995; Ewers et al. 2000; Li et al. 2015). High N availability could increase xylem
69 conduit diameter (Harvey and van den Driessche 1999), which likely enhances specific conductivity
70 (Hacke et al. 2010). However, wider conduits with N addition may increase the vulnerability to
71 embolism (Harvey and Van den Driessche 1999; Wheeler et al. 2005; Villar-Salvador et al. 2013;
72 Zhang et al. 2018). Previous studies detected that N fertilization significantly reduced leaf hydraulic
73 conductance, which in turn impacted whole-tree water use (Domec et al. 2009a), and drought altered
74 the partitioning of the resistance between leaves and other organs (Domec et al., 2009b). However,
75 the details of interactions between drought and N fertilization and how they affect tree hydraulic
76 traits remain unclear.

77 Previous studies have found synergy (Damatta et al. 2002), antagonism (Walters and Reich
78 1989), or no interaction (Correia et al. 1989; Kleiner et al. 1992) between drought stress and N
79 addition on the stress tolerance of plants. Dzedek et al. (2016) indicated that N deposition
80 aggravated the drought sensitivity of European beech seedlings, whereas Zhang et al. (2014) found
81 that N fertilization have a positive effect on water-use efficiency under low soil moisture conditions,
82 and Villar-Salvador et al. (2013) found that N addition and drought hardening exert opposite effects
83 on the stress tolerance of *Pinus pinea L.* seedlings. In addition, a number of studies demonstrated that
84 the effects of drought on plant water transport can be influenced by N addition. For example, low N
85 concentration of plant tissues may hinder drought tolerance (Saneoka et al. 2004; Andivia et al. 2011),
86 while high N may increase drought or cold susceptibility (Zhu et al. 2001; Islam et al. 2009; Lin et al.
87 2012). Many studies have revealed that N addition enhanced xylem conductance by increasing vessel
88 diameter (Hacke et al. 2001; Bucci et al. 2006; Watanabe et al. 2008; Plavcová and Hacke 2012), but
89 it is hard to predict the pattern of the interactions between drought and N addition from individual

90 effects. However, it is certain that N availability has large effects on plant hydraulic and functional
91 attributes under drought stress conditions, thereby influencing the water economy of trees (Driessche
92 1992; Lloret et al. 1999; Salifu and Timmer 2001; Trubat et al. 2011).

93 In this study, we investigated the responses of tree hydraulics to drought stress, N addition and
94 their interactions in seven-year-old saplings from a native forest. Specifically, we tested the
95 following two hypotheses: (i) N addition increases drought susceptibility, but species with different
96 wood types or leaf forms have different responses of water transport capacity to drought, N addition
97 and their interactions. A ring-porous species have greater hydraulic conductivity than a
98 diffuse-porous species, and ring-porous species with compound-leaves show unique responses to
99 simple-leaved species; (ii) different hydraulic responses to drought and N addition at both stems and
100 leaf levels associate with divergent xylem anatomical traits.

101 **2 Materials and methods**

102 **2.1 Descriptions of study site and tree species**

103 The experiment was conducted at the Research Station of Changbai Mountain Forest Ecosystems of
104 Chinese Academy of Sciences, located in Jilin province, China (128°28'E, 42°24'N), with an
105 elevation of 736 m above sea level. The site has a temperate continental climate, with mean annual
106 temperature of 3.6 °C and the monthly means ranging from -15.6 to 19.7 °C. Average annual
107 precipitation is 695 mm, of which about 60-70% is influenced by the monsoon from June to
108 September. Two dominant ring-porous tree species (*Quercus mongolica* and *Fraxinus mandshurica*)
109 and one diffuse-porous tree species (*Tilia amurensis*) were selected for the present study. Among two
110 ring-porous species, *Quercus mongolica* is a simple-leaved tree species, and *Fraxinus mandshurica*
111 is a compound-leaved species. They are all light-demanding temperate deciduous tree species that

112 can grow up to 30 m in height and 1 m in breast height diameter.

113 **2.2 Experimental design and treatments**

114 One-year old saplings were planted in individual pots in 2011. The pots were filled with forest
115 surface soils collected near the experimental site and the mean diameter of the soil interface was
116 34.25 cm for each pot (41 cm outer diameter \times 28 cm high). The mean concentrations of soil carbon
117 (C), nitrogen (N) and C/N ratio were 55.62 ± 2.45 g kg⁻¹, 4.27 ± 0.23 g kg⁻¹ and 13.05 ± 0.13 ,
118 respectively. Saplings of similar size (~1.5 m) were selected for each species. The potted saplings of
119 each species were divided into three groups: well-watered (maintained at field capacity which was
120 measured by cutting ring (oven-drying) method described by Zhu (1996) and O'Kelly and Sivakumar
121 (2014), which is based on removing soil moisture by oven-drying a soil sample at $105 \pm 2^\circ\text{C}$ until the
122 weight remains constant), moderate drought (MW, 40-50% field capacity) and severe drought (SW,
123 20-30% field capacity). Soil moisture was measured in each pot at 30 min intervals using capacitance
124 probes (Stevens Hydraprobe, Stevens Water Monitoring Systems, Inc., USA). Soil moisture was
125 measured and maintained between 0.38 and 0.40 for the well-watered condition. Each group was
126 subjected to four N addition levels to simulate N deposition intensity ranging from minimal nitrogen
127 (N0), low nitrogen (N1, 23 kg N ha⁻¹ year⁻¹), medium nitrogen (N2, 46 kg N ha⁻¹ year⁻¹) and high
128 nitrogen (N3, 69 kg N ha⁻¹ year⁻¹). Urea solutions with different N addition concentrations (0, 0.028,
129 0.056 and 0.084 mol l⁻¹) were sprayed into the pots once every month (45 ml per pot) from May to
130 October in 2017. Pots were placed in a 3.5-m-high transparent rain-out shelter in an open area to
131 maintain different drought stress conditions, and plants were watered every one or two days
132 according to the capacitance probes data to maintain relatively constant soil moisture. Six replicate
133 saplings were selected for each treatment.

134 **2.3 Leaf water potential measurements**

135 Leaf water potential was measured with a pressure chamber (Model 1505D, PMS Instrument
136 Company, USA) during consecutive sunny days in August in 2017. Leaf samples for predawn (Ψ_{pd})
137 and midday (Ψ_{md}) water potentials were collected before sunrise (06:00) and at midday
138 (12:00-14:00), respectively. Leaves were cut from saplings and immediately sealed in plastic bags
139 containing a moist towel and kept in a cooler until balancing pressures were determined in the
140 laboratory at the research station within 1 h of sample collection. Three different leaves from each of
141 the same four individuals per species and treatment were sampled for leaf water potential
142 measurements.

143 **2.4 Leaf hydraulic conductance**

144 Leaf hydraulic conductance (K_{leaf} , $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was measured in August 2017, determined as

$$145 \quad K_{leaf} = E / \Delta\Psi \quad (2)$$

146 Where E ($\text{mmol m}^{-2} \text{s}^{-1}$) is the average transpiration rate per unit leaf area measured using a portable
147 photosynthesis system (LI-6400XT, LI-COR Inc., USA) under saturating light. For each treatment,
148 the photosynthetic irradiance was maintained at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface and the leaf
149 cuvette temperature was maintained at $26 \pm 1.5 \text{ }^\circ\text{C}$. Measurements were made after the transpiration
150 rate was stable (~ 5 minutes) and subsequently five data points were collected. Nearby leaves were
151 covered with aluminum foil for at least 30 minutes prior to each measurement of water potential.
152 After measurement of transpiration rate, leaf samples and the covered leaves were cut immediately
153 from saplings and sealed in plastic bags containing a moist towel and transported to the laboratory to
154 measure leaf water potential. $\Delta\Psi$ (MPa) is the leaf water potential difference between covered and
155 nearby uncovered leaves. Four different leaves from each sapling and species were measured for

156 transpiration rate and leaf water potential.

