Seasonal Drought Induces Hydraulic Dysfunction, Not Carbohydrate Depletion, for Robinia Pseudoacacia in a Semi-humid Forest

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Research

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Abstract

Background

Shifts in rainfall patterns that are associated with climate change are likely to cause widespread forest decline in regions where droughts are predicted to increase in duration and severity. However, causes of forest decline and their physiological mechanisms remain unclear, particularly the roles of carbon metabolism and xylem function. To explore the response of hydraulic architecture and non-structural carbohydrates (NSC) traits under seasonal drought, we conducted a manipulation experiment in a *Robinia pseudoacacia* plantation in 2015 and 2016 in Loess Plateau of China. Sap-flow, leaf area index, water potential, non-structural carbohydrate concentrations, and hydraulics in different organs were measured.

Results

The mean pre-dawn and midday leaf water potential after two growing seasons of drought stress was significantly lower (-2.2 MPa and -2.7 MPa, respectively) than those of control trees (-1.5 MPa and -2.0 MPa, respectively). Drought stress accelerated the loss of conductivity, and promoted the formation of narrow hydraulic safety margins, which indicated that hydraulic failure could be a good predictor of “physiological drought” in trees when subjected to two growing seasons of drought. Both sugar and starch concentrations in stems and roots were similar in all trees throughout the drought period, which indicated that trees maintained good coordination between carbon supply and demand when confronted with two growing seasons of drought.

Conclusions

Our results emphasized that hydraulic failure plays the predominant role in causing tree death during highly intense drought, while whether “carbon starvation” occurs during tree mortality remains to be tested in longer (multi-year) but less intense drought.

Background

Reduced precipitation may be responsible for tree mortality events across different forest and woodland ecosystems (Fensham et al. 2009; Allen et al. 2010, 2015; Lopez et al. 2021). However, in many cases, mortality rates vary among species or functional types (Breshears et al. 2005; Fensham et al. 2009; Mitchell et al. 2014) suggesting differences in drought resistance among co-occurring species. Despite improved understanding on how water deficits affect plant functioning and physiological mechanisms (Mitchell et al. 2013; Anderegg et al. 2016), the controls on species survival under extreme drought remain poorly resolved and limit our ability to adequately predict future changes in ecosystem structure and function.

Recent researches have studied physiological mechanisms that have been linked to a plant's capacity to regulate carbon and water balance under drought conditions (Mitchell et al. 2014; Anderegg et al. 2012, 2016). Two main physiological mechanisms to explain the eventual drought-induced mortality of trees have been formalized by McDowell et al. (2008): hydraulic failure and carbon starvation. The framework suggests that, for any given drought event, both plant hydraulic strategy and meteorological conditions will define the 'physiological drought' experienced by the plant. Physiological drought is a function of plant regulation of water-use in response to declining soil water potential and thresholds associated with hydraulic- or carbohydrate-mediated mortality (Mitchell et al. 2013). For example, hydraulic failure occurs when plant water potential falls below a critical value, which causes xylem cavitation and conductivity loss (Adams et al. 2009). Consequently, failure to supply sufficient carbon substrate to metabolism following drought-related reductions in photosynthesis and increased use of NSC occur, theoretically leading to carbon starvation (McDowell 2008; 2011). This conceptual framework has been extensively studied in recent years (Mitchell et al. 2014; Adams et al. 2018; Weber et al. 2017; Tomasella et al. 2019; Li et al. 2020).

An initial experimental test of these mechanisms was conducted inside an environmentally-controlled greenhouse with transplanted *Pinus edulis* under two temperature regimes (Adams et al. 2009). Subsequent attempts postulated that tree mortality due to drought is a complex process that can occur by multiple mechanisms (Sala et al. 2010). For example, hydraulic failure is expected to proceed more rapidly leading to fast mortality in plants that keep their stomata open during drought (i.e., relatively anisohydric plants) (McDowell et al. 2008). Under severe drought, incapacity to regulate plant water status above critical thresholds will promote xylem cavitation and death through dehydration (Mitchell et al. 2013). Radial stem growth, transpiration, and leaf water potential are significantly affected during chronic drought and as a consequence of these drought responses, temporal patterns of NSC can occur (Mitchell et al. 2014). During mild or short droughts, however, NSC reserves have been found to increase in multiple tree species (Sala et al. 2010; Palacio et al. 2014). Therefore, carbon starvation could occur from a reduction in tree carbohydrate reserves below a survival threshold during severe drought if these resources became inaccessible through inhibition of conversion of stored starch to sugar (Knoblauch and Peters 2010; McDowell et al. 2011).

