Rapid response of the carbon balance strategy in *Robinia pseudoacacia* and *Amorpha fruticosa* to recurrent drought

Weiming Yan\(^a\), Yangquanwei Zhong\(^b\), Zhourping Shangguan\(^a,\ast\)

\(^a\) State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China

\(^b\) Center for Ecological and Environmental Sciences, Key Laboratory for Space Bioscience & Biotechnology, Northwestern Polytechnical University, Xi’an, 710072, China

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**Abstract**
Drought is becoming more severe and frequent in some regions due to climate change, which leads to carbon imbalance in plants and has gained significant attention. However, it remains unclear how the carbon balance responds to recurrent drought and recovery. To understand the carbon balance response to recurrent drought, we monitored dynamic changes in the physiological traits of two species during cycles of drought and recovery, *Amorpha fruticosa* and *Robinia pseudoacacia*, which are planted widely on the Loess Plateau. We found that the two species performed similarly in response to drought; both showed growth cessation and a reduction of carbon assimilation and respiration under cycles of drought and recovery. The soil water content (SWC) at the stress point of the fluorescence parameters was lower than those of gas exchange and water potential, which were higher in the second drought than in the first drought, except aboveground respiration, which may enhance the risk of drought-related mortality. After rewated, leaf photosynthesis recovered fully and root respiration increased; however, the aboveground carbon flux of the plants did not fully recover due to leaf shedding. In addition, drought caused a decrease in the stem diameter, which impeded phloem function and carbon translocation and redistribution, resulting in a decrease in non-structural carbohydrates in local plant tissues under drought in both species. Furthermore, *A. fruticosa* showed higher total non-structural carbohydrates. Our results suggest that plants that had experienced drought were more sensitive when faced with subsequent drought, and recurrent drought enhanced the risk of mortality in plants.

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1. Introduction

Drought is becoming more severe and frequent in some regions, which is reportedly due to climate change (Hoerling and Kumar, 2003; Myhre et al., 2013) and is the main reason for the carbon (C) imbalance in terrestrial ecosystems (Zhao and Running, 2010; Reichstein et al., 2013). Severe drought could result in a significant decline in net primary productivity in many different forest types and large-scale tree mortality events, which has received extensive attention in recent years (Breshears et al., 2009; Allen et al., 2010; Hicke and Zeppel, 2013); thus, it is necessary to study the response of plants’ C balance strategies to recurrent drought.

The underlying mechanisms of plant C imbalance caused by drought are the subject of ongoing research and have generated some debate (Leuzinger et al., 2009; McDowell, 2011). Plants cease growth and close the stomata to prevent water loss under drought conditions, which results in a simultaneous decrease in photosynthesis (McDowell et al., 2008; Zhao et al., 2013). However, maintenance respiration responds more slowly to drought than photosynthesis, resulting in a C deficit and forcing the plant to utilize stored carbohydrates (McDowell, 2011). If the C deficit persists for a long time, carbohydrates will be depleted and plants will experience C starvation (Sayer and Haywood, 2006; McDowell, 2011), resulting in plant mortality. In addition, the refilling of both embolized xylem conduits and woody growth after rewated are carbon-costly processes (Bucci et al., 2003; Salleo et al., 2009) that require the utilization of stored carbohydrates and result in C starvation. Thus, it is necessary to study the dynamics of plant C balance strategies in response to recurrent drought and recovery.

