

1 **Differential variation of NSCs in root branch orders of**
2 ***Fraxinus mandshurica* Rupr. seedlings across different**
3 **drought intensities and soil substrates**

4 **Li Ji^{1,2}, Yue Liu^{1,2}, Jun Wang¹, Zhimin Lu¹, Yuchun Yang^{1*}, Lijie Zhang^{3*}**

5 ¹ Jilin Academy of Forestry, Changchun 130033, China

6 ² Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of
7 Education, School of Forestry, Northeast Forestry University, Harbin 150040, China

8 ³ School of Forestry, Shenyang Agricultural University, Shenyang 110161, China

9 *** Correspondence:**

10 Yuchun Yang

11 yang-yu-chun@163.com;

12 Lijie Zhang

13 Zlj330@syau.edu.cn

14

15

16 Running Title: Response of Fine Root NSCs to Drought

17

18 Type of contribution: Original Research

19 Date of preparation: 8 April 2021

20 Number of text pages: 12;

21 Number of words: 6171;

22 Number of tables: 1 table;

23 Number of figures: 7 figures

24

25 **Abstract** : Non-structural carbohydrates (NSCs) facilitate plants adapt to drought
26 stress, could characterize trees growth and survival ability and buffer against external
27 disturbances. Previous studies have focused on the distribution and dynamics of NSCs
28 among different plant organs under drought conditions. However, discussion about the
29 NSC levels of fine roots in different root branch order were little, especially the
30 relationship between fine root trait variation and NSCs content. The aim of the study
31 is to shed light into the synergistic variation of fine root traits and NSC content in
32 different root branch order under different drought and soil substrate conditions.
33 2-year-old *Fraxinus mandshurica* Rupr. potted seedlings were planted in three
34 different soil substrates (humus, loam and sandy-loam soil) and conducted to four
35 drought intensities (CK, mild drought, moderate drought and severe drought) for two
36 months. With the increase of drought intensity, the biomass of fine roots decreased
37 significantly. Under the same drought intensity, seedlings in sandy-loam soil have
38 higher root biomass, and the coefficient of variation of fifth-order roots (37.4%, 44.5%
39 and 53.0% in humus, loam and sandy loam, respectively) is higher than that of
40 lower-order roots. With the increase of drought intensity, the specific root length (SRL)
41 and average diameter (AD) of all five orders increased and decreased, respectively.
42 The fine roots in humus soil had higher soluble sugar content and lower starch content.
43 Also, the soluble sugar and starch content of fine roots showed decreasing and
44 increasing tendency respectively. Soluble sugar and starch explain the highest degree
45 of total variation of fine root traits, that is 32.0% and 32.1% respectively. With
46 ascending root order, the explanation of the variation of root traits by starch decreased
47 (only 6.8% for fifth-order roots). The response of different root branch order fine root
48 morphological traits of *F. mandshurica* seedlings to resource fluctuations ensures that
49 plants maintain and constructure the root development by an economical way to
50 obtain more resources.

51 **Keywords** : NSCs; *Fraxinus mandshurica*; fine root; drought; soil substrates; root
52 traits

53 Introduction

54 In recent decades, forest decline and death caused by high temperatures and
55 extreme droughts, have occurred on a large scale worldwide (Allen et al., 2010; Choat
56 et al., 2012; Zhang et al., 2015; Martínez-Vilalta et al., 2016), and global climate
57 change is predicted tree death is becoming more and more serious, which inevitably
58 affect the carbon metabolism and balance in the plant and change its physiological
59 metabolic function (Choat et al., 2012; Adams et al., 2013). Non-structural
60 carbohydrates (NSCs), as important substances involved in the life process of plants,
61 are mainly composed of soluble sugars and starches, they largely reflect the carbon
62 supply status of plants and affect the growth and development of plants (Richardson
63 et al., 2013). Moreover, the size of their content can characterize the buffering
64 capacity to cope with the pressure of environmental stress (Xie et al., 2018). When
65 plants undergone drought stress, the stored NSCs can be used as a buffer to
66 temporarily supply plants for their growth and metabolism (Dietze et al., 2014). In
67 recent years, the different responses of NSCs among different plant tissues or organs
68 under drought stress were discussed deeply (Martínez-Vilalta et al., 2016; Furze et
69 al., 2019; Deng et al., 2020; He et al., 2020; Zhang et al., 2020). Many studies have
70 found that roots have the highest concentration of NSC except for trunk, which had an
71 important impact on NSC storage and distribution in trees (Dietze et al., 2014; Mei et
72 al., 2015; Ji et al., 2020). However, there have been few empirical investigations into
73 the variation of fine roots NSC (especially the functional root branch order) in
74 response to drought. Therefore, understanding the variation of the composition and
75 level of fine root NSC under drought conditions has great significance for better
76 recognition of the carbon balance and dynamics of plant survival and growth
77 (Hartmann and Trumbore, 2016).

78 Root plays a crucial role in plant growth and productivity, especially in
79 resource-constrained environments. The morphological and physiological plasticity of
80 root reflect an important mechanism for plants to obtain limited soil resources
81 (Ristova and Busch, 2014; Rogers and Benfey, 2015). Fine roots (≤ 2 mm) are the
82 main organ for water and nutrient absorption, and the most active and sensitive part of
83 the root system (McCormack et al., 2015; Ma et al., 2018). Many scholars pointed out
84 that changes in root starch under drought conditions are related to plant survival, and
85 root NSCs reserves play an important role in repairing embolism and preventing
86 self-death (Rodríguez-Calcerrada et al., 2017; Kannenberg et al., 2018). Oswald and
87 Aubrey (2020) observed that the root starch concentration of *Pinus palustris* in xeric
88 environments increased delayed in summer compared with mesic habitats. Starch
89 stored in the root system can promote root growth and maintain root osmotic potential,
90 ensuring plants can absorb more water (Ge et al., 2012; Camarero et al., 2016).
91 However, previous studies have mostly focused on the overall NSCs level of the root
92 system (including coarse roots and fine roots) (Hoch and Körner, 2003; Landhäuser
93 and Liefers, 2012; Hartmann, 2013; Yang et al., 2021), nevertheless, the response of
94 fine root NSCs with functional root branch orders to drought is rarely involved
95 (Aubrey and Teskey, 2018; Nikolova et al., 2020). Pregitzer et al. (2002) found that

96 the lower-order roots were produced later, the younger the age, the larger the
97 contribution, the lower-order roots have higher nitrogen content and the respiration
98 metabolism ability, and they are more sensitive and fragile than the higher-order roots.
99 Guo et al. (2008b) conducted a study on the root for twenty-three tree species in the
100 temperate of China, they pointed out that the first three roots of trees are generally
101 non-lignified, with primary structures and complete cortical tissues, and their
102 mycorrhizal colonization rate is relatively higher, and they mainly perform the
103 function of nutrient and water absorption. most of the fourth- and fifth-orders roots
104 have been lignified, and the cortex tissue has disappeared, with a continuous cork
105 layer and secondary xylem, which mainly play a role of transport and storage. In
106 addition, previous studies mostly compared the effects of single drought or severe
107 drought on the root system, but far too little attention had been paid to the gradient
108 studies (Hartmann et al., 2013; Kannenberg et al., 2018; Blackman et al., 2019; Zhang
109 et al., 2020). McDowell et al. (2008) speculated that trees only depleted NSC under
110 mild or moderate drought, while severe drought will cause the xylem to form
111 embolism while NSC is not depleted. Recently, a meta-analysis based on fifty-two
112 tree species around the world indicated that variations in plant NSCs were related to
113 drought intensity, and the net loss of carbohydrates from roots is the most obvious (He
114 et al., 2020). Therefore, this indicates a necessary to shed light into the response of
115 fine root NSCs exist among different root orders across drought intensity.

116 Root traits play a vital role in the acquisition and transportation of water and
117 nutrients. Therefore, they can strongly affect plant growth, survival and response to
118 climate change (Bardgett et al., 2014; Kong et al., 2014; Iversen et al., 2017).
119 Compared with leaf traits, the response of fine root traits to environmental changes
120 can reflect adaptation strategies for resource utilization and plant performance under
121 climate change (Bardgett et al., 2014; Warren et al., 2015). Moreover, root traits have
122 greater variability and uncertainty (Comas and Eissenstat, 2004). Although numerous
123 studies have reported the response of root traits to drought or water deficit (Comas et
124 al., 2013; Fort et al., 2017; Zhou et al., 2018; Zhou et al., 2019; Lozano et al.,
125 2020; Nikolova et al., 2020), few studies have focused on the synergistic changes and
126 relationships between root NSCs levels and root traits under stress condition (Ji et al.,
127 2020; Yang et al., 2021). Olmo et al. (2014) studied the drought resistance response of
128 ten woody tree species seedlings and found that SRL increased significantly under
129 drought conditions. It is a strategy that when water is limited, plants can build longer
130 root with less carbon. Indeed, increasing carbon input per unit produces a larger
131 surface area, length of fine roots and more fine roots, it could facilitate to optimize the
132 cost-benefit ratio of fine roots (Eissenstat et al., 2000; Ostonen et al., 2007b). Under
133 drought conditions, thicker roots with transport and storage functions tend to preserve
134 NSC (Konôpka et al., 2007; Yang et al., 2021), while thinner roots with absorption
135 function are severely affected by drought (Olmo et al., 2014). Yang et al. (2021) found
136 that significant correlation between the roots NSC concentration, root architecture and
137 SRL occurred in *Phyllostachys edulis* seedling under drought conditions, indicating
138 that the sensitivity of NSC concentration to drought supported the plasticity of root
139 architecture to a certain extent, and plants could build low-cost roots through more

140 carbon investment. In addition, when plants adapt to drought stress, they balance the
141 lack of tissue radial growth by increasing the concentration of NSC in the growing
142 parts (Dietze et al., 2014; Kannenberg et al., 2018). Therefore, exploring the coupled
143 relationship between the variation of fine root NSCs and root traits under drought
144 conditions will help us to further understand the response strategies of plants to water
145 deficit.