Robinia pseudoacacia is a fast-growing tree with profligate water use. It has been introduced widely for plantations in the Loess Plateau of China due to its potential to adapt to drought and low soil fertility, and because of its ability to fix nitrogen. However, soil desiccation has caused degradation of *R. pseudoacacia* forest plantations whereby mature trees have developed abnormally short trunks (Chen et al. 2010; Jia et al. 2017). Previous studies examined carbon starvation and hydraulic failure in potted experiments with *R. pseudoacacia* (Zhang et al. 2015; Yan et al. 2020). They found that only severe drought stress significantly decreased NSC reserves, but it increased the loss of conductivity, which reflected the intrinsic drought resistance of xylem tissues. However, no previous studies have measured both carbon stress and hydraulic stress in mature and drought-stressed trees directly, which makes it difficult to distinguish between the interpretations of carbon stress and hydraulic stress.

To address these shortcomings, we conducted a two growing seasons of experiment, and tested the relationships between drought stress, NSC dynamics, and plant-water relations in a mature forest setting. With this experimental design, we examined the response of hydraulic architecture and NSC components under two growing seasons of drought in *R. pseudoacacia* in a seasonally temperate forest in the Loess Plateau of China. We hypothesized that tight hydraulic regulation of water and carbon uptake reduces the risk of rapid hydraulic failure, but may also result in increased duration of the physiological drought, promoting depletion of nonstructural carbohydrates.

Methods

Site description

Our study was conducted at Yehe National Forestry Center in the Qishui watershed, which had a 12-year-old *R. pseudoacacia* plantation located in Fufeng County, Shaanxi Province, which is situated on the southern Loess Plateau in China (34.55°N, 107.90°E; 1080 m a.s.l.). The forest covers an area of 2980 ha. The soil in the area is classified as Gleyic Phaeozems, and according to the World Reference Base for Soil Resources, these soils are 11% sand, 20% clay, and 69% silt (Zhang et al. 2018). Stand age is ten, stand density is ~ 1300 trees ha^{-1} , mean tree height is 9.23 ± 1.22 m, and mean diameter at breast height (DBH) is 8.65 ± 0.86 cm, with an average leaf area index (LAI) of ~ 2.05 (based on digital hemispherical photographs). Throughout the region, artificial afforestation communities, which included *R. pseudoacacia* and *Pinus tabulaeformis*, were established during the end of the 20th century. *R. pseudoacacia* accounted for more than 90% of the forest coverage. The dominant understory vegetation was *Stipa bungeana* and *Artemisia argyi*, which had a coverage of 80% – 90%.

The area has a semi-humid, temperate, continental climate. Mean annual precipitation was 592 mm, 80 % of which falls between June and October based on 45 years of meteorological data (1971–2016). Mean annual temperature was 11.5°C, and mean monthly temperature ranged from -2°C in January to 26°C in July. From Fig. 1, 2015 can be considered a wet year, while 2016 was a normal year.

Experimental design

This study focused on the first 2 years of a large precipitation manipulation experiment that was established at the site in summer 2015 (Zhang et al. 2018). The experiment consisted of two treatments: drought and ambient control. Treatments were applied to 20×20 m plots (400 m^2) that contained 50 target trees each. The two treatments were replicated in three randomly selected blocks for a total of six experimental treatment plots. The blocks were at least 500 m apart, and each had the same soil type and similar topography. In the drought treatment, rainfall was reduced by approximately 50% relative to ambient using a system of plastic panels and plastic-lined gutters that were fixed to rails approximately 1 m high. Drainage tunnels (30 cm deep) were trenched to remove the effect of through-flow of soil water. To control for any temporary damage to roots from the trenching, the control plot was also trenched to the same depth.