157 **2.5 Xylem-specific conductivity**

158 At the end of August 2017, we measured xylem-specific conductivity of current-year branches in
159 each species and treatment (Sperry et al. 1987). Briefly, current-year branches ~ 30 cm in length
160 were cut off under water in the morning and transported to the laboratory immediately with the cut
161 end submerged in water at all times. Longitudinal sections were made to measure vessel length of
162 current-year branches; we found mean vessel length was 0.19, 0.13 and 0.11cm for *Q. mongolica*, *F.*
163 *mandshurica* and *T. amurensis*, respectively. Based on these observations, a stem segment ~5 cm in
164 length (~3 mm in diameter) was re-cut under water and removed to facilitate connection to the tubing
165 system, following best practices (Wheeler et al. 2013, Torres-Ruiz et al. 2015). Stem segments were
166 flushed with perfusion solution (1 mmol L⁻¹ CaCl₂ + 10 mmol L⁻¹ KCl) in the tubing system for
167 hydraulic measurements. The perfusion solution was filtered to 0.22µm pore diameter under 0.5 kpa
168 pressure by vacuum pump (Vacuubrand MD1, GMBH, Germany) to remove air. A constant hydraulic
169 head of 45 cm was used to generate a pressure that drove the solution flow through the segments.
170 Hydraulic conductivity (K_h , kg m s⁻¹ MPa⁻¹) was calculated as:

$$171 \quad K_h = \frac{J_V}{\Delta P / \Delta L} \quad (1)$$

172 Where J_V is the flow rate through the stem segment (kg s⁻¹), and $\Delta P / \Delta L$ is the pressure gradient
173 across the segment (MPa m⁻¹). Specific hydraulic conductivity (K_{sx} , kg m⁻¹ s⁻¹ MPa⁻¹) was calculated
174 as the ratio of K_h and the cross-sectional area of the xylem. All hydraulic samples were collected
175 within three sunny days and all hydraulic measurements were done in five days.

176 **2.6 Xylem anatomy**

177 Xylem anatomical measurements were performed on cross-sections of the collected current-year
178 stem samples and fully expanded leaves. Leaf anatomical observations were conducted on leaf main

179 veins. Paraffin sectioning and light microscopic observations methods were used according to the
180 procedure described in Li. (2009) and Spann et al. (2016). Briefly, current-year stem segments were
181 cut ~0.5 cm and leaves including main vein cut ~0.5×0.5 cm², then fixed in FAA (70% formalin–
182 acetic acid–alcohol, 43.5% ethanol 10% formalin 3% glacial acetic acid 43.5% 43.5% distilled water)
183 for at least 24h. Each specimen was dehydrated with increasing ethanol concentration series (30%,
184 50%, 70%, 85%, 90% and ethanol) and cleared with xylene (TP1020, Leica, Germany), embedded in
185 paraffin (HistoCore ArcadiaH+C, Leica, Germany) and cut into thin-sections (20 μm for stems and
186 12 μm for leaves) with an electronic rotation microtome (RM2245, Leica, Germany). The sections of
187 stems and leaves were stained with safranin-fast green and the slides were examined under a light
188 microscope (DM2500, Leica, Germany). Mean vessel diameter (D , μm) of each stem was estimated
189 based on measurements of lumen area of all vessel appearing in the analyzed area of each image and
190 the D of leaf main vein were estimated based the whole main vein. Vessel density (VD , no.mm⁻²) was
191 determined as the number of vessels per xylem area. ImageJ software was used for data calculation.

192 **2.8 Foliar C and N concentration, C/N ratio and leaf mass per area (LMA)**

193 Ten to fifteen fully expanded leaves were collected from the branches that were used for hydraulic
194 traits measurements. The foliage was photographed with a digital camera (WG-90, Pentax, Tokyo,
195 Japan). Leaf area was measured with ImageJ software from the images. The foliage was dried in an
196 oven at 65 °C for at least 72 h until dry mass was constant within 0.01 g and ground with a ball mill
197 (MM400, Retsch, Germany). Leaf mass per area was calculated as a ratio of dry mass to leaf area.
198 Total foliar C and N concentration were measured with an elemental analyzer (Vario EL, Elementar,
199 Germany) and C/N ratio were analyzed.

200 **2.9 Statistical analysis**

201 The SPSS 17 statistical package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.
202 Data were analyzed for normality and homogeneity of variance before further statistical analyses. A
203 three-way analysis of variance (ANOVA) was used to determine the interactive effects of drought
204 stress, N addition and tree species on each of eight hydraulic and anatomical traits (K_{sx} , K_{leaf} , Ψ_{pd} ,
205 Ψ_{md} , D_{stem} , VD_{stem} , D_{vein} and VD_{stem}). If warranted by significant results from the three-way ANOVA,
206 a series of two-way ANOVAs were employed for further analysis between drought stress and N
207 addition. If further warranted, a one-way ANOVA was applied to analyze the data for N addition
208 effects at different levels of drought stress for each species. Multiple comparisons among values
209 within each drought stress levels and species applying Tukey's HSD post hoc test. Correlations
210 between hydraulic conductivity in current-year stems and leaves with vessel diameter and density
211 were fitted using linear regressions, with standard errors as weight in the linear regression analyses.
212 Results were considered statistically significant at $p < 0.05$.

213

214 **3 Results**

215 N addition had no significant effects on K_{sx} ($p > 0.05$) but decreased K_{leaf} in *Q. mongolica* ($p = 0.041$)
216 and *T. amurensis* ($p = 0.021$) under well-watered conditions according to one-way ANOVA analysis.
217 N addition significantly increased foliar N concentration ($p < 0.05$) but decreased *LMA* ($p < 0.05$) in
218 all species under well-watered conditions. Treatment effects on foliar C concentration and C/N ratio
219 were dependent on species and watering regimes. No significant three-way interactive effects of
220 drought stress, N addition and tree species were found for K_{sx} , K_{leaf} , Ψ_{pd} and Ψ_{md} , while significant
221 interactions were found for xylem anatomical traits (D_{stem} , VD_{stem} and VD_{vein} ; $p < 0.001$, $p = 0.023$, p
222 < 0.001 , respectively; Table 1). The two-way interactive effects between drought stress and N

223 addition were significant for K_{leaf} ($p < 0.001$), Ψ_{pd} ($p < 0.001$) and xylem anatomical traits (D_{stem} ,
224 VD_{stem} , D_{vein} and VD_{vein} ; $p < 0.001$), while not for K_{sx} ($p > 0.05$; Table 1). Xylem anatomical traits
225 (D_{stem} , VD_{stem} and VD_{vein}) had significant two-way interactive effects between drought stress and tree
226 species ($p < 0.001$, $p = 0.01$, $p < 0.001$, respectively; Table 1), and these effects were also significant
227 in each tree species ($p < 0.05$; Table S2).

228 3.1 Responses of leaf water potential

229 N addition had no significant effects on Ψ_{pd} and Ψ_{md} in *Q. mongolica* and *F. mandshurica* under
230 well-watered and moderate drought, but decreased Ψ_{pd} (more negative) in these trees under severe
231 drought (Fig. 1). For *T. amurensis*, Ψ_{md} increased under well-watered conditions while Ψ_{pd} decreased
232 under moderate drought with low N addition (Fig. 1c). Drought stress significantly reduced Ψ_{pd} of all
233 three species ($p = 0.011$, 0.006 , 0.001 for *Q. mongolica*, *F. mandshurica* and *T. amurensis*,
234 respectively; Table S1), and the reduction of Ψ_{pd} was more pronounced than Ψ_{md} ($p = 0.026$, 0.202 ,
235 0.004 for *Q. mongolica*, *F. mandshurica* and *T. amurensis*, respectively).

236 3.2 Responses of leaf hydraulic conductance

237 K_{leaf} was significantly decreased with increasing drought stress in all species (Fig. 2, Table S1, $p <$
238 0.05). Under well-watered conditions, the K_{leaf} of *Q. mongolica* and *T. amurensis* were significantly
239 decreased with N addition, but not for *F. mandshurica* (Fig. 2). The K_{leaf} of *F. mandshurica* decreased
240 with increasing N addition under moderate drought, but K_{leaf} increased with N addition under severe
241 drought in *T. amurensis* (Fig. 2).