146 *Fraxinus mandshurica* Rupr. is one of the main timber species in northeastern
147 China. It has been proven that its root system (shorter primary root and developed
148 lateral roots) had a branch sequence of primary (non-woody) structure and developed
149 into a branch through secondary development. The branched order of woody roots is
150 sensitive to nutrients and water (Wei et al., 2009; Xia et al., 2010). In Jilin Province
151 China, *F. mandshurica* plantations are distributed in north-south latitudes, and the soil
152 types they inhabit are roughly divided into three categories, namely, humus, loam and
153 sandy-loam soil. Weemstra et al. (2017) found that the specific root length (SRL) and
154 root tissue density (RTD) of the fine roots of European beech and Norway spruce
155 were not significantly different in clay and sandy soil, but the dry mass of fine roots in
156 sandy (both species) was ten times than that in clay. Paudel et al. (2016) had studied
157 these interactions in the osmometer of the orchard and the clay sandy loam, and found
158 that soil type significantly affects the morphological traits of all root branch order of
159 *Citrus paradisi* Macf. The interaction of root system with soil quality and water will
160 cause changes in root growth, structure and function (Paudel et al., 2016). To our
161 knowledge, few studies have focused on the response of fine root NSC to drought
162 intensity gradient in different soil substrates, and even less is known regarding to the
163 coupled relationship between fine root NSC and traits. In this study, two-year-old *F.*
164 *mandshurica* potted seedlings with different drought intensities and soil substrates
165 were set up, and compare the variation and the coupled relationship of the fine root
166 traits and NSCs content of different root branch order of *F. mandshurica* seedlings
167 under different water and soil conditions. We hypothesize that 1) with the increase of
168 drought intensity, the specific root length of fine roots increases and the root diameter
169 decreases; the specific root length in humus soil is the lowest and the root diameter is
170 thicker; 2) with the increase of drought intensity, the variation of root carbon, nitrogen
171 and phosphorus is slight, but the variation of NSC will be obvious, and the root
172 soluble sugar content will decrease, and the starch content will increase; 3) root
173 morphological traits are closely related to the NSCs content.

174 **Materials and Methods**

175 **Experimental site and sapling preparation**

176 A controlled pot experiment was conducted at the Xinli Town, Jingyue
177 Development District, Changchun, which is located in Jilin Province, China (43°33'
178 N-44°41' N, 125°19' E-125°24' E). The location belongs to temperate continental
179 monsoon climate with a frost-free period of 140 days; and with mean annual rainfall
180 of 600-800 mm, which mainly falls from July to September and a mean annual

181 temperature of 4.6□.

182 The two-year-old *Fraxinus mandshurica* Rupr. seedlings of the Hongwei nursery
183 of Lushuihe Forestry Bureau, in Jilin Province were used as experimental materials.
184 They were transplanted into plastic pots (24 cm × 20 cm) in the April, 2017, and were
185 placed in the flat, open canopy before they started to bud. The cultured soil in pots
186 was filled with equal volumes of humus, loam and sandy-loam respectively, and the
187 humus was collected in coniferous and broad-leaved forest in Lushuihe Forestry
188 Bureau. The main soil type is an Eum-Orthic Anthrosol according to the Food and
189 Agricultural Organization soil classification system. The soil surface was well
190 ventilated throughout the experiment. At the experimental site, the pots were placed in
191 rows with 50 cm apart from the neighbors under full sunlight. They were kept
192 well-watered prior to the application of drought treatments, and the gravimetric soil
193 water content was initially maintained at field capacity. Fertilizer was not added
194 during the experiment period.

195 **Experimental design and sampling**

196 Before the beginning of the drought experiment, three soil substrates samples
197 were collected in July 2017 to determine soil total nitrogen, total phosphorus,
198 available phosphorus and soil physical structure, water content and field water
199 holding capacity. The one hundred-twenty pots of cultured *F. mandshurica* seedlings
200 were randomly selected from three different substrates to conduct a water control
201 experiments. The basal diameter and height of each seedlings were measured using a
202 vernier caliper with an accuracy of 0.01 mm and a tape measure with an accuracy of
203 0.1 cm prior to the application of drought treatments in July 2017, respectively.

204 The soil substrates and drought were conducted for two-factor complete
205 orthogonal design. Three soil substrates were set with four drought stress gradients,
206 the control (CK): approximately 80~85% of the maximum field water-holding
207 capacity; mild drought (T1): 60%~65%; moderate drought (T2): 40~55%; and severe
208 drought (T3): 20~25%. The total of 12 treatments, each treatment set 3 blocks,
209 selected 20 pots of seedlings in each block (selecting uniform seedlings). The detailed
210 description of the drought control refers to our previous study (Ji et al., 2020).

211 In each experimental block, ten seedlings were randomly selected and the roots
212 of the sampling were destructively sampled after two months of continuous drought
213 stress. Root were sorted carefully out of the soil, and root samples were washed free
214 of soil particles by deionized water until the branching structure of the roots can be
215 identified, and then put it into the labeled pocket and store it in a freezer (2-3°C).
216 Then divided the root sample into two parts: (1) root morphology analysis sample; (2)
217 chemical characteristic analysis sample. All samples were shipped back to the
218 laboratory on the same day and stored in a freezer at -20 °C. In this study, we only
219 measured live root samples, and the dead roots were picked and discarded.

220 **Soil physicochemical property**

221 The soil physical and chemical properties of the three soil substrates were

222 determined before drought stress (Table 1). The soil total nitrogen was determined by
223 Kjeldahl titration. The soil total phosphorus was determined by the sulfuric
224 acid-perchloric acid-molybdenum anti-colorimetric method (Yang et al., 2018). Soil
225 available phosphorus was extracted by double acid extraction, soil water content and
226 bulk density were determined by ring knife method (Yang et al., 2018), soil total
227 porosity, aeration porosity, water absorption multiple, water seepage rate and
228 evaporation rate reference (Wei et al., 2015) method determination.

229 **Fine root morphological and chemical traits**

230 In the laboratory, root samples for morphological analysis were carefully
231 dissected with forceps on the basis of branch order, following the procedure described
232 in Pregitzer et al. (2002) and Wang et al. (2006), with the distal nonwoody roots
233 regarded as first-order roots (1st-order root), and the next root segment is the
234 2nd-order roots. Then, root samples were scanned with an Expression 10000XL 1.0
235 scanner in Northeast Forestry University (Epson Telford Ltd, Telford, UK). The mean
236 diameter, total length and volume of root tips were determined with the root system
237 analyzer software (WinRhizo 2004b, Regent Instruments, Inc., Québec, Canada).
238 These root samples were oven-dried at 65°C to determine constant weight (nearest
239 0.0001 g) and calculated the specific root length (SRL), specific root surface area
240 (SRA) and root tissue density (RTD).

241 For root chemical analyses, as mentioned above, the fine root sample after
242 scanning was placed in a 65 °C and dried for 48 h to constant weight. The dried fine
243 root sample was ground and homogenized by using a Ball mill instrument (RETSCH
244 MM 400, Germany), and 1.00 g of the dry powder sample was weighed and pressed
245 with a FYD-20 electric tableting machine to a boat which was thickness of 6 mm and
246 a diameter of about 13 mm. For the pellet sample, the tableting conditions can be
247 adjusted according to the actual conditions. The tableting conditions of the test are
248 maintained at a pressure of 16 MPa for 3 min. The carbon, nitrogen and phosphorus
249 element in the root samples after tableting was measured by a J200 Tandem laser
250 spectroscopic element analyzer.

251 **Fine root NSCs concentration**

252 NSC concentration was defined as the sum of soluble sugar (SS) and starch (ST)
253 concentrations that were measured using the anthrone method (Yemm and Willis,
254 1954). Root sample (0.1000 g) was placed into a 10 ml centrifuge tube, and 2 ml of
255 80 % ethanol was then added. The mixture was incubated at 80°C in a shaking water
256 bath for 30 min and then centrifuged at 4000 rpm for 5 min. A further two extractions
257 from the pellets were carried out with 80% ethanol. The supernatant was retained,
258 combined, and stored at -20°C for soluble sugar determination.

259 Starch was extracted from the ethanol-insoluble pellet after ethanol was first
260 removed by evaporation. The starch in the residue was then released by boiling in 2
261 ml distilled water for 15 min. After cooling to room temperature, 2 ml 9.2 M HClO₄
262 was added, and the mixture was shaken for 15 min. Four milliliters of distilled water

263 were then added, and the mixture was centrifuged at 4000 rpm for 5 min. A further
264 extraction was carried out with 2 ml 4.6 M HClO₄. The supernatant was also retained,
265 combined, and stored at -20°C for starch determination.

266 Soluble sugar and starch determination were performed based on the absorbance
267 at 625 nm using the same anthrone reagent in a spectrophotometer (Yemm and Willis,
268 1954). Sugar concentration was calculated from the regression equations based on
269 glucose standard solutions and starch concentration by multiplying glucose
270 concentration with a conversion factor of 0.9 (Osaki et al. 1991).

271 **Data analysis**

272 Normality and variance homogeneity requirements were met, and no data
273 transformation was necessary. The Three-way ANOVA analysis of fine root traits and
274 NSC content was performed by SPSS19.0 (IBM Co., Armonk, NY, USA) and
275 examine the differences in fine root traits of seedlings between different treatments
276 (LSD, $\alpha=0.05$); Principal component analysis (PCA) and redundancy analysis (RDA)
277 were performed on the fine root traits of seedlings under different treatments using
278 Canoco software (Version 4.56, Biometris Plant Research International Wageningen,
279 The Netherlands). The Monte Carlo test was performed on the parameters in the RDA
280 analysis using the R software (vegan package) (R Core Team, 2018). A multiple linear
281 regression analysis was done using the Sigmaplot 12.5 software (Systat Software Inc.,
282 San Jose, California, USA) to analyze the influence of root traits on the fine root
283 NSCs (soluble sugar and starch) content in all treatments. All data are mean \pm
284 standard error (Mean \pm SE). All bar figures were drawn using Origin Pro 8.5
285 (OriginLab, Northampton, MA, USA).