In 2014, an instrumented flux tower was installed near the tower base which continuously measured major environmental variables (Ma et al. 2017). Soil volumetric water content (VWC) was measured continuously using eight electrically conductive sensors (EC-5, Decagon, USA). The sensors were installed around the trees at depths of 10, 20, 40, 60, 80, and 100 cm in one drought plot and one control plot, and measured from June 2015 to December 2016 (Zhang et al. 2018). The VWC were powered and controlled a data logger (CR1000 Campbell Scientific, Logan UT, USA) which scanned every 30 s, and this was recorded the value of averaging with 10 min.

Sap-flow and leaf area index

Six *R. pseudoacacia* trees were selected randomly for sap flow measurements from the drought and control plots. The thermal dissipation probe (TDP) method (Granier 1987) was used to measure the sap-flow density, and it was calibrated with the empirical equation established by Ma et al. (2017). We used 10-mm-long sensors, and the detailed information about the sensors is described in Peng et al. (2015). All of the TDP sensors were connected to a CR1000 data logger with an AM16/32 multiplexer (Campbell Scientific, Logan UT, USA). The temperature difference between the upper heated probe and the lower reference probe was measured every 30 s, and this was recorded the value of averaging with 10 min. The stand transpiration (Q , mm) was calculated as the product of sap-flow density and sapwood area of the plot. The sapwood area was determined by cutting neighboring trees and discriminating between the sapwood and the heartwood based on color differences.

The LAI for the two treatments was estimated indirectly twice a month at the center of each plot. The LAI was calculated in June–November 2015 and April–November 2016 by processing digital hemispherical photographs, which were collected under uniform sky conditions near dawn and sunset on clear days. Each photograph was analyzed using CAN-EYE 4.0 software (Demarez et al. 2008) to calculate an index of integrated light availability, expressed as percent open sky, at each measurement location (Ambrose et al. 2016). The camera and lens were Nikon D90 and Nikon AF DX 10.5mm f/2.8G ED, respectively.

Leaf water potential

Pre-dawn and midday leaf water potentials (ψ_{pd} and ψ_{md} , respectively) were measured once a month during the study. Five randomly-selected trees per plot were measured from May–October 2015 and May–October 2016. Two south-facing twigs with healthy foliage were collected from each selected tree before sunrise and immediately measured with a portable water potential pressure chamber (615D, PMS Instrument Company, Albany, OR, USA).

Native embolism and the vulnerability curves

In all cases, current year extension growth from the uppermost branches exposed to full sun was sampled. We limited the measured material to current year growth to avoid embolism that originated from conditions prior to the growing season (i.e. no freeze-thaw embolism) and to facilitate the cleanest cuts possible, thus reducing the possibility that damage to the xylem during harvest would affect our results. Zhang and Wang (2016) showed that the mean vessel length for *R. pseudoacacia* is 19.6 cm. In our study, therefore, branches longer than 50 cm were cut from the trees in the early morning and placed

immediately in black plastic bags to prevent dehydration. We also collected coarse root (diameter about 3 cm) segments longer than 40 cm which were immediately placed in black plastic bags. Branch and root samples were collected using the same protocol as above 'Leaf water potential measurements' occurred during October 2015 and October 2016, and for these measurements, we used the same sample trees as we used for the analysis of leaf water potential. Excised samples were immersed in water immediately and transported to the laboratory within 1 hour.

For branches and roots, we measured native and vacuum-infiltrated hydraulic conductivity. The exposed segments were cut underwater into 30-cm-long segments. The sections were attached to a tube that was filled with degassed and 0.2 μm -filtered 20 mmol/L KCl solution, and a gravitational pressure of 0.01 MPa was used to force the solution through the segments. Hydraulic conductivity was measured with a 0.1 mg precision balance. Then, we vacuum-infiltrated the samples for 1 h to refill any embolized conduits, and we re-measured conductivity (Kolb et al. 1996). We calculated percent loss of conductivity (*PLC*, %) as the percent difference between native and vacuum-infiltrated conductivity. Note that in all cases, measurement segments excised under water may prevent artifacts (Sperry 2013).

