

1 **Age-related water use characteristics of *Robinia***
2 ***pseudoacacia* on the Loess Plateau**

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23 **Abstract**

24 Understanding water use characteristics of revegetation species is crucial for
25 evaluating plant adaptability and guiding the sustainability of vegetation restoration in
26 semiarid regions. Ecological restoration projects have been implemented for decades
27 in degraded ecosystems, achieving significant changes in vegetation cover. However,
28 water use characteristics of the main tree species at different ages remain poorly
29 understood in such systems. We investigated water use characteristics of *Robinia*
30 *pseudoacacia* in plantations of different stand-age (18 and 30 years). The species is
31 the most widely planted tree in revegetation efforts on the Loess Plateau. The $\delta^2\text{H}$ and
32 $\delta^{18}\text{O}$ of xylem and soil water within 500 cm of the soil surface and the $\delta^{13}\text{C}$ values of
33 plant leaves were measured during two consecutive hydrological years. The results
34 showed that that water uptake proportions from across the soil columns changed in
35 18-yr *R. pseudoacacia* between a drier (2016) and wetter year (2017). In contrast,
36 shallow soil water was largely comparable in a stand of 30-yr *R. pseudoacacia* in
37 2016 and 2017, and similarly the pattern of water uptake by roots from the middle and
38 deep soil column was comparable. However, leaf-level water use efficiency (WUE_i)
39 of trees in the older plantation was higher during the wetter year, thereby partly
40 alleviating a low infiltration to precipitation ratio. These findings suggest that
41 different stand-age plantation trees have distinct water use characteristics and display
42 different responses to variations in precipitation. Older plantation trees respond to
43 increased water availability by increasing WUE_i instead of switching water sources.
44 This means that stand-age is an essential factor to be considered in ecological

45 restoration management, which can enhance the effectiveness of vegetation
46 restoration strategies. The study indicates useful input from research to management
47 throughout the continuity of restoration effort.

48

49 **Keywords:** O, H, C stable isotopes; plant water uptake; water use efficiency; stand
50 age; ecological restoration; Loess Plateau;

51

52 **1. Introduction**

53 Drylands are subjected to different forms of land degradation and future climate
54 change will accelerate the expansion of drylands (Huang et al., 2017). Land
55 degradation leads to the loss of biological or economic productivity and directly
56 affected the development of 250 million people. Ecological restoration represents the
57 main strategy for intervention to recover ecosystem services in degraded drylands
58 (Atkinson and Bonser, 2020). Healthy ecosystems are critical to promoting food and
59 water security, biodiversity conservation, poverty eradication, and achieving the
60 sustainable development goals (Blicharska et al., 2019). Water is the most important
61 limiting factor that affects plant survival, growth, and the sustainability of
62 revegetation in semiarid ecosystems (Austin et al., 2004; Wang et al., 2012).
63 Moreover, both climate models and long-term observations show the increasing air
64 temperatures and decreasing precipitation in most arid and semiarid regions (Huang et
65 al., 2017; IPCC, 2019). As current climate change is likely increasing the frequency of
66 severe soil droughts, detailed scrutiny of the water use characteristics of plants

67 becomes especially important regarding the species selected for revegetation
68 programs (Harrison et al., 2020; Grossiord et al., 2017).

69 Numerous physiological and physical characteristics influence water use
70 characteristics of plants in different ecosystems (Volkman et al., 2016), such as soil
71 water availability (Gow et al., 2020), the distribution and functioning of fine roots
72 (Wang et al., 2017b), and water demands of trees (Song et al., 2018). Specifically,
73 plants adjust water sources depending on soil water availability (Lanning et al., 2020).
74 Some plants shift to derive water sources from deep soil layers or groundwater when
75 shallow soil water is unavailable in the dry season (Wang et al., 2019a). The
76 distribution of fine roots affects the ability of plants to obtain water resources.
77 Deep-rooted species can obtain more steady water sources (e.g., deep soil water,
78 groundwater) than shallow-rooted species (McCole and Stern, 2007). Trees may
79 survive prolonged droughts by shift water uptake among existing roots rather than
80 growing new roots (Mackay et al., 2020). Fine roots are the first to be sacrificed under
81 extreme soil drought, thus reducing specific root length (Brunner et al. 2015). In
82 addition, plants at different growth stages have various water use characteristics due
83 to different water demands (Huo et al., 2018; Liu et al., 2015), which are associated
84 with factors such as biomass, leaf area index, and transpiration. One strategy is to
85 switch water sources depending on different life stages. For example, mature plants
86 may have deep roots and derive water from deeper sources than young plants, thereby
87 avoiding or minimizing the effects of drought during the dry season (Huo et al., 2018;
88 Kerhoulas et al., 2013). Another strategy is to avoid the effect of drought by leaf

89 shedding and remaining dormant during the dry season (Ellsworth and Sternberg,
90 2015). Therefore, understanding plant water use characteristics is critical to improve
91 the knowledge of ecological adaptability and soil-water-plant interactions in arid and
92 semiarid ecosystems.

93 Plant water use patterns and water use efficiency (WUE_i) are the two crucial
94 issues that reflect plant water use characteristics. Stable hydrogen and oxygen isotope
95 technique is a powerful and effective approach for identifying the water uptake
96 patterns of plants in semiarid ecosystems (Ding et al., 2020; Wang et al., 2020a). Plant
97 leaf $\delta^{13}C$ is widely used to indicate leaf-level WUE_i (Farquhar et al., 1989; Saugier et
98 al., 2012). Previous studies investigated water use characteristics of plants at different
99 stand-age in various semiarid ecosystems using the stable isotope technique (Zhou et
100 al., 2017; Song et al., 2018). For example, Zhou et al. (2017) found that young shrubs
101 (*Haloxylon ammodendron*) obtained water sources mainly from shallow soil, and
102 older shrubs obtained water mainly from permanent groundwater in a desert-oasis
103 ecotone. Song et al. (2018) explored seasonal variations in water sources used by
104 Mongolian pine trees of different ages, and found that different-aged trees obtained
105 soil water from the same depth. However, most of these studies focused on plant
106 water use patterns, and insufficient attention had been paid to WUE_i . There is limited
107 understanding of water use patterns and how WUE_i varies synergistically with
108 stand-age. In addition, little is known about the response of plant water use
109 characteristics to the variation of precipitation considering the future climate change
110 in semiarid regions.

111 The Loess Plateau is the largest and deepest loess deposit in the world with an
112 area of 640,000 km². The widespread ecosystem degradation and desertification
113 occurred due to the Millennia of land use and more recent population pressure. Massive
114 eco-restoration efforts were undertaken to decelerate the ecosystem degradation (Fu et
115 al., 2017). Large vegetation changes have occurred on the Loess Plateau in recent
116 decades since the ‘Grain for Green Project’ was implemented in 1999 (Fu et al., 2017).
117 Soil water resources are the only water source for plants, and water availability affects
118 the sustainability of revegetation on the semiarid Loess Plateau (Wang et al., 2017a;
119 Yu et al., 2020). The SWC of deep soil layers gradually decreased due to the depletion
120 by the trees with an increase in stand-ages. Soil desiccation formed in the deep layers
121 due to the utilization by revegetation species (Wang et al., 2011). Long-term soil
122 water deficit by revegetation species and soil desiccation led to the decline in the rate
123 of plant growth and forest degradation, causing dieback or mortality of the planted
124 trees. *Robinia pseudoacacia*, a main non-native planted tree species, is the most
125 widely planted for revegetation on the Loess Plateau (Zhang et al., 2015). Previous
126 studies explored seasonal variation in transpiration (Jiao et al., 2019) and the response
127 of soil water to rainfall events in *R. pseudoacacia* plantations (Chen et al., 2020).
128 However, these studies were limited to a specific stand age. Only few studies explored
129 the water uptake patterns of *R. pseudoacacia* (Zhao et al., 2020; Wang et al., 2020a),
130 and water use characteristics of *R. pseudoacacia* at different stand ages remain poorly
131 understood.

132 To fill the above knowledge gaps, this study investigated water use

133 characteristics of *R. pseudoacacia* at different stand ages using stable isotope
134 techniques ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) with monthly sampling during two consecutive
135 growing seasons on the semiarid Loess Plateau. The specific objectives of this study
136 were to (i) investigate water use characteristics of *R. pseudoacacia* at different
137 stand-ages and (ii) identify the sensitivity to the variation of precipitation in water use
138 characteristics of *R. pseudoacacia* at different stand ages. The two hypotheses were
139 that trees of young and mature plantations differed in (1) their water use
140 characteristics, and (2) their sensitivities to changes in precipitation.