Extreme meteorological events are predicted to appear more frequently due to climate change, such as severe summer droughts (Field et al., 2012; Myhre et al., 2013), which cause plants to experience recurrent drought. After a single drought, precipitation...
is usually abundant and rapid, leading to soil moisture fluctuations. Previous studies have indicated that many plants can recover during this rehydration process, including the full recovery of leaf gas exchange (Gallé et al., 2007; Brodribb et al., 2010). However, the C balance strategies of plants during recovery and after a second drought following a short recovery are unclear. Some previous studies have reported that plants can adapt to an abiotic stress environment when experiencing stress (Bruce et al., 2007; Walter et al., 2011) by changing the plant phenotype (Aubin-Horth and Renn, 2009), and changes in pigment content can prevent photodamage under drought conditions (Muné-Bosch and Alegre, 2000). The change in phenotype can result in a “stress memory”, which involves signaling proteins and transcription factors and protects plants when they are faced with recurrent drought (Bruce et al., 2007). Previous studies have also suggested that a single abiotic stress event can reduce the resilience of an ecosystem and cause the deterioration of the ecosystem when faced with recurrent stress (Scheffer et al., 2001). For example, Plaut et al. (2013) conducted a rainfall experiment in a forest and found that drought resulted in a downward spiral of the plants because trees were unable to utilize the intermittent soil water. Therefore, more frequent droughts may enhance the risk of drought-related mortality. Moreover, some studies have reported that plants showed greater vulnerability in plant communities faced with recurrent stress (Lloret et al., 2004; Mueller et al., 2005). The intensity and duration of drought change causes plant function by limiting photosynthesis in rainless periods, and changes in physiology and structure could alter the plants’ ability to utilize soil water after precipitation (Recso et al., 2009; Brodribb et al., 2010). Some physiological changes in response to drought could prevent plants’ immediate death; for example, leaf shedding could reduce the C demand and result in an improvement in the water status of the remaining foliage and the subsequent survival of the individual (McDowell et al., 2008; Sala et al., 2010). However, the mortality of the fine roots caused by drought could decrease the ability of a tree to utilize soil water when it becomes available. Xylem cavitation is expected to occur when water uptake by roots is insufficient to offset water loss from transpiration under drought, which would reduce the xylem hydraulic conductance and cause a lower leaf potential for gas exchange, eventually reducing the potential for C assimilation and leading to negative effects on plant growth (McDowell et al., 2008). Recent studies have investigated the effects of a single drought event on the C balance of a single species (Gallé et al., 2007; Jentsch et al., 2009) and of plant communities (Van Peer et al., 2004; Kreyling et al., 2008) and ecosystems (Noormets et al., 2008). The frequency and magnitude of droughts are expected to increase in the future; thus, it is necessary to better understand the effects of recurrent droughts on plant functions, such as the physiological mechanisms, C balance strategies and responses of different plants during and while recovering from drought (Blackman et al., 2009).

Two C3 woody legume seedlings, Amorpha fruticosa L. and Robinia pseudoacacia L., were widely planted on the Loess Plateau as pioneer afforestation species due to their high drought resistance. The large-scale afforestation of R. pseudoacacia has caused substantial problems, such as small old trees, which may be caused by the C imbalance caused by low soil water availability; however, this has not been observed in A. fruticosa. To determine how recurrent drought affects the ability of these two plants to utilize water and changes their C balance, we monitored dynamic changes in plant growth and the whole plant C balance during cycles of drought, recovery and a second drought. The objective of this study was to identify differences in plant growth and the whole-plant C balance as well as changes in non-structural carbohydrate (NSC) content in response to recurrent drought in these two species. We hypothesized that (1) plants that experience drought would exhibit an advanced stress point in the next drought cycle and that (2) the C distribution pattern of A. fruticosa would perform differently from that of R. pseudoacacia during recurrent drought.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at the Institute of Soil and Water Conservation in Yangling, Shaanxi Province (34°17′N, 108°04′E) from June to September 2015. The study site experiences a temperate and semi-humid climate; the mean annual temperature is 13 °C, and the mean annual precipitation is 632 mm, of which approximately 60% occurs in July–September.

Two-year-old seedlings of two deciduous woody legume species were studied, Amorpha fruticosa L. (shrub) and Robinia pseudoacacia L. (tree), which have been widely planted on the Loess Plateau as pioneer afforestation species. Seeds of both plants were sown in a nursery at the same time during the previous year. Three months before the start of the experiment, A. fruticosa (30–50 cm tall and 3–5 mm in diameter) and R. pseudoacacia (40–60 cm tall and 3–5 mm in diameter) were transplanted from the field to 400 L pots (980 × 760 × 680 mm, length × width × height) because small plants may affect the experimental results and undermine the purpose of an experiment (Poorter et al., 2012). The soil used in the study was collected from the 0 to 20 cm soil layer, and the physical and chemical properties of the soil are presented in Table 1.