Branch and root vulnerability curves (VCs) can be used to quantify the percentage loss of conductivity by air-injection method (Cai et al. 2010; Venturas et al. 2015). These partially debarked segments slightly longer than 30 cm were mounted in the double ended pressure collar. Each segment was flushed at 200 kPa water pressure for 30 min to remove embolism. The basal cut end was connected to a 1 m- long tube of approximately 25 mm in internal diameter, which is large enough to allow the escape of air bubbles coming out of the stem. The pressure was increased from 0 to 4 MPa, with each step followed by 10 min of relaxation, and flow was then measured at a constant background of 100 kPa air pressure until 88% of conductivity was lost. From the sigmoid shape of a VCs, the three variables were acquired: (i) the xylem pressure (ψ_x) that corresponded to 50% loss of hydraulic conductivity (ψ_{50}), the most commonly used index of embolism resistance; (ii) the air entry threshold (ψ_e) that corresponded to about 12% loss of conductivity, where PLC begins to rise sharply with declining ψ_x ; and (iii) $\psi_e - \psi_{50}$, which was defined as the hydraulic safety margin, where a larger value of $\psi_e - \psi_{50}$ indicated a more gradual rise of PLC after ψ_x had fallen below ψ_e (Meinzer et al. 2009).

NSC analysis

Using samples cut by a tree climber from fully sunlit twigs, five leaves were taken from the same trees as for *PLC* analysis. Samples of tree stem tissue were taken using a 4.3 mm diameter increment borer (Häglof Company Group, Långsele, Sweden) at breast height; this sampling was collected from five different trees to avoid excessive damage that would occur by repeated boring the same tree. The coarse roots (diameter > 5 mm) were also collected from different trees. All these samples were collected late in the morning and were immediately labeled and frozen by immersion in liquid nitrogen. Samples were collected in June and November 2015, and April to October 2016.

Here, NSCs are defined as starch plus soluble sugars, which included sucrose, glucose, and fructose. All samples were oven-dried at 70°C to a constant mass and then fine ground to a powder with a ball mill

(FOSS CT410, Sweden). The analyses for determining NSC followed Andrew et al. (2013). About 0.5 g of each plant organ powder was vacuum-infiltrated with 80% ethanol for 15 min, and then it was centrifuged at 7000 rpm for 10 min. A further two extractions were carried out with 80% ethanol. Supernatants were combined, filtered through a 0.45-µm syringe filter, and analyzed for soluble sugars. The ethanol-insoluble pellets were used to determine starch content.

The ethanol-soluble fractions were analyzed using a Waters Alliance high-pressure liquid chromatographic (HPLC) system (Milford, MA, USA) (Andrew et al. 2013). The separated soluble sugars were identified and quantified with known standards and expressed as per cent of dry matter.

Starch was extracted from the remaining dry matter in a boiling solution of 0.02 NaOH for 1 h, followed by hydrolysis to glucose with α-amylglucosidase (EC 3.2.1.3, Boehringer Mannheim Biochemicals, Mannheim, Germany). Glucose that was formed by hydrolysis was measured colorimetrically at 340 nm (spectrophotometer model 2550, Shimadzu, Japan). Starch concentrations were calculated from standard curves and expressed as per cent of dry matter.

Statistical analyses

A one-way analysis of variance (ANOVA) was used to test the effect of treatment on DBH, LAI, leaf water potential, *PLC*, and plant water use at each sampling date ($P < 0.05$). A one-way ANOVA was used to test the effect of treatment on the sugar and starch concentrations for each plant organ (leaf, stem, and root) at each sampling date ($P < 0.05$). Relationships between ψ_x (negative value) and *PLC* in stems and roots were fitted using a three-parameter, sigmoidal regression function (Wang et al. 2015; Zuo et al. 2012):

$$PLC = a / (1 + e^{-(x-x_0/b)})$$

All statistical analyses were performed using SPSS v.18.0 (SPSS, Inc., Chicago, USA).

Results

Plant water status

Daily stand transpiration (Q_d) was obtained for each measurement day. The Q_d ranged from 0 to 2.84 mm, and it exhibited pronounced diurnal variations (Fig. 2a). The daily average Q_d (1.06 mm day^{-1}) in the drought treatment was significantly lower than in the control (1.75 mm day^{-1}) ($P = 0.045$) due to the significant decrease in LAI (Fig. 2b). During the measurement period in 2015, total water use was similar between the drought treatment and the control. In 2016, however, total water use in the drought treatment (272 mm) was significantly lower than that of the control (378 mm) ($P = 0.038$) (Fig. 3).