242 3.3 Responses of xylem-specific conductivity

243 K_{sx} was variable among species and treatments, though the effects of drought and N addition on K_{sx}
244 were not statistically significant in most cases (Fig. 3). Overall, the mean xylem-specific

245 conductivity of current-year stems (K_{sx}) varied from 0.37 to 0.74, 0.09 to 0.30, and 0.06 to 0.28 kg
 246 $m^{-1} s^{-1} MPa^{-1}$ in *Q. mongolica*, *F. mandshurica* and *T. amurensis*, respectively (Fig. 3). Therefore,
 247 mean K_{sx} of *Q. mongolica* was more than double that of *F. mandshurica* and *T. amurensis*. K_{sx}
 248 increased with N addition under severe drought in *Q. mongolica* (Fig. 3a) and decreased with N
 249 addition under moderate drought in *T. amurensis* (Fig. 3c). Under drought stress, the K_{sx} of *Q.*
 250 *mongolica* increased with low and medium N addition, but the effects diminished or disappeared at
 251 higher N addition levels (Fig. 3a).

252 3.4 Responses of xylem anatomical traits

253 Xylem anatomical traits at both the stem and leaf levels varied substantially among the three tree
 254 species. Without treatment, *Q. mongolica* had higher D_{stem} (8.71 ± 0.53 μm ; Fig. 4a) and D_{vein}
 255 (5.83 ± 0.38 μm ; Fig. 5a) than *F. mandshurica* (7.43 ± 0.12 , 4.20 ± 0.11 μm ; Fig. 4b, 5b) and *T.*
 256 *amurensis* (7.38 ± 0.22 , 4.57 ± 0.05 μm ; Fig. 4c, 5c), and had significantly lower VD_{stem}
 257 ($359.81 \pm 30.92 mm^{-2}$; Fig. 4d) and VD_{vein} (651.86 ± 18.11 ; Fig. 5d) than *F. mandshurica*
 258 ($600.40 \pm 50.01 mm^{-2}$, $3087.43 \pm 60.13 mm^{-2}$; Fig. 4e, 5e) and *T. amurensis* ($908.65 \pm mm^{-2}$,
 259 $3721.00 \pm 137.17 mm^{-2}$; Fig. 4f, 5f).

260 N addition increased D_{stem} and D_{vein} (except D_{vein} in *Q. mongolica*) in all species, but decreased
 261 VD_{stem} in *T. amurensis* and VD_{vein} in all species under well-watered conditions (Fig. 4, 5). Without N
 262 addition, drought stress had significant effects on most xylem anatomical traits in *F. mandshurica*
 263 and *T. amurensis*, but not for *Q. mongolica* (Table S1). The interactions between drought stress and
 264 N addition were dependent on tree species and organs. For current-year stems, the changes of D were
 265 significant in *Q. mongolica* ($p = 0.038$; Fig. 4, Table S1) and *T. amurensis* ($p = 0.001$) while not for *F.*
 266 *mandshurica* ($p = 0.093$) under severe drought, but the changes of VD were significant in *F.*

267 *mandshurica* ($p = 0.008$) while not for *Q. mongolica* ($p = 0.769$) and *T. amurensis* ($p = 0.457$). For
268 leaf veins, D significantly increased in *Q. mongolica* ($p = 0.020$) and *F. mandshurica* ($p = 0.021$)
269 under moderate drought but decreased in *F. mandshurica* ($p = 0.037$) under severe drought (Fig. 5).
270 VD significantly decreased in *F. mandshurica* ($p = 0.009$) under moderate drought. However, N
271 addition significantly increased VD in leaves for all tree species under severe drought (Fig. 5).

272 **3.5 Responses of foliar C and N concentration, C/N ratio and leaf mass per area (LMA)**

273 Overall, the responses of foliar C and N concentration to drought, N addition and their interactions
274 depended on tree species (Fig. 6). Foliar C concentration was significantly increased under
275 well-watered conditions in *Q. mongolica* ($p = 0.018$; Fig. 6a) and *F. mandshurica* ($p = 0.026$; Fig.
276 6b), but no changes were observed in *T. amurensis* (Fig. 6c). We also found that foliar C
277 concentration significantly decreased in *Q. mongolica* under severe drought while not for other two
278 species. N addition significantly increased foliar N concentration in *Q. mongolica* and *F.*
279 *mandshurica* under differing watering regimes ($p < 0.05$; Fig. 6d, e). For *T. amurensis*, foliar N
280 concentration significantly increased under well-watered conditions ($p = 0.043$), but decreased in
281 moderate drought ($p = 0.011$), and exhibited no changes in severe drought (Fig. 6f). Foliar C/N ratio
282 significantly decreased under different treatments in *Q. mongolica* ($p < 0.05$; Fig. 6g). For *F.*
283 *mandshurica*, N addition decreased C/N ratio under moderate drought ($p = 0.001$; Fig. 6h). For *T.*
284 *amurensis*, the C/N ratio decreased with N addition under well-watered condition while it increased
285 under moderate drought (Fig. 6i). LMA was significantly decreased with N addition under
286 well-watered conditions in all species ($p < 0.05$; Fig. 7), but there were no effects on most values
287 under the interactions between N addition and drought stress. Only for *Q. mongolica*, N addition
288 increased LMA under severe drought ($p = 0.024$; Fig. 7a).

289

290 **4 Discussion**

291 Soil water and N availability can directly and indirectly affect plant water transport (Radin and
292 Ackerson 1981; Sterck et al., 2008; Reay et al., 2008). Consistent with our hypothesis, our results
293 showed that the selected three tree species diverged substantially in hydraulic traits coping with
294 different levels of drought and N addition, which was associated with different wood types, leaf
295 forms and anatomical traits. Compared with our diffuse-porous species (*T. amurensis*), ring-porous
296 species (*Q. mongolica* and *F. mandshurica*) had higher stem xylem hydraulic conductivity.
297 Consistently, we found the ring-porous species had wider stem vessel diameter. Ring-porous species
298 simultaneously produce leaves and wide earlywood vessels during a short period in the beginning of
299 seasonal growth, whereas for diffuse-porous species, vessel development occurs after the
300 development of the current-year leaves (Takahashi et al. 2013; Takahashi and Takahashi 2016; Kitin
301 and Funada 2016). Cambial activity and xylem element formation are strongly influenced by
302 environmental conditions (Fonti et al. 2007; Begum et al. 2012), which might affect hydraulic
303 function among woody types (Kitin and Funada 2016). Thus, further research on how cambial
304 phenology affects hydraulics and the interactions of drought and N addition is needed. N addition
305 decreased leaf hydraulic conductance of *Q. mongolica* and *T. amurensis*, but had no significant
306 effects on *F. mandshurica*. Reduced soil-to-leaf hydraulic conductance by increasing leaf area to
307 sapwood area ratio was observed by Bucci et al. (2006) under long-term N fertilization. The
308 insignificant responses of *F. mandshurica* may be related to different leaf forms and plant allometry.
309 *F. mandshurica* with compound-leaves had lower per leaflet area than simple-leaved species (*Q.*
310 *mongolica* and *T. amurensis*), and had faster growth associated with higher gas exchange capacity

311 (Yang et al. 2019). N addition might benefit this fast-growth, reflected in significantly increased
312 foliar N concentration even under different levels of drought stress. However, our results were
313 conducted using a short-term pot experiment, and might differ with field-grown plants due to
314 restricted rooting volume (McDowell et al. 2013), thus further long-term investigation in mature
315 trees is needed.

316 N addition significantly decreased K_{leaf} in *Q. mongolica* and *T. amurensis*, but had little effect
317 on K_{sx} in all species (Fig. 2, 3, Table S1). The divergence among species likely relate to different
318 stem xylem and leaf anatomical traits. Within species we found that N addition generally increased
319 stem vessel diameter (Fig. 4), which may improve hydraulic efficiency (Goldstein et al., 2013; Julio
320 et al., 2017). Previous studies have found that N enrichment enhances stem hydraulic conductivity
321 (Lovelock et al., 2004; Lovelock et al., 2006a; Lovelock et al. 2006b). This is inconsistent with our
322 results of K_{sx} across species. Because we found that K_{sx} was negatively related to VD_{stem} (Fig. 4),
323 which could diminish the increased hydraulic conductivity. On the other hand, stems with wider
324 vessel diameters under high N availability are more vulnerable to xylem cavitation (Hacke et al.,
325 2010; Plavcová et al., 2013). Plants can regulate stomatal closure to maintain water flow under
326 stressful conditions, which is associated with a reduction of K_{leaf} because of their direct coupling
327 (Vilagrosa et al. 2003; Brodribb et al. 2007). Vulnerability in leaf hydraulic conductance might drive
328 stomatal responses and thereby control water and carbon fluxes from leaves and canopies (Meinzer
329 & Grantz, 1990; Hacke et al., 2001). On the other hand, leaf conductance decline might contribute to
330 outside-xylem tissue vulnerability during dehydration (Scoffoni et al. 2017), which can protect the
331 stem xylem from tension, and make leaves more vulnerable to dehydration than stems (Sperry and
332 Ikeda 1997; Scoffoni and Sack 2017). Leaves play a critical role in the trade-off between carbon gain

333 and water loss, however, the responses of leaf conductance and resistance to multiple stressors
334 remain unclear and require further investigation.