141

142 **2. Materials and methods**

143 ***2.1 Study area and experimental sites***

144 The Yangjuangou catchment is located in the central region of the Chinese Loess
145 Plateau (36°42'45" N, 109°31'45" E). This catchment is a typical loess hilly gully
146 region with an area of 2.02 km². The soil is primary loessial soil derived from loess.
147 The soil depth is 50–200 m depending on topography. The bulk density of the surface
148 soil was 1.04–1.30 g cm⁻³. This area has a semiarid continental climate, with a mean
149 air temperature of 10 °C and a mean precipitation of 539 mm (1960–2017). Rainfall
150 has large interannual variability, and most of the precipitation is concentrated from
151 May to September. The original natural vegetation in the catchment was destroyed
152 due to the long-term disturbance caused by human activities. The current vegetation
153 of the catchment derives of near-to nature restoration including trees and shrubs, and
154 natural recovery of grass growth.

155 The forest species in this catchment is dominated by *R. pseudoacacia*, which was
156 planted in the later 1980s and 1999. *R. pseudoacacia* is a deciduous broad-leaved,
157 nitrogen-fixing tree species, and widely planted in the semi-arid Loess Plateau. Three
158 sample plots (10 m × 10 m) dominated by *R. pseudoacacia* were selected in each of
159 two plantations of different stand age (18- and 30-yr). These plantations had a
160 southeast aspect, middle-slope position, and similar slope gradients (~22°). The
161 different stand-age plantation had similar plantation densities (~1300 tree ha⁻¹). The
162 average heights of the 18- and 30-yr *R. pseudoacacia* plantations were 8.94 m and
163 9.28 m, respectively. The mean diameter at breast height (DBH) of 18- and 30-yr *R.*
164 *pseudoacacia* plantations was 11.43 cm and 12.36 cm, respectively. The understory
165 vegetation was mainly composed of *Artemisia sacrorum* and *Periploca sepium*.

166 ***2.2 Sample collection and isotope analysis***

167 Samples of soil and plants were collected monthly from May to September
168 during two consecutive growing seasons in 2016 and 2017. Three individual plants
169 were randomly selected for sampling in the different stand-age *R. pseudoacacia*
170 plantations on each sampling date. Three leaf and plant xylem samples from every
171 selected plant were collected in each stand-age tree per month. The fully sun-exposed,
172 mature and healthy leaves in the upper canopy from each selected plant were
173 collected in different directions on each sampling date. The leaves were mixed and
174 packed into craft paper bags and brought them back to the laboratory for measuring
175 the plant leaves $\delta^{13}\text{C}$. Plant xylem samples were obtained by cutting suberized twigs
176 from different canopy directions at the same height. Then, the outer bark and phloem

177 tissue were removed from these twigs, and immediately placed into glass vials, sealed
178 with parafilm, and kept frozen ($-20\text{ }^{\circ}\text{C}$). We collected a total of 60 xylem samples and
179 60 plant leaf samples during the study period.

180 In each stand-age plantation, three soil cores to a 500 cm depth were sampled
181 using a power auger at the same time as plant sampling for each stand-age plantation.
182 Soil samples from these cores were collected at 15 different depths: 0–10, 10–20, 20–
183 40, 40–60, 60–80, 80–100, 100–120, 120–150, 150–200, 200–250, 250–300, 300–350,
184 350–400, 400–450 and 450–500 cm. We collected a total of 900 soil samples during
185 the study period. The gravimetric soil water content (SWC, %) was determined by
186 drying soil samples for 24 h at $105\text{ }^{\circ}\text{C}$. Moreover, we collected 85 rainwater samples
187 with a polyethylene bottle and funnel in 2016 and 2017. The soil and rainwater
188 samples were stored frozen ($-20\text{ }^{\circ}\text{C}$) before isotopic analysis.

189 The xylem water and soil water were extracted by a cryogenic vacuum
190 distillation method described by Wang et al. (2019a). The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in soil water
191 and rainwater were measured using isotopic ratio infrared spectroscopy (IRIS)
192 (DLT-100, Los Gatos Research, Mountain View, USA) with an analytical accuracy of
193 $\pm 1.2\text{‰}$ for $\delta^2\text{H}$ and $\pm 0.3\text{‰}$ for $\delta^{18}\text{O}$. No contamination of organic matter was found
194 in soil water after quantifying applying the spectral contamination identifier
195 post-processing software. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in xylem water and the $\delta^{13}\text{C}$ in the plant
196 leaves were analysed using an isotope ratio mass spectrometer (IRMS) (MAT253,
197 Thermo Fisher Scientific, Bremen, Germany). The $^{18}\text{O}/^{16}\text{O}$ ratios in the xylem water
198 samples were measured using an automated equilibration unit (Gasbench II; Thermo

199 Fisher Scientific, Bremen, Germany) coupled to a MAT-253 mass spectrometer
200 (Thermo Fisher Scientific, Bremen, Germany). The xylem water samples were
201 reduced to H₂ gas using a fully automated chromium reduction system at 800 °C
202 (H/Device, Thermo Fisher Scientific) and the ²H/H ratios of this gas were then
203 measured with a MAT-253 mass spectrometer (Thermo Fisher Scientific, Bremen,
204 Germany). The isotopic analyses of carbon in the plant leaf were conducted with a
205 MAT-253 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled
206 to an automatic, online elemental analyzer (Flash EA1112, Thermo Electron, Milan,
207 Italy). The precision of the xylem water isotope using IRMS was ± 1‰ for δ²H and ±
208 0.2‰ for δ¹⁸O, and ± 0.15‰ for δ¹³C. The isotopic ratios in the water samples were
209 calculated as:

$$210 \quad \delta X = R_{sample} / R_{standard} - 1 \quad (1)$$

211 where X represents ²H, ¹⁸O, or ¹³C. R_{sample} and $R_{standard}$ are the ²H/¹H, ¹⁸O/¹⁶O or
212 ¹³C/¹²C ratios in the sample and standard, respectively. The standard material for ²H
213 and ¹⁸O is Vienna Standard Mean Ocean Water. Vienna Pee Dee Belemnite is the
214 standard material for ¹³C.

215 **2.3 Data analysis**

216 Soil water from different layers is considered to be the main water source for
217 plant species on the Chinese Loess Plateau because the plants have difficulty reaching
218 the groundwater (deeper than 60 m), and there was no irrigation during the study
219 period (Wang et al., 2017b). The depth of water uptake by the plants was estimated
220 using the graphical inference method. The relative proportional contributions of

221 different soil water sources to the xylem water were determined by the Bayesian
222 isotope mixing model MixSIAR (version 3.1.7) (Stock and Semmens, 2013). The
223 model used the raw isotopic values of xylem water of each plant as the 'mixture data'.
224 The means and standard deviations of the isotopic values of different soil layers were
225 used as the 'source data'. The potential fractionation was set to 0. More details about
226 the model settings can be found in a previous study (Wang et al., 2017b). For
227 subsequent analysis and comparison, the potential plant water sources were identified
228 as soil water from shallow (0–80 cm), middle (80–200 cm) and deep (200–500 cm)
229 soil depths according to the variability in SWC and the isotopic values of the soil
230 water.

231 We used mixed linear, random intercept models to test the differences in the
232 isotopic values of xylem water, soil water, and plant leaf, SWC between different
233 stand ages and sampling times. Soil depth and sampling times were set as fixed
234 effects and plot was a random effect. Differences in the $\delta^{13}\text{C}$ values of the plant leaves
235 and water source partitioning between different stand ages were analysed using
236 analysis of variance (ANOVA). Post hoc comparisons were based on Tukey's HSD.
237 The critical value of $p < 0.05$ was applied to determine statistical significance. All
238 statistical analyses were performed in R software (v.3.4.4, R Core Team, 2018).