286 **Results**

287 **Fine root biomass and morphological traits among root branch order**

288 Soil substrates, drought intensity and root order had significant differences in the
289 fine root biomass of *F. mandshurica* seedlings (Table S1). With the increase of
290 drought intensity, the fine roots biomass of seedling in the three substrates showed a
291 decrease progressively (Figure 1A~1E). The biomass of fifth-order roots had the
292 highest variation, which was 37.4%, 44.5% and 53.0% in humus, loam and sandy loam,
293 respectively (Figure 1E). Under the same drought treatment, the fine root biomass of
294 seedlings for all branch order was the highest in sandy-loam soil and the lowest in
295 humus soil. With the increase of drought intensity, the coefficient of variation in the
296 fifth-order root biomass among different soil substrates was the highest, which were
297 61.8% (in CK), 36.6% (in T1), 57.0% (in T2) and 40.4% (in T3) (Figure 1E).

298 Soil substrate, drought intensity and root order had significant effects on the
299 specific root length (SRL), specific root surface area (SRA) and root tissue density
300 (RTD) of *F. mandshurica* seedlings (Table S1). Under the same drought intensity, the
301 SRL and SRA of all branch order in the humus soil were the lowest, and showed a
302 significant decrease with ascending root order (Figure 2A~2C, Figure 2D~2F). The

303 average diameter (AD) and RTD of the fine roots of all branch order were the highest
304 and lowest in the humus and sandy-loam soil, respectively, and showed a significant
305 increase trend with ascending root order (Figure 2G~4I, Figure 2J~5L). With the
306 increase of drought intensity, the SRL and SRA of seedlings for all soil substrates
307 increased significantly. Compared with CK, the SRL and SRA under T3 treatment
308 increased significantly by 39.0% (variation range 19.1%~88.6%) and 22.3%
309 (variation range 9.9%~37.4%). Compared with CK, the RTD of seedlings in all the
310 three soil substrates was the lowest under T3 treatment, especially in the 1st root order,
311 which decreased by 13.7% (humus soil), 10.7% (loam soil) and 28.6% (sandy-loam)
312 respectively (Figure 2J). The AD of all root branch order was less affected by drought
313 intensity (Figure 2G~2I, Table S1).

314 **Fine root chemical traits among root branch order**

315 The fine root carbon, nitrogen and phosphorus contents of *F. mandshurica*
316 seedlings were significantly different among root branch orders. With ascending the
317 root orders, the fine root carbon and nitrogen content showed a decreasing trend,
318 while the fine root phosphorus content showed an increasing trend (Table S2, Figure
319 3A~3C and 4A~4C). The soil substrate had a significant effect on the fine root carbon
320 and nitrogen content of *F. mandshurica* seedlings (Table S2). The carbon and nitrogen
321 content of all branch orders in sandy-loam soil were significantly higher than those of
322 humus soil. The carbon and nitrogen content of 1st-order root in sandy-loam soil was
323 highest, which was 41.21 mg·g⁻¹ and 2.14 mg·g⁻¹, respectively (Figure 3A, 3B).
324 Drought had a significant effect on the fine root carbon and phosphorus content of *F.*
325 *mandshurica* seedlings, and the phosphorus content of first forth root order under T3
326 treatment was lower than that of CK (Figure 4C).

327 **Fine root NSCs content among root branch order**

328 Soil substrate, drought intensity and root order had significant effects on the
329 soluble sugar (SS), starch (ST) and total NSC content of the fine roots of *F.*
330 *mandshurica* seedlings (Table S3). Under the same drought intensity, the SS content
331 of seedlings in humus soil was the highest for all branch orders. With ascending root
332 order, the SS content of fine roots of seedlings in humus soil was 107.9%, 162.7%,
333 125.7%, 269.2% and 118.5% higher than those of sandy-loam soil, respectively (after
334 the average the intensity of the four droughts) (Figure 5A~5E). For all soil substrates,
335 the SS content of the fine roots of seedlings decreased with the increased drought
336 intensity. With ascending root orders, the SS content of fine roots under T3 treatment
337 was 51.3%, 58.1%, 62.1%, 68.7%, and 36.5% lower than those of CK, respectively
338 (after the average of the three soil substrates) (Figure 5A~5E). Under the same
339 drought intensity, seedlings in sandy-loam soil had the highest starch and total NSC
340 content for all root orders. The starch and total NSC content of lower-order roots in
341 different soil substrates varied greatly. The starch and total NSC content of the
342 1st-order and 2nd-order roots in sandy-loam soil were 276.1%, 231.1%, 195.8% and
343 186.7% higher than those of humus soil respectively (after averaging the intensity of

344 the four droughts) (Figure 5A~5E). For all soil substrates, the fine root starch content
345 generally increased with the increase of drought intensity. The starch and total NSC
346 content of the 1st-order roots of seedlings under T3 treatment were 58.6% and 43.4%
347 higher than those of CK, respectively (Figure 5A).

348 **Relationship among the fine root biomass, traits and NSCs content**

349 The root morphological and chemical traits of first five order root of *F.*
350 *mandshurica* seedlings under different drought intensities and soil conditions were
351 analyzed by redundancy analysis (RDA), the results showed that the first two axes of
352 the RDA explained approximately 65% of the total variations between all treatments
353 (Figure 6A~6E). The first and the second ordination axis indicated the variations of
354 fine roots morphological traits, and chemical traits and biomass, respectively. Soil
355 substrates and drought intensity in plots had a good degree of separation. The fine
356 roots morphological and chemical traits in all conditions were conducted partial
357 Monte Carlo test. For the 1st-order roots, SS and ST explained the highest degree of
358 total variation of fine root traits, which were 32.0% and 32.1%, respectively (Table
359 S4). With ascending root orders, the explanation of the variation of root traits by
360 starch decreased, only 6.8% (for 5th-order root) (Table S4).

361 The Pearson correlation analysis showed that the first five order root
362 morphological and chemical traits were significantly correlated with SS, SRL, SRA
363 and root nitrogen content are significantly negatively correlated with SS, while the
364 AD, RTD and root phosphorus content were significantly positively correlated with
365 SS (Figure 7A~7D).

366 **Discussion**

367 Our results highlighted several key findings related to NSC and fine root traits of
368 *F. mandshurica* seedlings under different soil substrates and drought intensities.
369 Firstly, with the increase of drought intensity, the fine roots biomass decreased
370 significantly. Under the same drought intensity, there had a higher biomass in
371 relatively poor sandy-loam soils, and the coefficient of variation for fifth-order roots
372 was higher than those of lower-order roots. Secondly, compared with chemical traits,
373 fine root morphological traits were more sensitive to soil substrates and drought
374 intensities. With the increase of drought intensity, the SRL and AD of all root orders
375 increased and decreased respectively. Finally, the fine roots in the humus soil had
376 higher soluble sugar content and lower starch content. With the increase of drought
377 intensity, the soluble sugar and starch content of the fine roots showed a decreasing
378 and increasing trend, and the NSC content of the fine roots significantly related to
379 root morphological traits. According to the rapid response of root morphology to
380 changes in the habitat environment, plants will maintain and construct the
381 development of underground organs in an economical way to obtain more resources
382 and nutrients.

383 **Response of root morphology and biomass to drought and soil substrate**

384 In our study, the fine root biomass of seedlings in sandy-loam soil (soil nutrient
385 relatively poor) was significantly higher than that in humus soil (soil nutrient
386 relatively rich), which was in line with those of previous studies (Hertel et al., 2013;
387 Poorter and Ryser, 2015). Weemstra et al. (2017) pointed out that the fine-root mass
388 and the growth rate of fine roots of both species of *Fagus sylvatica* and *Picea abies* in
389 sandy soil were three times and ten times higher than those of clay soil, despite the
390 family of tree species planted in different soils are not consistent. Hendricks et al.
391 (1993) proposed two hypotheses: 1) As nitrogen availability increases, the carbon
392 allocated to fine roots decreases, and the lifespan or turnover rate of fine roots
393 remains unchanged; 2) as nitrogen availability increases, the distribution to the carbon
394 in fine roots remains unchanged, the turnover rate increases, and the fine roots
395 biomass decreases. Both hypotheses believed that the increase in soil nutrient
396 availability will lead to a decrease in the fine root biomass. Liu et al. (2020) found
397 that the higher-order root biomass of *Pinus tabuliformis* seedlings under the 20% field
398 water holding capacity treatment was 33.3% lower than that under the 80% field
399 water holding capacity treatment. We found that the biomass of higher-order roots
400 (e.g., fifth-order) has the highest coefficient of variation under different soil substrates
401 and drought treatments. This result may be explained by the fact that the different
402 responses of fine roots of different root branch order to resource changes (Withington
403 et al., 2006; Guo et al., 2008a). Indeed, with ascending the root order, the diameter of
404 fine roots increased significantly (Pregitzer et al., 2002), and higher-order roots have a
405 large number of wooden vessels that could transport more nutrients to the
406 aboveground parts (Chapin III et al., 1990; Hishi and Takeda, 2005), and lower-order
407 roots were mainly responsible for the absorption of water and nutrients, and were
408 active in their growth and elongation (Chapin III et al., 1990; Noguchi et al., 2013).

409 The morphological traits of fine roots, such as AD, SRL, and RTD, are important
410 functional parameters that characterize or affect the water absorption efficiency and
411 ability of roots. In general, fine roots with smaller diameter and larger root length
412 have higher water absorption efficiency (Freschet and Roumet, 2017; Dhiman et al.,
413 2018; Ma et al., 2018). Root morphology characteristics responded to drought here in
414 accordance with our first hypothesis as well, that was the root system of seedlings in
415 humus soil had lower SRL, lower SRA, higher AD and RTD, these results were likely
416 to be related to the higher nutrient content in humus soil, which matched those
417 observed in earlier studies on the fine root traits of *Pinus tabuliformis*, *Larix gmelinii*
418 and *F. mandshurica* after nutrient availability increased (Liu et al., 2009; Wang et al.,
419 2013). Gruber et al. (2013) indicated that soil nitrogen deficiency usually promoted
420 the elongation of main roots and some lateral roots. Plants can increase the absorption
421 capacity of the root system in two different ways to adapt to water or nutrient
422 shortages, 1) increase root yield and maintain a larger absorption surface area
423 (acquisition strategy), or 2) increase the efficiency per unit mass absorption by
424 changing the morphology and physiological condition of the root system
425 (conservative strategy) (Löhmus et al., 2006; Ostonen et al., 2007a). In our study, with
426 the increase of drought intensity, the SRL and SRA of first five order roots increased
427 significantly, and the RTD decreased significantly, which was a strategy to water

428 deficit conditions of *F. mandshurica* seedlings. In addition, our results showed that the
429 difference in various morphological traits among root orders had reached a significant
430 level, indicating that the fine roots of *F. mandshurica* had a high degree of
431 morphological heterogeneity, which was consistent with the previous study of the
432 plasticity of the morphological traits of the root system from the perspective of orders
433 (Pregitzer et al., 1997; Pregitzer et al., 2002; Wang et al., 2006). Cortina et al. (2008)
434 found that *Pistacia lentiscus* seedlings could rapidly expand the length and surface
435 area of fine roots when exposed to drought stress, and shaped the fine roots to avoid
436 the damage of arid environment. In general, the larger the SRL or SRA and the
437 smaller the AD, the higher the water absorption efficiency of fine roots (Freschet and
438 Roumet, 2017; Dhiman et al., 2018). Our results emphasized the adaptation strategies
439 of the fine roots of *F. mandshurica* seedlings in three soil substrates to different
440 drought intensities.