335 The effect of N addition on K_{leaf} and K_{sx} among species depended on soil water availability. N
336 addition reduced K_{leaf} under moderate drought treatments and these effects were significant in *F.*
337 *mandshurica*, the compound-leaved species. A large difference in hydraulic vulnerability exists
338 between compound leaf petioles and leaflet laminas because compound-leaved tree species can shed
339 their leaflet when facing unfavorable environmental water or nitrogen conditions (Liu et al. 2015). N
340 addition increased K_{leaf} in *T. amurensis* under severe drought. These results could depend on wood or
341 leaf hydraulic architecture. Diffuse-porous trees have narrower vessels than ring-porous trees, which
342 are packed more densely per unit xylem area, especially in leaves (Fig. S1). As a result, the xylem of
343 diffuse-porous species generally stays functional longer since narrower vessels are less likely to be
344 lost to cavitation (Taneda and Sperry 2008). The non-monotonic responses of K_{sx} to N addition (first
345 increased then decreased) in *Q. mongolica* might be attributed to nutrient imbalance (Gleeson and
346 Good 2003; Templer 2013), which is consistent with decreased C/N ratio in our results. Low N may
347 have some benefit for water transport under water stress but high N increased drought susceptibility
348 by increasing vulnerability to xylem cavitation (Harvey and Van den Driessche 1999; Trubat et al.
349 2011).

350 The three-way interactive effects of drought stress, N addition and tree species were not
351 significant for K_{sx} , K_{leaf} and water potential, but were significant for most values of anatomical traits
352 (Table 1, three-way ANOVA), suggesting that hydraulic architecture responses to drought and N
353 varied strongly among species. In addition, we found divergence among plant organs in their
354 responses to the interactions of drought and N addition in terms of hydraulic efficiency, conductivity

355 and anatomical traits. The two-way interactive effects of drought stress and N addition for K_{sx} were
356 not significant, but these effects for K_{leaf} and Ψ_{pd} were significant. Although the leaf hydraulic system
357 represents less than 5% of the hydraulic pathway it accounts for 30-80% of the resistance to water
358 flow through the whole-plant (Sack and Holbrook, 2006; Domec et al., 2009a; Hao et al., 2013).
359 Leaves appear particularly sensitive to the interactive effects of drought and atmospheric N
360 deposition. In addition, the interactive effects between drought stress and N addition were significant
361 for all anatomical traits except VD_{stem} among the three species (Table S2, two-way ANOVA). These
362 results suggested that xylem hydraulic architecture was more susceptible to a combination of
363 multiple stress factors, thereby affecting water transport processes. Notably, stem xylem-conductivity
364 positively correlated with stem vessel diameter but leaf hydraulic conductance was negatively
365 correlated with main vein vessel diameter in *Q. mongolica* (Fig. 8). However, no similar
366 relationships were observed in the other two species (Fig. S2). *Q. mongolica* had lower vessel
367 density in both stems and leaves, thus vessel diameter seems more sensitive to N addition and
368 drought stress than vessel density. Ring-porous trees with simple leaves showed greater divergences
369 in this pattern between stem and leaves in our study, which may be less adaptive values for such
370 stress environments compared to compound-leaved trees (Song et al. 2017).

371

372 **Concluding remarks**

373 Divergences in hydraulic conductance between stem and leaves, in combination with xylem
374 anatomical traits, affected the interactions between drought and N addition across in juveniles of
375 three temperate tree species. The different responses of K_{sx} and K_{leaf} were associated with wood types
376 and leaf forms. Ring-porous trees had higher hydraulic efficiency than diffuse-porous trees, and for

377 ring-porous species, compound-leaved trees had greater adaptive significance to environmental stress
378 than simple-leaved trees. Drought stress decreased K_{leaf} , but N addition had conflicting effects that
379 depended on tree water status, perhaps due to the compensatory effect of N fertilization on xylem
380 anatomy. Leaves were more susceptible to N addition and drought stress than stems. The three-way
381 interactive effects of drought, N addition and tree species were significant for xylem anatomical traits,
382 such that hydraulic architecture was affected not only by drought and N addition, but also varied
383 across tree species. The divergence in hydraulics between stems and leaves indicates that some tree
384 species have different strategies at organ level for coping with interactive effects of drought and N
385 addition, and these may contribute to the overall plant survival strategy.

386

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394

395 **AUTHORS' CONTRIBUTIONS**

396 All authors contributed to the development of ideas, analysis of output results. H.Z. and F.Y.
397 conducted the experiment; H.Z., F.Y. and D.G. collected the data; H.Z., D.G., N.G.M. and H.D.A.
398 analysed the data; H.Z. and F.Y. wrote the manuscript. N.G.M. and H.D.A. provided valuable edits to

399 the manuscript and all authors contributed to revisions.

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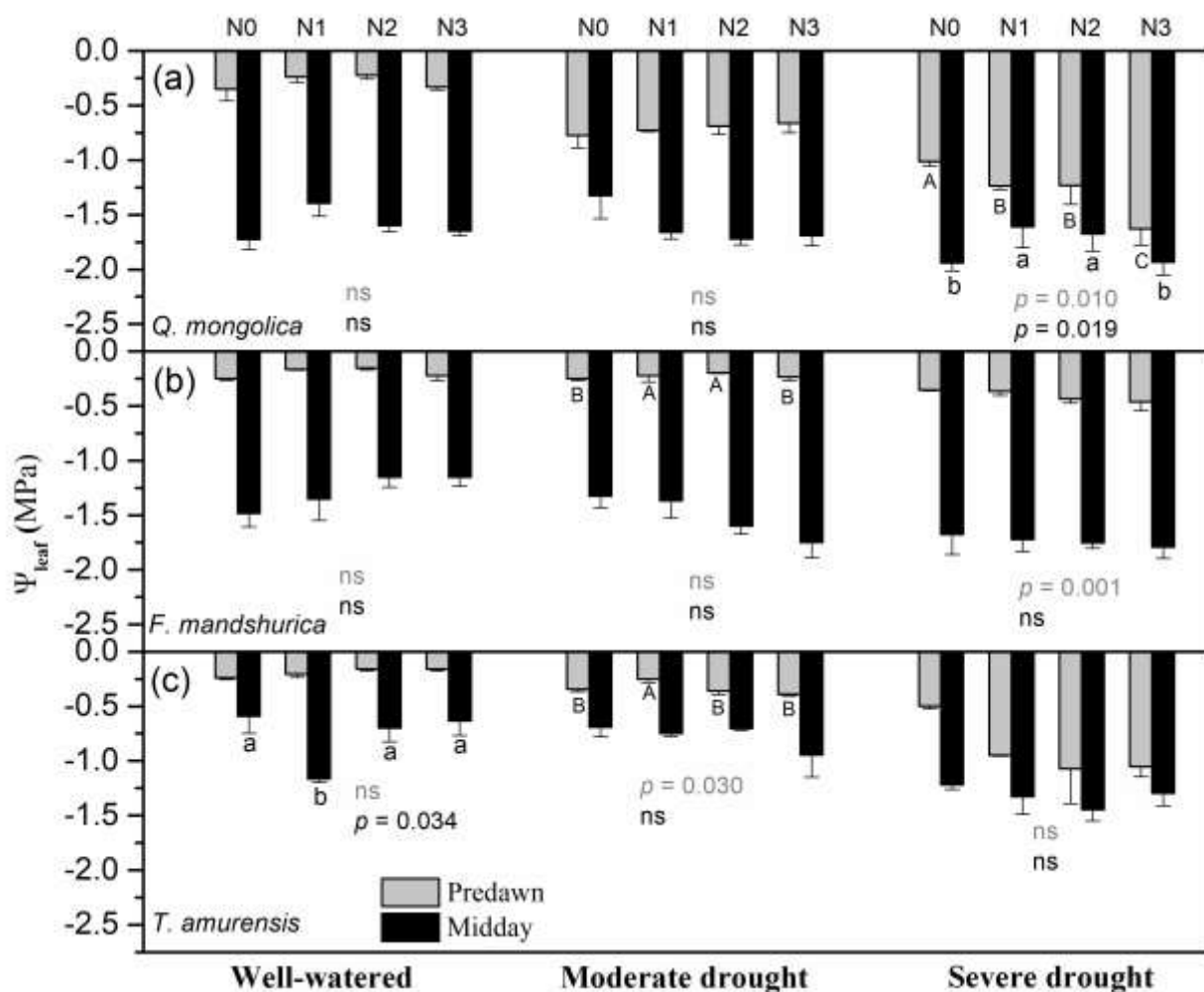
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607 Fig. 1. Responses of predawn and midday leaf water potential (Ψ_{leaf} , MPa) of (a) *Q. mongolica* (b) *F. mandshurica*
 608 (c) *T. amurensis* to different watering and N addition treatments. The error bar represents one standard error of all
 609 measurements for each individual tree species. Different letters indicate significant difference within each watering
 610 treatment for each species (multiple comparisons, $p < 0.05$).