As the drought treatment progressed, ψ_{pd} fluctuated between -0.6 and -2.6 MPa, with an average of -1.7 MPa. The ψ_{md} exhibited a steady decline throughout the drought and reached a mean minimum of -3.0 MPa after two growing seasons of drought (Fig. 4). Both ψ_{pd} and ψ_{md} in the drought treatment were significantly lower than in the control since June 2016 ($P = 0.036$). Control trees displayed comparatively tighter stomatal regulation, where monthly stand transpiration (Q_m) declined more rapidly with changes in ψ_{md} than in the drought (Fig. 5). These patterns were also reflected in the rate of water loss in response to declining water availability, as indicated by the slope of ψ_{md} compared with Q_m , which was larger for the control (50 mm) and significantly smaller for the drought (22 mm; Fig. 5).

Branches in the drought trees had significantly higher percentage loss of conductivity than their paired control baseline in 2016 ($P < 0.001$). Also, the percentage loss of conductivity of root segments in the drought trees (71%) was significantly higher than that of the control trees (37%) in 2016 ($P < 0.001$, Fig. 6). Thus, drought led to notable increases in loss of hydraulic function in this experiment, especially in roots. We also determined xylem ψ_{50} and ψ_e of branches and roots for the control and drought plots. After two growing seasons, ψ_{50} in stems and roots exhibited approximately -1.8 and -1.4 MPa in control trees, respectively, compared with -1.2 and -0.8 MPa in drought trees, respectively. The ψ_e in stems and roots showed similar patterns. The hydraulic safety margin expressed as $\psi_e - \psi_{50}$ in the control was 40% – 70% higher compared with the drought plots (Fig. 7).

Patterns of NSC

The seasonal changes in sugar and starch concentrations, which varied by 50% – 90%, were much larger than any differences associated with the drought treatment (Fig. 8). As the 2016 growing season progressed, leaf sugar concentration increased on average from 3.2–6.6%, but starch decreased on average from 5.9–3.1% (Figs. 8a and 8b). For stems and roots, sugar and starch concentrations increased consistently throughout the growing season.

Different tree tissues (leaves, stems, and roots) showed different responses in NSC concentration to the progression of the drought (Fig. 8). At the start of the drought experiment, leaf sugar concentrations were similar for droughted and control trees (means 2.5 ± 0.2 and $2.6 \pm 0.2\%$ dry mass, respectively, $P = 0.169$). For droughted trees, we found a significant decline in leaf sugar concentration over the course of the experiment (Fig. 8a, $P = 0.048$). This pattern was also found in leaf starch (Fig. 8b). Neither the concentrations of soluble sugar nor starch were depleted significantly in stems and roots from the drought treatment relative to the control (Fig. 8c, d, e, f).

Discussion

Hydraulic responses to drought

To examine the role of hydraulics responses to drought, we measured native and vacuum-infiltrated hydraulic conductivities of stems and roots. Our data showed that two growing seasons of drought could lead to notable increases in loss of hydraulic function of root and branch segments (Fig. 6). It seems that hydraulic failure may have been induced by xylem tension exceeding cavitation thresholds due to progressive water loss, for example, through leaf and cuticular tissues, or when fast decline in water potential occurred (Fig. 4, Sevanto et al. 2014). In addition, in our study, we have identified two alternative hydraulic safety parameters (ψ_{50} and ψ_e) referenced to species-specific values of daily minimum leaf water potential (Fig. 7). Physiologically, attainment of ψ_{50} indicates that nearly catastrophic hydraulic failure has already occurred and that the risk of further runaway embolism is high because xylem pressure is operating along the steepest portion of the vulnerability curve (Tyree and Sperry 1988). Thus, the relevance of ψ_{50} as an indicator of resistance to hydraulic failure may be restricted to extreme episodic drought conditions under which it becomes physically impossible for the stomata to constrain xylem pressure above or around ψ_e (Meinzer et al. 2009). In the present study, for branches and roots, ψ_{50} and ψ_e in droughted trees were higher than in the control (Fig. 7), which was consistent with the acclimatisation of these traits to drought (Anderegg et al. 2016). These results indicated that branches and roots of *R. pseudoacacia* may regularly experience a substantial loss of their conductivity even when soil water availability is not severely restricted.