441 **Effects of drought and soil substrate on root NSC**

442 Soluble sugar is an important osmotic adjustment substance for plants to tolerate
443 arid environment. It could reflect the drought status of plants. The variation of its
444 concentration changes can adjust the osmotic pressure of cells in plants to maintain
445 normal physiological activities to adapt to drought stress (Quentin et al., 2015). In this
446 study, compared with sandy-loam soil, the soluble sugar content of fine roots
447 (especially lower-order roots) in humus soil significantly increased, the starch content
448 significantly decreased, the NSC significantly decreased, and the soluble sugar-starch
449 ratio increased significantly. The development of fine roots accelerates the
450 consumption of NSC, and the roots increase the ratio of soluble sugar-starch ratio to
451 cope with stress conditions. The increase in the ratio is beneficial for plants to adjust
452 osmotic potential to maintain the transport channel between leaves and roots, and
453 improve water transport efficiency (Sala et al., 2012). Some studies had pointed out
454 that lower-order roots might obtain greater carbon investment than higher-order roots,
455 because the main function of lower-order roots is to absorb water and nutrients
456 (absorptive root), while higher-order roots are responsible for transporting nutrients
457 and supporting the entire root (Liu et al., 2020). Our results showed that after two
458 months period of drought, the lower-order roots of *F. mandshurica* seedlings had
459 more NSC content than the higher-order roots. This result implied that when carbon
460 was limited, *F. mandshurica* preferentially allocate carbon to thin roots rather than
461 thick roots. It supported the results of studies in hybrid poplar *Populus×canadensis* cv.
462 *Eugeneii* (Kosola et al., 2001) and *Pinus tabuliformis* (Liu et al., 2020). Under
463 carbon-limiting conditions, plants give priority to the distribution for fine roots. On
464 the one hand, they could reduce carbon consumption (higher-order roots consume
465 more carbon than lower-order roots). On the other hand, maintaining fine roots to
466 absorb more water and nutrient (Finér et al., 2011). Liu et al. (2020) conducted the
467 short-term drought stress on *Pinus tabuliformis* seedlings and found that the amount
468 of ¹³C allocated to the first three roots 120 days after isotope labeling was
469 significantly higher than that of the control in the moderate and mild drought, while

470 the amount of ^{13}C allocated to fifth-order roots was significantly higher in the control.
471 Some studies had suggested that carbon starvation caused by drought stress may only
472 exist in the belowground organ and had little to do with the aboveground part.
473 Specially, thick roots are essential to alleviate the decline in the NSC content of the
474 entire plant caused by drought (Hartmann, 2013; Kannenberg et al., 2018), drought
475 will cause the loss of phloem function, making the aboveground and underground
476 parts uncoupled. When plant is subject to drought stress, the NSC content of the
477 aboveground organs will increase or remain stable for a short time, while the NSC
478 content of the root will decrease significantly (Sevanto et al., 2014).

479 Galvez et al. (2011) found that drought significantly increased the concentration
480 of soluble sugar and starch in the fine roots of *Populus tremuloides* seedlings. Our
481 results supported the second hypothesis, that was with the increase of drought
482 intensity, the variation of root NSCs was higher than that of root chemical traits, root
483 SS content decreased, starch and total NSC content increased. A possible explanation
484 for this might be that the path of NSC to the fine roots of *F. mandshurica* seedlings
485 was blocked after two months drought stress, causing the carbohydrates produced by
486 leaf photosynthesis to not be transported to the belowground organ. The fine roots
487 could only rely on regulating their own NSC levels to deal with drought stress (Dietze
488 et al., 2014). However, our inference needs to be further verified by combining the
489 dynamic changes of fine root NSC content under different periods of drought
490 treatment. Our results revealed the interaction of different soil substrates and drought
491 intensities on the variations of first five root orders NSCs of *F. mandshurica*
492 seedlings.

493 **Linking the fine root traits to the NSCs level**

494 The root system is the organ responsible for absorbing water, and is the first
495 responder to various stresses. Given that the root tends to grow in the moist soil, it can
496 minimize the impact of water shortage (Brunner et al., 2015; Weemstra et al., 2016).
497 Under drought stress conditions, more carbohydrates are allocated to lower-order
498 roots to promote root structure and growth (Liu et al., 2020). Despite there is evidence
499 that thick roots increase NSC accumulation under drought conditions (Yang et al.,
500 2016), whether the variation of fine root traits is directly related to NSC accumulation
501 remains to be explored. Our previous research pointed out that the root tip length and
502 diameter of the seedlings (three broad-leaved tree species in the temperate zone)
503 under drought conditions explained a higher variation of starch and soluble sugar (Ji
504 et al., 2020). In this study, drought reduced the root biomass of *F. mandshurica*
505 seedlings, changed the root traits of all root orders, and promoted the absorption of
506 water and nutrients. This response may be related to the increased demand for osmotic
507 active C compounds under drought conditions. NSC can provide fuel for root
508 respiration and is an important substrate for root growth and physiological regulation
509 (George et al., 2003; Xu et al., 2008). In this study, SRL and SRA of all samples were
510 significantly negatively correlated with the soluble sugar content of fine roots, and
511 AD and RTD were significantly positively correlated with starch content, which

512 confirms our third hypothesis. When the diameter is smaller, the level of NSC
513 required to build and maintain a thin root per unit length of the plant would be lower
514 than that of the thicker root (Guo et al., 2004). Ma et al. (2018) pointed out that the
515 root diameter of herbaceous plants were thinner than woody plants, so more carbon
516 could be easily distributed to the roots under drought conditions. Maguire and Kobe
517 (2015) pointed out that drought stress may directly increase root mortality by
518 depleting starch and sugar reserves, and indirectly inhibited the transport of
519 photosynthetic products to roots (Hasibeder et al., 2014). Therefore, our results
520 suggested that changes in NSCs caused by changes in environmental conditions were
521 related to the variation of fine root morphological traits.

522 **Conclusion**

523 This project is the first comprehensive investigation of the interactive response
524 of root branch order and fine root NSCs levels of *F. mandshurica* seedlings to drought
525 intensity and soil substrate. Our results confirm that the variation of higher-order fine
526 root biomass was higher than that of lower-order roots under different soil substrates
527 and drought intensities. Secondly, the fine roots of the seedlings in the humus soil
528 (first fifth root orders) have higher soluble sugar content and lower starch content.
529 With the increase of drought intensity, the soluble sugar and starch contents of the fine
530 roots showed decrease and increase trends respectively. The variation of fine root
531 NSCs content was related to the variation of root morphological traits (SRL, SRA,
532 AD, and RTD) induced by drought and soil substrate, rather than root chemical traits.
533 This study reveals the adaptation strategies of *F. mandshurica* seedlings to drought
534 under different soil substrate conditions, thereby enhancing the understanding of the
535 construction and maintenance of the root system of *F. mandshurica*, and contributing
536 to optimize soil water management in *F. mandshurica* plantations. In further research,
537 it is necessary to combine ¹³C isotope labeling technology to more deeply reveal the
538 mechanism of carbohydrate distribution among different root branch orders in a
539 prolonged periods drought.

540

541 **Author Contributions:** LJ and YY conceptualized the main question. LJ, JW and ZL
542 conducted the field work. LJ and YL collected data and performed the data analyses.
543 LJ wrote the manuscript. LJ, YY and LZ revised the manuscript. All authors read and
544 approved the manuscript.

545 **Funding:** This research was funded by the National Key Research & Development
546 Program of China (2017YFD0600605) and the Fundamental Research Funds for the
547 Central Universities (2572019AA07). Li Ji was supported by a scholarship granted
548 from China Scholarship Council (No. 201906600038).

549 **Acknowledgments:** We gratefully acknowledge the managers and workers of nursery
550 for their logistic assistance with this project. We sincerely thank Wanying Cui, Sijia
551 Liu, Jianhua Bi and Linlin Cao for helping with field work.

552 **Conflicts of Interest:** The authors declare no conflict of interest.