239

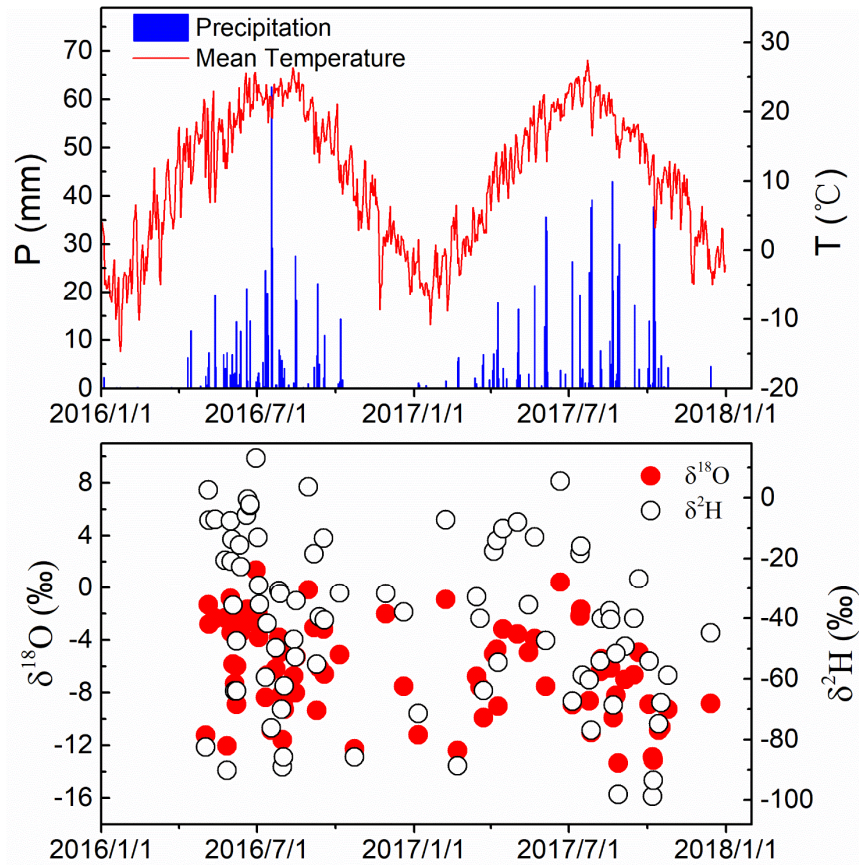
240 **3. Results**

241 *3.1 Meteorological factors and isotopic composition of precipitation*

242 The total precipitation in 2016 and 2017 was 473.6 and 689.7 mm, respectively.

243 Most of the precipitation was concentrated in the growing season (Fig. 1). The total
244 precipitation in 2016 was 12.14% lower than the multiyear mean (1961–2017)
245 precipitation (539.9 mm) and the growing season precipitation amount was 432.6 mm.
246 However, the total precipitation in 2017 was 27.83% higher than the average annual
247 precipitation and 69.71% of the precipitation (480.8 mm) occurred during the growing
248 season. The growing season precipitation in 2017 was 11.14% higher than that in
249 2016. The ratio of precipitation to potential evapotranspiration in the growing season
250 was 0.46 in 2016 and 0.77 in 2017. The mean temperature in 2016 and 2017 was 10.7
251 and 10.1 °C, respectively. These values are higher than that of average annual
252 temperature (10 °C). The monthly mean temperature during the growing season was
253 19.6 °C in 2016 and 19.2 °C in 2017.

254 The isotopic compositions of the precipitation showed a large fluctuation during
255 the study period (Fig. 1). The mean $\delta^{18}\text{O}$ of the precipitation in 2016 was -5.40‰ ,
256 ranging from -1.37 to -12.03‰ , and the mean $\delta^2\text{H}$ of the precipitation in 2016 was
257 -36.92‰ , ranging from -13.06 to -90.25‰ . The $\delta^{18}\text{O}$ value of the precipitation in
258 2017 ranged from -13.36 to 0.44‰ with an average of -7.31‰ , and the average $\delta^2\text{H}$
259 was -47.03‰ , ranging from -98.19 to 5.51‰ . The degree of isotopic variation in
260 precipitation was higher in 2016 than that in 2017. The average isotopic value of the
261 precipitation in 2017 was more depleted than that in 2016.

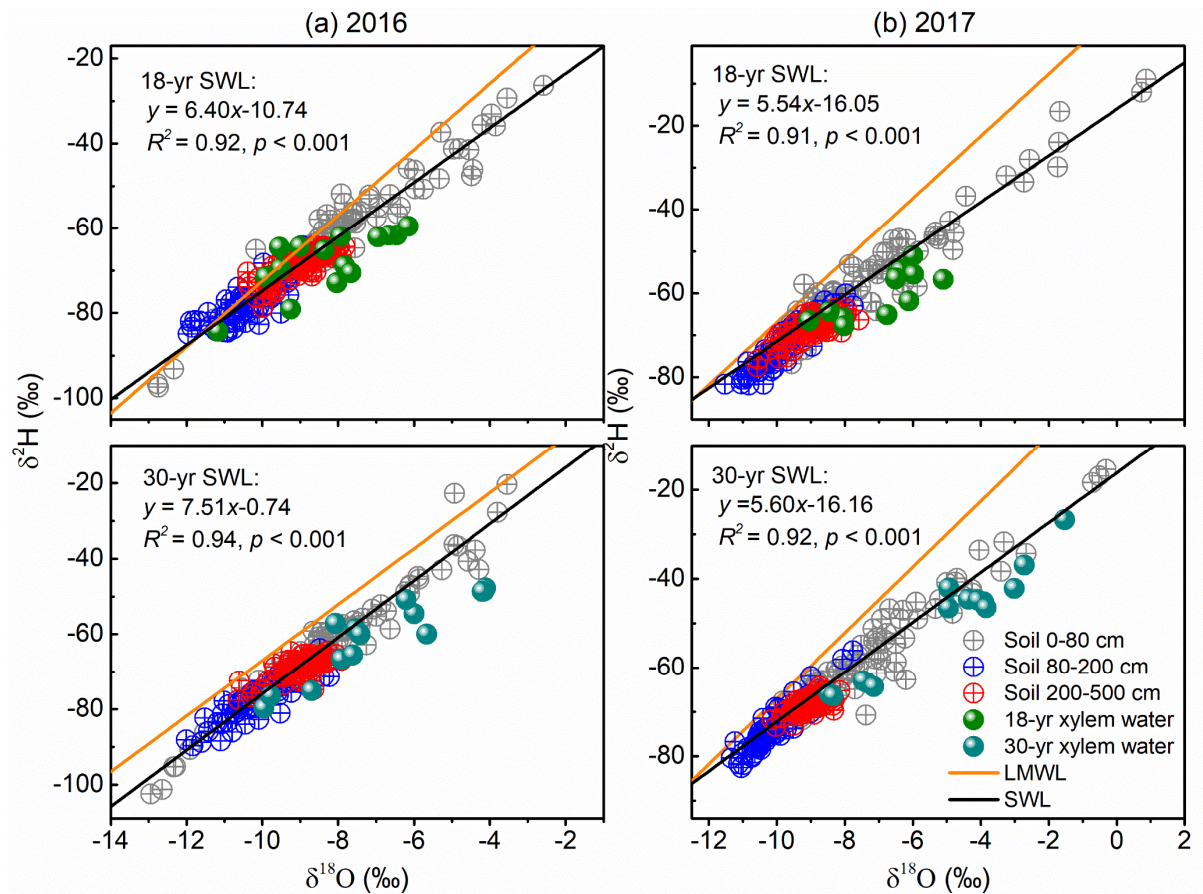


262

263 **Fig. 1.** Variations in precipitation, mean air temperature, and isotopic values ($\delta^2\text{H}$, $\delta^{18}\text{O}$) in
 264 precipitation at a daily timescale from 2016 to 2017.

265 The local meteoric water line (LMWL) was fitted based on the isotopic values of
 266 the precipitation. The soil water evaporation line (SWL), which was fitted based on
 267 the isotopic values of the soil water, had a lower slope value than that of the LMWL
 268 during the study period (Fig. 2). All the SWLs during the study periods are plotted to
 269 the right of the LMWL. Moreover, the slope of the SWL in the studied *R.*
 270 *pseudoacacia* plantations had higher values in 2016 than that in 2017. The $\delta^{18}\text{O}$ and
 271 $\delta^2\text{H}$ values of 18- and 30-yr *R. pseudoacacia* were within the range of the soil water
 272 isotopic values (Fig. 2).

273



274

275 **Fig. 2.** The relationships of isotopic values of soil water and xylem water from 18- and 30-yr *R.*

276 *pseudoacacia* in 2016 and 2017. LMWL is the local meteoric water line (2016: $y = 7.76x + 5.14$,

277 $R^2 = 0.91$, $p < 0.01$; 2017: $y = 7.41x + 7.14$, $R^2 = 0.91$, $p < 0.01$). SWL is the soil water

278 evaporation line based on isotopic values of soil water.