The plants were assessed during drought cycles and divided into two treatments: well-watered (80% field capacity) and drought-rewatered-drought treatments. A completely randomized design was used, and six replicates with four plants each were planted. All of the plants were well watered before the experiment onset. The control plants were watered every other day, and drought was induced by ceasing to water plants until the net photosynthetic rate was decreased to close to zero or until the predawn water potential (Ψp) decreased to between –3.0 and –3.5 MPa before rewatering, which took 35 and 28 days in A. fruticosa and R. pseudoacacia, respectively. The turgor loss points of the two species were –1.25 and 1.22 MPa, respectively. Then, the drought plants were rewatered until net photosynthesis had almost completely recovered; recovery took seven days. Additionally, 40L, 20L and 20L of water was added on the first, fourth and seventh days of the recovery stage, respectively. The soil water content (SWC) after rewatering can be seen in Fig. 1b. Subsequently, the plants were kept without water for approximately 42 days, which constituted the second drought cycle. Three replicates were used to measure physiological parameters, such as water potential, gas exchange, chlorophyll fluorescence, soil respiration, and total plant C exchange parameters. The drought and control plants were identical during the entire study. The other three replicates were

<table>
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<th>Table 1</th>
<th>The physical and chemical properties of the soil used in this study.</th>
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<td>Property</td>
<td>Value</td>
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<tr>
<td>Taxonomy</td>
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<tr>
<td>Texture</td>
<td>2000–50 μm (g kg⁻¹)</td>
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<td>50–2 μm (g kg⁻¹)</td>
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<td>&lt;2 μm (g kg⁻¹)</td>
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<tr>
<td>Soil total phosphorus (g kg⁻¹)</td>
<td>0.69</td>
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sampled for NSC content analysis. One plant was sampled in each replicate at each time point, for a total of three plants per sampling. The sampled plants were divided into leaves, branches, stems, bark and roots, and then oven-dried at 105 °C for 30 min and at 75 °C to a constant mass. All of the samples were ground into a uniformly fine powder and sieved through a 1-mm mesh screen.

### 2.2. Measurements

The SWC was measured with an SWC reflectometer probe (CS650-L, Campbell Scientific, Australia). The soil moisture at 10 and 40 cm was recorded every 30 min, and the average SWC was calculated. The mean daily temperature, relative air humidity and SWC are shown in Fig. 1.

Throughout the experiment, the leaf $\Psi_p$ and gas exchange were measured on sun-exposed leaves on the upper crowns of the plants on a sunny day, for which at least three plants per replicate were randomly selected. Spot $\Psi_p$ measurements were performed between 05:00 and 06:00 h using a PMS 600 pressure chamber (PMS Instruments Company, Albany, USA). Gas exchange traits were measured in at least two leaves per plant selected during 09:00–11:00 h using the Li-Cor model 6400 system (Lincoln, NE, USA). Fully expanded, mature leaves at the upper crowns were selected and marked for the gas exchange measurements on each sunny day or the second day after each rainy day during the experimental period, and adjacent leaves were selected the leaf $\Psi_p$ measurements. The saturating photosynthetic photon flux density was between 1000 and 1500 μmol m−2 s−1 in the leaf chamber during the measurement periods. The temperature, CO2 concentration and relative humidity inside the leaf cuvette were always close to ambient air values.

Measurements of leaf chlorophyll fluorescence were performed on the same leaf with gas exchange by a portable pulse amplitude-modulated fluorometer on at least two leaves per plant from 09:00–11:00 h (FMS-2.02 system, Hansatech Instruments, Norfolk, UK). The Fm′, Fo′ and Fs were recorded after 20 min of darkness, and the following parameters were determined and calculated: $F_{v}/F_{m}$ (the maximum quantum efficiency of photosystem II); non-photochemical quenching (NPQ, heat dissipation), which was calculated as $(Fm-Fm′)/Fm′$; and photochemical quenching $(qP)$, which was calculated with the following formula: $qP = (Fm′-Fs)/(Fm′-Fo′)$ (Baker and Rosenqvist, 2004).

Repeated chamber measurements were performed using an LI-8100 automated soil CO2 flux measurement system and an LI-8150 multiplexer with 8100-104 long-term chambers (Li-Cor Inc., Lincoln, NE, USA), and a polyvinyl chloride collar (20 cm inner diameter, 11 cm height) was installed in the center of each plot to measure the temporal variation in the CO2 flux. The collar was inserted 6–8 cm into the soil to allow the roots to grow into it, and it was kept free of falling leaves. Soil respiration (SR) was recorded continuously every 0.5 h during the measuring day. The chamber was closed for 120 s, and the linear increase in the CO2 concentration in the chamber was used to estimate the SR. The mean SR of one day was calculated during the experiment. The soil used in the study was air-dried soil with the plant litter removed. The 5 cm soil temperature and water content were measured using a thermocouple probe and a moisture meter (EC5, Decagon, USA), respectively.