553 **References**

- 554 Adams, H. D., Germino, M. J., Breshears, D. D., Barron-Gafford, G. A.,
555 Guardiola-Claramonte, M., Zou, C. B., and Huxman, T. E. (2013).
556 Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during
557 drought-induced tree mortality reveal role for carbon metabolism in mortality
558 mechanism. *New Phytol.* 197, 1142-1151.
- 559 Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier,
560 M., Kitzberger, T., Rigling, A., Breshears, D. D., and Hogg, E. H. (2010). A
561 global overview of drought and heat-induced tree mortality reveals emerging
562 climate change risks for forests. *For. Ecol. Manag.* 259, 660-684.
- 563 Bardgett, R. D., Liesje, M., and Vries, F. T., De (2014). Going underground: root traits
564 as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692-699.
- 565 Blackman, C. J., Creek, D., Maier, C., Aspinwall, M. J., Drake, J. E., Pfautsch, S.,
566 O'grady, A., Delzon, S., Medlyn, B. E., and Tissue, D. T. (2019). Drought
567 response strategies and hydraulic traits contribute to mechanistic
568 understanding of plant dry-down to hydraulic failure. *Tree Physiol.* 39,
569 910-924.
- 570 Brunner, I., Herzog, C., Dawes, M. A., Arend, M., and Sperisen, C. (2015). How tree
571 roots respond to drought. *Front. Plant Sci.* 6, 547.
- 572 Camarero, J. J., Sangüesa-Barreda, G., and Vergarechea, M. (2016). Prior height,
573 growth, and wood anatomy differently predispose to drought-induced dieback
574 in two Mediterranean oak species. *Ann. For. Sci.* 73, 341-351.
- 575 Chapin Iii, F. S., Schulze, E., and Mooney, H. A. (1990). The ecology and economics
576 of storage in plants. *Ann. Rev. Ecol. Syst.* 21, 423-447.
- 577 Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.
578 J., Feild, T. S., Gleason, S. M., and Hacke, U. G. (2012). Global convergence
579 in the vulnerability of forests to drought. *Nature* 491, 752-755.
- 580 Comas, L., Becker, S., Cruz, V. M. V., Byrne, P. F., and Dierig, D. A. (2013). Root
581 traits contributing to plant productivity under drought. *Front. Plant Sci.* 4, 442.
- 582 Comas, L., and Eissenstat, D. (2004). Linking fine root traits to maximum potential
583 growth rate among 11 mature temperate tree species. *Funct. Ecol.* 18,
584 388-397.
- 585 Cortina, J., Green, J., Baddeley, J., and Watson, C. (2008). Root morphology and
586 water transport of *Pistacia lentiscus* seedlings under contrasting water supply:
587 a test of the pipe stem theory. *Environ. Exp. Bot.* 62, 343-350.
- 588 Deng, X., Xiao, W., Shi, Z., Zeng, L., and Lei, L. (2020). Combined effects of drought
589 and shading on growth and non-structural carbohydrates in *Pinus massoniana*
590 Lamb. seedlings. *Forests* 11, 18.
- 591 Dhiman, I., Bilheux, H., Decarlo, K., Painter, S. L., Santodonato, L., and Warren, J. M.
592 (2018). Quantifying root water extraction after drought recovery using
593 sub-mm in situ empirical data. *Plant Soil* 424, 73-89.
- 594 Dietze, M. C., Sala, A., Carbone, M. S., and Al., E. (2014). Nonstructural carbon in
595 woody plants. *Ann. Rev. Plant Biol.* 65, 667-687.
- 596 Eissenstat, D., Wells, C., Yanai, R., and Whitbeck, J. (2000). Building roots in a
597 changing environment: implications for root longevity. *New Phytol.* 147,

- 598 33-42.
- 599 Finér, L., Ohashi, M., Noguchi, K., and Hirano, Y. (2011). Fine root production and
600 turnover in forest ecosystems in relation to stand and environmental
601 characteristics. *For. Ecol. Manag.* 262, 2008-2023.
- 602 Fort, F., Volaire, F., Guillioni, L., Barkaoui, K., Navas, M. L., and Roumet, C. (2017).
603 Root traits are related to plant water use among rangeland Mediterranean
604 species. *Funct. Ecol.* 31, 1700-1709.
- 605 Freschet, G. T., and Roumet, C. (2017). Sampling roots to capture plant and soil
606 functions. *Funct. Ecol.* 31, 1506-1518.
- 607 Furze, M. E., Huggett, B. A., Aubrecht, D. M., Stolz, C. D., Carbone, M. S., and
608 Richardson, A. D. (2019). Whole tree nonstructural carbohydrate storage and
609 seasonal dynamics in five temperate species. *New Phytol.* 221, 1466-1477.
- 610 Galvez, D. A., Landhäusser, S., and Tyree, M. (2011). Root carbon reserve dynamics
611 in aspen seedlings: does simulated drought induce reserve limitation? *Tree*
612 *Physiol.* 31, 250-257.
- 613 Ge, T. D., Sun, N. B., Bai, L. P., Tong, C. L., and Sui, F. G. (2012). Effects of drought
614 stress on phosphorus and potassium uptake dynamics in summer maize (*Zea*
615 *mays*) throughout the growth cycle. *Acta Physiol. Plant.* 34, 2179-2186.
- 616 George, K., Norby, R. J., Hamilton, J. G., and Delucia, E. H. (2003). Fine-root
617 respiration in a loblolly pine and sweetgum forest growing in elevated CO₂.
618 *New Phytol.* 160, 511-522.
- 619 Gruber, B. D., Giehl, R. F., Friedel, S., and Von Wirén, N. (2013). Plasticity of the
620 Arabidopsis root system under nutrient deficiencies. *Plant Physiol.* 163,
621 161-179.
- 622 Guo, D., Mitchell, R. J., Withington, J. M., Fan, P. P., and Hendricks, J. J. (2008a).
623 Endogenous and exogenous controls of root life span, mortality and nitrogen
624 flux in a longleaf pine forest: root branch order predominates. *J. Ecol.* 96,
625 737-745.
- 626 Guo, D. L., Mitchell, R. J., and Hendricks, J. J. (2004). Fine root branch orders
627 respond differentially to carbon source-sink manipulations in a longleaf pine
628 forest. *Oecol.* 140, 450-457.
- 629 Guo, D. L., Xia, M. X., Wei, X., and Al, E. (2008b). Anatomical traits associated with
630 absorption and mycorrhizal colonization are linked to root branch order in
631 twenty-three Chinese temperate tree species. *New Phytol.* 180, 673-683.
- 632 Hartmann, H., and Trumbore, S. (2016). Understanding the roles of nonstructural
633 carbohydrates in forest trees - from what we can measure to what we want to
634 know. *New Phytol.* 211, 386-403.
- 635 Hartmann, H., Ziegler, W., and Trumbore, S. (2013). Lethal drought leads to reduction
636 in nonstructural carbohydrates in Norway spruce tree roots but not in the
637 canopy. *Funct. Ecol.* 27, 413-427.
- 638 Hartmann, H., Ziegler, W., Trumbore, S. (2013). Lethal drought leads to reduction in
639 nonstructural carbohydrates in Norway spruce tree roots but not in the canopy.
640 *Funct. Ecol.* 27, 413-427.
- 641 Hasibeder, R., Fuchslueger, L., Fritz, K., Richter, A., and Bahn, M. (Year). "Summer

- 642 drought alters dynamics of carbon allocation to roots and root respiration in
643 mountain grassland", in: *EGU General Assembly Conference Abstracts*,
644 12991.
- 645 He, W., Liu, H., Qi, Y., Liu, F., and Zhu, X. (2020). Patterns in nonstructural
646 carbohydrate contents at the tree organ level in response to drought duration.
647 *Glob. Change Biol.* 26, 3627-3638.
- 648 Hendricks, J. J., Nadelhoffer, K. J., and Aber, J. D. (1993). Assessing the role of fine
649 roots in carbon and nutrient cycling. *Trends Ecol. Evol.* 8, 174-178.
- 650 Hertel, D., Strecker, T., Müller-Haubold, H., and Leuschner, C. (2013). Fine root
651 biomass and dynamics in beech forests across a precipitation gradient—is
652 optimal resource partitioning theory applicable to water-limited mature trees?
653 *J. Ecol.* 101, 1183-1200.
- 654 Hishi, T., and Takeda, H. (2005). Dynamics of heterorhizic root systems: protoxylem
655 groups within the fine-root system of *Chamaecyparis obtusa*. *New Phytol.*
656 167, 509-521.
- 657 Hoch, G., and Körner, C. (2003). The carbon charging of pines at the climatic treeline:
658 a global comparison. *Oecol.* 135, 10-21.
- 659 Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T.,
660 Kattge, J., Roumet, C., Stover, D. B., Soudzilovskaia, N. A., and
661 Valverde-Barrantes, O. J. (2017). A global Fine-Root Ecology Database to
662 address below-ground challenges in plant ecology. *New Phytol.* 215, 15-26.
- 663 Ji, L., Attaullah, K., Wang, J., Yu, D., Yang, Y., Yang, L., and Lu, Z. (2020). Root
664 traits determine variation in nonstructural carbohydrates (NSCs) under
665 different drought intensities and soil substrates in three temperate tree species.
666 *Forests* 11, 415.
- 667 Kannenberg, S. A., Novick, K. A., and Phillips, R. P. (2018). Coarse roots prevent
668 declines in whole-tree non-structural carbohydrate pools during drought in an
669 isohydric and an anisohydric species. *Tree Physiol.* 38, 582-590.
- 670 Konôpka, B., Noguchi, K., Sakata, T., Takahashi, M., and Konôpková, Z. (2007).
671 Effects of simulated drought stress on the fine roots of Japanese cedar
672 (*Cryptomeria japonica*) in a plantation forest on the Kanto Plain, eastern Japan.
673 *J. For. Res.* 12, 143-151.
- 674 Kong, D. L., Ma, C. E., Zhang, Q., Li, L., Chen, X. Y., Zeng, H., and Guo, D. L.
675 (2014). Leading dimensions in absorptive root trait variation across 96
676 subtropical forest species. *New Phytol.* 203, 863-872.
- 677 Kosola, K. R., Dickmann, D. I., Paul, E. A., and Parry, D. (2001). Repeated insect
678 defoliation effects on growth, nitrogen acquisition, carbohydrates, and root
679 demography of poplars. *Oecol.* 129, 65-74.
- 680 Löhmus, K., Truu, J., Truu, M., Kaar, E., Ostonen, I., Alama, S., Kuznetsova, T.,
681 Rosenthal, K., Vares, A., and Uri, V. (2006). Black alder as a promising
682 deciduous species for the reclaiming of oil shale mining areas. *WIT*
683 *Transactions Ecol. Environ.* 94.
- 684 Landhäusser, S. M., and Lieffers, V. J. (2012). Defoliation increases risk of carbon
685 starvation in root systems of mature aspen. *Trees* 26, 653-661.

