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

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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 rewatered, 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 rewatered 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

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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 (Munné-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 cause changes in 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 (Resco 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 C_3 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 plots 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 (Ψ_p) 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, 40 L, 20 L and 20 L of water was added on the first, fourth and seventh days of the recovery stage, respectively. The soil water content (SWC) after rewatered 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 1
The physical and chemical properties of the soil used in this study.

Property	Value
Taxonomy	Udic Haplustalf
Texture	
2000–50 μm (g kg^{-1})	64
50–2 μm (g kg^{-1})	694
<2 μm (g kg^{-1})	342
Bulk density (g cm^{-3})	1.27
pH (H$_2$O)	8.30
Water-holding capacity (%)	22.8
Soil organic carbon (g kg^{-1})	7.45
Soil total nitrogen (g kg^{-1})	0.84
Soil total phosphorus (g kg^{-1})	0.69