279 **3.2 Isotopic compositions of soil water and xylem water**

280 In 2016, the mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of soil water in 18-yr *R. pseudoacacia*

281 plantations were -67.66‰ and -8.90‰ , respectively, compared with corresponding

282 values of -68.27‰ and -8.92‰ in 30-yr *R. pseudoacacia* plantations. The mean

283 isotopic values of soil water in 18-yr (-8.48‰ for $\delta^{18}\text{O}$, -64.72‰ for $\delta^2\text{H}$) and 30-yr

284 *R. pseudoacacia* plantations (-8.64‰ for $\delta^{18}\text{O}$, -65.42‰ for $\delta^2\text{H}$) were higher in

285 2017 than that in 2016. The isotopic composition of water in the middle soil layers

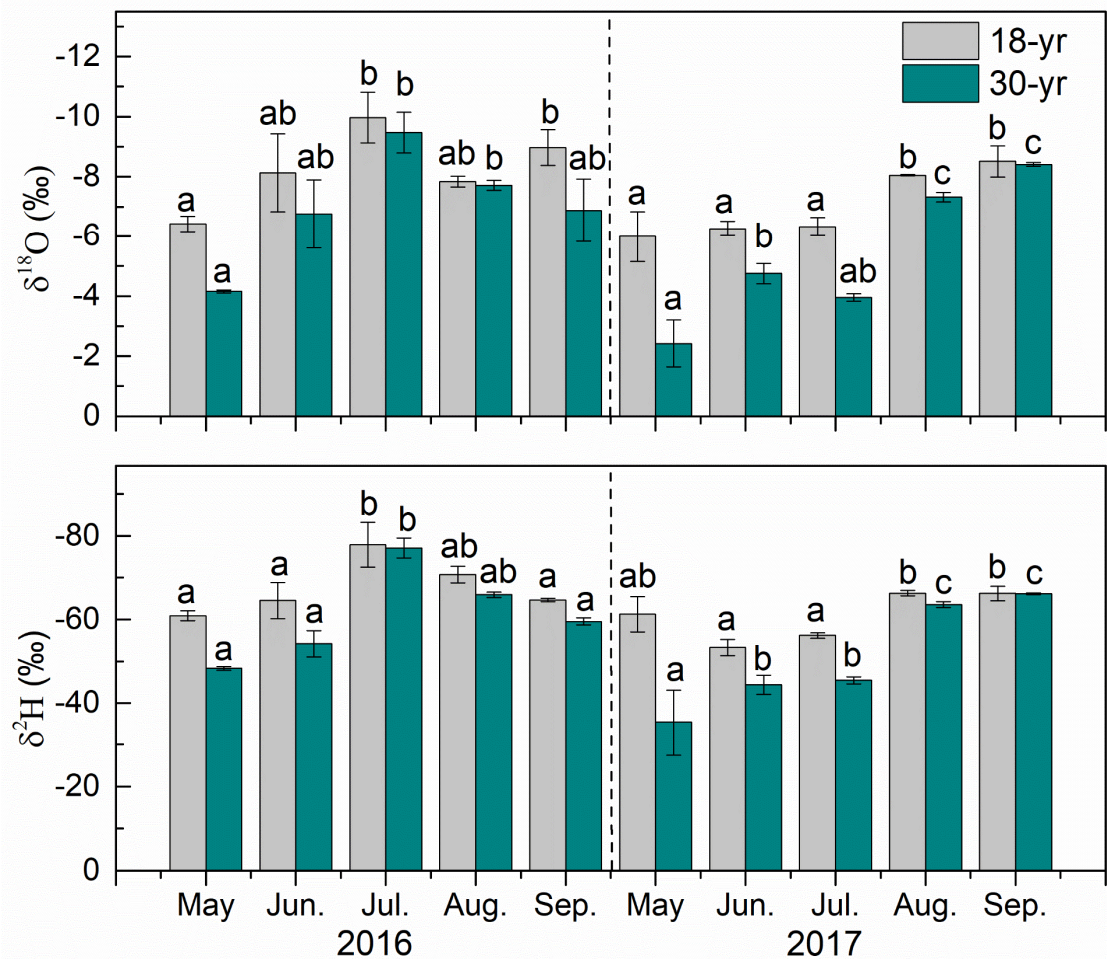
286 was more negative than that in the shallow and deep soil layers during the study
 287 period (Table 1). Soil water isotopes in different stand-age *R. pseudoacacia*
 288 plantations showed significant differences ($p < 0.01$) with depth and season, but there
 289 was no significant difference in the soil water isotopes between the two stand ages in
 290 2016. In 2017, the soil water isotopes in 18-yr *R. pseudoacacia* plantations changed
 291 significantly ($p < 0.05$) with depth and season. However, there was no significant
 292 difference ($p = 0.30$ for $\delta^2\text{H}$, $p = 0.26$ for $\delta^{18}\text{O}$) in soil water isotopes among different
 293 seasons, and there was a statistically significant difference ($p < 0.001$) among
 294 different depths in the 30-yr *R. pseudoacacia* plantations. Only the water isotopes in
 295 the middle soil layers differed significantly ($p < 0.05$) between the different-aged *R.*
 296 *pseudoacacia* plantations.

297 **Table 1** Variation characteristics of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in xylem and soil water during the study period.
 298 Data are shown as the mean \pm 1SD.

	2016		2017	
	18-yr	30-yr	18-yr	30-yr
Xylem water $\delta^{18}\text{O}$	-8.30 ± 1.43	-7.00 ± 1.89	-7.03 ± 1.15	-5.37 ± 2.30
0–80 cm soil water $\delta^{18}\text{O}$	-7.67 ± 1.11	-8.04 ± 1.23	-6.93 ± 1.22	-6.98 ± 0.93
80–200 cm soil water $\delta^{18}\text{O}$	-10.35 ± 0.26	-10.06 ± 0.74	-9.79 ± 0.45	-10.15 ± 0.32
200–500 cm soil water	-8.96 ± 0.29	-8.91 ± 0.36	-8.90 ± 0.26	-9.02 ± 0.13
Xylem water $\delta^2\text{H}$	-67.84 ± 7.04	-61.02 ± 10.38	-60.65 ± 5.75	-50.96 ± 12.72
0–80 cm soil water $\delta^2\text{H}$	-58.73 ± 8.16	-61.16 ± 9.81	-54.47 ± 7.76	-55.26 ± 5.25
80–200 cm soil water $\delta^2\text{H}$	-77.48 ± 2.09	-76.99 ± 4.51	-72.73 ± 2.24	-73.93 ± 2.72
200–500 cm soil water $\delta^2\text{H}$	-68.56 ± 1.67	-68.39 ± 2.51	-67.91 ± 1.02	-68.22 ± 0.78

299 The isotopic composition of xylem water changed with seasons and stand-ages.
 300 The isotopic values of xylem water in 18-yr *R. pseudoacacia* were lower than those of
 301 30-yr *R. pseudoacacia* during the study period (Fig. 3). The $\delta^2\text{H}$ of xylem water in

302 18-yr *R. pseudoacacia* ranged from -77.90 to -60.92‰ (mean \pm 1SD, $-67.84 \pm$
 303 7.04‰), the $\delta^{18}\text{O}$ ranged from -9.97 to -6.42‰ with an average of -8.30‰ , and the
 304 mean isotopic values of xylem water in 30-yr *R. pseudoacacia* were -61.02‰ for $\delta^2\text{H}$
 305 and -7.00‰ for $\delta^{18}\text{O}$ during the growing season in 2016. The respective average
 306 isotopic compositions of 18- and 30-yr trees were -60.65 and -50.96‰ for $\delta^2\text{H}$, and
 307 -7.03 and -5.37‰ for $\delta^{18}\text{O}$ during the growing season in 2017 (Table 1). There were
 308 significant differences ($p < 0.05$) in the xylem water isotopes between 18- and 30-yr
 309 *R. pseudoacacia* during the study period.