The aboveground C exchange of the plants was measured using an LI-8100 attached to a soil chamber in which the standard soil chamber was replaced by a 250 L transparent Perspex chamber from 09:00–11:00 h. The air in the chamber was mixed using small fans. To measure the aboveground C exchange, the transparent chamber was placed on smooth plywood at approximately 10 cm above the soil surface, and the bottom rim of the transparent chamber was sealed with a rubber seal strip to prevent air leakage. The aboveground fixed CO2 (APconv) was measured for 130 s after allowing the chamber to equilibrate for approximately 20 s after the chamber was placed on the plywood. The first 70 s of the measurement was used as an estimate of the
AP$_n$ rate. After the AP$_n$ rate measurement, the chamber was vented and repositioned, which was followed by the measurement of the aboveground respiration (AR) rate with the chamber covered by a thick layer of opaque tarpaulin (Street et al., 2007; Williams et al., 2014).

The total NSC content was calculated as the sum of the starch and soluble sugar contents, which were determined using the anthrone method with minor modifications (Yemm and Willis, 1954; Luo et al., 2015). Sugar and starch determinations were performed spectrophotometrically at 625 nm. The sugar and starch contents were presented as g g$^{-1}$ of dry matter. The C concentrations was assayed by dichromate oxidation (Bao, 2000).

Changes in the stem radius were monitored with an automated DD dendrometer (Ecomatik GmbH, Dachau, Germany) with a resolution of 0.1 µm. The dendrometer was mounted 5 cm above the ground on the stems of plants of both species. Stem radius changes were continually recorded at 1 min intervals. Diameter variations in stems can be used as a reliable predictor of phloem hydration (Zwieniecki and Holbrook, 2009; Hartmann et al., 2013b). In addition, the bark tissue was very thin in these young saplings; thus, the thickness of the bark was neglected (Hartmann et al., 2013b).

2.3. Statistical analyses

The first day that the values of the physiological traits significantly declined relative to the average values of the previous days was defined as the stress point (SP) (t-test, $P < 0.05$). It was assumed that no drought stress occurred before the SP and that any difference in physiological traits reflected the influence of drought alone. An independent samples t-test was used to test for statistical significance of the SWC at the SP, and one-way ANOVAs were used to test for statistical significance at the 95% confidence level using SPSS software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Treatment effects on stem diameter variation

The stem diameter increased before the SWC decreased to 9.05% in R. pseudoacacia and 12.13% in A. fruticosa. Then, a shrinkage of the mean daily stem diameter was observed before the plants were rewatered in the first drought, which showed a decrease of 208.37 µm in R. pseudoacacia and 132.18 µm in A. fruticosa, indicating a decline in the hydration of the phloem and the xylem (Fig. 2). After rewatered, the stem diameter increased significantly and then began to shrink when the SWC decreased to 11.98% in R. pseudoacacia and 10.12% in A. fruticosa. The daily stem diameter variation was higher when the SWC was higher during both drought cycles. The two species had different growth patterns when subjected to recurrent drought, the rate of stem diameter increase in R. pseudoacacia in the rewatered stage was similar to that of the control plants; however, in A. fruticosa, the rate of stem diameter increase was lower than in the control plants.

3.2. Leaf gas exchange, water potential and chlorophyll fluorescence parameters

Leaf gas exchange ($P_n$ and $g_s$) decreased rapidly when watering was stopped in both recurrent drought cycles (Fig. 3). Reewatered caused instantaneous increases in $P_n$, which showed no difference from the control ($P > 0.05$) in either species; however, the $g_s$ of R.
pseudoacacia did not fully recover to the control treatment value (P < 0.05) (Fig. 3b). Pn, g, during the two drought cycles dropped to close to zero (i.e., the physiological death point) when the SWC was 7.58% in the first cycle and 6.72% in the second cycle for R. pseudoacacia and 8.05% in the first cycle and 7.67% in the second cycle for A. fruticose. The $\Psi_p$ showed no significant difference when the SWC was higher than 10.82% in the first drought cycle and rapidly recovered in R. pseudoacacia, with no difference compared to the control (P > 0.05); the $\Psi_p$ showed a significant decrease when the SWC was lower than 13.21% in the second drought. The $\Psi_p$ of A. fruticosa showed a similar trend to that of R. pseudoacacia. Specifically, it showed a significant decrease when the SWC decreased to 11.17% and 15.15% in the first and second drought cycles, respectively, and fully recovered after three days of recovery. The SWC parameters of Pn, g, and $\Psi_p$ at the SP were higher in the second drought cycle than those during the first drought cycle (Table 2).