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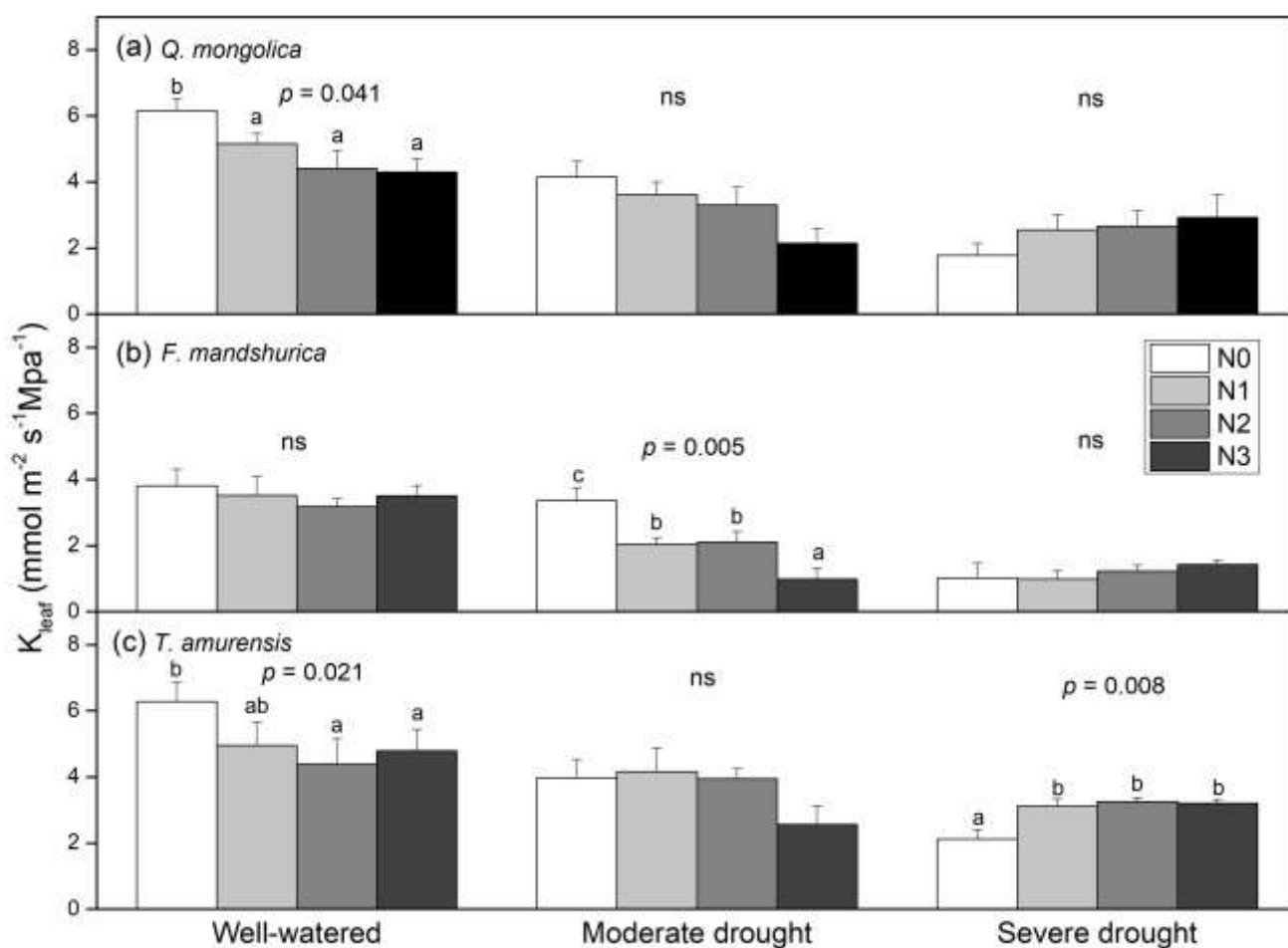
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618 Fig. 2. Responses of leaf hydraulic conductance (K_{leaf} , $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) of (a) *Q. mongolica* (b) *F. mandshurica*
 619 (c) *T. amurensis* to different watering and N addition treatments. The error bar represents one standard error of all
 620 measurements for each individual tree species. Different letters indicate significant difference within each watering
 621 treatment for each species (multiple comparisons, $p < 0.05$).
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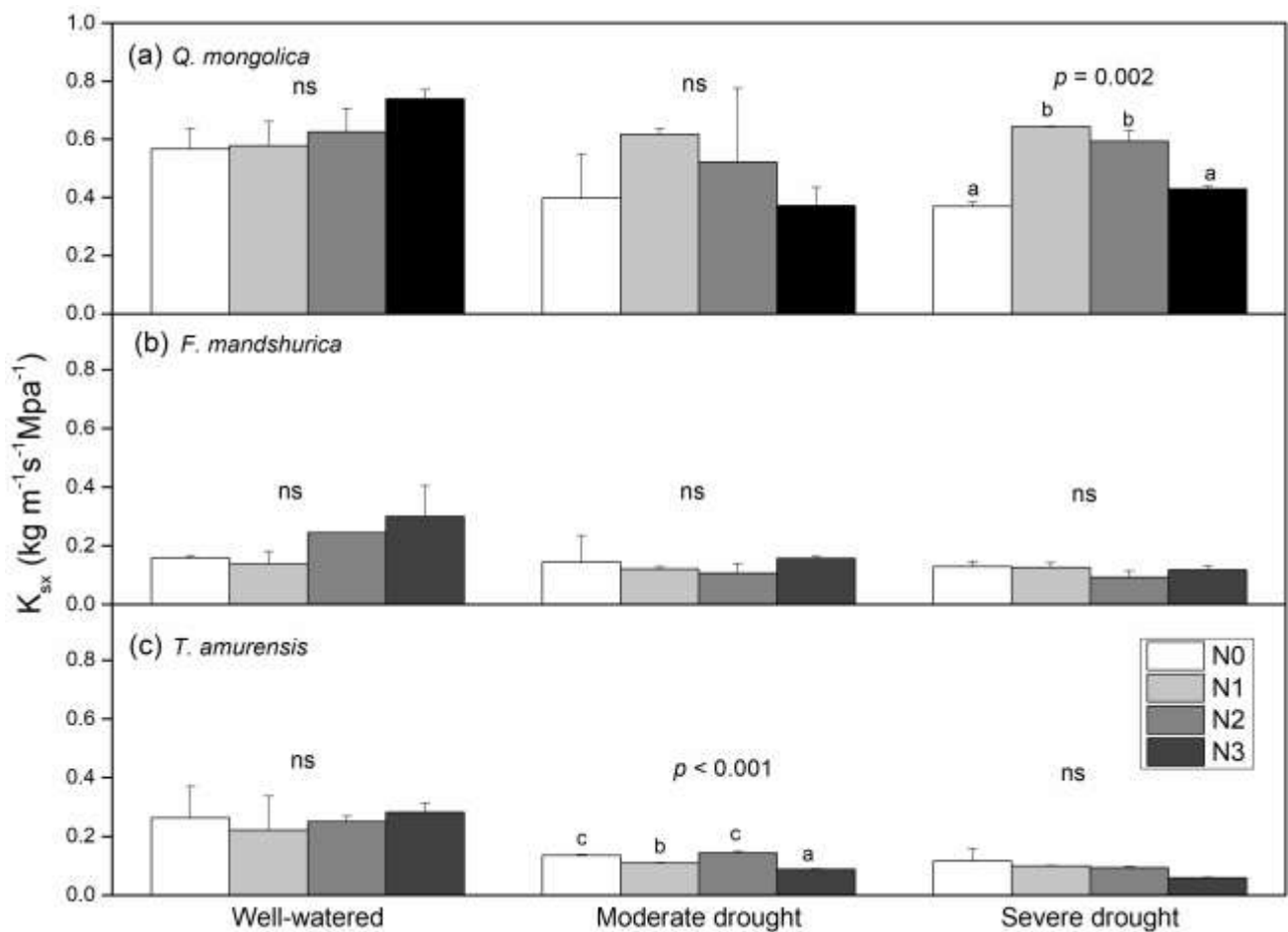
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629 Fig. 3. Responses of xylem-specific conductivity (K_{sx} , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) of (a) *Q. mongolica* (b) *F. mandshurica* (c)
 630 *T. amurensis* to different watering and N addition treatments. Trees were watered to field capacity (well-watered),
 631 40-50% of field capacity (moderate drought), or 20-30% of field capacity (severe drought). Nitrogen addition
 632 treatments were zero (N0), +23 (N1), +46 (N2), and +69 $\text{kg N ha}^{-1} \text{year}^{-1}$ (N3). Each error bar represents one
 633 standard error of all measurements for each individual tree species. Different letters indicate significant difference
 634 within each watering treatment for each species (multiple comparisons, $p < 0.05$).