- 686 Liu, J. L., Mei, L., Gu, J. C., Quan, X. K. and Wang, Z. Q. (2009). Effects of nitrogen
687 fertilization on fine root biomass and morphology of *Fraxinus mandshurica*
688 and *Larix gmelinii*: A study within-growth core approach. *Chin. J. Ecol.* 28,
689 1-6. (in Chinese)
- 690 Liu, Y., Li, P., Xiao, L., Wang, W., Yu, K., and Shi, P. (2020). Heterogeneity in
691 short-term allocation of carbon to roots of *Pinus tabulaeformis* seedlings and
692 root respiration under drought stress. *Plant Soil* 452, 359-378.
- 693 Lozano, Y. M., Aguilar-Trigueros, C. A., Flaig, I. C., and Rillig, M. C. (2020). Root
694 trait responses to drought are more heterogeneous than leaf trait responses.
695 *Funct. Ecol.* 34, 2224-2235.
- 696 Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R. D., Eissenstat, D. M., McCormack, M.
697 L., and Hedin, L. O. (2018). Evolutionary history resolves global organization
698 of root functional traits. *Nature* 555, 94-97.
- 699 Maguire, A. J., and Kobe, R. K. (2015). Drought and shade deplete nonstructural
700 carbohydrate reserves in seedlings of five temperate tree species. *Ecol. Evol.* 5,
701 5711-5721.
- 702 Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.
703 I., and Lloret, F. (2016). Dynamics of non-structural carbohydrates in
704 terrestrial plants: a global synthesis. *Ecol. Monogr.* 86, 495-516.
- 705 McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W.,
706 Guo, D., Helmisaari, H. S., Hobbie, E. A., Iversen, C. M., and Jackson, R. B.
707 (2015). Redefining fine roots improves understanding of below-ground
708 contributions to terrestrial biosphere processes. *New Phytol.* 207, 505-518.
- 709 McDowell, N., Pockman, W., Allen, C., and Al, E. (2008). Mechanisms of plant
710 survival and mortality during drought: why do some plants survive while
711 others succumb to drought? *New Phytol.* 178, 719-739.
- 712 Mei, L., Xiong, Y., Gu, J., Wang, Z., and Guo, D. (2015). Whole-tree dynamics of
713 non-structural carbohydrate and nitrogen pools across different seasons and in
714 response to girdling in two temperate trees. *Oecol.* 177, 333-344.
- 715 Nikolova, P. S., Bauerle, T. L., Häberle, K.-H., Blaschke, H., Brunner, I., and
716 Matyssek, R. (2020). Fine-root traits reveal contrasting ecological strategies in
717 european beech and norway spruce during extreme drought. *Front. Plant*
718 *Sci.* 11, 1211.
- 719 Noguchi, K., Nagakura, J., and Kaneko, S. (2013). Biomass and morphology of fine
720 roots of sugi (*Cryptomeria japonica*) after 3 years of nitrogen fertilization.
721 *Frontiers in plant science* 4, 347.
- 722 Olmo, M., Lopez-Iglesias, B., and Villar, R. (2014). Drought changes the structure
723 and elemental composition of very fine roots in seedlings of ten woody tree
724 species. Implications for a drier climate. *Plant Soil* 384, 113-129.
- 725 Ostonen, I., Lõhmus, K., Helmisaari, H.-S., Truu, J., and Meel, S. (2007a). Fine root
726 morphological adaptations in Scots pine, Norway spruce and silver birch along
727 a latitudinal gradient in boreal forests. *Tree Physiol.* 27, 1627-1634.
- 728 Ostonen, I., Lõhmus, K., Helmisaari, H., Truu, J., and Meel, S. (2007b). Fine root
729 morphological adaptations in Scots pine, Norway spruce and silver birch along

- 730 a latitudinal gradient in boreal forests. *Tree Physiol.* 27, 1627-1634.
- 731 Oswald, S. W., and Aubrey, D. P. (2020). Xeric Tree Populations Exhibit Delayed
732 Summer Depletion of Root Starch Relative to Mesic Counterparts. *Forests* 11,
733 1026.
- 734 Paudel, I., Cohen, S., Shaviv, A., Bar-Tal, A., Bernstein, N., Heuer, B., and Ephrath, J.
735 (2016). Impact of treated wastewater on growth, respiration and hydraulic
736 conductivity of citrus root systems in light and heavy soils. *Tree Physiol.* 36,
737 770-785.
- 738 Poorter, H., and Ryser, P. (2015). The limits to leaf and root plasticity: what is so
739 special about specific root length? *New Phytol.* 206, 1188-1190.
- 740 Pregitzer, K. S., Deforest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., and
741 Hendrick, R. L. (2002). Fine root architecture of nine North American trees.
742 *Ecol. Monog.* 72, 293-309.
- 743 Pregitzer, K. S., Kubiske, M. E., Yu, C. K., and Hendrick, R. L. (1997). Relationships
744 among root branch order, carbon, and nitrogen in four temperate species.
745 *Oecol.* 111, 302-308.
- 746 Quentin, A. G., Pinkard, E. A., Ryan, M. G., Tissue, D. T., L Scott, B., Adams, H. D.,
747 Pascale, M., Jacqueline, M., Landh?Usser, S. M., and André, L. (2015).
748 Non-structural carbohydrates in woody plants compared among laboratories.
749 *Tree Physiol.* 35, 1146-1165.
- 750 Richardson, A. D., Carbone, M. S., Keenan, T. F., Czimczik, C. I., Hollinger, D. Y.,
751 Murakami, P., Schaberg, P. G., and Xu, X. (2013). Seasonal dynamics and age
752 of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytol.*
753 197, 850-861.
- 754 Ristova, D., and Busch, W. (2014). Natural variation of root traits: from development
755 to nutrient uptake. *Plant Physiol.* 166, 518-527.
- 756 Rodríguez□Calcerrada, J., Li, M., López, R., Cano, F. J., Oleksyn, J., Atkin, O. K.,
757 Pita, P., Aranda, I., and Gil, L. (2017). Drought□induced shoot dieback starts
758 with massive root xylem embolism and variable depletion of nonstructural
759 carbohydrates in seedlings of two tree species. *New Phytol.* 213, 597-610.
- 760 Rogers, E. D., and Benfey, P. N. (2015). Regulation of plant root system architecture:
761 implications for crop advancement. *Curr. Opin. Biotechnol.* 32, 93-98.
- 762 Sala, A., Woodruff, D. R., and Meinzer, F. C. (2012). Carbon dynamics in trees: feast
763 or famine? *Tree Physiol.* 32, 764-775.
- 764 Sevanto, S., Mcdowell, N. G., Dickman, L. T., Pangle, R., and Pockman, W. T. (2014).
765 How do trees die? A test of the hydraulic failure and carbon starvation
766 hypotheses. *Plant Cell Environ.* 37, 153-161.
- 767 R Core Team. (2018). R: a language and environment for statistical computing. R
768 Foundation for Statistical Computing, Vienna.
- 769 Wang, G., Fahey, T. J., and Xue, S. (2013). Root morphology and architecture respond
770 to N addition in *Pinus tabulaformis*, west China. *Oecol.* 171, 583-590.
- 771 Wang, Z., Guo, D., Wang, X., Gu, J., and Mei, L. (2006). Fine root architecture,
772 morphology, and biomass of different branch orders of two Chinese temperate
773 tree species. *Plant Soil* 288, 155-171.

- 774 Warren, J. M., Hanson, P. J., Iversen, C. M., Kumar, J., Walker, A. P., and
775 Wullschleger, S. D. (2015). Root structural and functional dynamics in
776 terrestrial biosphere models—evaluation and recommendations. *New Phytol.*
777 205, 59-78.
- 778 Weemstra, M., Mommer, L., Visser, E. J., Van Ruijven, J., Kuyper, T. W., Mohren, G.
779 M., and Sterck, F. J. (2016). Towards a multidimensional root trait framework:
780 a tree root review. *New Phytol.* 211, 1159-1169.
- 781 Weemstra, M., Sterck, F. J., Visser, E. J., Kuyper, T. W., Goudzwaard, L., and
782 Mommer, L. (2017). Fine-root trait plasticity of beech (*Fagus sylvatica*) and
783 spruce (*Picea abies*) forests on two contrasting soils. *Plant and Soil* 415,
784 175-188.
- 785 Wei, X., Li G. Y., and Lv, L. (2015). Water and nutrient preservation of agri-forest r
786 esidues used as nursery matrix. *Scientia Silvae Sinicae* 51, 26-34. (in Chinese)
- 787 Wei, X., Wang, Z. Q., Zhang, G. Z., Chen, H. B. and Wang, J. (2009). Physiological
788 and biochemical responses of different order roots in *Fraxinus mandshurica*
789 seedlings to drought stress. *Scientia Silvae Sinicae* 45, 16-21. (in Chinese)
- 790 Withington, J. M., Reich, P. B., Oleksyn, J., and Eissenstat, D. M. (2006).
791 Comparisons of Structure and Life Span in Roots and Leaves among
792 Temperate Trees. *Ecol. Monogr.* 76, 381-397.
- 793 Xia, M., Guo, D., and Pregitzer, K. S. (2010). Ephemeral root modules in *Fraxinus*
794 *mandshurica*. *New Phytol.* 188, 1065-1074.
- 795 Xie, H., Yu, M., and Cheng, X. (2018). Leaf non-structural carbohydrate allocation
796 and C: N: P stoichiometry in response to light acclimation in seedlings of two
797 subtropical shade-tolerant tree species. *Plant Physiol. Biochem.* 124, 146-154.
- 798 Xu, X., Kuzyakov, Y., Wanek, W., and Al., E. (2008). Root-derived respiration and
799 non-structural carbon of rice seedlings. *Eur. J. Soil Biol.* 44, 22-29.
- 800 Yang, N., Ji, L., Salahuddin, Yang, Y., and Yang, L. (2018). The influence of tree
801 species on soil properties and microbial communities following afforestation
802 of abandoned land in northeast China. *Eur. J. Soil Biol.* 85, 73-78.
- 803 Yang, Q., Zhang, W., Li, R., Xu, M., and Wang, S. (2016). Different responses of
804 non-structural carbohydrates in above-ground tissues/organs and root to
805 extreme drought and re-watering in Chinese fir (*Cunninghamia lanceolata*)
806 saplings. *Trees* 30, 1863-1871.
- 807 Yang, Z., Cao, Y., Zhao, J., Zhou, B., Ge, X., Li, Q., and Li, M. (2021). Root response
808 of moso bamboo (*Phyllostachys Edulis* (Carrière) J. Houz.) seedlings to
809 drought with different intensities and durations. *Forests* 12, 50.
- 810 Yemm, E. W., and Willis, A. J. (1954). The estimation of carbohydrates in plant
811 extracts by anthrone. *Biochem. J.* 57, 508-514.
- 812 Zhang, P., Zhou, X., Fu, Y., Shao, J., Zhou, L., Li, S., Zhou, G., Hu, Z., Hu, J., and
813 Bai, S. H. (2020). Differential effects of drought on nonstructural carbohydrate
814 storage in seedlings and mature trees of four species in a subtropical forest.
815 *For. Ecol. Manag.* 469, 118159.
- 816 Zhang, T., Cao, Y., Chen, Y., and Liu, G. (2015). Non-structural carbohydrate

817 dynamics in *Robinia pseudoacacia* saplings under three levels of continuous
818 drought stress. *Trees* 29, 1837-1849.
819 Zhou, G., Zhou, X., Nie, Y., Bai, S. H., Zhou, L., Shao, J., Cheng, W., Wang, J., Hu, F.,
820 and Fu, Y. (2018). Drought-induced changes in root biomass largely result
821 from altered root morphological traits: Evidence from a synthesis of global
822 field trials. *Plant Cell Environ.* 41, 2589-2599.
823 Zhou, M., Wang, J., Bai, W., Zhang, Y., and Zhang, W. H. (2019). The response of
824 root traits to precipitation change of herbaceous species in temperate steppes.
825 *Funct. Ecol.* 33, 2030-2041.