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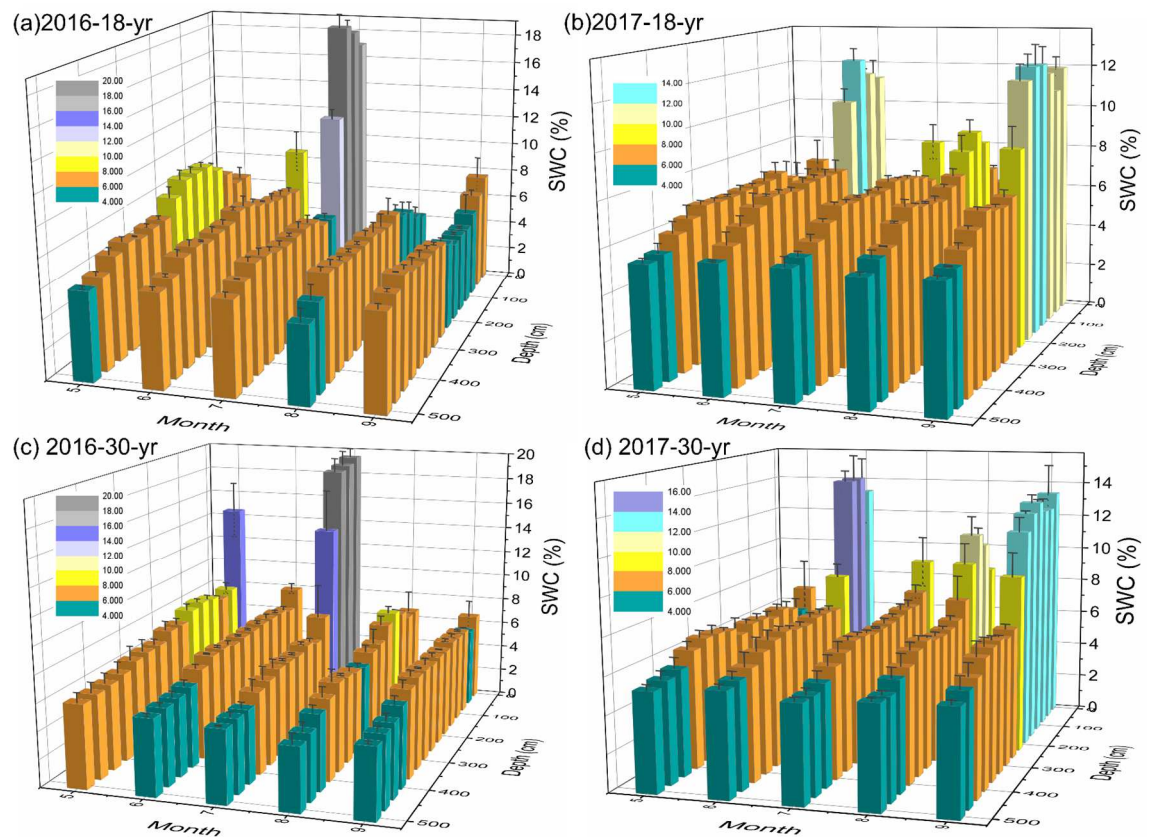
311 **Fig. 3.** Seasonal variation in isotopic compositions of xylem water in 18- and 30-yr *R.*

312 *pseudoacacia* in 2016 and 2017. Different lowercase letters represent significant differences

313 among sampling months at the $p < 0.05$ level. The error bar is the standard deviation.

314 **3.3 Variations in SWC and water uptake patterns**

315 The SWC in different stand-age *R. pseudoacacia* showed clear vertical
316 and seasonal variations during the study period (Fig. 4). The SWC in the shallow
317 soil layers (0–80 cm) had a higher degree of variation than the SWC in the 200–
318 500 cm soil layers. The SWC in the shallow soil layers showed higher variations with
319 season due to the influence of precipitation and evaporation. However, the SWC in
320 the 200–500 cm soil layer showed a lower degree of variation with sampling month
321 (Fig. 4). In the growing season of 2016, both the shallow and deep SWC differed
322 significantly ($p < 0.01$) between the 18- and 30-yr *R. pseudoacacia*
323 plantations. However, a significant difference was not observed for the SWC in
324 the middle soil layers. In the growing season of 2017, there were significant
325 differences in the shallow ($p < 0.01$), middle ($p < 0.01$) and deep SWC ($p < 0.01$)
326 between 18- and 30-yr *R. pseudoacacia* plantations. In addition, the shallow,
327 middle and deep SWC in the 30-yr *R. pseudoacacia* plantations displayed no
328 significant changes between 2016 and 2017. However, the deep SWC of 18-yr *R.*
329 *pseudoacacia* plantations in 2017 was significant lower ($p < 0.001$) than that in 2016,
330 and this difference was not found in the shallow and middle SWC. Moreover, the
331 shallow and deep SWC of the 30-yr *R. pseudoacacia* plantations did not change
332 significantly among seasons, while the SWC in the middle soil layers exhibited
333 significant differences ($p < 0.05$) among seasons. The shallow and deep SWC of
334 the 18-yr *R. pseudoacacia* plantations changed significantly ($p < 0.05$) among
seasons, but not for middle SWC.

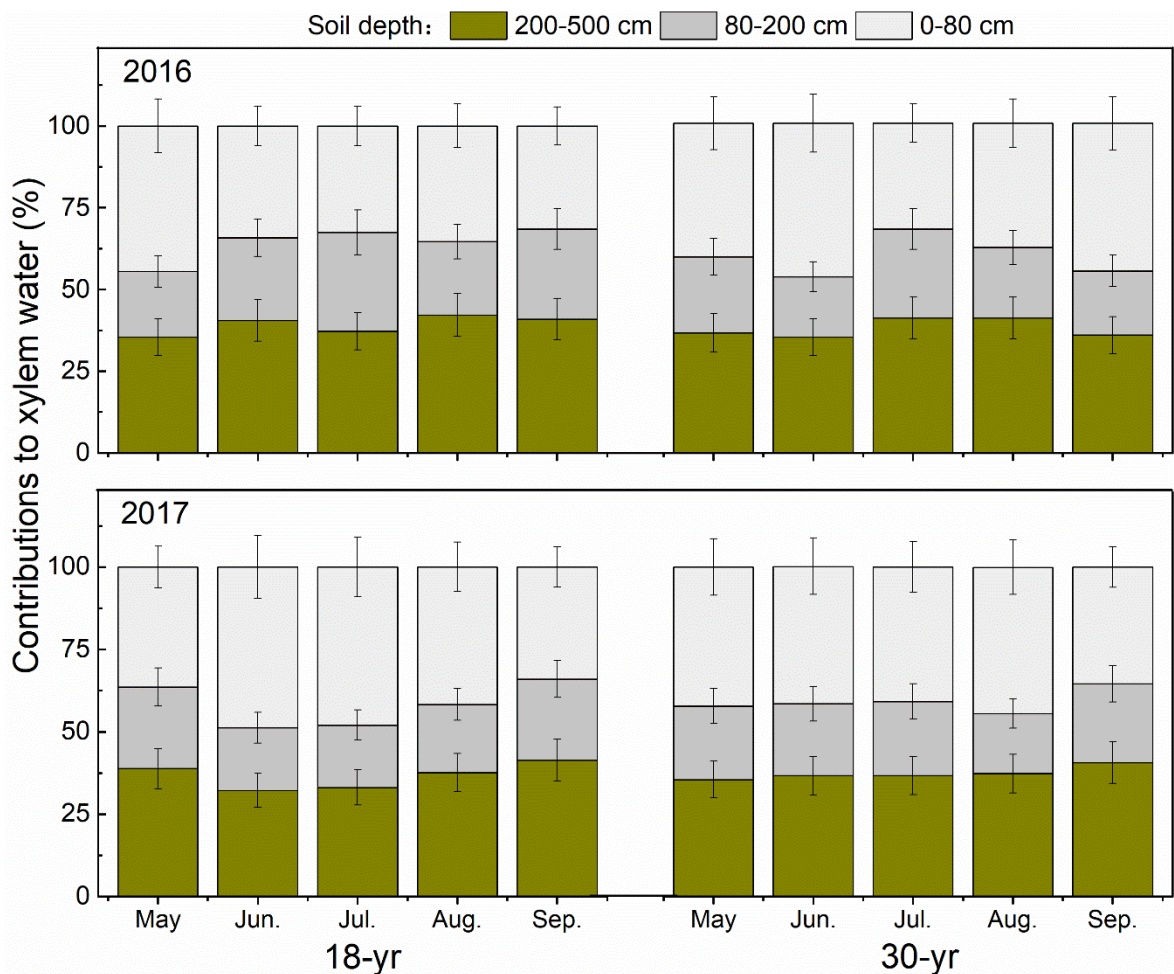


336

337 **Fig. 4.** Seasonal variation in the soil water content (SWC, %) from 18- and 30-yr *R. pseudoacacia*
 338 plantations in 2016 and 2017. The error bar represents the standard deviation.