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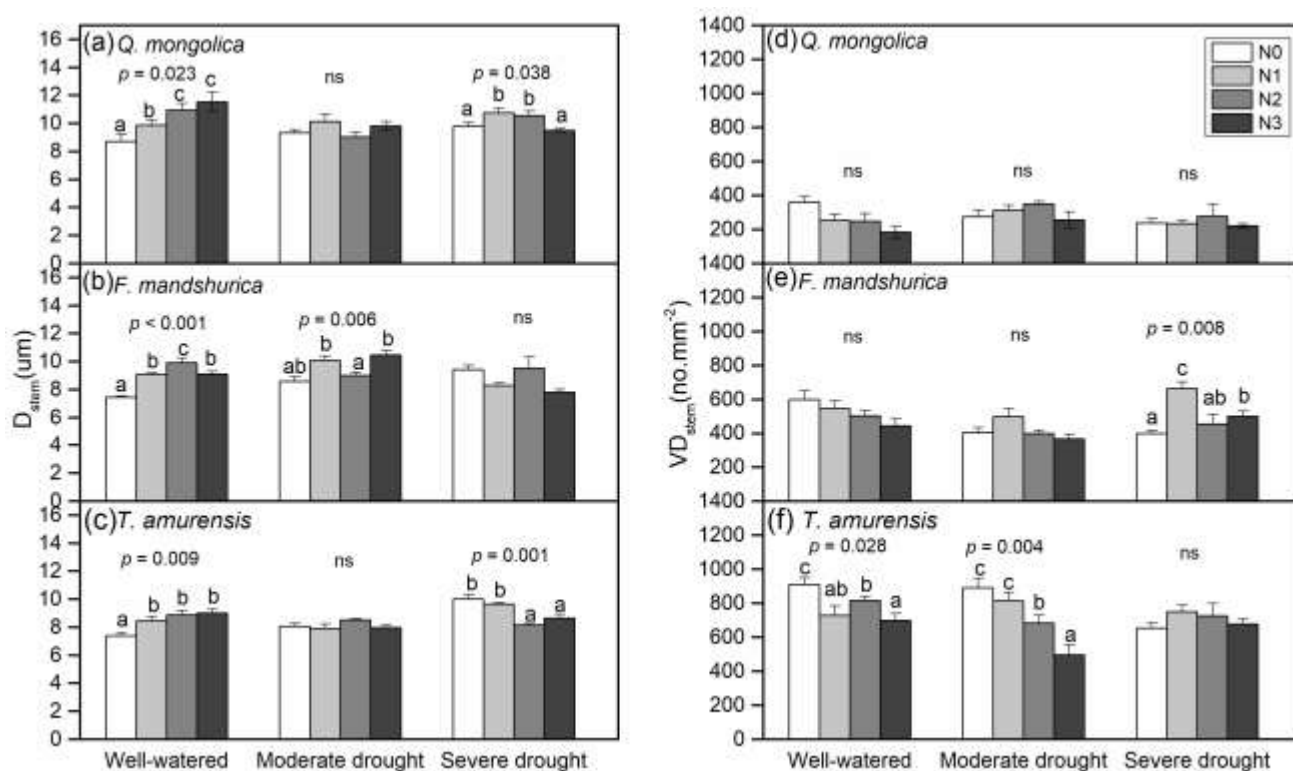
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641 Fig. 4 Responses of mean vessel diameter (D , μm) and vessel density (VD , no. mm^{-2}) of current-year stems of *Q.*
 642 *mongolica*, *F. mandshurica* and *T. amurensis* to different watering and N addition treatments. The error bar
 643 represents one standard error of all measurements for each individual tree species. Different letters indicate
 644 significant difference within each watering treatment for each species (multiple comparisons, $p < 0.05$).
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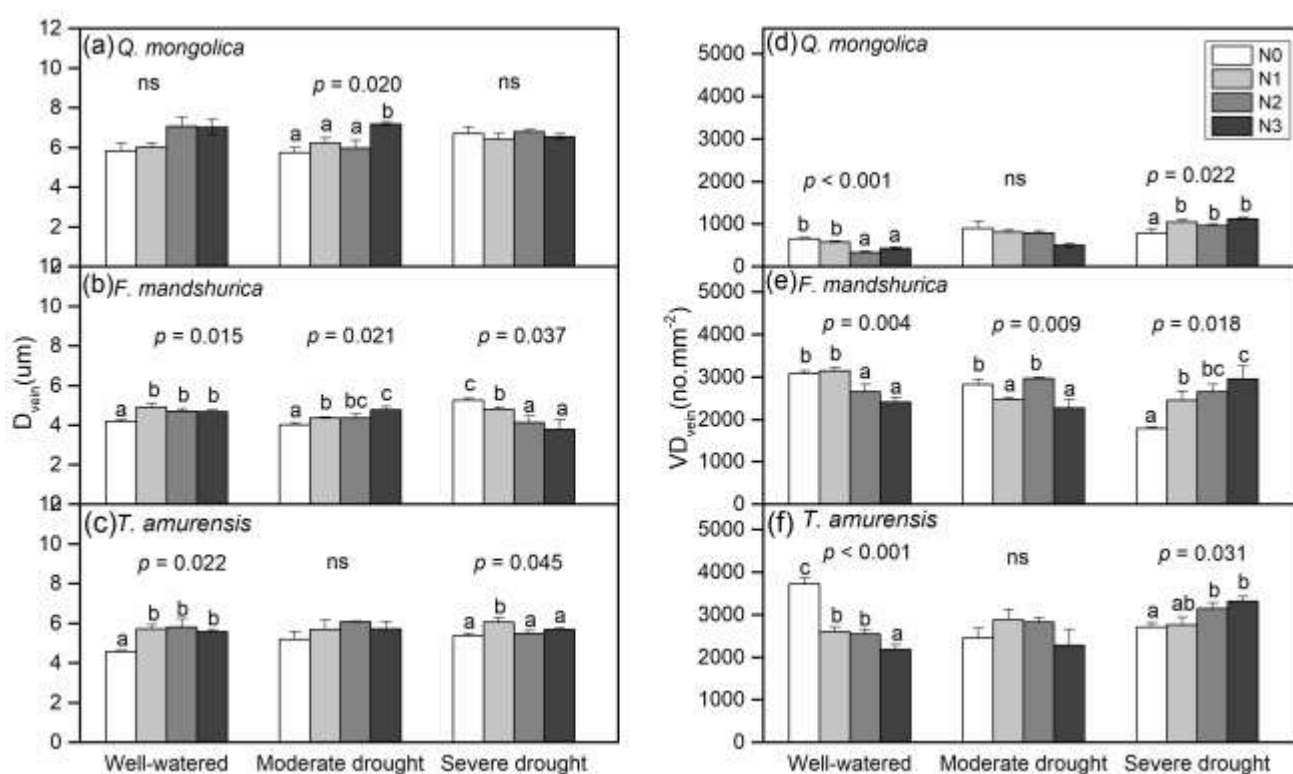
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655 Fig. 5 Responses of mean vessel diameter (D , μm) and vessel density (VD , no. mm^{-2}) of leaves of *Q. mongolica*, *F.*
 656 *mandshurica* and *T. amurensis* to different watering and N addition treatments. The error bar represents one
 657 standard error of all measurements for each individual tree species. Different letters indicate significant difference
 658 within each watering treatment for each species (multiple comparisons, $p < 0.05$).