The fluorescence parameters did not change significantly until the SWC decreased below 10.41% and 10.52% in the first drought cycle in R. pseudoacacia and A. fruticose, respectively (Table 2), and then the $F_r/F_m$ and qP significantly decreased, and the NPQ increased as the SWC declined further (Fig. 4). In addition, the SWC parameters of $F_r/F_m$ and qP at the SP were higher in the second drought cycle than those during the first cycle in R. pseudoacacia. The change in the $F_r/F_m$, qP and NPQ was greater in R. pseudoacacia in the later stage of the second drought than that observed during the first drought; however, there was no significant change in the second drought in A. fruticosa.

3.3. Aboveground net C exchange of the plants and soil respiration

In the first drought cycle, the APn and AR of R. pseudoacacia decreased significantly when the SWC was lower than 11.64% and 14.16% (Fig. 5a), respectively, and the SWC at the SP of the APn and

<table>
<thead>
<tr>
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<th>R. pseudoacacia</th>
<th>A. fruticosa</th>
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<tbody>
<tr>
<td>$\Psi_p$</td>
<td>10.82</td>
<td>10.72</td>
</tr>
<tr>
<td>Pn</td>
<td>15.01</td>
<td>14.16</td>
</tr>
<tr>
<td>g</td>
<td>15.01</td>
<td>14.76</td>
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<tr>
<td>$F_r/F_m$</td>
<td>11.64</td>
<td>14.16</td>
</tr>
<tr>
<td>qP</td>
<td>9.79</td>
<td>9.72</td>
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<tr>
<td>NPQ</td>
<td>8.38</td>
<td>9.96</td>
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Table 2

The soil water content (SWC%) at the stress point (SP) for the predawn water potential ($\Psi_p$), photosynthetic rate (Pn), stomatal conductance (g), aboveground fixed CO2 rate ($F_r/F_m$), released CO2 rate (AR), soil respiration (SR), the maximum quantum efficiency of photosystem II ($F_r/F_m$), photochemical quenching (qP) and non-photochemical quenching (NPQ) in both species and drought cycles. *Indicates a difference between the two drought cycles, and – indicates the SP was not detectable in the drought.
AR in *A. fruticosa* were 10.72% and 12.91%, respectively, indicating that the AR was more sensitive than the APₙ in the first drought. In the second drought, the SWC at the SP of the APₙ in *R. pseudoacacia* and *A. fruticosa* were 13.21% and 12.92%, respectively, which was higher than that in the first drought; however, the SWC at the SP of the AR in the second drought was lower than that in the first drought in both species.

Drought also strongly inhibited root development and microbial activity. The control plants had well-developed root systems, in which the SR increased with the plant growth (Fig. 6). The SR decreased during both drought cycles, and was more sensitive than the other parameters to decreases in the SWC. The SWC at the SP was 15.01% and 16.41% of the SR in *R. pseudoacacia* and *A. fruticosa* in the first drought, respectively, and then it decreased slowly as the SWC decreased further. Interestingly, the SR values of both species increased sharply after rewatered, and were higher than the SR when the experiment began. Similar to the other traits, the SWC at the SP of the SR was lower in the first drought than in the second drought (Table 2), with values of 16.45% and 18.14% for *R. pseudoacacia* and *A. fruticosa* in the second drought, respectively.