339 In the growing seasons of 2016, the mean water uptake fractions of 18-yr *R.*
 340 *pseudoacacia* were 35.66% for shallow soil layers, 25.10% for middle soil layers, and
 341 39.24% for deep soil layers. The average proportional contributions of soil water from
 342 shallow, middle, and deep layers water to xylem water of 30-yr *R. pseudoacacia* were
 343 40.34%, 21.86%, and 37.80%, respectively. Similar patterns of plant water uptake
 344 were observed in 2017 (Fig. 5). The mean fraction of shallow soil water used by
 345 18-yr *R. pseudoacacia* in 2017 was 17.2% higher than that in 2016. However, the
 346 water uptake fractions from shallow soil water for 30-yr *R. pseudoacacia* were
 347 relatively stable between the growing seasons of 2016 and 2017 (Fig. 5). The
 water uptake

348 fractions of both 18- and 30-yr *R. pseudoacacia* showed no significant differences
 349 among sampling dates in 2016 and 2017. Moreover, for both 18- and 30-yr *R.*
 350 *pseudoacacia*, the proportional contributions of soil water from shallow, middle, and
 351 deep layers to xylem water exhibited no significant differences during the study
 352 period.



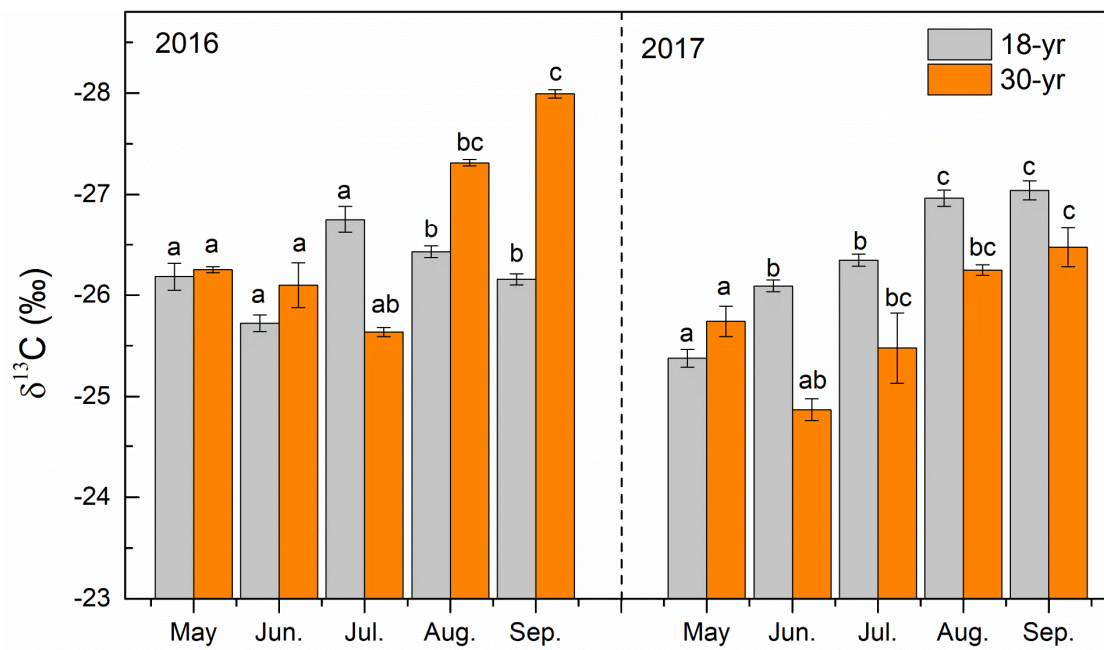
353

354 **Fig. 5.** Seasonal variation in the proportional contributions of soil water from shallow (0–80 cm),
 355 middle (80–200 cm) and deep (200–500 cm) layers to xylem water of 18- and 30-yr *R.*
 356 *pseudoacacia* during the study period. The error bar represents the standard deviation.

357 **3.4 Variation in $\delta^{13}C$ values of plant leaves**

358 The leaf $\delta^{13}C$ values of the studied plants varied with sampling time (Fig. 6). In

359 2016, the mean leaf $\delta^{13}\text{C}$ values of 18- and 30-yr *R. pseudoacacia* were -26.25‰ and
 360 -26.66‰ , respectively. In 2017, the leaf $\delta^{13}\text{C}$ values of 18-yr *R. pseudoacacia* ranged
 361 from -27.14 to -25.31‰ , with the average value of -26.36‰ , and the mean leaf $\delta^{13}\text{C}$
 362 values of 30-yr *R. pseudoacacia* was -25.76‰ . The leaf $\delta^{13}\text{C}$ values of 30-yr *R.*
 363 *pseudoacacia* was higher than that of 18-yr *R. pseudoacacia* except for May (Fig. 6).
 364 There were significant differences ($p < 0.05$) in the leaf $\delta^{13}\text{C}$ values between 18- and
 365 30-yr *R. pseudoacacia* during the growing season in 2017 but not 2016. In addition,
 366 the leaf $\delta^{13}\text{C}$ values of 30-yr *R. pseudoacacia* in 2017 showed significantly ($p < 0.001$)
 367 higher than those in 2016, but this difference was not found in 18-yr *R. pseudoacacia*.
 368 Moreover, the leaf $\delta^{13}\text{C}$ values of 30-yr *R. pseudoacacia* changed significantly with
 369 sampling months ($p < 0.001$) in 2016 and 2017. The leaf $\delta^{13}\text{C}$ values of 18-yr *R.*
 370 *pseudoacacia* varied significantly with sampling months ($p < 0.001$) in 2017 but not
 371 2016.



372
 373 **Fig. 6.** Seasonal variation in leaf $\delta^{13}\text{C}$ values of 18- and 30-yr *R. pseudoacacia* during the study

374 period. Different lowercase letters represent significant differences among sampling times at the p
375 < 0.05 level. The error bar represents the standard deviation.

376

377 **4. Discussion**

378 ***4.1 Differences in water uptake patterns of *R. pseudoacacia* at different stand-age***

379 The different stand-age *R. pseudoacacia* plantation showed different water use
380 patterns during the study period. The isotopic compositions of xylem water in 30-yr *R.*
381 *pseudoacacia* approached that of shallow soil water (Fig. 2), suggesting that it was
382 mainly derived from shallow soil water during the growing season. The MixSIAR
383 model also predicted that 30-yr *R. pseudoacacia* absorbed 40.34% and 40.88% from
384 shallow soil water in 2016 and 2017, respectively. These values were higher than
385 those for soil water from the middle and deep layers (Fig. 5). Plant water uptake
386 patterns were associated with the vertical distribution of fine roots (Wang et al.,
387 2017b). Previous study showed that the mature tree had a high fine-root area density
388 in the shallow soil layers (Zhang et al., 2018), causing the higher fractions of shallow
389 soil water was absorbed by 30-yr *R. pseudoacacia*. However, 18-yr *R. pseudoacacia*
390 derived the highest proportion (39.24%) of water from deep soil layers in 2016 and
391 41.78% from shallow soil layers in 2017. Our results showed that the proportional
392 contributions of soil water from shallow, middle, and deep layers to xylem water did
393 not display significant differences between the two stand-ages. However, Huo et al.
394 (2018) found that jujube trees of different ages differed significantly with respect to
395 water uptake: older jujube trees were more dependent on deep soil water than young

396 trees in semiarid plantations in China. Previous research reported that the water
397 demand of *R. pseudoacacia* increases with growth and the water uptake depth shifts
398 from shallow to deeper soil layers. Further, when deep soil water consumption
399 reaches a certain level, the water absorption depth returns to the upper layer (Nan et
400 al., 2019).

401 In this study, the 30-yr *R. pseudoacacia* used more water from shallow soil layer
402 than 18-yr *R. pseudoacacia* in 2016, but this capacity may have been offset by
403 increased rainfall. The growing season precipitation in 2017 was 11.14% higher than
404 that in 2016, and 18-yr *R. pseudoacacia* used more shallow soil water than 30-yr *R.*
405 *pseudoacacia*. Moreover, with an increase in rainfall, the utilization fraction of
406 shallow soil water by 18-yr *R. pseudoacacia* in 2017 was 17.2% higher more than that
407 in 2016. However, the proportional contributions of shallow soil water for 30-yr *R.*
408 *pseudoacacia* were relatively stable during the two years (40.34%, 40.88% for 2016
409 and 2017, respectively).