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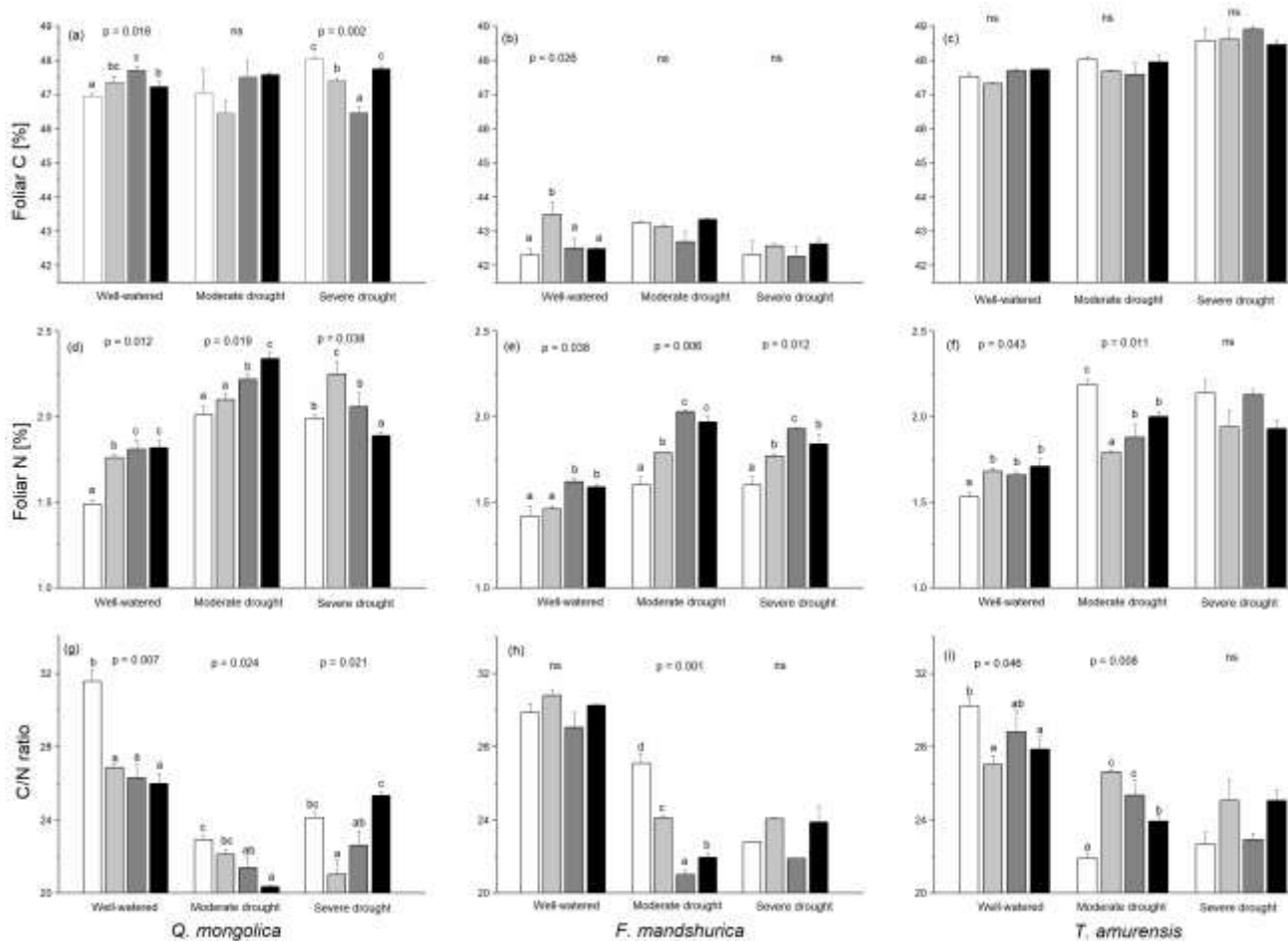
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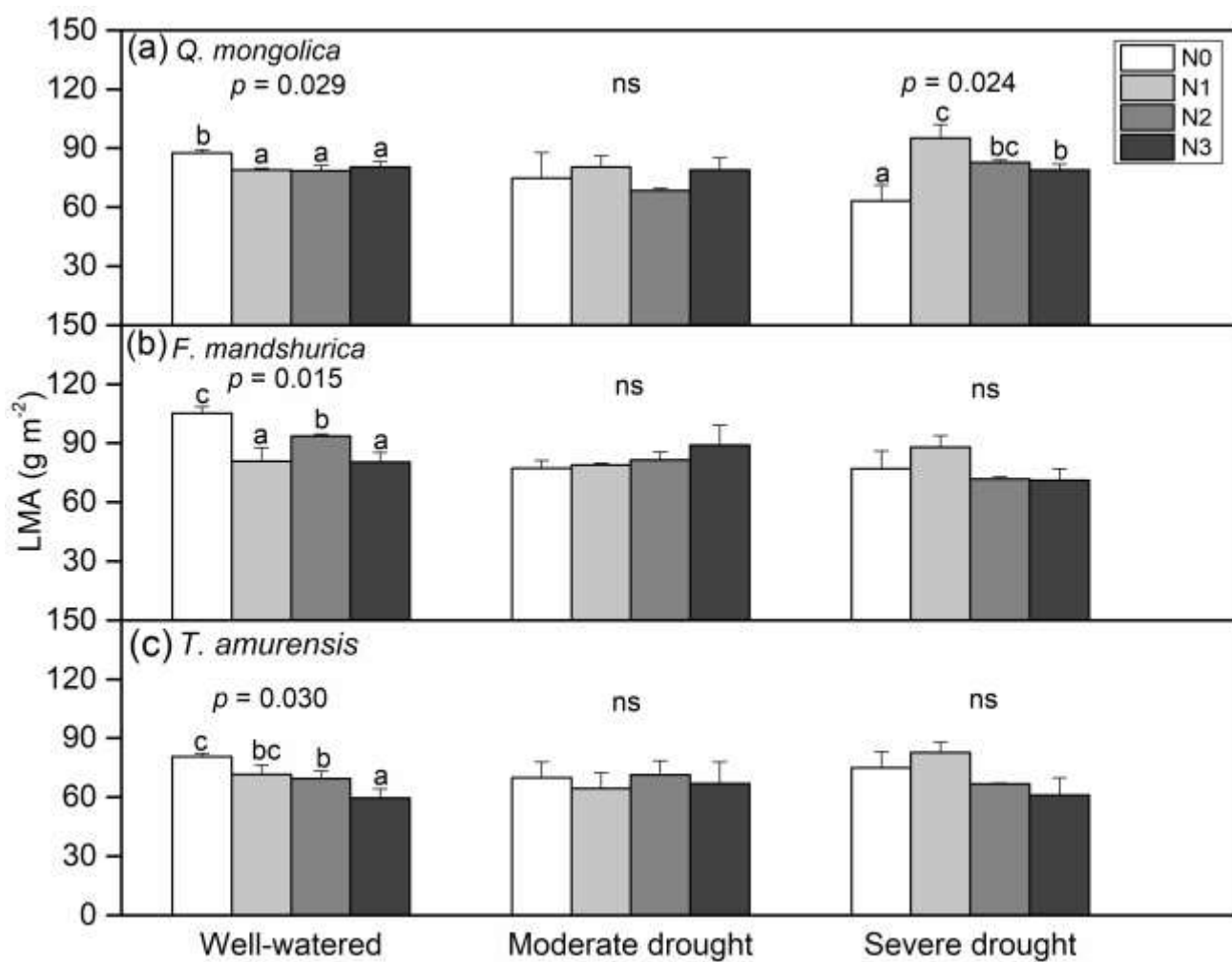
669 Fig. 6 Responses of foliar C concentration (%; a, b, c), N concentration (%; d, e, f) and C/N ratio (g, h, i) of *Q.*
 670 *mongolica* (a, d, g), *F. mandshurica* (b, e, h) and *T. amurensis* (c, f, i) to different watering and N addition
 671 treatments. Nitrogen addition treatments were zero (N0), +23 (N1), +46 (N2), and +69 kg N ha⁻¹ year⁻¹ (N3). Each
 672 error bar represents one standard error of all measurements for each individual tree species. Different letters
 673 indicate significant difference within each watering treatment for each species (multiple comparisons, $p < 0.05$).
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680 Fig. 7 Responses of leaf mass per area (*LMA*) of *Q. mongolica* (a, d, g), *F. mandshurica* (b, e, h) and *T.*
 681 *amurensis* (c, f, i) to different watering and N addition treatments. Each error bar represents one standard error of
 682 all measurements for each individual tree species. Different letters indicate significant difference within each
 683 watering treatment for each species (multiple comparisons, $p < 0.05$).