3.4. Recurrent drought effects on the NSC and organic C content

The C pools in the plants were different in various tissues and were also affected by drought. The soluble sugar content was higher in the bark and root tissues in both species, and the starch content was higher in the roots, as was the total NSC content. We also found that the starch and total NSC contents were higher in *A. fruticosa* than in *R. pseudoacacia* overall (Fig. 7). In addition, the soluble sugar and starch and the total NSC concentration decreased in the leaves, branches and bark of *R. pseudoacacia* after the first drought, but showed a slight increase in the stems. Furthermore, the organic C content was increased in the various tissues after the first drought, except in the roots. However, in *A. fruticosa*, the leaves and bark showed a decrease in the soluble sugar and starch and the total NSC concentration after the first drought. In addition, the organic C content was increased in the various tissues, except in the leaves. The soluble sugar in the stems, bark and roots decreased after rewatered in both species. In contrast, the starch content was increased, and the total NSC content showed a different response after rewatered. In addition, the organic C content varied in both species after rewatered; in particular, the organic C content was increased in the leaves, stems and roots of *R. pseudoacacia*, whereas it was decreased in these tissues in *A. fruticosa*. The soluble sugar in the branches, stems, bark and roots showed an increase in both species after the second drought cycle, and the starch in the leaves, branches and roots showed a decrease. Furthermore, the total NSC showed a similar response in both drought cycles.

4. Discussion

Soil water availability is one important factor that limits terrestrial biological activity in an ecosystem (Huxman et al.,...
2004), and the importance of soil water availability is increasing as variations in precipitation patterns due to climate change (Easterling et al., 2000; Hoerling and Kumar, 2003) may cause droughts to become more frequent and severe in some regions (Myhre et al., 2013). Thus, it is necessary to better understand the effects of drought on plant functions, especially the underlying mechanisms.

Fig. 5. Time courses of the aboveground fixed CO₂ and released CO₂ rates of the drought R. pseudoacacia and A. fruticosa plants during recurrent drought cycles.

Fig. 6. Time courses of the soil respiration in the control and drought R. pseudoacacia and A. fruticosa pots during recurrent drought cycles.
of the responses of plants to recurrent drought (Blackman et al., 2009).

4.1. Advanced stress point in recurrent drought

All the studied physiological traits showed significant changes as the drought progressed during recurrent drought; however, the sensitivity of the various physiological traits differed among the traits and between the two species and the drought cycles. The results showed that the stem growth response was more sensitive than the other physiological traits, especially in R. pseudoacacia, as the stem increase rate began to decrease during the third day of the experiment compared to that in the control plants. This result supports previous findings that plant growth is often the first process to be affected due to the acute sensitivity of cell turgor and the effects of cell division, enlargement and differentiation (Galvez et al., 2011; Mitchell et al., 2014). In addition, the SWC at the SP of the fluorescence parameters was lower than the other traits (Table 2), which indicated that the PSII reaction centers were more strongly affected by the lower SWC (Maxwell and Johnson, 2000; Woo et al., 2008; Bresson et al., 2015).

During drought stress, C assimilation should be mainly responsible for the C balance because it shows larger decreases than respiration under drought conditions (Zhao et al., 2013). Plants close the stomata to prevent water loss under drought, which can reduce the CO2 diffusion in and out of the leaves, causing declines in Pn and assimilation (Flexas et al., 2006; Yan et al., 2016). Consistent with previous studies (Adams et al., 2009, 2013; Hartmann et al., 2013a), we observed an earlier decline in the AR compared to the APn under drought. The APn in the drought-exposed plants significantly decreased until 10 and 20 days and declined to the minimum level after 28 and 35 days in R. pseudoacacia and A. fruticosa (Fig. 5), respectively. The earlier decrease in the AR during drought may be mainly caused by a turgor-mediated decrease in plant growth (Muller et al., 2011), which occurred in R. pseudoacacia but not in A. fruticosa (Fig. 2). In addition, a direct reduction in mitochondrial respiratory capacity could also decrease the AR (Atkin and Macherel, 2009). We found that the SWC at the SP of the APn was lower than that for the AR and SR (Table 2) in the first drought, which indicated that the drought might have caused an NSC surplus during early drought because the drought caused greater reductions in growth and