410 These results indicated that water uptake patterns of 18-yr *R. pseudoacacia* were
411 sensitive to the variation of precipitation. This was consistent with our hypothesis (2)
412 that different stand-age *R. pseudoacacia* showed various sensitivity to changes in
413 precipitation in water uptake patterns. Our findings were similar to those of previous
414 studies (Rose et al., 2003; Schwinning et al., 2005). McCole and Stern (2007) found
415 that trees mainly derive water from shallow soil layers when surface soil water is
416 highly available. However, our results disagreed with those of a previous study (Huo
417 et al., 2018) in which the proportional contributions of shallow soil water to jujube

418 trees of different ages did not increase with the rainfall. Goldsmith et al. (2012)
419 reported that different ages of plant species derived water from the same soil layer in a
420 seasonally dry tropical montane cloud forest. There were several possible
421 explanations for this different pattern from the findings reported in previous studies
422 (Goldsmith et al., 2012; Huo et al., 2018; Song et al., 2018). First, the sensitivity to
423 the variation of precipitation in plant water uptake patterns exhibited species-specific.
424 Different plant species had divergent eco-physiological characteristics related to water
425 uptake, such as the distribution and functioning of fine roots (Wang et al., 2017b),
426 recovery ability of photosynthesis after drought stress (Zunzunegui et al., 2018), more
427 rapid leaf turnover, and greater allocation to roots. Moreover, the habitat
428 environments where plants are located may also cause this difference pattern. Habitat
429 environment reflects the differences in water and heat conditions and soil properties,
430 such as sandy land of Inner Mongolia (Song et al., 2018), seasonally dry tropical
431 montane (Goldsmith et al., 2012). In addition, the methods associated with stable
432 isotopes for determining plant water source partitioning also cause this different
433 pattern. For example, SIAR model were used in Huo et al (2018), IsoSource model
434 was applied in Song et al (2018), and MixSIAR model was used in this study. The
435 performances of different isotopic approaches to quantify plant water uptake have
436 been confirmed (Rothfuss and Javaux, 2017; Wang et al., 2019b).

437 The water uptake fractions of 30-yr *R. pseudoacacia* from shallow, middle, and
438 deep soil layers were comparable during the study periods (Fig. 5), indicating
439 the 30-yr *R. pseudoacacia* did not show plasticity in water uptake pattern. There are
some

440 possible explanations for this interesting phenomenon. Normally, plants' roots obtain
441 resources from shallow soil layers (when available) because the energy costs are
442 lower, and both water and nutrient availability are generally high in the upper layers
443 (Schenk, 2008). Although the growing season precipitation in 2017 was 11.14%
444 higher than that in 2016, the shallow SWC in 30-yr *R. pseudoacacia* plantations
445 hardly changed ($p>0.05$), increasing by only 2% compared to 2016. The most of the
446 precipitation were short-duration heavy rainfall during the growing seasons (Fig. 1).
447 The infiltration excess overland flow was the dominant runoff generation process in
448 this region (Kang et al., 2001). The combined precipitation from three heavy rainfall
449 events accounted for 46.05% of the total rainfall during the growing season of 2017.
450 During these short-duration heavy rainfall events, most of the rainfall was quickly lost
451 in the form of runoff. Thus, despite the increase in rainfall during the growing seasons,
452 there was no significant increase ($p>0.05$) in shallow SWC in 30-yr *R. pseudoacacia*
453 plantations (Fig. 4). Therefore, *R. pseudoacacia* did not absorbed more shallow soil
454 moisture and kept relatively stable in water uptake patterns. In addition, the difference
455 in water uptake patterns of different stand-age of *R. pseudoacacia* may be associated
456 with the distribution and functioning of fine roots which played an essential role in
457 water, nutrient absorption of the plants. Previous study showed that root system of
458 younger trees had higher sensitivity to water (Wang et al., 2020b). The fine roots
459 distribution of different stand-ages trees needs further investigation to provide more
460 insights to the variation of water uptake patterns under different precipitation.
461 Furthermore, a time lag effect may be existed between the variation of plant water

462 sources following precipitation increases and the identification of its consequences.
463 Previous studies detected a time lag between water shortage and plants' responses
464 (Vicente-Serrano et al., 2013; Huo et al., 2018). While it was not possible to collect
465 data with sufficient time resolution to investigate this time lag effect in the current
466 study, future studies are desirable focusing on better spatial and temporal resolutions
467 of stable isotopes in water and plants.

468 ***4.2 Differences in plant leaf $\delta^{13}\text{C}$ values of different stand-age of *R. pseudoacacia****

469 The leaf $\delta^{13}\text{C}$ values of 30-yr *R. pseudoacacia* were significant ($p < 0.05$) higher
470 than that of 18-yr *R. pseudoacacia* in 2017. This indicated that there was a higher
471 leaf-level WUE_i in 30-yr *R. pseudoacacia* plantation than the 18-yr *R. pseudoacacia*
472 plantation during the study period of 2017 since the $\delta^{13}\text{C}$ value of plant leaves is
473 positively correlated with the leaf-level WUE_i (Farquhar et al., 1989). The leaf $\delta^{13}\text{C}$
474 values of 30-yr *R. pseudoacacia* displayed a significant change with the sampling
475 time ($p < 0.01$) (Fig. 6), suggesting that the leaf-level WUE_i varied with the growing
476 season. Some meteorological factors, such as temperature, light, precipitation, and
477 atmospheric CO_2 , can influence the leaf $\delta^{13}\text{C}$ values of plants by affecting stomatal
478 conductance (g_s) during photosynthesis (Farquhar et al., 1989). In our study, the
479 different stand-age *R. pseudoacacia* plantations were located close to each other, and
480 the variation in atmospheric temperature, precipitation, and light was small in these
481 plantations. In addition, the studied plantations had closed canopies, and the gradient
482 in the atmospheric CO_2 concentrations under the canopy did not vary significantly and
483 could be ignored. Therefore, temperature, light, precipitation, and atmospheric CO_2

484 concentrations had little effect on the difference in carbon isotope compositions
485 between 18- and 30-yr *R. pseudoacacia*. Stand-age was an essential factor which
486 impacts plant photosynthesis. *R. pseudoacacia* plantations reached their mature stage
487 around 30 years (Kou et al., 2016) and 18-year *R. pseudoacacia* plantations was
488 vigorous growth period on the Loess Plateau. Previous studies showed that juvenile *R.*
489 *pseudoacacia* plantations had higher average daily stomatal conductance and ratio of
490 intercellular (C_i) to ambient (C_a) CO_2 , carboxylation efficiency compared to mature
491 plantations (Zheng et al., 2011), indicating different stand-age *R. pseudoacacia*
492 plantation had divergent physiological characteristics. Therefore, stand-age
493 plantations may lead to the difference in the leaf-level WUE_i of *R. pseudoacacia*. Our
494 results were consistent with previous studies (Zhang and Jin, 2016; Zheng et al., 2011)
495 which found WUE was higher in the mature forests compared with that in the juvenile
496 plantations in the Loess Plateau. Moreover, the competition for above-ground
497 resources may also cause the difference in leaf-level WUE_i of *R. pseudoacacia*.
498 Nitrogen, phosphorus, and water in soil were essential factor for plant growth and
499 survival. Nitrogen as a belowground resource did not impact on the growth
500 partitioning of *R. pseudoacacia* due to its ability to fix atmospheric nitrogen.
501 Phosphorus and water as below-ground resources were the key factors affecting
502 growth partitioning and competition of *R. pseudoacacia* (Carl et al., 2018). In our
503 study, the shallow SWC of 30-yr *R. pseudoacacia* plantations was higher significantly
504 ($p < 0.01$) than those in 18-yr *R. pseudoacacia* both in 2016 and 2017. Previous
505 studied showed mature *R. pseudoacacia* plantations had higher phosphorus compared

506 to juvenile plantations (Kou et al., 2016). Higher phosphorus and water led to increase
507 the competition for above-ground resources, mainly sunlight (Carl et al., 2018), which
508 may cause the difference in leaf-level WUE_i of different stand-age *R. pseudoacacia*.