Differential variation of NSCs in root branch orders of *Fraxinus mandshurica* Rupr. seedlings across different drought intensities and soil substrates

Li Ji^{1,2}, Yue Liu^{1,2}, Jun Wang¹, Zhimin Lu¹, Yuchun Yang^{1*}, Lijie Zhang^{3*}

¹ Jilin Academy of Forestry, Changchun 130033, China

² Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, School of Forestry, Northeast Forestry University, Harbin 150040, China

³ School of Forestry, Shenyang Agricultural University, Shenyang 110161, China

*** Correspondence:**

Yuchun Yang

yang-yu-chun@163.com;

Lijie Zhang

Zlj330@syau.edu.cn

Running Title: Response of Fine Root NSCs to Drought

Type of contribution: Original Research

Date of preparation: 8 April 2021

Number of text pages: 12;

Number of words: 6171;

Number of tables: 1 table;

Number of figures: 7 figures

Table 1 Physicochemical properties of three soil substrates

Soil property	Humus	Loam	Sandy-Loam
Bulk density (g·cm ⁻³)	1.18±0.02b	1.35±0.02a	1.32±0.02a
Total porosity (%)	53.19±1.12b	58.90±0.91a	36.72±0.95c
Aeration porosity (%)	25.61±0.48a	23.52±0.53a	13.01±0.99b
Water absorption capacity	0.23±0.01b	0.26±0.01a	0.18±0.01c
Penetrate rate(g·min ⁻¹)	4.49±0.04a	4.01±0.12b	3.04±0.18c
Evaporation rate(g·h ⁻¹)	0.59±0.01a	0.50±0.01b	0.37±0.01c
Total nitrogen (mg·g ⁻¹)	7.09±0.78a	3.11±0.05b	1.20±0.03c
Total phosphorus (mg·g ⁻¹)	0.71±0.04a	0.34±0.09b	0.32±0.01b
Available phosphorus (mg·kg ⁻¹)	13.10±0.82a	5.04±0.21b	12.75±0.69a

Note: The different letters in the same line indicate significant difference among the different treatments ($P<0.05$).

Differential variation of NSCs in root branch orders of *Fraxinus mandshurica* Rupr. seedlings across different drought intensities and soil substrates

Li Ji^{1,2}, Yue Liu^{1,2}, Jun Wang¹, Zhimin Lu¹, Yuchun Yang^{1*}, Lijie Zhang^{3*}

¹ Jilin Academy of Forestry, Changchun 130033, China

² Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, School of Forestry, Northeast Forestry University, Harbin 150040, China

³ School of Forestry, Shenyang Agricultural University, Shenyang 110161, China

*** Correspondence:**

Yuchun Yang

yang-yu-chun@163.com;

Lijie Zhang

Zlj330@syau.edu.cn

Running Title: Response of Fine Root NSCs to Drought

Type of contribution: Original Research

Date of preparation: 8 April 2021

Number of text pages: 12;

Number of words: 6171;

Number of tables: 1 table;

Number of figures: 7 figures

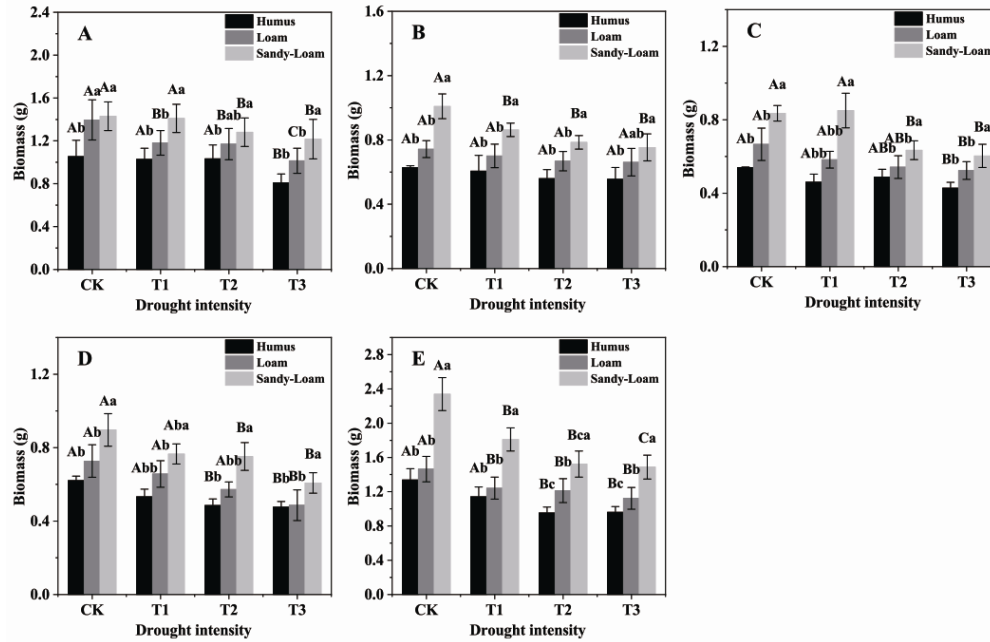


Figure 1 The fine root biomass of *F. mandshurica* seedlings in different drought intensities and soil substrates. The different lowercase letters denote significant differences among soil substrates ($P < 0.05$). The different uppercase letters denote significant differences among drought intensities ($P < 0.05$). (A), 1st order root; (B), 2nd order root; (C), 3rd order root; (D), 4th order root; (E), 5th order root. CK, control; T1, mild drought; T2, moderate drought; T3, severe drought.

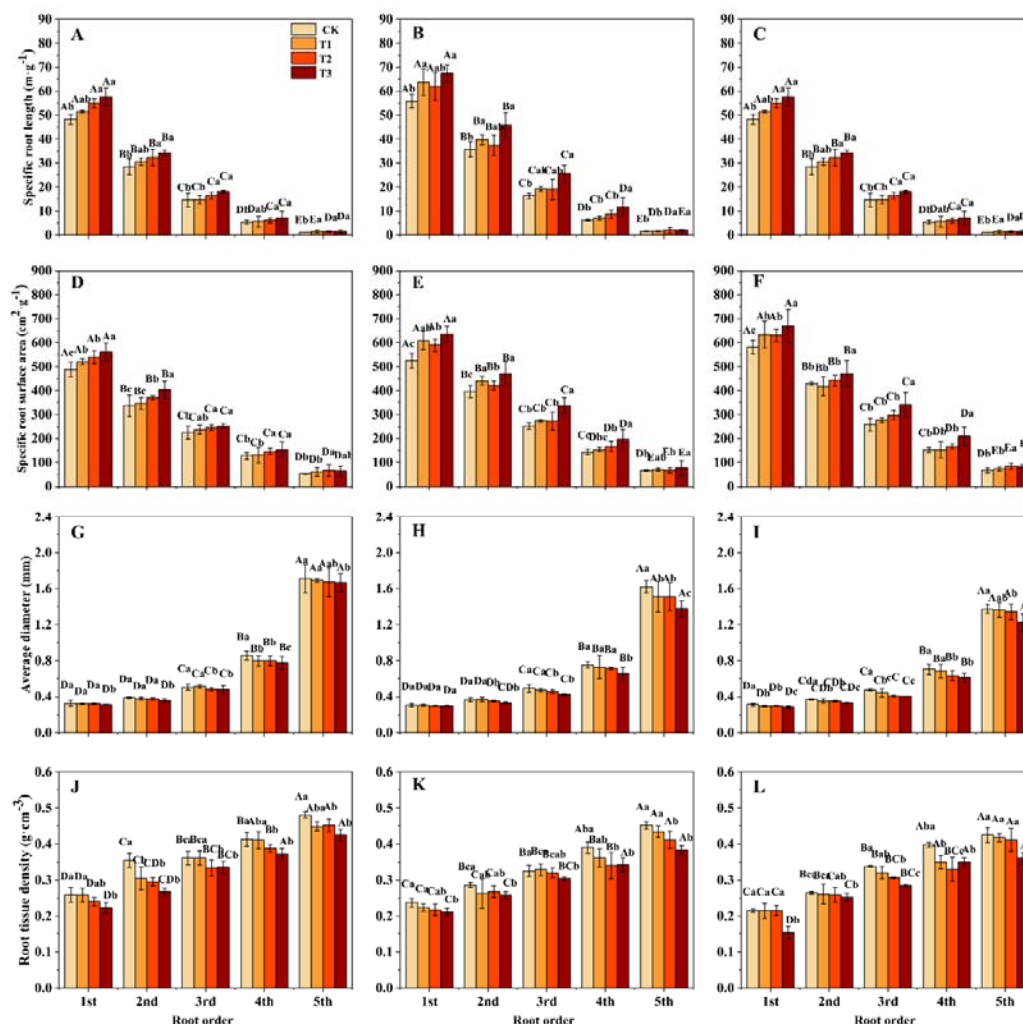


Figure 2 The specific root length (A, B, C), specific root surface area (D, E, F), average diameter (G, H, I), root tissue density (J, K, L) of *F. mandshurica* seedlings in different drought intensities and soil substrates. The different lowercase letters denote significant differences among drought intensities ($P < 0.05$). The different uppercase letters denote significant differences among root branch order ($P < 0.05$). (A, D, G, J), humus soil; (B, E, H, K), loam soil; (C, F, I, L), sandy-loam soil; CK, control; T1, mild drought; T2, moderate drought; T3, severe drought.