![Fig. 7. Non-structural carbohydrate (NSC) concentrations (in g g⁻¹ of dry biomass) for soluble sugar and starch, total NSC and organic carbon (C) concentration (in g kg⁻¹) during the experimental period in different plant tissues. “Before” indicates the beginning of the experiment, “drought 1” indicates the end of the first drought cycle, “rewatered” indicates 7 days after recovery, and “drought 2” indicates the end of the second drought cycle. The different lowercase letters indicate a significant difference at P < 0.05. The lowercase letters above and below symbols represent A. fruticosa and R. pseudoacacia, respectively.](image-url)
maintenance respiration than the APn \( (\text{Hummel et al., 2010; McDowell, 2011; Pinheiro and Chaves, 2011}) \). The lower APn in the later stage of drought was also affected by the total number of leaves because the drought caused leaf shedding in \( K. \text{pseudocacia} \) and \( A. \text{frutcosa} \) of 4.2 g and 0.99 g per plant in the first drought and 4.6 g and 1.8 g per plant in the second drought, respectively. In the recovery stage, although the \( P_{\text{n}} \) at the leaf level almost recovered, the \( AP_{\text{n}} \) could not fully recover due to leaf shedding.

The SR is inhibited during both drought cycles \( (\text{Bryla et al., 1997; Huang and Fu, 2000; Thorne and Frank, 2009}) \). Roots are the first plant organ affected by soil drying; both the root growth and nutrient uptake of plants decline \( (\text{Espeleta and Eisenstat, 1998; Eisenstat et al., 1999}) \), as well as the root cell integrity \( (\text{Huang et al., 2005}) \). Moreover, lower microbial activity, higher root mortality \( (\text{Harper et al., 2005}) \) and a reduction in the photosynthetic C supply \( (\text{Huang et al., 2005; Flexas et al., 2006; Atkin and Macherel, 2009}) \) contribute to a further reduction in the SR during drought. However, the SR had a stimulating effect at the recovery stage, which was consistent with previous studies that showed that after a rewetting event, the SR increased rapidly \( (\text{Sponseller, 2007; Aanderud et al., 2011}) \) due to an increase in soil microbial and root activity. The later increase in the SR during the recovery stage was mainly due to increased root activity and biomass. However, lower SR rates were observed at a later stage of drought in the second drought cycle than in the first, primarily due to leaf shedding, which caused lower maintenance respiration \( (\text{Scagel and Andersen, 1997}) \). In addition, we found that the SWC at the SP of the SR was earlier than the leaves’ physiological traits, indicating that the SR was the most sensitive to the drought because the roots were the first plant tissue affected by soil drought \( (\text{Eisenstat et al., 1999}) \).

Although the two species examined here are morphologically different, a few general patterns occurred regarding the plants’ responses during recurrent drought stress. The physiological traits did not significantly decrease at the onset of treatment but showed a sharp decrease as the SWC decreased further, which indicated that the decline of the SWC in early stage of the experiment did not affect the physiological traits in the first drought \( (\text{Figs. 3 and 4}) \). However, the results showed different physiological responses between the two drought cycles in both species. In particular, the SWC at the SP in the first drought was lower than that in the second drought cycle, except the AR \( (\text{Table 2}) \). These findings indicated that plants respond to drought more rapidly to subsequent drought because the accumulation of proteins and transcription factors involved the drought process, which can enhance the response rate to subsequent drought stress \( (\text{Bruce et al., 2007; Boyko and Kováčik, 2011; Backhaus et al., 2014}) \) and also optimize C partitioning for osmotic adjustment to adapt to subsequent drought \( (\text{Hartmann et al., 2013b}) \). The lower SWC at the SP of the AR in the second drought observed in this study may be because the plants’ response to drought, which involves signaling proteins and transcription factors, was stronger during the second drought to protect themselves \( (\text{Bruce et al., 2007}) \); however, all these metabolic processes require metabolite and energy from respiration. In addition, this less sensitive response in recurrent drought of the AR to the SWC caused the plants to utilize the restored C and may have enhanced the risk of drought-related mortality \( (\text{Plaut et al., 2013}) \).