In our study, the hydraulic safety margin $\psi_{50} - \psi_e$ indicated a progressively more gradual slope of the VC after the air entry threshold had been crossed. This can be regarded as another hydraulic safety parameter of defence against catastrophic xylem failure once stomatal regulation can no longer prevent xylem pressure from entering the steeper portion of the vulnerability curve (Meinzer et al. 2009). The specific xylem structural features responsible for the observed behavior of $\psi_{50} - \psi_e$ are not currently known. Most likely they relate to the incidence of safety features in the population of cells contributing to embolism avoidance, which could include not only vessels, but also parenchyma and fibre cells (Meinzer et al. 2009; Adams et al. 2018). In the present study, the branches and roots operated with narrow (< 1 MPa) hydraulic safety margins, which suggested that *R. pseudoacacia* were exposed to the risk of tree decline during two growing seasons of drought (Nardini et al. 2013).

NSC during the drought

The responses of NSC during drought represent the balance among the assimilation, respiration, and export of carbon across different source and sink tissues (da Costa et al. 2010). We demonstrated that after two growing seasons of water deficit, *R. pseudoacacia* showed no significant change in NSC concentrations in the roots and stems (Fig. 8). Similar NSC phenomena across drought trees have already been shown in some regions (Anderegg et al. 2012; Adams et al. 2013, 2018; Dickman et al. 2014; Zhang et al. 2020). The maintenance of NSC concentrations during drought conditions suggested that the sustainability of growth during drought did not occur at the expense of depleted carbon stores. However, Zhang et al. (2015) reported that *R. pseudoacacia* had significantly lower NSCs under severe drought (i.e., 6% soil gravimetric water content) in potted experiments. In our study, despite two growing seasons of drought, the use of NSCs was unaltered in roots and stems, which implied that *R.*

pseudoacacia did not draw significantly upon their NSC reserves to buffer against the short-term effects of soil moisture deficit. Many past investigations have documented no change, and often even an increase, of C reserves in trees exposed to drought (Anderegg et al. 2012; Sevanto et al. 2014). An explanation for such phenomenon is that growth and respiration demand for C increases faster than photosynthetic activity; a response known as source limitation (Korner 2003; Weber et al. 2017). By contrast, Wiley and Helliker (2012) proposed that higher C storage can help species to better survive environmental stress like drought, and increased NSC concentrations might thus even exacerbate C limitation to growth.

However, leaf carbohydrate resources were diminished during drought as leaf NSCs declined by 26% compared with the control, but not in roots and branches (Fig. 8), which indicated a proportionally larger drawdown of leaf NSC after two growing seasons of drought. These differences may be related to characteristics of carbon allocation and transport. Under optimum conditions, tissues near to the leaves, the major carbon source, appear to be favored in carbon allocation at the expense of other distant tissues (Jordan and Habib 1996). Under drought stress, however, trees tend to allocate more carbon to roots and branches to facilitate water absorption and survival (Chapin et al. 1990). For a continuous controlled drought pot experiment, the synthesis of starch was inhibited under drought stress, and the activity of hydrolases was accelerated, which resulted in a decrease in starch (Zhang et al. 2015). The decline in starch may have indicated that there was a relatively small carbohydrate pool for leaves that maintained only a small buffer when the demand for carbon became larger than the supply.