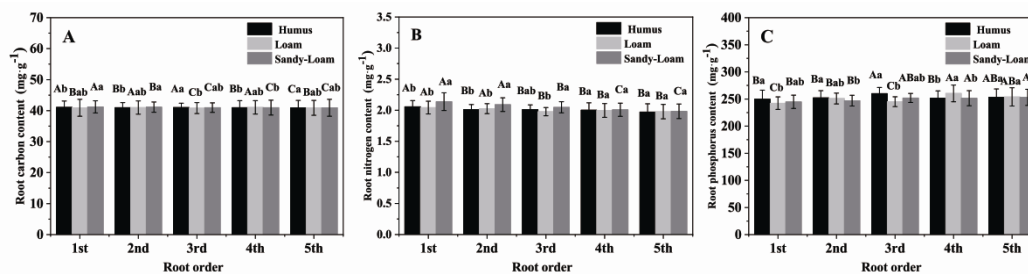


Figure 3 The root carbon (A), nitrogen (B) and phosphorus (C) of *F. mandshurica* seedlings in different soil substrates. The different lowercase letters denote significant

differences among soil substrates ($P < 0.05$). The different uppercase letters denote significant differences among root branch order ($P < 0.05$).

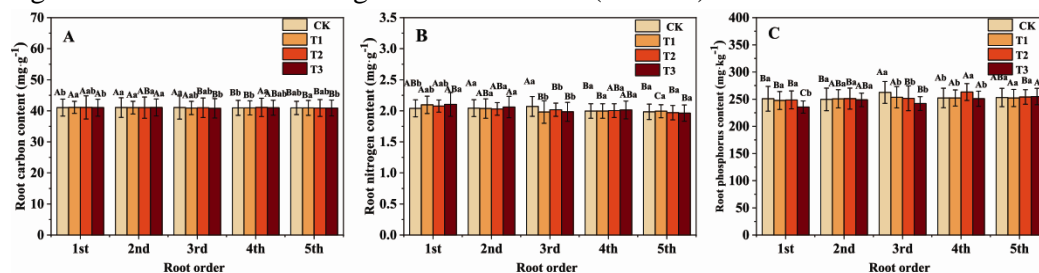


Figure 4 The root carbon (A), nitrogen (B) and phosphorus (C) of *F. mandshurica* seedlings in different drought intensity. The different lowercase letters denote significant differences among drought intensities ($P < 0.05$). The different uppercase letters denote significant differences among root branch order ($P < 0.05$).

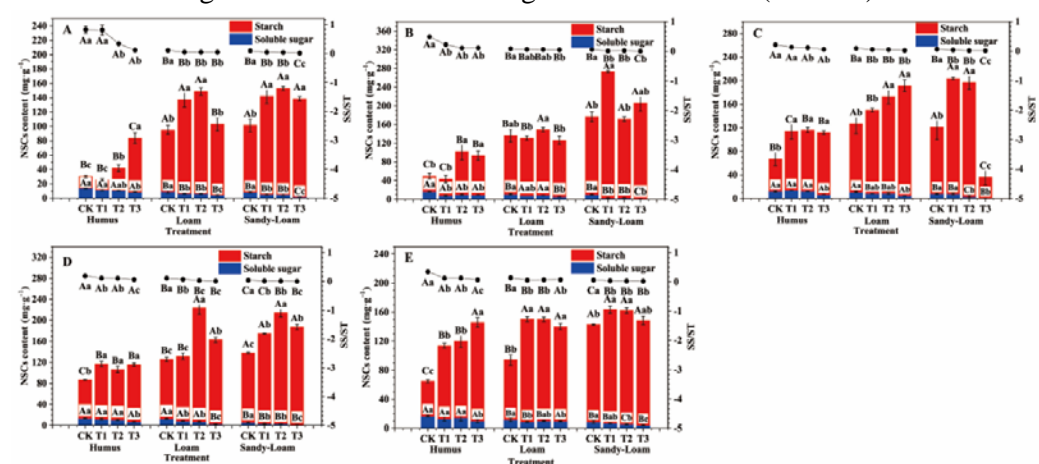


Figure 5 The fine root NSCs content of *F. mandshurica* seedlings in different drought intensities and soil substrates. The histogram represents the soluble sugar and starch content, and the line chart represents the soluble sugar-starch ratio (SS/ST). The different lowercase letters denote significant differences among soil substrates ($P < 0.05$). The different uppercase letters denote significant differences among drought intensities ($P < 0.05$). (A), 1st order root; (B), 2nd order root; (C), 3rd order root; (D), 4th order root; (E), 5th order root. CK, control; T1, mild drought; T2, moderate drought; T3, severe drought.

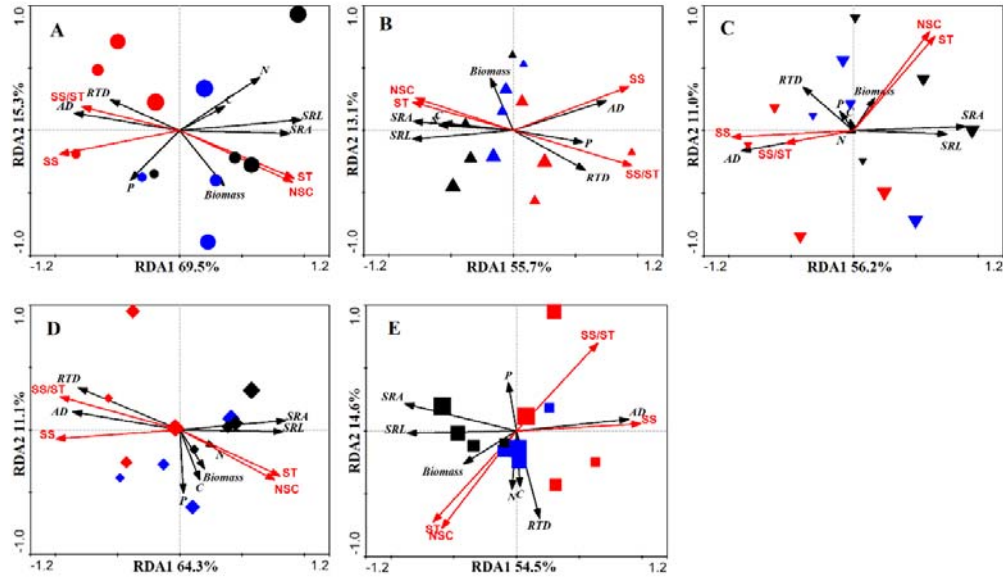


Figure 6 Redundancy analysis of fine root morphological and chemical traits in different drought intensity and soil substrates. (A), 1st-order root; (B), 2nd-order root; (C), 3rd-order root; (D), 4th-order root; (E), 5th-order root. Red symbols, humus; Blue symbols, loam; Black symbols, sandy-loam. SRL, specific root length; SRA, specific root surface area; AD, average diameter; RTD, root tissue density; SS, soluble sugar; ST, starch; C, root carbon; N, root nitrogen; P, root phosphorus.

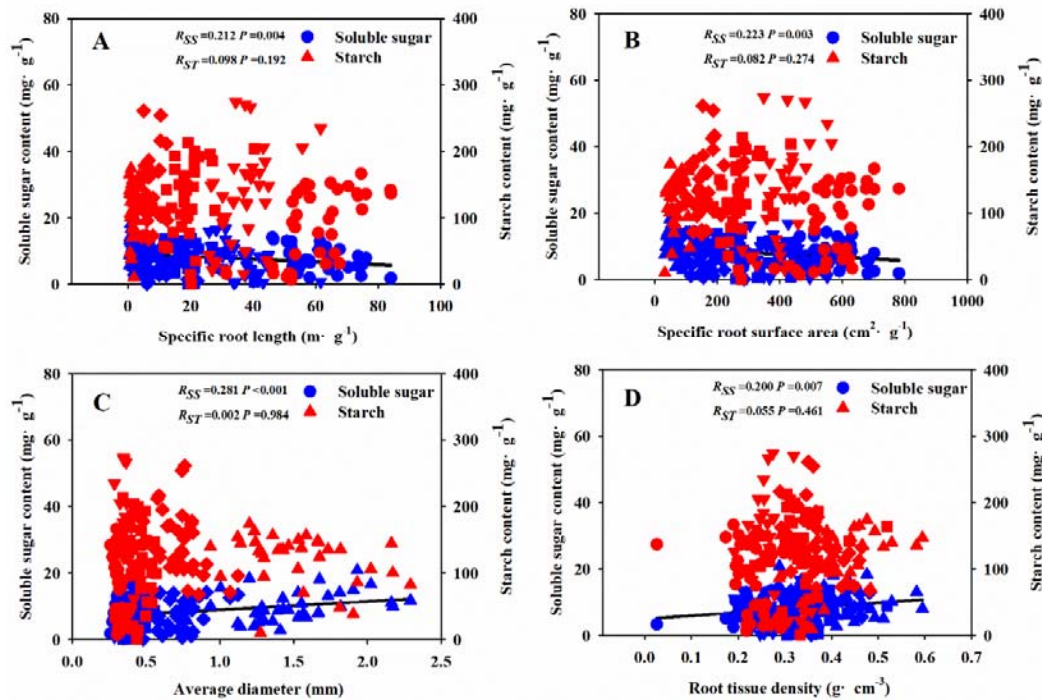


Figure 7 Relationships between soluble sugar, starch concentration and fine root morphological traits (SRL (A), SRA (B), AD (C) and RTD (D)) during the whole experiment period. R: correlation coefficient. SS, soluble sugar; ST, starch; circle

represents 1st-order root; down-triangle represents 2nd-order root; square represents 3rd-order root; diamond represents 4th-order root; up-triangle represents 5th-order root;