509 The leaf $\delta^{13}C$ values of 18-yr *R. pseudoacacia* showed no significant difference
510 between 2016 and 2017, implying that the leaf-level WUE_i remained relatively stable.
511 However, the leaf $\delta^{13}C$ values of 30-yr *R. pseudoacacia* in 2017 were significantly
512 higher than those in 2016 ($p < 0.001$), indicating that leaf-level WUE_i of 30-yr
513 plantation would appear higher in a wet year than in a dry year. This result was
514 consistent with a previous study in the same catchment (Wang et al., 2020a) and
515 indicated that *R. pseudoacacia* displayed opportunistic water uptake strategy.
516 However, some previous studies showed that leaf $\delta^{13}C$ values had a significant
517 negative correlation with precipitation or soil water availability in arid and semi-arid
518 regions (Ale et al., 2018; Zheng et al., 2007). Some reasons may explain this
519 phenomenon that old plantation had higher leaf-level WUE_i in a wetter year. WUE_i is
520 defined as the ratio of photosynthetic rate over g_s , and photosynthetic rate can be
521 expressed as $(g_s / 1.6) * (C_a - C_i)$ (Farquhar et al., 1989). The maximal carboxylation
522 rate (V_{cmax}), a measure of photosynthetic capacity, is usually well correlated with
523 nutrient availability (i.e., nitrogen and phosphorus) (Maroco et al., 2002; Prior et al.,
524 2003). Previous studies reported that a wetter condition can promote phosphorus
525 availability for plants in the semi-arid Loess Plateau (Wu et al., 2019; Xu et al., 2020),
526 thus increasing V_{cmax} . The increase of V_{cmax} would lead to a further drawdown of C_i
527 and result in an increase in $C_a - C_i$. The proportional increase in photosynthetic rate

528 might outweigh that in g_s , therefore ensuring an increase in WUE_i . However, we need
529 to further verify this physiological mechanism in the future research. In addition, the
530 mature tree had a high fine-root area density in the shallow soil layers (Zhang et al.,
531 2018), which accordingly dried more rapidly than the deeper soil, causing the increase
532 of leaf-level WUE_i of mature trees. Furthermore, soil water content significantly
533 affects stomatal conductance during the process of photosynthesis, which
534 subsequently influences the carbon isotope ratios of plants, especially in arid and
535 semiarid regions (Farquhar et al., 1989). Long-term vegetation restoration consumed a
536 large amount of soil water, and when the water was suddenly replenished, this may
537 have had a stimulating effect, leading to an increase in the leaf-level WUE_i of plants.
538 However, the specific mechanism remains to be further explored.

539 ***4.3 Implications for afforestation***

540 Water availability is an important factor for successful vegetation restoration in
541 arid and semiarid regions (Wang et al., 2012). Higher precipitation does not
542 necessarily result in larger water availability because runoff drains much of water
543 from the short-duration heavy rainfall due to the low infiltration in semiarid Loess
544 Plateau. Limited precipitation is the only water source for plant growth because the
545 groundwater level is deep (Jia et al., 2017) and most plant roots cannot reach the
546 groundwater of the semiarid Loess Plateau. Artificial forestation has caused excessive
547 consumption of soil water and has resulted in dry soil layers due to inappropriate
548 species use and planting density (Wang et al., 2011). Therefore, the sustainability of
549 revegetation must consider plant water uptake patterns. Our results showed that water

550 uptake patterns of 18-yr *R. pseudoacacia* were more sensitive to changes in
551 precipitation than those of 30-yr *R. pseudoacacia*, and their leaf-level WUE_i differed
552 significantly during the study period (Fig. 6). The different stand-age plantation
553 trees exhibited the specific effect of water use characteristics response to
554 different precipitation. In the same catchment, Jiao et al. (2016) found stand
555 transpiration of different stand-age black locust trees was significantly different. A
556 previous study also showed that the depths of soil water consumed by *R.*
557 *pseudoacacia* gradually increased (100–500 cm) with increasing forest stand age
558 (Nan et al., 2019). In our study, the deep SWC of 18- and 30-yr *R.*
559 *pseudoacacia* plantations showed a significant difference ($p < 0.01$).
560 Therefore, in future afforestation activities, stand-age is an essential factor to
561 be considered to implement the corresponding management measures, which
562 can strengthen the effectiveness of vegetation restoration strategies. In
563 forestland dominated by *R. pseudoacacia*, with the increase in the restoration stage,
564 this species consumed high amounts of deep soil water. Even in the year with high
565 rainfall, the water source used by 30-yr *R. pseudoacacia* did not change significantly
566 (Fig. 4), implying that *R. pseudoacacia* may reach the critical point of growth
567 decline in 30 years. Moreover, the 30-yr *R. pseudoacacia* displayed dieback and
568 some branches had no leaves or withered during the field investigation. In the
569 Fuxian district of the Loess Plateau, Jia et al. (2017) found that the height of *R.*
570 *pseudoacacia* increased over time before age 20 years, and the rate of plant
571 growth showed a downward trend during the following 20 years, the branches
appeared to be increasingly death. Nan et al. (2019) also found that the heights of 20-
yr and 40-yr *R.*

572 *pseudoacacia* were only 600 cm and 900 cm, respectively and the DBH was
573 approximately 35 cm and 58 cm, respectively. In our study, the average heights of 18-
574 and 30-yr *R. pseudoacacia* were 894 cm and 928 cm, respectively, and the
575 corresponding DBH was 11.34 cm and 12.36 cm. Therefore, it is necessary to carry
576 out appropriate management measures for forestland after a certain restoration stage.
577 Some water retention measures can be implemented, for example water-fertilizer pits
578 (60 cm × 60 cm × 40 cm) were dug along the contour lines, with the direction parallel
579 to the slope on the steep forestland. Water-fertilizer pits can intercept runoff and
580 deliver rainwater to deep soil layers. In addition, appropriate logging is required in
581 high-density areas to maintain the balance of the water source supply and the needs of
582 plant growth (Jia et al., 2017). Some restoration methods, such as natural grassland
583 and abandoned farmland, has little effect on soil water regardless of afforestation age
584 (Zhang et al., 2016). The choice of shallow-rooted herbs and natural restoration may
585 be the optimal choices for vegetation restoration on the Loess Plateau. Many *R.*
586 *pseudoacacia* plantations may exacerbate soil water deficits, which is not conducive
587 to the sustainability of vegetation restoration. When the mean annual precipitation
588 was below 520 mm, the soil water in the 100–300 cm layers was equivalent to or
589 below the permanent wilting point (Wang et al., 2017a), therefore, *R. pseudoacacia*
590 should be planted where the mean annual precipitation exceeds 520 mm.

591

592 **5. Conclusions**

593 This study provides insight into seasonal water use characteristics of

594 different-aged *R. pseudoacacia* plantations (18- and 30-yr) on the Loess Plateau using
595 stable isotope techniques ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) combined with the MixSIAR model.
596 The results showed that although the growing season precipitation in 2017 increased
597 by 11.14% compared to 2016, the proportional contributions of shallow soil water to
598 xylem water in 30-yr *R. pseudoacacia* were comparable between the two years
599 (40.34%, 40.88% for 2016 and 2017, respectively), but the water uptake fraction of
600 shallow soil water by 18-yr *R. pseudoacacia* in 2017 was 17.2% higher than that in
601 2016, tracking the precipitation increase with the shallow soil moisture increased by
602 7.60%. These results indicated that the water uptake patterns of 18-yr *R.*
603 *pseudoacacia* were sensitive to the variation of precipitation. The leaf $\delta^{13}\text{C}$ values of
604 18-yr *R. pseudoacacia* showed no significant difference between 2016 and 2017.
605 However, the leaf $\delta^{13}\text{C}$ values of 30-yr *R. pseudoacacia* in 2017 were significantly
606 higher ($p < 0.001$) than those in 2016, suggesting that increased precipitation could
607 stimulate leaf-level WUE_i , and older plantations respond to increased water
608 availability by increasing WUE_i instead of switching water sources. SWC was the
609 main factor that caused the differences in the $\delta^{13}\text{C}$ values of plant leaves between
610 different-aged *R. pseudoacacia*. Overall, the plantation trees exhibited distinct age
611 specific water use characteristics in response to differences in precipitation and
612 resulting water content across soil layers. This study provides insight into revegetation
613 dynamics in semiarid ecosystems. The stand age of plant species should be considered
614 to implement the corresponding management measures to strengthen the effectiveness
615 of vegetation restoration strategies in afforestation activities.

616

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626

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