### 4.2. Different C distribution patterns in recurrent drought

When the free mobile NSC cannot meet the requirements of normal plant growth, C starvation may occur \( (\text{McDowell et al., 2011}) \). Although the threshold at which NSC availability fails to meet a plant’s growth demand is not defined, drought results in a larger decline in C assimilation without a concomitant reduction in C consumption \( (\text{McDowell et al., 2011}) \). In this study, C assimilation and respiration decreased with drought as expected \( (\text{Figs. 5 and 6}) \), and C assimilation showed a greater decline than respiration. In addition, the decrease in stem diameter \( (\text{Fig. 2}) \) suggested that the stem water potential was very low as the SWC decreased further \( (\text{Offenthaler et al., 2001}) \), although a phloem water deficit could explain 90% of the diurnal variation in stem diameter \( (\text{minus radial growth}) \) due to the link between xylem water potential and phloem functioning \( (\text{Zweifel et al., 2005}) \). The reduction in stem diameter in both species indicated a severe impact of phloem function and a strong C limitation \( (\text{no C left for stem growth}) \) or a shift in C allocation from plant structural growth to maintenance respiration during this period in drought-exposed plants \( (\text{Offenthaler et al., 2001; Hartmann et al., 2013b}) \). A lack of phloem transport would impede C translocation because the translocation of stored C from aboveground to belowground requires phloem loading and transport \( (\text{Hötttä et al., 2009}) \); thus, the impairment of phloem function would affect the C translocation and redistribution, resulting in C depletion in local tissues rather than whole-plant C starvation. Stem growth increased suddenly and substantially after rewatered, indicating relief from the limitation of phloem function caused by drought \( (\text{Hartmann et al., 2013b}) \) and suggesting a recovery of the C translocation and distribution pattern of the phloem.

In the present study, we found that the C concentrations in different issues showed no significant difference \( (\text{Fig. 7}) \); however, the soluble sugar content was higher in the bark and roots, and the total NSC content was significantly higher in the roots in both species. Furthermore, the results also showed that the soluble sugar, starch and total NSC content were higher in \( A. \text{frutcosa} \), which suggests a greater chance of survival under drought \( (\text{O’Brien et al., 2014}) \). The different C distributions in the different tissues suggest a different C allocation and balance under drought, which also indicates a switch in carbohydrates from growth to maintenance induced by drought to balance carbohydrate accumulation and plant growth \( (\text{Galvez et al., 2011}) \). We found an indication of soluble sugar depletion in the leaves, branches and bark after the first drought because soluble sugar is preferentially used by plants \( (\text{Klein et al., 2014}) \), whereas the roots showed an increase in soluble sugar but a decrease in starch, indicating that the roots began to utilize the starch and demonstrating a C deficit in the roots \( (\text{Galvez et al., 2011}) \). A decline in total NSC was observed in leaves due to maintenance respiration and a larger decrease in photosynthesis. In addition, the impeded phloem transport during drought was likely also responsible for the decreased NSC in the branches, bark and roots because impeded phloem affected the C translocation from source to sink and forced the sink tissues to progressively rely more on local C reserves for respiration and even on other respiration substrates, such as lipids \( (\text{Tcherkez et al., 2003; Hartmann et al., 2013b}) \). The depletion in the leaves, branches and bark indicated that the NSC could not meet the normal growth requirements of these tissues, which is consistent with previous results \( (\text{Muller et al., 2011; Hartmann et al., 2013b; Mitchell et al., 2014}) \). After rewatered, we observed an increase in NSC in the leaves due to the recovery of photosynthesis and an increase in roots and bark, which suggested recovery of C translocation and phloem function. However, a decrease in soluble sugar was observed in the branches, stems, bark and root tissues, mainly caused by the recovery of tissue growth because the soluble sugar could be used quickly for plant growth \( (\text{Klein et al., 2014}) \). The increase in starch indicated the synthesis of carbohydrates after rewatered was stored in the form of starch, which could be decomposed to soluble sugars for osmoprotection and osmoregulation when plants suffered drought \( (\text{Galvez et al., 2011}) \).
5. Conclusions

In conclusion, the two species studied here showed reduced leaf gas exchange and limited growth as the SWC decreased. However, the responses of both species differed between the first and second drought. The SWC at the SP for the fluorescence parameters was lower than that of the other physiological traits, and all of the SWC fluorescence parameters at the SP were higher in the second drought cycle than in the first, except the AR. As expected, both the APn and AR were reduced under drought. Although the leaf PA recovered fully, the APn did not fully recover due to leaf shedding. Moreover, the SR had a stimulatory effect at the recovery stage. In addition, drought strongly reduced the stem diameter, which impeded phloem function and caused a decrease in the NSC concentration in the plant tissues in both drought cycles, specifically the leaves, branches and bark showed NSC depletion after the drought. Finally, a decrease in soluble sugar was observed in the branch, stem, bark and root tissues after rewetted due to recovery of tissue growth.

Data accessibility

All the data used in this manuscript are presented in the manuscript.

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