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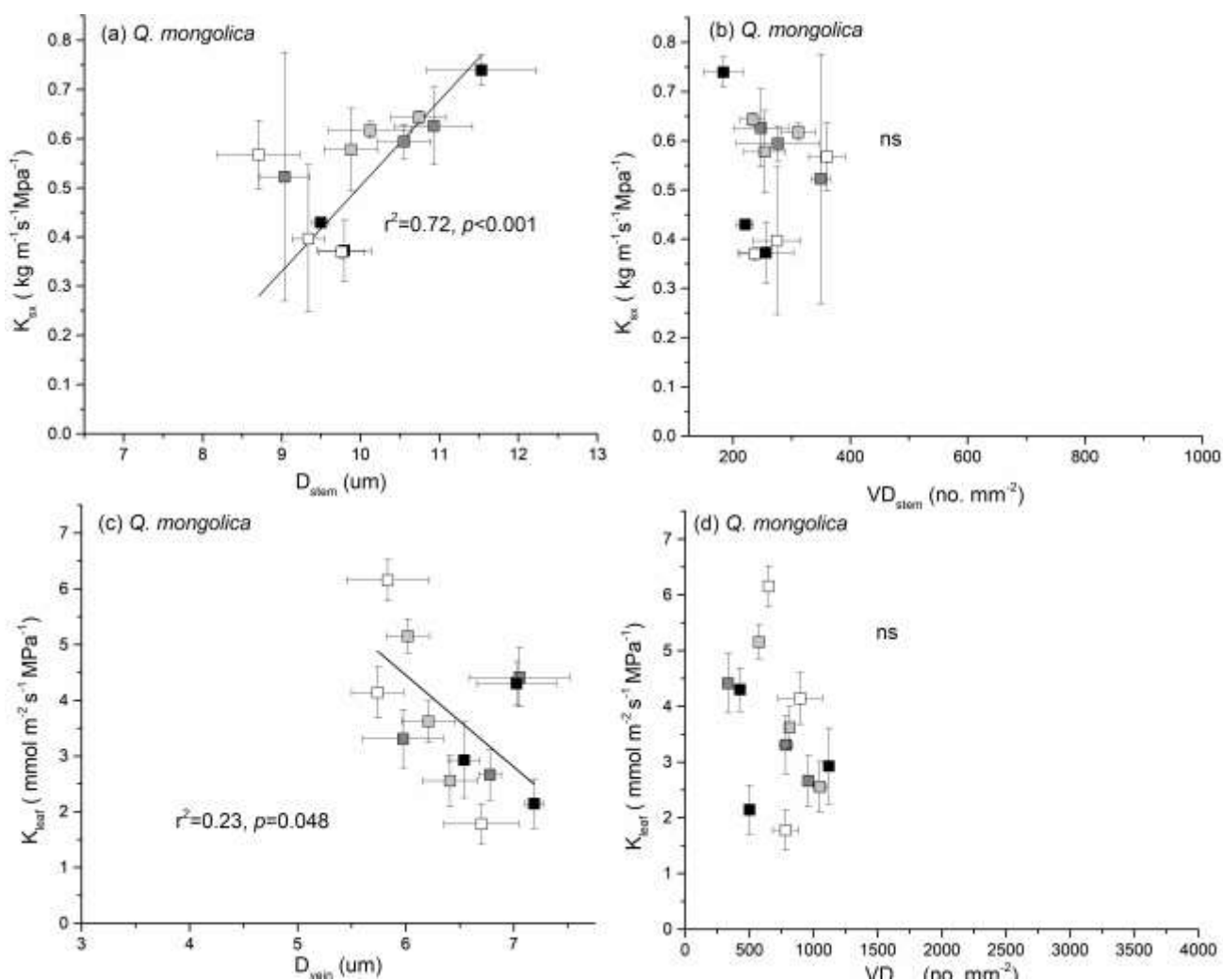
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691 Fig. 8 Correlations between xylem-specific conductivity (K_{sx}) with mean vessel diameter (D_{stem} , a) and vessel
 692 density (VD_{stem} , b) in current-year stem of *Q. mongolica*; and correlations between leaf hydraulic conductance (K_{leaf})
 693 with mean vessel diameter (D_{vein} , c) and vessel density (VD_{vein} , d) in main vein of *Q. mongolica*. The color of the
 694 point represents N addition levels (white, N0; light gray, N1; dark gray, N2; black, N3). The error bar represents
 695 one standard error of all measurements for each individual tree species. Linear regression is fitted to the individual
 696 data in each panel.



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700 Table 1 Results (F and P-value) of three-way ANOVA on the effects of drought stress, N addition and tree species
 701 on eight key hydraulic traits of stems and leaves. Significance at $p < 0.05$ are presented in bold. K_{sx} , xylem-specific
 702 conductivity, K_{leaf} , leaf hydraulic conductance, Ψ_{pd} , predawn leaf water potential, Ψ_{md} , midday leaf water potential,
 703 D_{stem} , mean vessel diameter of current-year stem, VD_{stem} , vessel density of current-year stem, D_{vein} , mean vessel
 704 diameter of leaf vein, VD_{vein} , vessel density of leaf vein.
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Dependent variable		Drought stress	N addition	Tree species	Drought stress × N addition	Drought stress × Tree species	N addition × Tree species	Drought stress × N addition × Tree species
K_{sx}	<i>F</i>	13.093	0.860	126.581	1.639	0.552	1.647	0.524
	<i>p</i>	< 0.001	0.471	< 0.001	0.165	0.699	0.163	0.885
K_{leaf}	<i>F</i>	85.935	46.027	46.027	6.864	0.239	0.673	0.673
	<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.001	0.915	0.771	0.771
Ψ_{pd}	<i>F</i>	247.192	4.412	84.403	9.931	29.717	1.407	1.209
	<i>p</i>	< 0.001	0.007	< 0.001	< 0.001	< 0.001	0.216	0.297
Ψ_{md}	<i>F</i>	25.415	0.634	52.487	1.908	2.775	1.549	1.449
	<i>p</i>	< 0.001	0.596	< 0.001	0.091	0.033	0.165	0.171
D_{stem}	<i>F</i>	1.777	7.435	56.643	13.434	9.157	1.780	3.758
	<i>p</i>	0.176	< 0.001	< 0.001	< 0.001	< 0.001	0.115	< 0.001
VD_{stem}	<i>F</i>	4.643	12.376	393.062	5.986	5.008	3.837	2.167
	<i>p</i>	0.013	< 0.001	< 0.001	< 0.001	0.01	0.002	0.023
D_{vein}	<i>F</i>	1.047	6.058	180.905	6.020	1.534	3.733	1.430
	<i>p</i>	0.356	0.001	< 0.001	< 0.001	0.201	0.003	0.173
VD_{vein}	<i>F</i>	3.503	2.699	798.932	18.634	11.198	1.556	3.933
	<i>p</i>	0.035	0.052	< 0.001	< 0.001	< 0.001	0.173	< 0.001

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