Interplay between hydraulics and carbohydrates

We examined carbon and water status in *R. pseudoacacia* to develop an understanding of the interdependence of carbon- and water-use strategies. In contrast to existing conceptual models (Amthor and McCree 1990), we found that there was not necessarily a generalized plant response to drought that was characterized by accumulation of carbohydrates during drought (Mitchell et al. 2014). Our results showed that droughted *R. pseudoacacia* trees succumbed to pronounced hydraulic failure (i.e., relatively rapid decline in hydraulic conductivity) (Fig. 6, Fig. 7), but not to carbon starvation (i.e., lower carbohydrate concentrations in stems and roots) (Fig. 8), which suggests a decoupling of hydraulic failure and carbon starvation (McDowell 2011). The decoupling strategy of *R. pseudoacacia* may have increased the capacity of hydraulics to recover from the two growing seasons of drought (Galiano et al. 2011). Generally, carbohydrates supposed to play an important role in refilling embolized conduits (Secchi 2011), and if the carbohydrates can be transported to where they are needed, this may prevent hydraulic failure. However, embolized conduits diminish the hydraulic capacity of the plant, which in turn can limit its photosynthetic capability (Sperry 2000). This may exhaust carbohydrate reserves, which implies that prolonged drought may cause hydraulic failure and limit carbohydrate utilization (McDowell 2011).

Conclusions

The interdependence of carbon- and water-use strategies can help us to understand the physiological mechanisms that underlie plant responses during water deficit. Following two growing seasons of drought, our results suggested that the *R. pseudoacacia* experienced hydraulic dysfunction, but not carbohydrate depletion. The hydraulic failure was reflected mainly in the accelerating loss of hydraulic conductivity, lower water potential and xylem transpiration, and the narrow hydraulic safety margins. Furthermore, trees maintained a fairly good coordination between carbon supply and carbon demand when confronted with two growing seasons of drought. Overall, drought stress affected hydraulics more than it affected carbon storage. Our results provide a means of identifying the response of physiological mechanisms during two growing seasons of drought. Our findings also emphasized that hydraulic failure plays the predominant role in causing tree death during highly intense drought, while whether "carbon starvation" occurs during tree mortality remains to be tested in longer (multi-year) but less intense drought.

Declarations

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Author's contributions

All authors contributed to the present paper preparation. ZQY developed methodology, analysed and interpreted the data and wrote the paper. JXX contributed to conceptualization, data collection, methodologies development, manuscript writing and reviewing. SMA was involved in project management tasks and final manuscript review. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

(a) Temporal variation in measured values of precipitation at the study site and (b) soil volumetric water content (0-1 m) in the drought and control treatments from June 2015 to November 2016. The gray area indicates the period of sap-flow measurement.

Figure 2

(a) Temporal variation in measured values of daily stand transpiration and (b) leaf area index (LAI) in *R. pseudoacacia* from June 2015 to November 2016 in the drought and control treatments. Error bars indicate the standard deviation.

Figure 3

Total water use measured for *R. pseudoacacia* stands in 2015 and 2016 in the drought and control treatments. Error bars indicate the standard deviation.

Figure 4

(a) Progression of pre-dawn and (b) mid-day leaf water potential in *R. pseudoacacia* from June 2015 to November 2016 in the drought and control treatments. Asterisks denote significant treatment differences based on pairwise comparisons ($P < 0.05$). Error bars indicate the standard deviation.

Figure 5

Relationships between mean mid-day, leaf water potential (Ψ_{md}) and monthly stand transpiration (Q_m) for the drought and control treatments. Each treatment data set was fitted with a linear regression ($P < 0.05$); Droughted trees: $Q_m = -22.56\Psi_{md} - 25.08$, $R^2 = 0.42$; Control trees: $Q_m = -50.32\Psi_{md} - 50.82$, $R^2 = 0.55$.

Figure 6

Pair-wise difference in native embolism for (a) branches (b) and roots of *R. pseudoacacia* in the drought and control treatments. Different lowercase letters indicate significant differences between treatments within each year, and different uppercase letters indicate significant differences between years within each treatment ($P < 0.05$). Error bars indicate the standard deviation.

Figure 7

Typical xylem VCs that shows the relationship between the percent loss of conductance for branches (a, b) and roots (c, d) and xylem pressure for the drought and control treatments. The xylem pressures that correspond to 50% loss of conductance (Ψ_{50}) and the air entry threshold (Ψ_e) are shown. The difference between Ψ_{50} and Ψ_e (shaded area) corresponds to a “hydraulic safety margin”.

Figure 8

Temporal changes in NSC for the different plant organs (± 1 SE) in the drought and control treatments. Soluble sugars (a, c, e) and starch concentrations (b, d, f). Asterisks denote significant treatment differences at each sampling date based on pairwise comparisons ($P < 0.05$).